



ISSN (P): 3105-7071  
ISSN (O): 3105-708X  
INNOV STEAM RES EDU  
WWW.TUFSTEAM.COM  
<https://doi.org/10.63793/ISRE>

# INNOVATIONS IN STEAM: RESEARCH & EDUCATION

An International Journal

VOLUME 3 ISSUE 2 2025

ARTICLES: 25030201-25030205

# Innovations in STEAM: Research & Education

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**Innovations in STEAM: Research and Education** (acronym ISRE; abbreviated Innov STEAM Res Edu) publishes peer reviewed papers on all facets of STEAM (Science, Technology, Engineering, Arts, Management). The journal publishes reviews (submitted as well as solicited), full-length research articles, short communications, case studies, extension articles, product registration etc. Submissions made for consideration by ISRE must be original and must not be considered for publication elsewhere simultaneously.

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## Fabrication of an Extended Electric Bike

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### METADATA

#### Paper history

Received: 20 February 2025

Revised: 27 June 2025

Accepted: 29 August 2025

Published online: 26 September 2025

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#### Keywords

Hybrid electric vehicle

Urban bike

Energy efficiency

Fabrication

Fossil fuel

#### Citation

Shahid M, Hafeez MA, Khan MU, Anns M, Abdullah M, Arif Z (2025) Fabrication of an extended electric bike. *Innovations in STEAM: Research & Education* 3: 25030201.

<https://doi.org/10.63793/ISRE/0026>.

### ABSTRACT

**Background:** The fabrication of an Extended Electric Bike (EEB) has been developed as a practical solution for low-income communities. It aims to reduce pollution and ensure compliance with government regulations. Surveys were conducted to gather feedback on electric and hybrid bikes, which supported the design process.

**Objective:** The objective of EEB is to create a user-friendly hybrid bike that utilizes both fossil fuel (petrol) and batteries, providing a sustainable and convenient mode of transportation.

**Methodology:** The system was configured to utilize a petrol-powered alternator for charging onboard batteries, which subsequently supplied power to a brushless direct current (BLDC) motor for propulsion. Before the operation, the batteries were fully charged to ensure continuous performance. During operation, the alternator recharged the batteries, thereby extending usage time without the need for external charging. In cases of battery depletion, the petrol engine served as a backup power source. An Arduino microcontroller was employed to regulate system functions, including real-time monitoring of battery status and automatic switching between petrol and battery power.

**Results:** The fabricated design successfully integrated petrol and battery power. The alternator-based charging system eliminated the need for external charging, while the Arduino-controlled unit ensured efficient power management and enabled Internet of Things (IoT) functionality.

**Conclusion:** The EEB design effectively addressed transportation challenges by offering a dual-power system that reduces pollution, supports low-income communities, and enhances user convenience through smart control features.

## INTRODUCTION

In many developing countries, energy resources are limited, and environmental protection is often not given priority (Zahedi *et al.* 2025). Internal combustion (IC) motorcycles are a major source of pollution, releasing carbon dioxide, carbon monoxide, sulfur oxides, nitrogen oxides, and lead (Sugiarto *et al.* 2025). These emissions create serious environmental problems. Electric motorcycles provide an alternative by supplying power directly to the motor rather than relying on fuel combustion. Unlike IC engines, they do

not consume fuel or produce exhaust when idling. However, electric motorcycles also have limitations. Under heavy load, the battery drains more quickly, which reduces speed and can eventually stop the bike. In addition, the absence of portable charging systems makes them less practical, and without such systems, their use becomes restricted. The concept of an extended electric bike has the potential to address these issues by improving performance, reliability, and efficiency. At the global level, electric vehicles are gaining attention because of both economic and environmental concerns. Rising oil demand, increasing fuel



prices, and the effects of climate change have accelerated this shift. The transport sector is one of the largest contributors of greenhouse gases, including CO<sub>2</sub> and CH<sub>4</sub>. In recent years, environmental awareness and the search for cleaner energy alternatives have grown to a stage where they cannot be ignored. As a result, electric power in transportation is expanding, reflecting the global move toward sustainable and pollution-free mobility. In Pakistan, the demand for fuel-efficient and environmentally friendly motorcycles is particularly high because a large part of the population lives in urban areas. Vehicles that combine conventional engines with electric motors can reduce fuel use and emissions. Fossil fuel, mainly petrol, is still widely used, but the country is not entirely dependent on it. New technologies such as regenerative braking, where the electric motor on the wheel reduces vehicle speed while recharging the battery, further lower the power demand (Sheu 2020; Nosratzadehi *et al.* 2025).

Although electric vehicles are more efficient than IC engine motorcycles, their higher cost and lower speed have limited their adoption. Increasing production can make them more affordable and attractive to users. A recent advancement is the extended electric bike, which has strong potential to expand its access to sustainable and pollution-free mobility. The addition of Internet of Things (IoT) features enhances this design by allowing real-time monitoring, GPS tracking, route information, emission detection, and advanced security systems. These advantages demonstrate the promise of IoT-based extended electric bikes in addressing transportation challenges (Hadayat *et al.* 2025).

## MATERIALS AND METHODS

The extended model of the electric bike is designed to operate on the principle of dual-drive functionality, utilizing two independent power sources. The motor receives energy first from a battery and subsequently from an alternator.

### *Battery source*

The motor is powered by a 20Ah battery operating at 48V. Dry-cell batteries manufactured by YUASA were employed for this purpose. The battery consists of eight individual 12V cells connected in series as four sets of two cells. This configuration enables the system to deliver 48V and 20A under full operating conditions. The battery supplies power to the motor, thereby propelling the vehicle.

### *Charging methods*

Two distinct charging methods were adopted. The first is the plug-in charging method, utilizing electricity from the grid, which is readily available. Charging requires 4–5 hours and consumes approximately 1.5 units of electricity to reach full capacity. When fully charged, the vehicle can travel approximately 60 kilometers at maximum speed while

carrying loads up to 250 kg. The maximum speed achieved under these conditions is 60 km/h. Upon ignition, the controller evaluates the charge status and displays the remaining power. If the charge level falls below 15%, the controller automatically initiates engine ignition. The second method is alternator-based charging. In this configuration, the alternator derives mechanical power from the engine once it is activated. The alternator supplies power to both the engine and the battery while simultaneously recharging the latter.

A boost converter was integrated to maximize charging efficiency by amplifying the current and filtering harmonic distortions. The dynamo, coupled with the motor, enables charging during motion. Four LED indicators reflect charging status, with each light representing 25% capacity. Once the battery reaches full capacity, the engine disengages automatically, and the battery assumes exclusive operation of the motor. The tachometer is monitored by the controller to assess vehicle speed, enabling real-time adjustment of motor revolutions per minute. A GPS module was incorporated to provide continuous location tracking, enhancing safety and security (Fig. 1).

### *Construction methodology of the extended electric bike*

The fabrication methodology of EEB is presented in Fig. 1. The dual-drive bicycle is powered by a 48V, 20Ah battery assembled from eight series-connected 12V cells. Charging can be achieved through plug-in connection, requiring 4–5 hours, or via the alternator powered by the vehicle's engine. The alternator provides direct energy to the motor while recharging the battery. A boost converter enhances efficiency by regulating voltage and eliminating harmonics. Once the battery attains full charge, the system automatically transitions to battery-only operation. The controller regulates motor speed according to variations in voltage and current, while the GPS module provides continuous location tracking for improved safety.

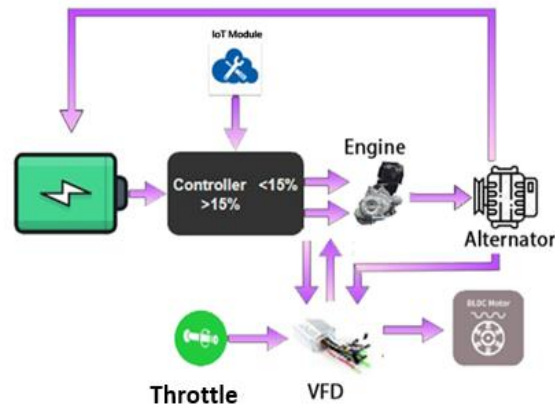
### *Component analysis*

During construction, an integrated electrical framework was established to facilitate the reliable performance of the EEB. When the system is activated, the electric mode becomes operational. The electrical configuration comprises several core components:

**BLDC hub motor:** A 1000W, 48V hub motor was employed. Hub motors have become increasingly popular for lightweight electric vehicles, including e-bikes and scooters, due to their efficiency and compact design (Siddique *et al.* 2020). Load-bearing capacity and resistance forces were considered during motor selection.

**Controller for BLDC hub motor:** A 1000W, 48V speed controller was utilized to regulate the hub motor. Brushless DC motors offer advantages over brushed motors due to electronic commutation, which allows efficient current





**Fig. 1:** Methodology of the system

**Table 1:** Specifications of BLDC Hub motor

Specifications	Ratings
Power	1000 W
Torque	18 Nm
Peak torque	83 Nm
Voltage Operation	48 V
Current	23 A
Limiting current	38 A
RPM	510

**Table 2:** Specifications of Controller

Specifications	Rating
Voltage	48 V
Power	1000 W
Phase Angle	120/60°
Limiting current	38 A

**Table 1** Specifications of a dry battery

Specifications	Rating
Voltage	48 V
Maximum current	20 A
Charging time	3 h
Weight	17.6 kg
Dimension	5.95×2.56×3.7 inches

switching. The controller enables starting, stopping, reversing, and precise regulation of torque and speed. Specifications of the controller are provided in Table 1–2.

**Dry battery:** A 12V, 9Ah sealed dry battery with high energy density and leak-proof design was adopted. Such batteries are widely used in UPS, CCTV, and fire monitoring systems. In EEB, a combination of these units was arranged to provide 48V/20Ah, with a discharge capacity of 20A per hour. The batteries were positioned beneath the seat to conserve space and maintain balance, thereby supporting eco-friendly operation.

**Charger (AC to DC):** The charger employs a front-end AC-DC converter, allowing connection to a residential AC supply (Hazarathaiyah *et al.* 2019). This ensures accessibility for routine charging. Specifications are shown in Table 3.

**Alternator:** An alternator powered by fuel was integrated into the system. This component supplies energy when the battery charge is insufficient, ensuring uninterrupted operation. Placement of the alternator was in the carburetor position of the base motorcycle, making it a critical feature of the extended system.

**Boost converter:** The boost converter stabilizes voltage, filters harmonics, and improves energy transfer efficiency, ensuring continuous battery charging during engine operation.

**Self-start motor:** A self-starting mechanism was implemented to enable ignition without external assistance. Once combustion is initiated, inertia sustains engine cycles without requiring repeated starter use.

**GPS module:** The GPS module, integrated as part of the IoT-based security system, provides anti-theft protection, remote locking, and real-time location monitoring. The module disables the motor within 10 sec if unauthorized movement occurs. Additional features include monitoring of charging status and battery condition.

**Bike frame:** The Honda Pridor was selected as the structural base. This motorcycle is equipped with an overhead cam four-stroke engine, refined suspension, improved aerodynamics, and a durable frame. Its robust design made it suitable for integration with the extended electric system.

### *Mechanical design and modeling*

Positioning of electrical and mechanical components presented a key challenge, as integration was required without compromising aesthetic appearance. Careful design and adjustments were implemented to arrange components in a manner that maintained both structural appeal and functional efficiency.

### *Speed parameters controller*

Nominal power=35 kg  
 Nominal voltages=48 V  
 Nominal current=30 A  
 Efficiency = 90%  
 Protection voltage=60 V

### *Torque calculations*

Weight of the bike=80 kg  
 One person's average weight=70 kg  
 Batteries weight=20 kg  
 Alternator weight=5 kg  
 Total weight= $m \times g$ .....(1)  
 Total weight=175×9.81  
 Total weight=1715 Newtons

### *Force required to displace the body*

Rolling friction between rubber and coal tar=0.05

$$F = \mu \times \text{Total weight acting downward} \dots\dots\dots (2)$$

$$F = 0.05 \times 1715$$

$$F = 85.75$$

$$\text{Wheel diameter} = 45.75 \text{ cm}$$

$$\text{Wheel radius} = 22.875 \text{ cm}$$

$$\text{Torque} = r \times F \dots\dots\dots (3)$$

$$\text{Torque} = 0.227 \times 85.75 \text{ cm}$$

$$\text{Torque} = 19.4 \text{ Nm}$$

$$\text{On one wheel, it will be} = 9.73 \text{ Nm}$$

#### Wind load estimation

The maximum velocity of the design

$$V(\text{max}) = 70 \text{ km/h}$$

$$V(\text{max}) = 19.66 \text{ m/s}$$

$$\text{Wind pressure} = \text{constant} \times \text{wind density} \times$$

$$V(\text{max})^2 \dots\dots\dots (4)$$

$$\text{Wind Pressure} = 0.5 \times 1.2 \times 361$$

$$\text{Wind Pressure} = 216.6 \text{ N/m}^2$$

$$\text{Total drag force}$$

$$\text{Total Drag Force acting on the structure} = 19.79 \text{ N}$$

$$\text{Torque load to resist the wind load} = 225 \times 19.79/4$$

$$\text{Torque load to resist the wind load} = 1113.18 \text{ N-mm}$$

Considering the Frictional load and Inertial load 10% each

$$\text{Total torque} = \text{derive torque} + \text{wind load torque} + \text{frictional torque} + \text{inertial torque} \dots\dots\dots (5)$$

$$M(t) = 19.4 + 1.1 + (0.1 \times 19.74) + (0.1 \times 19.74)$$

$$M(t) = 24.38 \text{ Nm}$$

Total tractive effort method for calculating torque

$$\text{Gross vehicle weight} = m \times g \dots\dots\dots (6)$$

$$\text{Gross vehicle weight} = 105 \times 9.81$$

$$\text{Gross vehicle weight} = 1030 \text{ N}$$

The weight on each vehicle is derived

$$W = 1030/2$$

$$W = 515 \text{ N}$$

$$\text{Radius of wheel} = 22.83 \text{ cm}$$

$$\text{Desired top speed} = 20 \text{ km/h}$$

$$\text{Desired to speed} = 5.5 \text{ ms}^{-1}$$

$$\text{Desired acceleration time} = 40 \text{ sec}$$

$$\text{Working surface} = \text{Coal tar}$$

#### Acceleration force

Acceleration Force (FA) is the force necessary to accelerate from a stop to maximum speed in the desired time. The vehicle will perform as designed regarding tractive effort and acceleration; it must calculate the required wheel torque (TW) based on the tractive effort.

$$FA = (\text{Gross weight vehicle} \times V_{\text{max}}) / (g \times \text{Time required}) \dots\dots\dots (7)$$

$$FA = ((1715 \times 5.5)) / ((9.81 \times 40))$$

$$FA = 24.06 \text{ W}$$

$$\text{Wheel motor torque}$$

$$TW = \text{Resistive torque} + \text{Accelerating torque} + \text{wind torque} \dots\dots\dots (8)$$

$$TW = 20 + 15.3 + 12.8 = 48.19 \text{ Nm}$$

#### Motor calculations

$$\text{Power of motor} = \text{torque} \times \text{speed} \dots\dots\dots (9)$$

$$P = 48.19 \times 16.6$$

$$P = 800 \text{ W}$$

$$\text{Motor selected} = 1000 \text{ W}$$

$$\text{Factor of safety} = 0.25$$

$$\text{Battery calculations}$$

$$\text{Battery} = 48 \text{ V}$$

$$\text{Battery ampere per hour} = 20 \text{ Ah}$$

$$\text{Total power} = 1000 \text{ W}$$

$$\text{Battery back-up}$$

$$\text{Battery time} = (V \times I) / 1000 \dots\dots\dots (10)$$

$$\text{Battery time} = (48 \times 20) / 1000$$

$$\text{Battery time} = 1 \text{ hour (at full speed at full load)}$$

$$\text{Charging calculations}$$

$$\text{Power of adopter} = V \times I \dots\dots\dots (11)$$

$$\text{Power of adopter} = 48 \times 7$$

$$\text{Power of adopter} = 336 \text{ W}$$

$$\text{Time to charge} = 1000/336$$

$$\text{Time to charge} = 3 \text{ h}$$

#### Charging at the alternator

$$\text{Power for charging} = P \times I \dots\dots\dots (12)$$

$$\text{Power for charging} = 48 \times 1.1$$

$$\text{Power} = 528 \text{ W}$$

$$\text{Time} = 1000/528$$

$$\text{Time} = 1.8 \text{ hour}$$

$$\text{Efficiency} = 800/1000 = 80\%$$

$$\text{Alternator calculation}$$

$$\text{Total power} = 1000 \text{ W} + 528 \text{ W}$$

$$\text{Total power} = 1528 \text{ W}$$

$$\text{Alternator voltage} = 24 \text{ V}$$

$$\text{Alternator ampere} = 90 \text{ at full speed}$$

$$\text{At optimum speed} = 65 \text{ A}$$

#### Bike mileage

$$\text{Mileage at petrol} = 45 \text{ km (in 1 L of petrol and 20\% charge of batteries)}$$

$$\text{In that 20\%, the bike can run 18 km}$$

$$\text{Total gross mileage at petrol and battery charge} = 60 \text{ km (battery) and 63 km (petrol)}$$

$$\text{Total mileage} = 60 + 63 = 123 \text{ km}$$

#### Security through IoT-based GPS tracking

GPS tracking devices are employed to monitor and record the location of an object, most commonly when installed in automobiles as part of vehicle tracking systems. Although it shares certain similarities with car navigation systems, the two technologies serve distinct purposes. Navigation systems primarily display the driver's current location on a digital



map and provide route guidance to a selected destination, whereas GPS trackers focus on recording a vehicle's position and travel history. The tracking device transmits collected GPS data wirelessly to an external platform such as a computer, smartphone, or tablet. A typical GPS module provides live tracking, playback of completed rides, mileage summaries, and other trip-related details. Additional features often include remote locking capability, information regarding route start and end times, duration of travel, and maximum speed achieved. Notifications on speed limit violations and engine ON/OFF status can also be transmitted via SMS. The associated application displays all these parameters in a user-friendly format. Functionally, GPS trackers rely on satellites to determine precise location. By employing trilateration with signals from three or more Global Navigation Satellite System (GNSS) satellites, the device calculates latitude, longitude, elevation, and time. Power for these trackers is generally supplied through the onboard diagnostics (OBD) connector, cigarette lighter port, accessory socket, or an internal rechargeable battery. The data collected were subsequently transmitted to specialized software, where they were aggregated, stored, and analyzed for interpretation and further application.

## SIMULATION AND RESULTS

A voltmeter was used to measure values in a hardware simulator, which was then simulated using MATLAB R2021b and the TRINAMIC trainer. A boost converter and a BLDC hub motor were both tested in this setup. The input and output voltages of the boost converter were measured with a voltmeter in order to test its operation and to measure voltage regulation. Some of the operational parameters of the BLDC hub motor were measured during simulation, such as speed, current, input power, torque, output power, and efficiency. These were measured with the help of the voltmeter, TRINAMIC trainer, and other necessary equipment. The general aim of the simulation was to examine the dynamic operation of the BLDC hub motor, focusing on speed variation, directional control, current variation over time, and the maximum achievable speed under load (Table 4–9).

### BLDC Hub Motor Simulation

The BLDC hub motor was simulated in various stages. The speed torque relationship, as shown in Fig. 2–6, was examined in the first stage. The second stage included applying changing torque values to the motor and measuring the obtained speeds. The information gathered was utilized in determining the relationship between motor speed and torque output, as seen in Fig. 7. The simulation was able to replicate varying operating conditions by altering torque values in various ranges.

The third phase examined the interaction between torque and current consumption. Varying torque loads were

**Table 2:** Specification of AC to DC charger

Specifications	Rating
AC voltage	220 volts
DC voltages	55 volts
Maximum current	20 Amperes

**Table 3:** Specification of the alternator

Specifications	Rating
Voltage	24 V
Current	90 A

**Table 4:** Specification of the boost converter

Specifications	Rating
Power	1000 W
Torque	18 Nm
Peak Torque	83 Nm
Voltage	48 V
Current	23 A
Limiting current	38 A

**Table 5:** Self-start motor specification

Specifications	Rating
Voltage	12 V
Current	5 A

**Table 6:** Specification of the GPS module

Specification	Rating
Operating voltage	12 V
Current	1 mA
GPS positioning precision	10 m
Temperature	-20°C to 70°C
RH	20–80%
Dimensions	6.7 cm*3.88cm*1.15cm
Weight	0.125 kg

**Table 9:** Overall comparison of three bikes

Design parameters	Extended electric bike	Electric bike	IC engine bike
Maximum speed	60 km/h	60-65 km/h	100 km/h
Lifetime of battery	5-7 years	5-7 years	N/A
Physical weight	105 kg	72 kg	96 kg
Energy used	Electrical energy or chemical energy of petrol to convert into mechanical energy	Electrical energy	The chemical energy of petrol + chemical gasoline energy to converted into mechanical energy
Manufacturing cost	Rs. 70,000	Rs. 95,000	Rs. 130,000
Time of charging	2.5 h	2.5 h	N/A
per km cost	Rs. 1.4	Rs. 0.5	Rs. 2.38
Noise during driving	60-100 dB	60-80 dB	80-100 dB
Maintenance cost	Slightly less	Low	Slightly high
Braking power	Good	Good	HIGH
Fuel required	YES	NO	YES
Efficiency	Best	Good	Best
Effects on environment	Slight	No	Yes
Emission of gases	Slight	No	Yes
The capacity to carry the load	High	Slightly high	Very High

imposed upon the motor, and current was measured. The linear correlation between current and torque, as shown in



Fig. 2: Boost converter



Fig. 2: Fabricated Extended Electric Bike



Figure 3: Placement of part



Fig. 4: GPS-based IoT module

Fig. 8, sheds light on motor efficiency and control methods. A fourth test was then performed with the TRINAMIC trainer to test motor performance at full load. This trainer allowed precise measurement, in-depth simulations, and real-time feedback. Advanced control algorithms, accurate data measurement, and light-to-heavy loading flexibility enabled the system to produce realistic performance for motors. The velocity-load characteristic is presented in Fig.

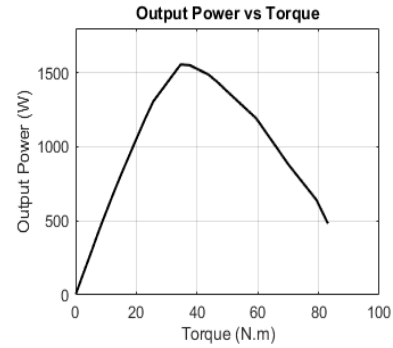


Fig. 6: Output vs torque

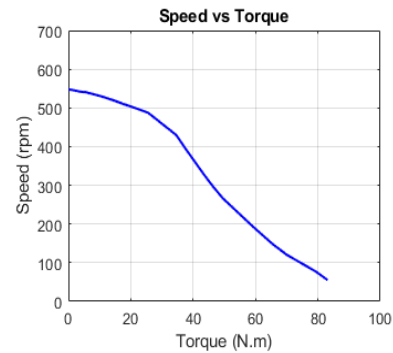


Fig. 1: Speed vs. torque

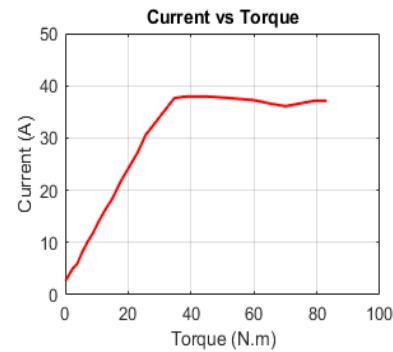
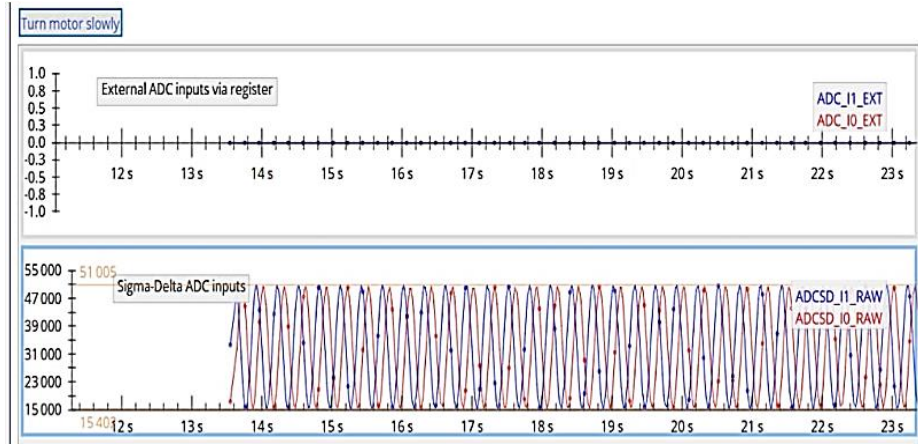


Fig. 8: Speed vs. current

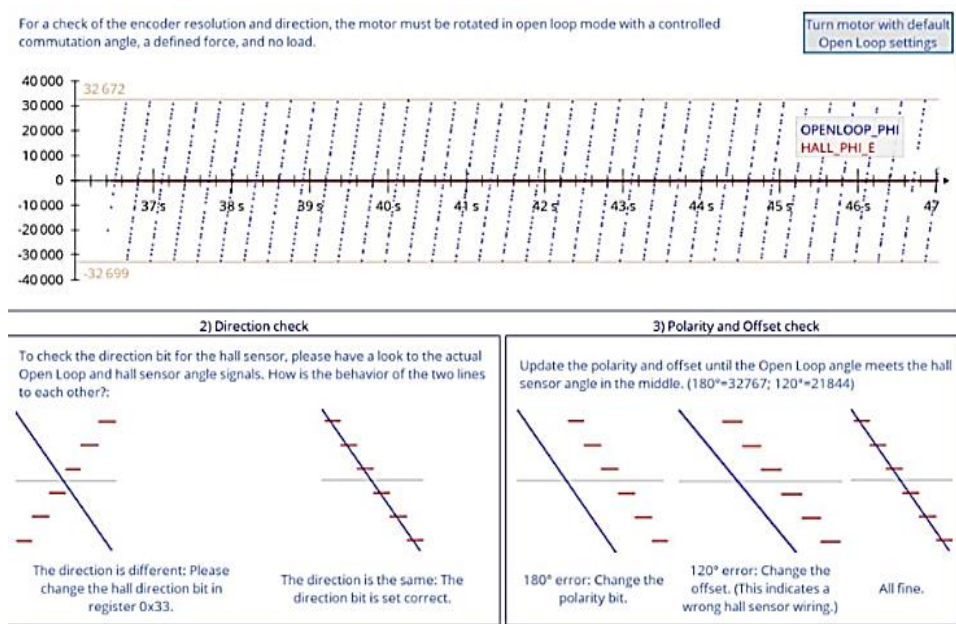
9. In stage five, direction control of the BLDC hub motor was tested via the TRINAMIC trainer (Fig. 10).

#### Boost Converter Simulation

Sufficient supply for the BLDC hub motor and battery charging requirements. In the simulation, a graph was obtained showing output current and voltage versus input. The primary role of the boost converter in this system was to elevate the voltage from 24 V to 55 V, ensuring a voltage that demonstrated the converter's efficiency across varying conditions. The converter exhibited stable performance when maintaining an output voltage of 48 V, a current



**Fig. 5:** Velocity simulation at full load



**Fig. 6:** Directional control of motor

adequate to power the motor load, and voltage stability under transient conditions. Fig. 11 presents the simulation of the boost converter.

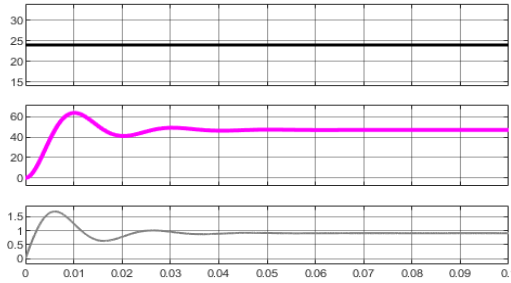
#### *Practical Results of IoT-Integrated GPS Module*

Simulation results also highlighted the integration of the Burj Track application with the Internet of Things (IoT) and GPS systems. The module provided real-time monitoring of the vehicle's location and performance. Features included mileage tracking, ride playback, and live updates on parameters such as fuel consumption, engine ON/OFF times, and total distance traveled. When connected to a mobile device, the system displayed comprehensive information regarding the vehicle's operation. Fig. 12 and

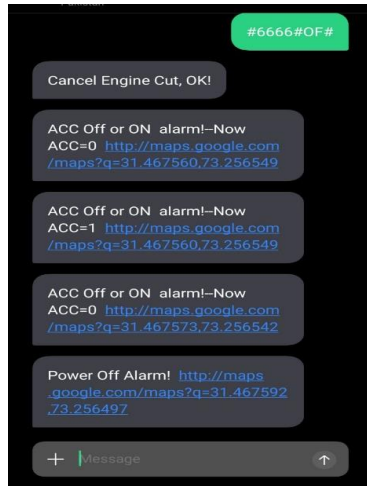
13 illustrate simulation results of the Burj Track application under IoT control, demonstrating its potential to enhance both monitoring and security of the EEB system.

#### **CONCLUSION**

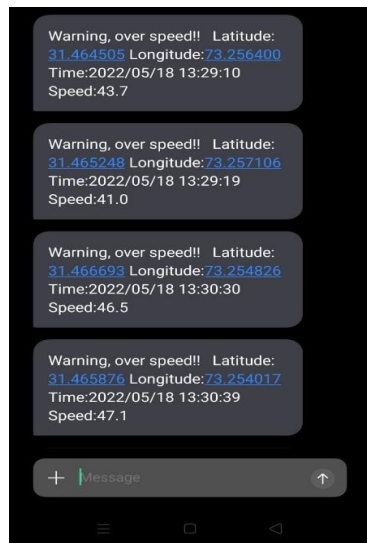
In an increasingly resource-constrained and polluted environment, technologies that optimize motor performance while reducing operational cost and environmental burden are needed for sustainable, low-cost, and pollution-free mobility. The integration of alternator-assisted battery charging and IoT-enabled GPS monitoring significantly improves the overall utility of the system. The primary objective of this work was to maximize efficiency at minimal cost, while the secondary objective was to alleviate



**Fig.11:** Boost Converter Simulation



**Fig. 12:** Engine on/off message



**Fig. 7** Speed limit warning message

environmental strain. Battery units, designed for ease of replacement, ensure continuous energy availability, and gasoline provides a supplementary source of power when necessary. Furthermore, the IoT-enabled GPS enhances vehicle security and provides essential data for system

management. Overall, this approach combines affordability, sustainability, and operational safety, thereby contributing to both user convenience and environmental conservation.

## ACKNOWLEDGMENT

We thank the Department of Mechatronics and Control Engineering faculty and staff at UET Faisalabad Campus for providing the necessary guidance and lab facilities.

## AUTHOR CONTRIBUTIONS

MS contributed to conceptualization, methodology design, data curation, and drafting of the manuscript. MAH performed the experimental setup, conducted performance analysis, and validated the results. MUK provided critical review, and technical guidance. MA, MA and ZA carried out simulations, data interpretation, and figure preparation.

## CONFLICTS OF INTEREST

No conflict of interest among the authors to declare

## DATA AVAILABILITY

Data will be made available on a fair request to the corresponding author

## ETHICS APPROVAL

Not applicable to this paper.

## FUNDING SOURCE

Self-funded.

## REFERENCES

- Hazarathaiha P, Reddy YAK, Reddy PVB, Sreenivasulu M (2019) Design and fabrication of a hybrid electric bike. *International Journal of Applied Engineering Research* 14: 930–935.
- Hidayat MN, Nugroho A, Munir AF, Putri RI (2025) Internet of things (IoT) based monitoring system for hybrid-powered E-bike charging station. *International Journal of Power Electronics and Drive Systems* 16: 243–250. <https://doi.org/10.11591/ijpeds.v16.i1.pp243-2>.
- Nithya Kumari R, Bari A, Achar GV, Kumar RA, Harshavardhan K (2018) Smart motorbike integration using IoT. *International Journal of New Technologies in Science and Engineering* 5: 235–240.
- Nosratzadeh H, Bhowmick D, Rios Carmona AB, Thompson J, Thai T, Pearson L (2024) A scoping review of the design and characteristics of e-bike financial incentives. *Transport Reviews* 44: 149–171. <https://doi.org/10.1080/01441647.2024.2419884>.
- Sugiarto M, Herdiyanto YD, Prasetyo H, Karyanto Y, Sadili R, Hardianto D (2025) Air optimization on motorcycle intake manifold towards work performance and exhaust gas emissions. *Jurnal Teknologi Transportasi dan Logistik* 6: 85–94. <https://doi.org/10.52920/jttl.v6i1.420>.
- Zahedi R, Sadeghitabar E, Khazaei M, et al. (2025) Potentiometry of wind, solar and geothermal energy resources and their future perspectives in Iran. *Environment, Development and Sustainability* 27: 15311–15337. <https://doi.org/10.1007/s10668-024-04633-2>



REVIEW ARTICLE



# Interacting Environmental Factors Affect Targeted Milk Thistle Metabolomic Profile and other Growth Components: A Review

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## METADATA

### Paper history

Received: 28 February 2025

Revised: 30 June 2025

Accepted: 20 November 2025

Published online: 26 October 2025

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(Muhammad Bilal Hafeez)

### Keywords

Cancer

Hepatitis

Biotic stress

Abiotic stress

Metabolites

### Citation

Hafeez MB (2025). Interacting environmental factors affect targeted milk thistle metabolomic profile and other growth components: A review. *Innovations in STEAM: Research & Education* 3: 25030202.

<https://doi.org/10.63793/ISRE/0027>

## ABSTRACT

**Background:** Milk thistle (*Silybum marianum* L. Gaertn.) is a medicinally important herb of the family Asteraceae. Its achenes contain the active compound silymarin, which has gained significant attention in the pharmaceutical industry for its hepatoprotective properties, including protection against hepatotoxic agents and stimulation of liver regeneration. However, biosynthesis and accumulation of active ingredients are strongly influenced by environmental variability.

**Objective:** To provide an inclusive overview of the physiological and phenotypic variations in milk thistle under different abiotic and biotic stresses, with a particular focus on silymarin synthesis and accumulation.

**Methodology:** A literature-based review was conducted, compiling information from available sources regarding germination, growth behavior, and secondary metabolite production of milk thistle under variable environmental conditions.

**Results:** Milk thistle not only serves as a medicinally valuable plant but also behaves as a noxious weed. Its germination, growth, and metabolite accumulation, especially silymarin, are highly affected by environmental factors. Despite available research, the full potential of this plant under diverse environmental conditions remains underexplored.

**Conclusion:** Milk thistle can adapt and grow under diverse conditions. However, stresses such as salinity, temperature, and rainfall adversely affect its growth and development, particularly silymarin production. Understanding the physiological responses and secondary metabolite production of milk thistle under different environmental stresses is crucial for optimizing its medicinal use and managing its weed potential in agriculture.

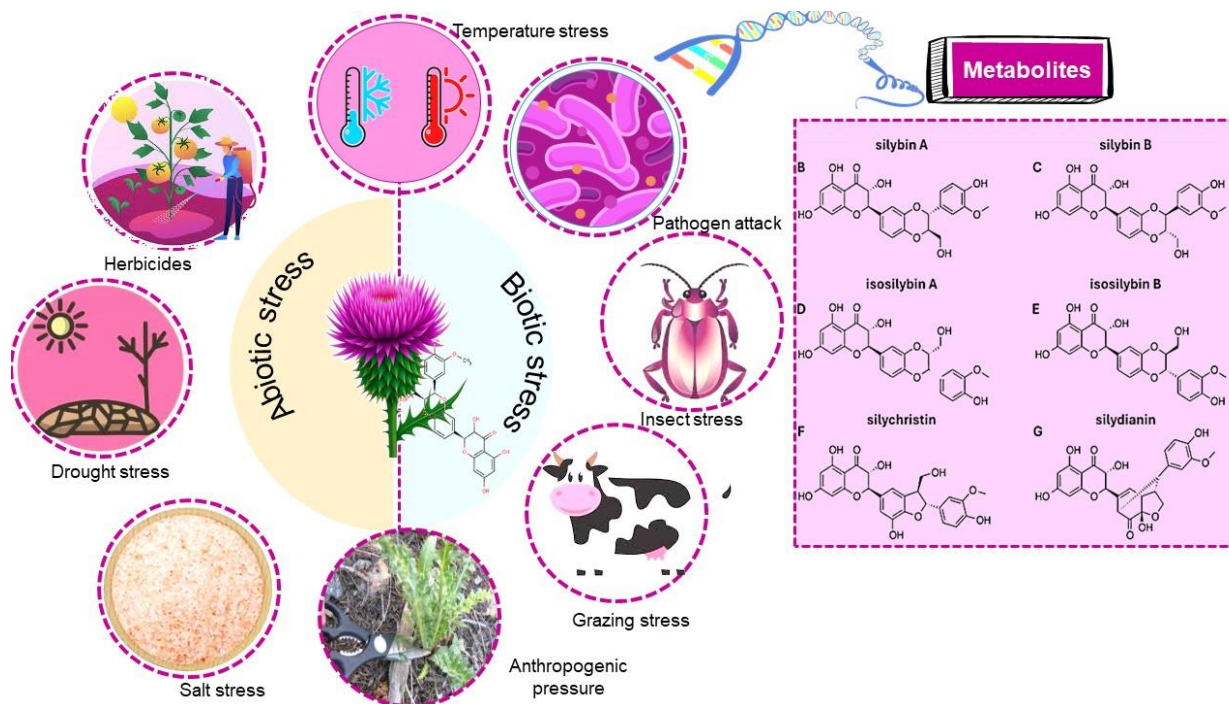
## INTRODUCTION

Cells, organs, tissues, and metabolic functions at different developmental stages respond differently to environmental conditions. Environmental stresses pose serious challenges to agriculture by increasing consumption demands, limiting land availability, and reducing plant-derived medicinal product yields. Abiotic stresses exert considerable influence on the synthesis of secondary metabolites (Jaleel *et al.* 2007, Zahra *et al.* 2022). Milk thistle (*Silybum marianum* L. Gaertn.) is distributed across several countries but is specifically indigenous to Mediterranean regions. It grows at diverse altitudes, ranging from 700 to 1100 m, and thrives in sub-mountainous to coastal areas (Morazzoni and Bombardelli 1995). It can tolerate a wide range of pH but grows best at 5.5 to 7.6 (Andrzejewska and Sadowska

2008). As a dietary supplement, it is ranked among the top ten and is widely used for liver- and bile-related diseases (Kurkin 2003). Its achenes contain 20–35% fatty oil (Ramasamy and Agarwal 2008). Its oil is rich in vitamins (El-Mallah *et al.* 2003). Medicinally, milk thistle is used to treat gallbladder and various liver diseases (Abenavoli *et al.* 2010). It also hinders cholesterol biosynthesis, reduces certain cancer risks, and inhibits leukotriene production. Smith *et al.* (2008) reported that silymarin sales reached approximately 16.6 million USD in 2018 due to the presence of bioactive compounds. Its medicinal importance lies in the active compound silymarin, an isomeric mixture of flavonolignans including silychristin, silydianin, isosilybin, and silybin (Afshar *et al.* 2014). Silybin, a major component of silymarin, is in high demand due to its anti-carcinogenic properties. Silymarin stabilizes cell







**Fig. 1:** Illustration of abiotic and biotic stresses affecting milk thistle

membranes, prevents hepatotoxic damage and stimulates liver regeneration (Fraschini *et al.* 2002).

The production of secondary metabolites is genetically and environmentally regulated, varying across plant families. Such metabolites enable plants to cope with severe environmental stresses and are used as therapeutic agents. Metabolic pathways and active substances are also severely affected by environmental stresses (Bohnert *et al.* 1995). Their biosynthesis and accumulation depend strongly on soil properties (Selmar and Kleinwachter 2013). Silymarin content in achenes is influenced by both genotypic variation and environmental conditions (Ghavami and Ramin 2008). Interestingly, silybin content was reported to be higher in cultivated ecotypes, whereas isosilybin, silydianin, and silychristin levels were higher in native ecotypes (Radjabian *et al.* 2008). Milk thistle can adapt and grow under diverse conditions. However, stresses such as salinity, temperature, and rainfall adversely affect its growth and development, particularly silymarin production (Fig. 1). However, no comprehensive review is present on the effect of biotic and abiotic stresses on milk thistle production and metabolites synthesis. Being one of the most important medicinal plants for treating liver diseases in humans, understanding its ecophysiological behavior is crucial for promoting large-scale cultivation. This review applies a nonlinear regression model to describe milk thistle's responses under different stresses and to highlight the challenges faced in its cultivation and utilization. Such insights can guide future research and support sustainable production.

## ABIOTIC STRESSES

### *Milk thistle under salinity stress*

Germination of plants faces a life-threatening challenge in salt marshes and saline desert areas, leading to the mortality of germinating plants. However, different plant species have their own salinity tolerance mechanisms (Brady and Weil 1996). In general, satisfactory achene germination of milk thistle was recorded up to 6 dS/m salinity stress. A 50% reduction in achene germination and seedling emergence was reported at a salinity level of 9 dS/m. Significant reductions in the number of leaves per plant, main capitulum per plant, achene weight per capitulum, achene weight per plant, and 1000 achene weights were observed at 9 dS/m salinity stress. However, at the 15 dS/m salinity level, plants still produced achenes, but the yield was one-third compared to the control. At low salinity (< 9 dS/m), milk thistle shows limited growth and no effect on grain yield compared to control plants, which is why it is categorized as a facultative halophyte (Ghavami and Ramin 2007). Sedghi *et al.* (2010) recorded a severe reduction of growth attributes of milk thistle seedlings under salinity, including plumule and radicle length, plumule fresh and dry weight, and germination percentage with increasing salinity. Maximum reduction was observed at 10 dS/m. Kashmir *et al.* (2016) documented that salinity levels up to 100 mM had a non-significant effect on germination and growth-related parameters of milk thistle, but concentrations higher than

Xiao-fang *et al.* (2000) also found that germination percentage decreased with increasing salinity. Similarly, Ghanbari *et al.* (2013) reported that shoot and root growth were negatively affected under salinity in milk thistle. Moreover, Solouki *et al.* (2015) also reported that increasing  $\text{Na}^+$  concentration decreased germination time, germination percentage, seedling number, coefficient of germination time, radicle length, vigor index, seedling length, plumule fresh weight, and radicle fresh weight. Safikhan *et al.* (2018) reported that salt stress, especially salinity levels of 8 and 12 dS/m, decreased growth as well as other biochemical attributes, including chlorophyll content, carbohydrates, enzymatic activity, and proline concentration. Hydrogen peroxide ( $\text{H}_2\text{O}_2$ ) concentration also increased, indicating stress severity. It was concluded that salt stress, especially under 8 and 12 dS/m, decreased growth characteristics and chlorophyll content, while proline, carbohydrates, enzymatic activity, and  $\text{H}_2\text{O}_2$  concentration increased in milk thistle leaves (Fig. 2). Little information has been published regarding the correlation of active substances, yield components, and grain yield under salinity stress in milk thistle (Omidbaigi and Nobakht 2001). Maximum oil content was observed up to 6–9 dS/m salinity level; however, further increase in salinity levels gradually decreased oil content. The positive effect of salinity stress on achene silymarin and silybin content has also been reported (Ghavami and Ramin 2008). Similarly, Zahra *et al.* (2021a; 2021b; 2022) also reported that silymarin content was enhanced under salinity stress, while severely deteriorating all growth and yield parameters.

In crux, salinity stress adversely affects the growth and yield attributes. Moreover, salinity stress increased the production of silymarin and silybin, which are medicinally important phytochemicals present in the achene. However, further research is required to explore its metabolic shifts under a saline environment.

#### *Milk thistle and drought stress*

Worldwide, plant growth and development are severely affected by drought stress, especially in arid and semi-arid regions (Afshar *et al.* 2014). Deliri *et al.* (2010) worked on different milk thistle ecotypes and observed that ecotypic differences are highly significant in relation to drought stress. They emphasized that decreases in chlorophyll content, dry weight, root volume, and root tolerance index, along with an increase in electrolyte leakage, are related to drought stress severity. Moreover, Afshar *et al.* (2015) also noted that silymarin content increases in drought-affected milk thistle achenes. Furthermore, they elaborated that the amount of silybin increased under water stress, which is a more biologically active compound compared to others. Zahir *et al.* (2014) found enhanced accumulation of total flavonoids and phenolic content in drought-affected milk thistle. Malekzade *et al.* (2011) proved that milk thistle oil increased under drought stress. A high content of

unsaturated fatty acids accumulated under severe drought stress. Ghassemi-Golezani *et al.* (2017) reported that under water stress, harvest index, 1000 achene weight, achene yield per plant, number of achenes per plant, and plant biomass decreased. Furthermore, oil percentage and yield also decreased; however, flavonoid content increased in water-stressed milk thistle.

Essential oil levels are significantly reduced under acute water stress. Afshar *et al.* (2016) evaluated that relative water content, stem diameter, leaf dry weight, and leaf area remained unaffected under moderate drought and were only affected under severe drought stress. They observed a decrease of about 19 and 44% in photosynthesis under moderate and severe drought stress, respectively. Moreover, Zahir *et al.* (2014) elaborated that water deficiency inhibited shoot and root growth; however, total phenolic content, total protein, antioxidant enzymes, and flavonoids increased under drought stress (Table 1). The potential use of drought stress is to enhance the production of active compounds, especially phenolic compounds (Bettaieb *et al.* 2009). It has been observed that under drought stress, the total flavonoid and phenolic content increase in milk thistle (Zahir *et al.* 2014). A significant increase in silymarin accumulation and synthesis was observed in milk thistle achenes under drought stress. So, severe and moderate drought stress enhanced silymarin by 4 and 17% respectively, compared to the control. Under drought conditions, silymarin, silychristin, isosilybin, and silybin also increased, but decreased silydianin content (Afshar *et al.* 2015). A decrease in grain yield of milk thistle was also reported under drought, so the enhanced concentration of silymarin is not economically beneficial according to Afshar *et al.* (2014). In conclusion, drought stress causes a severe reduction of all the growth and yield-related traits but enhances its medicinally important secondary metabolite production. The production of these metabolites is stress stress-relieving strategy, but their metabolic profile characterization under mild stress may play a plausible role in uplifting its economic benefits.

#### *Milk thistle under temperature stress*

Temperature is an important abiotic factor that influences plant growth and development. Rahman *et al.* (2016) documented that temperature changes have regulatory effects on plant height, number of flowers per plant, number of achenes per plant, and crop yield per hectare. Milk thistle achene germination, germination percentage, and the number of seedlings at 15 °C were higher compared to 25 or 35°C. Germination percentage was about 95 and 70% under 15 and 35°C, respectively (Ghavami and Ramin 2007). Kashmir *et al.* (2016) reported that 25°C (optimum temperature) resulted in higher growth and germination rates; however, lower (15°C) and higher temperatures (40°C) resulted in poor germination and growth. Pourreza and Bahrani (2012) reported that temperatures ranging from



**Table 1:** Changes in silymarin content under different stresses

Stress	Change in silymarin contents	References
Salinity stress	Silymarin ↑; Silybin ↑	Ghavami and Ramin (2008)
Drought stress	Silymarin ↑	Afshar <i>et al.</i> (2014); Afshar <i>et al.</i> (2015); Shawky (2015)
	Silymarin + silybin A & B ↑	Afshar (2015)
	Silymarin ↓	Malekzade <i>et al.</i> (2011)
	Oil ↑	Zahir <i>et al.</i> (2014)
Density	Silymarin ↓	Afshar (2014)
	Silymarin ↑	Azizi <i>et al.</i> (2018); Katar <i>et al.</i> (2013)
	Silymarin ↑	Rio-Celestino <i>et al.</i> (2006)
Heavy metal	Silymarin is not affected	Zheljazkov (2006)
Herbicides	Silymarin ↑	Zheljazkov (2006)
Metribuzin	Silymarin ↓	Zheljazkov (2006)
bentazon		
Higher population	Not affected —	Omer <i>et al.</i> (1993)

**Fig. 2:** Milk thistle necrosis under salinity stress

21–27°C were effective in enhancing germination percentage. Heidari *et al.* (2014) used three varieties of milk thistle to confirm cardinal temperatures related to germination response. They concluded that varietal and temperature differences are of prime importance, especially regarding germination rate, reciprocal time to 50% germination, germination uniformity, germination percentage, and time to 5, 10, 50, 90, and 95% germination. They suggested that the optimum temperature for milk thistle growth is 28–29.5°C.

#### *Milk thistle under heavy metal stress*

Milk thistle often faces heavy metal stress due to its cosmopolitan nature. Khatamipour *et al.* (2011) reported that cadmium toxicity affected germination rate, germination percentage, seedling growth, fresh and dry weight of shoot and root, and shoot and root length of milk thistle. They also concluded that all concentrations of

cadmium (Cd) slightly increased the shoot/root ratio and proline content. Moreover, results indicated that roots were more affected by Cd than shoots. Several researchers reported that milk thistle can grow well in contaminated soils with heavy metals such as zinc (Zn), manganese (Mn), copper (Cu), lead (Pb), chromium (Cr), and Cd (Zheljazkov and Nikolov 1996), and even tolerates the radioactive element cesium (Cs). Zheljazkov and Nikolov (1996) reported that Zn accumulated mainly in leaves and stems, while Mn, Cu, Pb, and Cd accumulated in leaves and roots. Achene yield decreased by 16% under heavy metal stress compared to the control. It was noted that the species can accumulate zinc and lead and can also relocate them to the harvestable parts. For this reason, Del Rio-Celestino *et al.* (2006) suggested that milk thistle is not a hyperaccumulator. However, silymarin content remained unaffected under heavy metal stress (Zheljazkov and Nikolov 1996). According to Ikram *et al.* (2025), arsenic (As) stress increased silymarin production up to 80%, and they suggested that increasing its production plays a pivotal role in neutralizing stress and initiating tolerance mechanisms.

## BIOTIC STRESSES

### *Effect of insect attack on milk thistle*

Milk thistle is susceptible to insect attack. For instance, Goeden (1971) noticed that an assemblage of phytophagous insects fed or reproduced on milk thistle plants, but apparently no deleterious effect was observed on the root, stem, or reproductive parts of milk thistle. *Rhinocyllus conicus* (weevil) attacks thistle genera *Onopordum*, *Carduus*, *Cirsium*, and *Silybum* (Fig. 3) (Goeden and Ricker 1974). *R. conicus* larvae were also found in the achene tissues and achene heads of milk thistle (Coombs *et al.* 1996). Clarke and Walter (1993) observed that *Nezara viridula* infects milk thistle in Queensland, Australia. Abdel-Moniem (2002) reported the presence of the achene head weevil (*Larinus latus* Herbst) on milk thistle. They noted that weevil achene larvae have an injurious effect on the flower head. A single larva can destroy all the achenes of a flower head ranging from 2 to 3 cm in diameter. In Greece and Iran, *Aphis fabae cirsiacanthoidis* and *Dysaphis lappae cynarae* are well-known aphids (Fig. 3) that attack milk thistle plants (Kavallieratos *et al.* 2007; Rezwani 2008). Khan *et al.* (2009) observed that caterpillars of *Spodoptera* sp. damage leaves at the end of flowering. Snails are pests recorded frequently in wet weather conditions. Abdel-Moniem (2002) reported a reduction in achene heads by *L. latus*. Dodd (1989) pointed out that weevils have restricted oviposition and low-density larvae per capitulum, with little effect on prolonged flowering of milk thistle. Scientists in Israel focused on dense plant occurrence near ant nests and achene dispersal by ants. Ants move the achene into their nest and remove the oily body (elaiosome) to feed their



**Fig. 3:** *Rhinocyllus conicus* attack on milk thistle

larvae, which increases milk thistle vigor and germination (Gabay *et al.* 1994).

#### *Pest and disease attack*

Pest and disease attacks on plants not only affect growth but also yield. Like other plants, milk thistle is also infected by various pests and microbes. *Septoria silybi* is a fungus that interferes with photosynthesis and causes leaf lesions (Moscow and Lindow 1989). Roche (1991) observed that *S. silybi* infects milk thistle plants during daylight when there is a high humidity inoculation period, but rare infestation was observed when light was excluded. The reason behind this infestation is related to the requirement for open stomata for pathogen penetration in milk thistle leaves. Berner *et al.* (2002) observed that the rust fungus *Puccinia punctiformis* is a pathogen of Canada thistle but often affects milk thistle. El-Elimat *et al.* (2014) isolated *Aspergillus iizukae* from the leaves of milk thistle. Souissi *et al.* (2005) suggested that *Microbotryum silybum* (a smut fungus) is a naturally occurring pathogen of *Silybum marianum* (Tamouridou *et al.* 2018). Moscow and Lindow (1989) observed *S. silybi* infection in milk thistle plants over several years in central California. Saccardo (1884) and Oudemans (1923) reported that *S. silybi* is the only pathogen on the sole host of milk thistle. Moscow and Lindow (1989) conducted a detailed experiment on *S. silybi*-infected milk thistle and reported that it can survive under dry periods when rain and dew are inadequate. A very low inoculum of *S. silybi* spores is enough to cause considerable infection, leading the leaves to become necrotic. Under high inoculum, severe disease was observed with numerous necrotic leaves that reduced plant growth and eventually killed the plant (Jamali 2015). *Puccinia cruchetiana*, *P. tyrimni*, *P. mariana* and *P. laschii* also cause infestation in milk thistle (Brandenburger 1985).

Kováčiková and Kubínek (1986) noted that milk thistle is severely infected by the *Fusarium* genus. Cwalina-Ambroziak *et al.* (2012) reported approximately six species of this genus that infect milk thistle. Šafránková *et al.* (2015) observed mildew, *Golovinomyces orontii* on milk thistle during the vegetation period. Besides, gray mold (*Botrytis cinerea*) was observed during the rainy season. At the achene ripening stage, vast infestation was observed on stems, leaves, and anthodia. Additionally, they observed the presence of *Fusarium* and *Rhizoctonia* sp. on milk thistle roots and *Rhizoglyphus* sp. infection on roots and root collars. Milk thistle is also a host for cucumber mosaic virus (Souissi *et al.* 2005) and tomato spotted virus (Chatzivasilioni *et al.* 2001). Furthermore, Chatzivasilioni *et al.* (2001) observed that it is also a host for TSWV (tomato spotted wilt virus).

#### *Weed attack and milk thistle productivity*

One of the most important limiting factors in the production of milk thistle is the lack of weed control (Topalov *et al.* 1983). Zheljaskov *et al.* (2006) noted 16 species, of which the most abundant were green foxtail (*Setaria viridis* L. Beauv.), bermudagrass (*Cynodon dactylon* L. Pers.), and redroot pigweed (*Amaranthus retroflexus* L.). The most observed perennials were monocotyledonous johnsongrass (*Sorghum halepense* L. Pers.), Canada thistle (*Cirsium arvense* L.), motherwort (*Leonurus cardiaca* L.), and bindweed (*Convolvulus arvensis* L.). Other weed species included large crabgrass (*Digitaria sanguinalis* L.), prostrate pigweed (*Amaranthus blitoides* S. Wats.), velvetleaf (*Abutilon theophrasti* Medik.), common cocklebur (*Xanthium strumarium* L.), black nightshade (*Solanum nigrum* L.), prostrate knotweed (*Polygonum aviculare* L.), wild buckwheat (*P. convolvulus*), jimsonweed (*Datura stramonium* L.), and common lambsquarters (*Chenopodium album* L.). They also documented that the highest infestation was found in untreated milk thistle plants.

#### *Animal attack*

The achene bank for milk thistle is very limited (Sofer-Arad *et al.* 2007), and continuous grazing might control this species within a few years (Fig. 4). The density of milk thistle is severely affected by cattle grazing, including rotational and continuous grazing (De Bruijn and Brok 2006). However, Sofer-Arad *et al.* (2007) observed that cattle grazing may be associated with higher thistle frequency in the mid-eastern rangelands. Spines in milk thistle deter cattle, thus hampering grazing (Danin and Yom-Tov 1990). Grazing milk thistle is toxic for cattle due to lethal and high content of nitrates (Clark County Noxious Weed Program; CCNWP 2015). Campbell *et al.* (1979) reported that goats can limit milk thistle biomass and reduce achene production. Goats will graze on milk thistle, but less than 1% of achenes pass through their digestive tract (Sindel





**Fig. 4:** Animal attack on milk thistle

1991). Vinograd *et al.* (2011) reported that in Israel, milk thistle is a dominant weed and cannot be grazed by sheep and goats.

#### *Milk thistle and population density*

Population density and row spacing between plants have a significant effect on growth, yield, and active compounds of milk thistle. Austin *et al.* (1988) maintained eight plants per pot (18 cm diameter pot) and found the highest shoot yield after 6 weeks of plantation, while a decrease in shoot yield was observed with increased density of plants per pot. Gabucci *et al.* (2002) noted that higher population density decreased achene yield, number of blooms per plant, and bloom diameter. Belitz and Sams (2007) observed that achene yield decreased when population density increased, showing a negative correlation between yield, mature achene counts, bloom diameter, number of blooms per plant, and population density. Contrarily, Duran Katar *et al.* (2013) reported that higher population density increased achene yield and silymarin content in milk thistle. They found a higher yield (83.13 kg ha<sup>-1</sup>) and silymarin (1.413 kg ha<sup>-1</sup>) at a sowing density of 40,000 plants ha<sup>-1</sup>. Omidbaigi *et al.* (2003) concluded that 50 × 30 cm is the most suitable density for milk thistle. Recently, Azizi *et al.* (2018) observed the tallest plants, the highest grain, and biological yield at 8 plants m<sup>-2</sup> density. Moreover, they noted that population density had no impact on silymarin concentration. However, Omer *et al.* (1993) noted that narrow row spacing of approximately 25 cm increased achene yield but decreased flavonolignan and oil content compared with 50 cm spacing in milk thistle. They also found that row spacing greater than 25 cm significantly increased silymarin, isosilybin, silychristin, and silybin concentrations.

#### *Anthropogenic activities*

Milk thistle is a noxious weed that is harmful to economic and environmental resources; therefore, plants are targeted for eradication. It has pappi-bearing achenes that are easily pollinated even before harvest, thus emerging as a weed for the next crops. In North America, it is classified as a noxious weed in Washington (category A), Oregon (category B), and Texas (category S2) (Plant Protection and Quarantine 2002), but no case has been reported from Canada (USDA-ARS 2005). A limiting factor for milk thistle production is weed control. Milk thistle is very sensitive to herbicides used for other crops (Topalov *et al.* 1983). Parsons (1973) noted that it is very easy to eradicate milk thistle plants with several herbicides; however, large flowering and rosette plants are difficult to kill. Shimi *et al.* (2006) observed that clopyralid (0.24 kg ha<sup>-1</sup>) can control 94% of milk thistle growth. In cereals, 2,4-D ester and MCPA, 2,4-D amine can be used to control milk thistle (Department of Primary Industries, Water and Environment, 2008). Zand *et al.* (2007) noted that bromoxynil plus MCPA at 560 g ha<sup>-1</sup>, metsulfuron plus sulfosulfuron at 36 g ha<sup>-1</sup>, and chlorsulfuron at 10.5 g ha<sup>-1</sup> suppressed milk thistle achene production. So, these herbicides also affect the milk thistle production.

## CONCLUSION

Milk thistle (*Silybum marianum* L. Gaertn.) is a valuable medicinal plant recognized for its hepatoprotective, antioxidant, and anti-inflammatory properties, primarily attributed to its bioactive compound silymarin. Despite its therapeutic potential, milk thistle faces several abiotic and biotic stresses, including drought, salinity, heavy metals, insect pests, diseases, weeds, and grazing pressure, which significantly affect its growth, yield, and active constituents. Research findings indicate that appropriate agronomic practices, such as optimized population density, nutrient management, and protective measures against pests and weeds, can improve its productivity and phytochemical composition. Furthermore, its classification as both a medicinal resource and a noxious weed highlights the dual challenges in its management. Overall, milk thistle represents a promising plant species with significant pharmaceutical and ecological importance, but sustainable cultivation strategies are essential to maximize its benefits while minimizing its invasive potential.

## DATA AVAILABILITY

Not applicable to this paper

## ETHICS APPROVAL

Not applicable to this paper.

## FUNDING SOURCE

Not applicable to this paper.

## REFERENCES

- Abdel-Moniem ASH (2002) The seed-head weevil *Larinus latus* Herbst (Coleoptera: Curculionidae) as a new record in Egypt on the milk thistle *Silybum marianum* (L.) (Asteraceae: Compositae). *Archives of Phytopathology and Plant Protection* 35: 157–160. <https://doi.org/10.1080/03235400214210>.
- Abenavoli L, Capasso R, Milic N, Capasso F (2010) Milk thistle in liver diseases: past, present, future. *Phytotherapy Research* 24: 1423–1432. <https://doi.org/10.1002/ptr.3207>.
- Abenavoli L, Izzo AA, Milić N, Cicala C, Santini A, Capasso R (2018) Milk thistle (*Silybum marianum*): A concise overview on its chemistry, pharmacological, and nutraceutical uses in liver diseases. *Phytotherapy Research* 32: 2202–2213. <https://doi.org/10.1002/ptr.6171>.
- Afshar RK, Chaichi MR, Ansari Jovini M, Jahanzad E, Hashemi M (2015) Accumulation of silymarin in milk thistle seeds under drought stress. *Planta* 242: 539–543. <https://doi.org/10.1007/s00425-015-2265-9>.
- Afshar RK, Chaichi MR, Asareh MH, Hashemi M, Liaghat A (2014) Interactive effect of deficit irrigation and soil organic amendments on seed yield and flavonolignan production of milk thistle (*Silybum marianum* L. Gaertn.). *Industrial Crops and Products* 58: 166–172. <https://doi.org/10.1016/j.indcrop.2014.03.043>.
- Afshar RK, Hashemi M, DaCosta M, Spargo J, Sadeghpour A (2016) Biochar application and drought stress effects on physiological characteristics of *Silybum marianum*. *Communications in Soil Science and Plant Analysis* 47: 743–752. <https://doi.org/10.1080/00103624.2016.1146752>.
- Andrzejewska J, Sadowska K (2008) Effect of cultivation conditions on the variability and interrelation of yield and raw material quality in milk thistle (*Silybum marianum* L. Gaertn.). *Acta Scientiarum Polonorum Agricultura* 7: 3–11.
- Austin MP, Fresco LFM, Nicholls AO, Groves RH, Kaye PE (1988) Competition and relative yield: Estimation and interpretation at different densities and under various nutrient concentrations using *Silybum marianum* and *Cirsium vulgare*. *Journal of Ecology* 76: 157–171. <https://doi.org/10.2307/2260460>.
- Azizi K, Nazari Alam J, Faizian M, Heydari R (2018) Effect of climate and density on different populations of milk thistle (*Silybum marianum* L.). *Iranian Journal of Field Crop Science* 49: 65–75. <https://doi.org/10.22059/ijfcs.2017.226910.654273>.
- Belitz AR, Sams CE (2007) Effect of population density on growth, yield, and flavonolignan content in milk thistle (*Silybum marianum*). *Acta Horticulturae* 756: 251–258. <https://doi.org/10.17660/ActaHortic.2007.756.26>.
- Berner DK, Paxson LK, Bruckart WL, Luster DG, McMahon MB, Michael JL (2002) First report of *Silybum marianum* as a host of *Puccinia punctiformis*. *Plant Disease* 86: 1271. <https://doi.org/10.1094/PDIS.2002.86.11.1271B>.
- Bettaieb I, Zakhama N, Wannes WA, Kchouk ME, Marzouk B (2009) Water deficit effects on *Salvia officinalis* fatty acids and essential oils composition. *Scientia Horticulturae* 120: 271–275. <https://doi.org/10.1016/j.scienta.2008.10.016>.
- Bohnert HJ, Nelson DE, Jensen RG (1995) Adaptations to environmental stresses. *Plant Cell* 7: 1099–1111. <https://doi.org/10.2307/3870060>.
- Brady NC, Weil RR (1996) *The Nature and Properties of Soils*, 11<sup>th</sup> edn. Upper Saddle River, NJ: Prentice Hall, Inc.
- Brandenburger W (1985) *Parasitische Pilze an Gefäßpflanzen in Europa*. Stuttgart: Spektrum Akademischer Verlag.
- Campbell MH, Holst PJ, Auld BA, Medd RW (1979) Control of three pasture weeds using goats. In: *Proceedings of the 7th Asian-Pacific Weed Science Society Conference*, Sydney, Australia, 26–30 November 1979. Asian-Pacific Weed Science Society, Sydney, 201–205. Available at: <https://catalogue.nla.gov.au/catalog/2937787>.
- Chatzivasilioni EK, Bourakas I, Drossos E, Eleftherohorinos I, Jenser G, Peter D, Katis (2001) Weeds in greenhouses and tobacco fields are differentially infected by tomato spotted wilt virus and infested by its vector species. *Plant Disease* 85: 40–46. <https://doi.org/10.1094/PDIS.2001.85.1.40>.
- Clark County Noxious Weed Program (2015) *Milk thistle (Silybum marianum)*. Clark County Noxious Weed Program. Available at: <https://clark.wa.gov/sites/default/files/dept/files/publicworks/Vegetation/Milk%20Thistle%20Control%20Sheet.pdf>.
- Clarke AR, Walter GH (1993) Variegated thistle (*Silybum marianum* (L.)): a non-crop host plant of *Nezara viridula* (Hemiptera: Pentatomidae) in Southeastern Queensland. *Journal of the Australian Entomological Society* 32: 81–83. <https://doi.org/10.1111/j.1440-6055.1993.tb00549.x>.
- Coombs E, Turner C, Rees N (1995) Milk thistle *Silybum marianum*. In: Rees N, Quimby P, Piper G, Coombs E, Turner C, Spencer N, Knutson L (eds) *Biological Control of Weeds in the West*. Western Society of Weed Science in cooperation with USDA-ARS, Montana Department of Agriculture and Montana State University.
- Cwalina-Ambroziak B, Wierzbowska J, Damszel M, Bowszys T (2012) The effect of mineral fertilization on achenes yield and fungal communities isolated from the stems of milk thistle *Silybum marianum*. *Acta Scientiarum Polonorum Hortorum Cultus* 11: 157–168.
- Danin Y, Yom-Tov Y (1990) Ant nests as primary habitats of *Silybum marianum* (Compositae). *Plant Systematics and Evolution* 169: 209–217. <https://doi.org/10.1007/BF00937675>.
- De Bruijn SL, Bork EW (2006) Biological control of Canada thistle in temperate pastures using high-density rotational cattle grazing. *Biological Control* 36: 305–315. <https://doi.org/10.1016/j.biocontrol.2005.10.007>.
- Del Rio-Celestino MD, Font R, Moreno-Rojas R, De Haro-Bailon A (2006) Uptake of lead and zinc by wild plants growing on contaminated soils. *Industrial Crops and Products* 24: 230–237. <https://doi.org/10.1016/j.indcrop.2006.06.013>.
- Deliri R, Shokrpour M, Asghari A, Esfandiari E, Sharifi RS (2010) Assessment of milk thistle ecotypes for drought resistance in a hydroponic system. *Journal of Science and Technology of Greenhouse Culture* 1: 9–18.
- Department of Primary Industries, Water and Environment (2008) *Variegated Thistle (Silybum marianum L.) Control Guide*. Hobart: Department of Primary Industries, Water and Environment.
- Dodd J (1989) Phenology and seed production of variegated thistle, *Silybum marianum* (L.) Gaertn., in Australia in relation to mechanical and biological control. *Weed Research* 29: 255–263. <https://doi.org/10.1111/j.1365-3180.1989.tb00910.x>.
- Duran Katar K, Arslan Y, Subasi I (2013) Effect of different plant densities on growth and yield of milk thistle [*Silybum marianum* (L.) Gaertn.] grown under ecological conditions of Ankara, Turkey. *Research on Crops* 14: 304–310.
- El-Elmat T, Raja HA, Graf TN, Faeth SH, Cech NB, Oberlies NH (2014) Flavonolignans from *Aspergillus izukae*, a fungal endophyte of milk thistle (*Silybum marianum*). *Journal of Natural Products* 77(2): 193–199. <https://doi.org/10.1021/np400955g>.
- El-Mallah MH, El-Shami SM, Hassanein MM (2003) Detailed studies on some lipids of *Silybum marianum* seed oil. *Asian Journal of Weed Technology* 54: 397–402. <https://doi.org/10.3989/gya.2003.v54.i4.227>.
- Fraschini F, Demartini G, Esposti D (2002) Pharmacology of silymarin. *Clinical Drug Investigation* 22: 51–65. <https://doi.org/10.2165/00044011-200222010-00007>.
- Gabay R, Plitmann U, Danin A (1994) Factors affecting the dominance of *Silybum marianum* L. (Asteraceae) in its specific habitats. *Flora* 189: 201–206. [https://doi.org/10.1016/S0367-2530\(17\)30594-7](https://doi.org/10.1016/S0367-2530(17)30594-7).
- Gabucci L, Curioni A, Garcia M, Urrutia ME (2002) Seeds production in milk thistle crop. *Acta Horticulturae* 569: 121–128. <https://doi.org/10.17660/ActaHortic.2002.569.20>.
- Ghanbari F, Zarie B, Pour-Aboughadarch A, Kordai S (2013) Investigation of 5-aminolevulinic acid (ALA) effects on seed germination and seedling growth of *Silybum marianum* under salinity stress. *International Journal of Biosciences* 3(9): 95–101. <https://doi.org/10.12692/ijb/3.9.95-101>.

- Ghassemi-Golezani K, Ghassemi S, Yaghoobian I (2017) Improving oil and flavonoid contents of milk thistle under water stress by salicylic acid. *Advances in Horticultural Science* 31: 19–23. <https://doi.org/10.13128/ahs-20721>.
- Ghavami N, Ramin AA (2008). Grain yield and active substances of milk thistle as affected by soil salinity. *Communications in Soil Science and Plant Analysis* 39: 2608–2618. <https://doi.org/10.1080/00103620802358672>
- Ghavami, N., & Ramin, A. A. (2007). Salinity and temperature effects on seed germination of milk thistle. *Communications in Soil Science and Plant Analysis* 38: 2681–2691. <https://doi.org/10.1080/00103620701662901>.
- Goeden RD (1971) The phytophagous insect fauna of milk thistle in Southern California. *Journal of Economic Entomology* 64: 1101–1104. <https://doi.org/10.1093/jee/64.5.1101>.
- Goeden RD, Ricker DW (1974) Imported seed weevils attack Italian and milk thistles in Southern California. *California Agriculture* 28: 8–9.
- Heidari Z, Kamkar B, Sinaki JM (2014) Determination of cardinal temperatures of milk thistle (*Silybum marianum* L.) germination. *Advances in Plants and Agriculture Research* 1: 172–178. <https://doi.org/10.15406/apar.2014.01.00027>.
- Ikram M, Pirzada AS, Zulfat M, Khan A and Saqib MN (2025) *Silybum marianum* (milk thistle): as antioxidant, alleviating pollution-induced oxidative stress in the presence of heavy metals contamination. *Global Research Journal of Natural Science and Technology* 3: 25–33. <https://doi.org/10.53762/grjnst.03.03.02>.
- Jaleel CA, Manivannan P, Sankar B (2007) Water deficit stress mitigation by calcium chloride in *Catharanthus roseus*: Effects on oxidative stress, proline metabolism and indole alkaloid accumulation. *Colloids and Surfaces B Biointerfaces* 60: 110–116. <https://doi.org/10.1016/j.colsurfb.2007.06.006>.
- Jamali S (2015) First report of *Septoria silybi* associated with leaf blotch of *Silybum marianum* from Iran. *Plant Science Today* 2: 21–23. <https://doi.org/10.14719/pst.2015.2.1.82>.
- Kashmir S, Khan MA, Shad AA, Marwat KB, Khan H (2016) Temperature and salinity affect the germination and growth of *Silybum marianum* Gaertn and *Avena fatua* L. *Pakistan Journal of Botany* 48: 469–476.
- Kavallieratos NG, Tomanović Z, Sarlis GP, Vayias BJ, Zikić V, Emmanouel NE (2007) Aphids (Hemiptera: Aphidoidea) on cultivated and self-sown plants in Greece. *Biology* 62: 335–344. <https://doi.org/10.2478/s11756-007-0056-x>.
- Khalil-ur-Rahman, Jahan N, Ahmed Z, Shakeel A, Aslam HKW, Sakandar HA, Raza MS (2016) Behavioral pattern of milk thistle (*Silybum marianum* L.) agronomic parameters to variable temperature and land zones of Punjab. *Journal of Global Innovations in Agricultural and Social Sciences* 4: 15–22.
- Khan MA, Blackshaw RE, Marwat KB (2009) Biology of milk thistle (*Silybum marianum*) and the management options for growers in north-western Pakistan. *Weed Biology and Management* 9: 99–105. <https://doi.org/10.1111/j.1445-6664.2009.00326.x>.
- Khatamipour M, Piri E, Esmailian Y, Tavassoli A (2011) Toxic effect of cadmium on germination, seedling growth and proline content of milk thistle (*Silybum marianum*). *Annals of Biological Research* 2: 527–532.
- Kováčiková E, Kubínek J (1986) Fading of the milk thistle (*Silybum marianum* L. Gaertn.). *Proceedings of the Institute of Scientific and Technical Information for Agriculture, Plant Protection* 22: 217–222.
- Kurkin VA (2003) Saint-Mary thistle: A source of medicine. *Pharmaceutical Chemistry Journal* 37: 189–202. <https://doi.org/10.1023/A:1024782728074>.
- Malekzade M, Mirmazloum SI, Angoran HR, Alirezalu A, Radacsi P (2011) Climate change: challenges and opportunities in agriculture. In: *AGRISAFE Final Conference Proceedings*, Budapest, Hungary, 21–23 March 2011. 187–190. Available at: <https://www.cabidigitallibrary.org/doi/pdf/10.5555/20133092387>.
- Morazzoni P, Bombardelli E (1995) *Silybum marianum* (*Carduus marianus*). *Fitoterapia* 66: 3–42.
- Moscow D, Lindow SE (1989) Infection of milk thistle (*Silybum marianum*) leaves by *Septoria silybi*. *Phytopathology* 79: 1085–1090. <https://doi.org/10.1094/Phyto-79-1085>.
- Omer EA, Refaat AM, Ahmed SS, Kamel A, Hammouda FM (1993) Effect of spacing and fertilization on the yield and active constituents of milk thistle, *Silybum marianum*. *Journal of Herbs Spices and Medicinal Plants* 1: 17–23. [https://doi.org/10.1300/J044v01n04\\_04](https://doi.org/10.1300/J044v01n04_04).
- Omidbaigi R, Karimzadeh G, Koshki MH (2003) A study on the influence of sowing date and plant density on the productivity of *Silybum marianum* (L.) Gaertn. and characteristics correlation. *Iranian Journal of Science and Technology* 1: 203–212.
- Omidbaigi R, Nobakht A (2001) Nitrogen fertilizer affecting growth, seed yield and active substances of milk thistle (*Silybum marianum*). *Pakistan Journal of Biological Sciences* 11: 1345–1349. <https://doi.org/10.3923/pjbs.2001.1345.1349>.
- Oudemans CAJA (1923) *Enumeratio Systemica Fungorum*, Vol. 4. The Hague: Apud Martinum Nijhoff.
- Parsons WT (1973) *Noxious Weeds of Victoria*. Melbourne: Inkata Press.
- Plant Protection and Quarantine (2002) Federal Noxious Weed List. *USDA Animal and Plant Health Inspection Service*. Available at: [http://plants.usda.gov/cgi-bin/federal\\_noxious.cgi](http://plants.usda.gov/cgi-bin/federal_noxious.cgi).
- Pourreza J, Bahrani A (2012) Estimating cardinal temperatures of milk thistle (*Silybum marianum*) seed germination. *American-Eurasian Journal of Agricultural and Environmental Sciences* 12: 1030–1034.
- Radjabian T, Rezazadeh SH, Fallah-Huseini H (2008) Analysis of silymarin components in the seed extracts of some milk thistle ecotypes from Iran by HPLC. *Iranian Journal of Science and Technology* 32: 141–146.
- Ramasamy K, Agarwal R (2008) Multitargeted therapy of cancer by silymarin. *Cancer Letters* 269: 352–362. <https://doi.org/10.1016/j.canlet.2008.03.053>.
- Rezwani A (2008) A new species, and a new record of a subspecies belonging to the genus *Dysaphis* (Homoptera: Aphididae) from Iran. *Journal of the Entomological Society of Iran* 27: 23–26.
- Roche C (1991) Milk thistle (*Silybum marianum* (L.) Gaertn.). *Pacific Northwest Extension Publication* no. PNW382. Washington State University/Oregon State University.
- Saccardo PA (1884) *Sylloge Fungorum*, Vol. 3. Berlin: Friedlander & Sohn.
- Safikhani S, Khoshbakht K, Chaichi MR, Amini A, Moteszarehadeh B (2018) Role of chitosan on the growth, physiological parameters and enzymatic activity of milk thistle (*Silybum marianum* L. Gaertn.) in a pot experiment. *Journal of Applied Research on Medicinal and Aromatic Plants* 10: 49–58. <https://doi.org/10.1016/j.jarmap.2018.06.002>.
- Šafránková I, Koláčková P, Růžicková G (2015) The pathogens of milk thistle (*Silybum marianum* [L.] Gaertn.) in the Czech Republic. *Journal of Advances in Agriculture* 5: 538–545. <https://doi.org/10.24297/jaa.v5i1.4264>.
- Saller R, Meier R, Brignoli R (2001) The use of silymarin in the treatment of liver diseases. *Drugs* 61: 2035–2063. <https://doi.org/10.2165/00003495-200161140-00003>.
- Sedghi, M, Gholipouri a, Sharifi RS (2008)  $\gamma$ -Tocopherol accumulation and floral differentiation of medicinal pumpkin (*Cucurbita pepo* L.) in response to plant growth regulators. *Notulae Botanicae Horti Agrobotanici Cluj-Napoca* 36: 80–84. <https://doi.org/10.15835/nbha361101>.
- Selmar D, Kleinwachter K (2013) Stress enhances the synthesis of secondary plant products: The impact of stress-related over-reduction on the accumulation of natural products. *Plant and Cell Physiology* 54: 817–826. <https://doi.org/10.1093/pcp/pct054>.
- Shimi P, Poorazar R, Jamali M, Bagherani-Torshiz N (2006) Evaluating clopyralid as a broadleaf herbicide in canola fields of Iran. *Pakistan Journal of Weed Science Research* 12: 307–311.
- Sindel BM (1991) A review of the ecology and control of thistles in Australia. *Weed Research* 31: 189–201. <https://doi.org/10.1111/j.1365-3180.1991.tb01758.x>.
- Smith T, Gillespie M, Eckl V, Knepper J, Reynolds CM (2019) Herbal supplement sales in US increase by 9.4% in 2018. *HerbalGram* 123: 62–73.



- Sofer-Arad S, Kigel H, Henkin Z and Kol M (2007) Reproduction and dispersion of annual thistles in natural pastures. In: *Proceedings of the 15th Meeting Israeli Association for Range Science*, March 14–15 2007, Ramat Hanadiv.
- Solouki H, Rezvani Moghaddam P, Phiroozjaee GK (2015) Investigating the seed germination characteristics of milk thistle (*Silybum marianum* L.) affected by magnetic field, sodium chloride and hydro-priming. *Advances in Plants and Agriculture Research* 2: 1–6. <https://doi.org/10.15406/apar.2015.02.00070>.
- Souissi T, Berner DK, Smallwood EL (2005) First report of smut caused by *Microbotryum silybum* on inovythistle. *Plant Disease* 89: 1242. <https://doi.org/10.1094/PD-89-1242A>.
- Tamouridou AA, Pantazi XE, Alexandridis T, Lagopodi A, Kontouris G, Moshou D (2018) Spectral identification of disease in weeds using multilayer perceptron with automatic relevance determination. *Sensors* 18: 2770. <https://doi.org/10.3390/s18092770>.
- Topalov V, Dechev I and Pehlivanov M (1983) Crop production of blessed thistle. In: Topalov VD, Dechev I and Pehlivanov M (eds) *Crop Production*. Sofia: Zemizdat Press, 421–424.
- USDA-ARS Agricultural Research Service (2005) Noxious weeds in the US and Canada. Available at: [http://invader.dbs.umt.edu/noxious\\_weeds/](http://invader.dbs.umt.edu/noxious_weeds/).
- Vinograd A, Zaady E, Kigel H (2011) Dynamics of plant populations in abandoned sheep corrals: processes and management. In: *Proceedings of the 19th Meeting Israeli Association for Range Science*, Ramat Hanadiv, Israel, 14–15 March 2011, 48–49.
- Xiao-Fang S, Qingsong Z, Youlinag L (2000) Regulation of salt tolerance of cotton plants at seedling emergence stage by soaking seeds in pix (DPC) and CaCl<sub>2</sub> solutions. *Jiangsu Journal of Agricultural Sciences* 16: 204–207.
- Zahir A, Abbasi BH, Adil M, Anjum S, Zia M, Ihsan-Ul-Haq (2014) Synergistic effects of drought stress and photoperiods on phenology and secondary metabolism of *Silybum marianum*. *Applied Biochemistry and Biotechnology* 174: 693–707. <https://doi.org/10.1007/s12010-014-1098-5>.
- Zahra N, Wahid A, Hafeez MB, et al. (2021a) Plant growth promoters mediated quality and yield attributes of milk thistle (*Silybum marianum* L.) ecotypes under salinity stress. *Scientific Reports* 11: 23200. <https://doi.org/10.1038/s41598-021-02435-4>.
- Zahra N, Wahid A, Hafeez MB, Shaukat K, Shahzad S, Shah T, Alyemeni MN (2022) Plant growth promoters alleviate oxidative damages and improve the growth of milk thistle (*Silybum marianum* L.) under salinity stress. *Journal of Plant Growth Regulation* 41: 3091–3116. <https://doi.org/10.1007/s00344-021-10498-w>.
- Zahra N, Wahid A, Shaukat K, Hafeez MB, Batool A, Hasanuzzaman M (2021b) Oxidative stress tolerance potential of milk thistle ecotypes after supplementation of different plant growth-promoting agents under salinity. *Plant Physiology and Biochemistry* 166: 53–65. <https://doi.org/10.1016/j.plaphy.2021.05.042>.
- Zand E, Baghestani MA, Soufizadeh S, Eskandari A, PourAzar R, Veysi M et al. (2007) Evaluation of some newly registered herbicides for weed control in wheat (*Triticum aestivum* L.) in Iran. *Crop Protection* 26: 1349–1358. <https://doi.org/10.1016/j.cropro.2006.10.011>.
- Zheljazkov V, Nikolov S (1996) Accumulation of Cd, Pb, Cu, Mn and Zn by *Silybum marianum* L. on polluted soils. *Acta Horticulturae* 426: 297. <https://doi.org/10.17660/ActaHortic.1996.426.35>.
- Zheljazkov VD, Zhelnov I, Nedkov NK (2006) Herbicides for weed control in blessed thistle (*Silybum marianum*). *Weed Technology* 20: 1030–1034. <https://doi.org/10.1614/WT-05-135.1>.



## The Impact of Smart Safe City Initiatives on Crime Reduction in Punjab

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### METADATA

#### Paper history

Received: 31 March 2025

Revised: 12 June 2025

Accepted: 20 July 2025

Published online: 13 September 2025

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(Muhammad Murtaza Chishti)

#### Keywords

Security

Crime

Security policies

Artificial intelligence

#### Citation

Chishti MM (2025) The impact of smart Safe City initiatives on crime reduction in Punjab. *Innovations in STEAM: Research & Education* 3: 25030103.

<https://doi.org/10.63793/ISRE/0028>

### ABSTRACT

**Background:** Constant technological developments have promoted the growth of Smart Safe Cities, which integrate surveillance, artificial intelligence, and data-based policing under one umbrella to enhance urban security.

**Objective:** The study investigates the effects of smart Safe City programs on street crime in Punjab. This research contributes to the urban security policy debate by providing insight into the roles performed by Smart Safe Cities in preventing crime and the guidelines that need to be adopted to further strengthen crime prevention strategies.

**Methodology:** Crime data before and after the introduction of smart surveillance technologies was examined to evaluate the effectiveness of CCTV monitoring, automated emergency response, predictive policing, and real-time tracking. A mixed-method approach was employed, incorporating crime statistics and police records to identify shifts in crime patterns.

**Results:** Analysis revealed a considerable reduction in street crime, improved police response rates, and heightened public perceptions of safety. Persistent challenges include system integration, privacy concerns, and allocation of resources.

**Conclusion:** The research adds to the debate on urban security policy by highlighting the contribution of Smart Safe Cities to crime prevention and by outlining necessary guidelines for strengthening crime strategies. Surveillance initiatives must also adhere strictly to Islamic ethical principles.

### INTRODUCTION

Smart Safe Cities are those that employ information and communication technologies (ICT) to enhance security and improve the quality of life of residents. ICT includes surveillance systems, data analysis, communication networks, and emergency response systems. Smart Safe Cities are mainly concerned with crime prevention, situational awareness, improving the efficiency of crime investigation, quicker responses to emergencies, data-driven policing, enhancing public trust, and traffic management. Predictive analytics are used to identify crime hotspots and deploy resources in advance, while real-time surveillance of public spaces detects suspicious behavior and potential threats. Digital evidence helps in identifying suspects and building cases, while emergency response systems rapidly coordinate services and optimize resource deployment and communication. Data-driven policing directs strategy and resource allocation, while crime trend tracking identifies areas for improvement. Public confidence is strengthened

through greater transparency and accountability from the police, along with community engagement and dialogue. Traffic management also benefits from technology-based enforcement of regulations and improved traffic flow control. Overall, Smart Safe Cities aim to make urban areas safer and more secure by enhancing the efficiency of law enforcement and emergency services (Ristvej 2020).

Growing street crime tendencies are new challenges in cities worldwide, particularly in Punjab, Pakistan. These are driven by socio-economic factors, policing issues, and evolving offending practices. Street crime has increased rapidly, with a growing proportion of new perpetrators. Offenders are using technology to conduct and coordinate activities such as snatching, mugging, robbery, street violence, car theft, and extortion. Challenges in managing crime and justice include poverty, unemployment, income inequality, urbanization, limited resources, corruption, and difficulties in gathering and analyzing evidence. Technological innovations also pose risks, as they can be misused by criminals. Community distrust further





contributes to underreporting and a lack of cooperation in investigations. Gang addiction fuels street offenses, while gang activity introduces elements of organized crime (Shah 2021). The Punjab Safe Cities program intends to make urban spaces more secure through technology, including video surveillance, rapid police response, and management through smart city planning. However, safety is not only about control and surveillance; it also involves trust, justice, and respect for rights and privacy. Islamic ethics guide that technology should be employed in service to society with integrity, honesty, and compassion. Surveillance, for instance, should not be used as an instrument of espionage or discriminatory targeting, but rather to prevent harm and encourage justice. Islam teaches both the protection of life and respect for privacy.

Sheikhupura, one of the developing urban centers in Punjab, Pakistan, is a major industrial and cultural town of strategic importance. Its growing population and rising crime rate pose serious challenges. The city is affected by increasing street crime, gang violence, socio-economic problems such as poverty, unemployment, and drug addiction, as well as broader law and order issues. Development brings both opportunities and challenges, requiring a multifaceted approach that includes effective law enforcement, socio-economic development, and community participation. Urbanization and population growth in Punjab have been accompanied by a sharp rise in street crimes such as snatching, robbery, and highway robbery. Criminal activity increased by 28.56% in 2023, with 1,063,518 crimes recorded compared to 759, 816 in 2022 (The News, 2024). Conventional policing has been unable to curb these crimes, leading the government to introduce the smart Safe City project. This initiative combines digital monitoring, recognition technologies, and predictive analysis to improve urban security and is currently being piloted in 18 cities across Pakistan, including Sheikhupura, Gujrat, Jhelum, Okara, and Taxila (Shahid *et al.* 2024). While preliminary results in Sheikhupura are promising, challenges remain regarding the capacity of law enforcement agencies, the efficiency of the surveillance network, public trust, and long-term sustainability. This study helps in reducing street crime, strengthening law enforcement capacity, and ensuring long-term viability in Punjab. Through this comprehensive qualitative research procedure, the study was able to construct an integrated and detailed picture of the impact of Smart Safe Cities on street crime in Sheikhupura, providing valuable insights for policy and practice.

## MATERIALS AND METHODS

The research utilized a convergent parallel mixed-methods design to study the impact of Smart Safe Cities on street crime in Punjab, using the case study of Sheikhupura. In this design, data from qualitative and quantitative approaches were collected simultaneously, analyzed separately, and

then merged to derive valid conclusions. The quantitative aspect of the study involved structured questionnaires and the analysis of crime statistics, with a sample size of 40 respondents. Crime statistics were compared between May and December 2024, after the implementation of smart city programs. Descriptive and inferential statistics were employed to examine the responses from the questionnaire, while crime rate analysis compared crime patterns before and after the introduction of smart city technology.

The study also analyzed the effect of Smart Safe Cities on street crime in Sheikhupura through a comprehensive qualitative research approach. The research philosophy followed interpretivism, as social reality was constructed from individual and collective experiences. A single case study of Sheikhupura was adopted as a representative example of Punjab's smart Safe City implementation. Data collection techniques included semi-structured interviews with opinion leaders such as police officials, residents, businesspersons, community leaders, and Safe City Authority officials. Focus group discussions (FGDs) were conducted to identify recurring experiences and perceptions regarding street crime and the Safe City initiative. Observational studies in public spaces were carried out to assess public behavior, CCTV surveillance, and police command center operations. Document analysis involved reviewing police crime reports, statistics, policy documents, local press articles, media reports, and minutes of meetings related to community affairs. Data analysis included transcription of interviews and FGDs, followed by thematic analysis to establish recurring and patterned themes. Coding schemes were used to categorize and organize data, supported by qualitative data analysis software. Narrative analysis was employed to highlight stakeholders lived experiences and how participants made sense of street crime and the Safe City initiative. Triangulation was used to enhance the validity and reliability of the study. Ethical considerations included informed consent, anonymity and confidentiality, data protection, reflexivity, and community feedback. Sampling techniques used were purposive sampling, snowball sampling, and maximum variation sampling. Limitations included subjectivity, limited generalizability, and restricted access to sensitive data and key stakeholders.

## MULTIPLE APPROACHES TO THE EFFECTIVENESS OF SMART SAFE CITY INITIATIVES IN PUNJAB

### *Theoretical frameworks*

**Crime Prevention through Environmental Design (CPTED):** It is a proactive crime prevention strategy that emphasizes the design and management of the physical environment to minimize the occurrence of crime and fear. CPTED applies simple principles like natural surveillance, natural access control, territorial reinforcement,

maintenance, and activity support. CPTED works by enhancing the perceived danger of being caught and decreasing the perceived gain from committing a crime. This approach renders criminals to carry out activities covertly. CPTED can be used in residential areas, business districts, public parks, schools, and transportation centers. The advantages of CPTED are a decrease in crime rate, improvement in safety and security, better quality of life, and better community harmony. Generally, CPTED seeks to create safer environments through management and careful design.

**Routine Activity Theory (RAT) & Rational Choice Theory:** Routine Activity Theory (RAT) and Rational Choice Theory are two of the most established criminology theories that attempt to account for crime. RAT emphasizes situational circumstances such as a motivated offender, an attractive target, and the absence of a guardian, stressing the significance of daily routines and behavior in providing opportunities for crime. Rational Choice Theory takes it for granted that people make choices based on balancing likely rewards and costs for performing a particular act. In crime, criminals exhibit criminal behavior when they reap rewards exceeding costs, based on the perceived risk of crime benefit, arrest, and punishment. Both theories offer useful knowledge regarding crime origin and assist in explaining and acting on criminality (Rege 2014).

Predictive policing is one of the cornerstones of smart city policing, utilizing advanced algorithms and data analysis to forecast where and when crime will occur. It enables police to strategically deploy resources, prevent crime, and intervene before it happens. Data-driven prediction utilizes algorithms to analyze enormous amounts of information, including historic crime patterns, demographic information, weather, and social media. RTCCs enable rapid response to events, establish the capacity for information sharing, and enhance operational coordination. Challenges exist, however, including algorithmic bias, data privacy, transparency, and accountability. Technology-driven community policing focuses on integrating technology into traditional community policing strategies, enhancing cooperation and trust between law enforcement and the populace (Joh 2019). Data-driven performance management improves accountability and efficiency by quantifying police performance and identifying areas for improvement. This data-driven decision-making informs resource allocation, policy development, and training programs, promoting evidence-based policing practices. Integrated smart city platforms combine various technologies and data platforms for public safety. Networked systems connect traffic management, environmental sensing, and emergency response. By prioritizing transparency, accountability, and community engagement, law enforcement agencies can leverage smart city technologies to create safer, equitable, and resilient communities. However, ethical, legal, and social issues must be addressed (Springs 2024). Therefore,

the future success of smart city technologies in public safety depends on balancing innovation with ethical safeguards, ensuring that advancements not only reduce crime but also uphold public trust and social equity.

**Comparative analysis of smart policing strategies in Lahore, Islamabad, and international cities:** Smart policing programs are being implemented globally. Lahore and Islamabad, cities of Pakistan, are focusing on technology to increase citizen safety and enforcement efficiency. However, these cities also have their own challenges, such as urbanization, population boom, and crime trends. The police policy of Lahore is focused on integrating technology for surveillance and traffic control, whereas Islamabad's Safe City Project employs CCTV cameras to deter crime. Insufficiency in infrastructure, shortcomings in capacity development, and non-availability of resources impede high-end technology long-term induction. Islamabad's strategy is predominantly human-oriented; it added reliance on electronic media for reporting offenses. However, there is a need to address the gaps in data compatibility, digital forensics, data analytics capacity building, and security arrangements while maintaining the balance with civilian freedoms (Agha 2016). The empirical gap in the performance of technology-driven strategies in street crime control in Punjab's Safe City initiatives is vast. Despite gargantuan investments in such programs, particularly in Lahore, there is a lack of stringent empirical data that paralyzes evidence-based policy formulation, resource distribution, and, therefore, expected public security results. Safe City schemes, whose main purpose is the extensive use of CCTV networks, have been introduced as part of a wider agenda for crime reduction, situational awareness, and rapid police response. However, the reality on the ground is different. Eyewitness accounts suggest that while Safe City programs may have improved traffic control and the monitoring of certain high-profile crimes, their impact on reducing the overall rate of street crime remains doubtful. This is due to several factors:

- 1) Peer-reviewed, systematic evidence for regular comparisons of the effects of Safe City programs on street crimes in Punjab is not extensive.
- 2) Proper and robust crime statistics, as well as utilization statistics on the application of Safe City technology, are rarely available, therefore preventing unrestricted analysis.
- 3) It is challenging to design effective studies that separate the impact of technology-based crime prevention measures from other factors, such as socioeconomic patterns and police policies, that also influence crime reduction.
- 4) While CCTV surveillance may discourage crime in some areas, it will also displace crime to other, less surveilled places. This "surveillance paradox" necessitates higher sophistication in the theoretical comprehension of the spatial dynamics of crime.
- 5) It is challenging to guarantee problem-free

operation and upkeep of advanced technological infrastructure, particularly in power-scarce and technologically backward environments.

- 6) The success of Safe City initiatives depends on having the capacity to obtain citizens' cooperation and trust, yet violation of privacy and misuse of surveillance information destroy trust.
- 7) Civil liberties and privacy are preoccupied with intrusion and privacy abuse.
- 8) Effective control mechanisms and well-established legal standards can ensure ethical and responsible use of technology-driven policing techniques.

To overcome these challenges and improve the performance of Safe City programs, efforts should focus on bridging the research gap. This requires investment in empirical research, better data disclosure and access, sound policymaking, qualitative and interdisciplinary studies, addressing ethical and legal issues, and the conduct of comparative studies within other cities in Pakistan and abroad. With research and evidence-based policy, Punjab can ensure that Safe City initiatives are effectively reducing street crime and enhancing public security (Khan 2025). The limited research on AI in law enforcement is due to its novelty, constant changes, ethical and privacy concerns, and the complexity of public trust. Factors such as transparency, perceived fairness, bias, discrimination, and data security require sophisticated research methodologies. A recent report by UNICRI on "Not Just Another Tool" provides valuable insight into global public perceptions of AI in law enforcement. It highlights the public's cautious optimism, coupled with ethical concerns surrounding privacy, discrimination, and real-time decision-making. To address the research gap, it is crucial to conduct more empirical studies, promote interdisciplinary collaboration, involve communities in the development and implementation of AI-based policing strategies, and develop ethical guidelines and regulations. By prioritizing research and public engagement, AI-based policing can be used in a way that promotes public safety and builds public trust (Schiff 2025). Crime prevention through Environmental Design (CPTED) is a cross-disciplinary tool that applies to urban and architectural design to prevent crime. It addresses natural surveillance, natural access control, territorial reinforcement, maintenance, and activity support. CPTED operates on the ability of potential offenders' behavior to be manipulated by making environments less risky for crime to be accomplished. It may be used in residential neighborhoods, business buildings, public parks and squares, schools, and transport terminals. Benefits are decreased crime rates, heightened security and safety, enhanced quality of life, and improved community cohesion. CPTED is a crime prevention strategy that addresses the role played by the environment in influencing the behavior of humans (Cozens 2005). Techno-enabled policing strategies in smart cities utilize technology and data-driven policing to enhance public safety and community-police relations. Techno-

enabled policing strategies focus on proactive and predictive crime prevention and response by applying data analytics, machine learning, and statistical algorithms to predict the possibility of crime. Some of the challenges are ensuring fair access to technology, managing data creation, and privacy guarantees. Data-Driven Policing and Performance Management measure police performance, determine where they must improve, and increase accountability. Integrated Smart City platforms combine several technologies and data platforms to provide an integrated view of public safety challenges, facilitate coordinated emergency response, and increase efficiency (Araujo 2017).

Smart safety cities embrace technology and evidence-led approaches for enhancing public safety, quality of life, and security perception among citizens and visitors. Smart Safety Cities embrace proactive prevention and best practices in security management for reducing crime, enhancing emergency response, enhancing road safety, engaging the community, and making fact-based decisions. Predictive analytics are used for identifying crime hotspots, enhancing surveillance, and reducing response time. Emergency services are supplemented by communications and real-time information systems, and smart traffic management systems reduce accidents and congestion. Public safety reporting infrastructure and up-to-date information are made available to citizens through online public spaces, and a culture of public and community safety. Data-driven decision making improves quality of life on average by reducing crime and improving response times (Tutak 2023).

#### *Practical framework*

Artificial intelligence (AI) is transforming surveillance, real-time monitoring, and online policing. AI platforms are capable of sifting through large amounts of audio and video information in real time, detecting patterns and anomalies that will elude human operators. It is thus automated and enabled to operate with little need for constant human oversight. AI is used to identify and prevent cybercrimes like hacking, identity theft, and fraud. AI-based security software can help detect malicious traffic and prevent cyberattacks. Ethical concerns are present, mainly regarding privacy, civil rights, and algorithmic discrimination. Privacy of the individual needs to be preserved while handling data privacy (Gautam 2025). Smart policing of safe cities has changed through technological advances, urban expansion, and advances in criminal thinking. Information technology and data systems prompted data-led policing, converging resources upon the basis of crime trends and rates. AI and predictive policing ramped up analysis and foresight. Mobile applications, social media platforms, body-worn cameras, online crime mapping, and public dashboards are utilized for information sharing and reporting. Data privacy, security, transparency, accountability, ethical considerations, community outreach, and technology

availability are issues of concern (Yamin 2020). The initial deployments of smart city technology in Europe, Asia, and North America set the stage for advanced systems today. These early projects were centered on using technology to solve urban problems, increase efficiency, and raise the quality of life. Europe was the pacesetter in integrated and sustainable solutions, with initiatives such as Amsterdam's "Amsterdam Smart City," Barcelona's "Smart City Barcelona," Copenhagen's "Green Energy and Transportation," and Tokyo's "Yokohama." These cities demonstrated the capability of technology in optimizing urban use of resources, reducing carbon emissions, and maximizing resource management. Asian cities, driven by economic development and rapid urbanization, saw early adoption of the Smart City in infrastructure development and technology. Singapore's "Intelligent Nation 2015" initiative laid the foundation for its own Smart City vision, bridging technology with various aspects of city life, including transport, public services, and security (Martin 2018). South Korea's Songdo city and Yokohama of Japan have pioneered integrated Smart City growth, taking advantage of the most advanced technologies, including sensor networks and big data analytics. North American municipalities like New York City's "311" and Chicago's "Array of Things" also focused on technology. Toronto started smart traffic infrastructure and open data to enhance city services. Such early steps towards smart cities focused on infrastructure, evidence-based decision-making, citizens' participation, integrated solutions, sustainability, and privacy, thus providing substantial lessons for other cities across the world (Dameri 2017).

## CASE STUDIES ON THE GLOBAL STAGE

Singapore, London, and New York City employ intelligent policing methods to improve city safety. Singapore employs CCTV networks, facial recognition, and data analysis in preventing crime, London employs data policing in crime spot identification, and the Metropolitan Police Service employs people engagement through online discussion and openness. New York City employs CompStat models in tracking crime patterns and commander accountability. Real-Time Crime Center applies information to ready response and coordination. But their social and ethical considerations must be argued so that they can be appropriately addressed (Calder 2016). Smart policing is an international trend that leverages technology to maximize situational intelligence, decrease crime, and eliminate inefficiency (Table 1, Fig. 1). Singapore has developed sophisticated sensor systems and real-time analytics, while New York City has integrated information from multiple sources for investigation. Challenges such as data privacy, algorithmic bias, cyber-attacks, public trust, and digital space divide, and ethics are also there. Success depends on public confidence, application context, use of technology, and participation of the people. Technology and social

intervention must be used responsibly and sustainably to prevent crime (Khan 2024).

The Punjab Safe Cities Authority (PSCA) is a Pakistani government program that looks to increase public safety, policing, and crime prevention. It was initiated in to bring an integrated surveillance system to major cities, with the first city being the metropolis of Lahore. The Punjab government and international partners finance the project, combining technology with police forces, traffic management systems, and intelligence agencies. PSCA also assists public communication through helplines, mobile apps, and campaigns (Maguire 2012). PSCA uses high-definition CCTV cameras in cities to identify and track down suspects with facial recognition software. Big Data Analytics and AI assist the system in predicting crime hotspots and pre-emptive measures. PSCA operates emergency call centers to reduce response time and improve emergency management. It also focuses on cybersecurity, preventing internet fraud, cybercrime, and economic crimes. PSCA performs awareness programs to raise awareness concerning crime prevention methods and emergency response. Overcoming technical challenges, PSCA has been an enormous contributor to crime prevention and law enforcement in Punjab.

The Punjab Safe City Authority (PSCA) is a technology-driven program for enhancing security, law and order, and public safety in Punjab. Created under the Punjab Safe Cities Ordinance, it collaborates with the Punjab Police to introduce new surveillance, data processing, and quick response systems. PSCA's short-term objectives are enhanced vigilance, real-time monitoring of crime, smart traffic management, improved emergency response services, and evidence-based policing. It also operates the Punjab Police Integrated Command, Control, and Communication Centre (PPIC3) and employs predictive policing and big data for predicting crime patterns. The PSCA and Punjab Police are extending surveillance to rural areas, creating AI-based crime prediction models, increasing cybersecurity, and creating public awareness by engaging with the public (Gilling 2010).

## PUNJAB SAFE CITIES AUTHORITY AND CRIME COMPARISONS

The PSCA is the most significant organization in Pakistan's public safety and security, utilizing high-tech systems such as CCTV surveillance, crime-prediction systems based on AI, converged emergency response systems, a central PPIC3 hub, an e-challan system for facilitating traffic policing, and a virtual police station for women. These services aim to revolutionize policing, improve public safety, strengthen emergency response capacity, enable data-driven decision-making, and improve traffic management. The main goals of the PSCA are to secure cities using technology-driven prevention of crime, detection, and response (Basthikodi 2024). It utilizes

predictive analytics to supplement its proactive policing. It is a step in the direction of a policing and public safety revolution. Predictive analytics is used for crime hotspot mapping, risk assessment, resource allocation, and trend identification, and optimization of response times. By examining previous crime statistics, trends, and patterns, PSCA is able to predict where most of the future crimes will take place, thereby allowing police resources to be allocated where they are needed the most. With the use of machine learning and AI algorithms, it can discern patterns and associations in the data and predict future criminal behavior. Its advantages are maximized crime prevention, maximized resource use, enhanced public safety, and enhanced policing. Ethical issues of discrimination and bias must be addressed. Precision in the data is required since the quality of the output is based on the quality of the data. Finally, it is involved in crime prevention to make communities safer using predictive analytics. Ethical concerns and accuracy of data need to be ensured to allow responsible and transparent utilization of these systems (Montasari 2023). Finally, predictive analytics plays a vital role in crime prevention by supporting safer communities. Its effectiveness, however, depends greatly on the accuracy and reliability of the data being utilized. Ethical concerns, including issues of privacy, fairness, and accountability, must be carefully addressed to ensure responsible application. Transparency in processes and decisions is equally important for building public trust and legitimacy.

### **CASE STUDIES OF SMART SAFE CITIES IN PUNJAB: LAHORE, RAWALPINDI, MULTAN, AND FAISALABAD**

Smart policing is being extended to urban cities in Punjab, including Lahore, Rawalpindi, Multan, and Faisalabad. This modernization includes Safe City Projects, which use CCTV surveillance networks, command and control centers, and AI for real-time monitoring and crime detection. Smart Police Stations are being created to improve efficiency and citizen participation. Data-policing helps identify crime hotspots, forecast trends, and enhance resource deployment. Technology integration helps law enforcers curb crimes, such as property-related crimes. However, challenges such as data privacy, algorithmic bias, infrastructure constraints, public trust, and cost constraints need to be addressed for the responsible and ethical use of these technologies (Hong 2022).

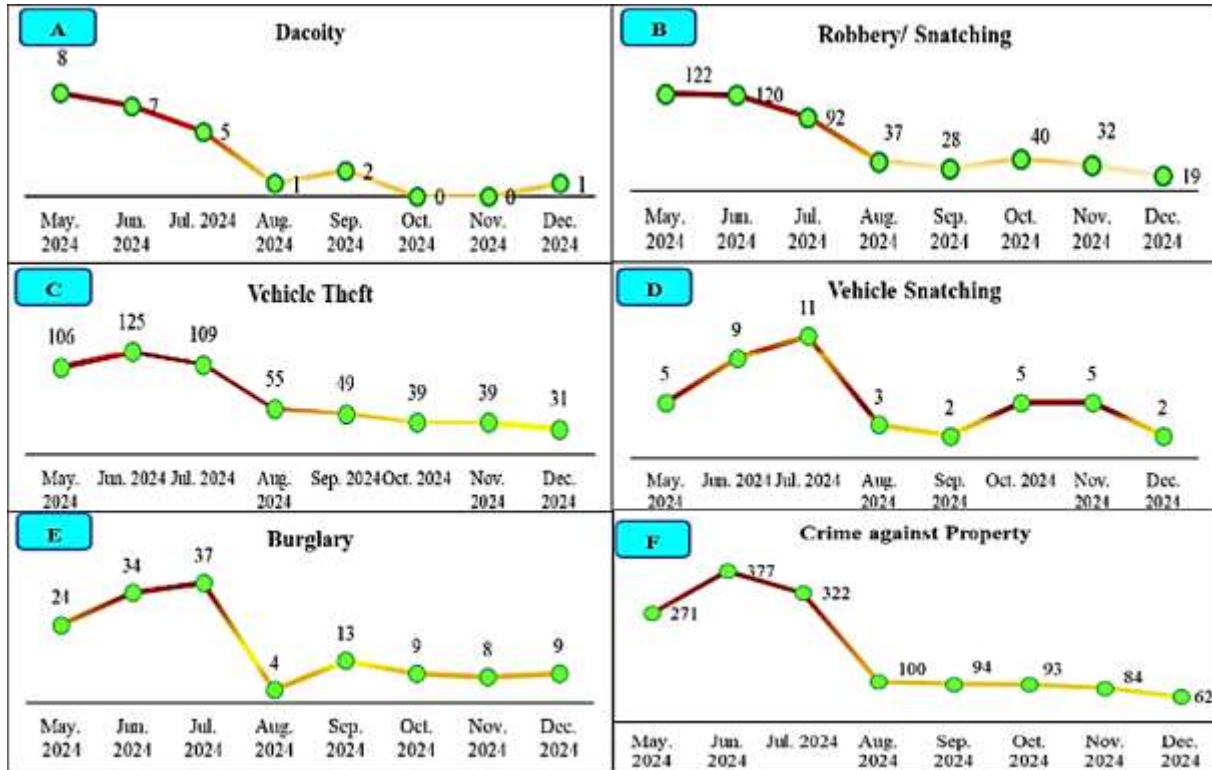
Street crime rate analysis in urban Punjab cities is a complex process involving knowledge of socio-economic determinants and data availability. High crime rates are determined by poverty, unemployment, income inequality, population density, and urbanization. Crime rates, place, demographic information, police response time, arrest and clearance, and socio-economic information are the key predictors. Statistical modeling and Geographic Information

Systems (GIS) are employed to identify trends and patterns over time. Data sources employed include the official Punjab Police website, Punjab Bureau of Statistics, and Pakistani research institutions and universities (Haider 2015). "The key indicators are the decrease in crime rate, clearance rate, response time, public safety perception, and arrest rate. The research will establish whether the intervention through the Smart City is successful or not, whether it leads to decreases in crime rates in specific locations, or whether there are partial results (Tariq 2024). Sheikhpura, a district of the province of Punjab in Pakistan, once well known for its past glory and richness in agriculture, was faced with an ugly situation: an outbreak of street crime, supremacy of gang culture, and failure of traditional models of policing. This convergence of causes produced a climate of insecurity and fears that has destabilized the social fabric and quelled the growth of the district. To get to the root causes of this crisis, one would have to explore the field of socio-economic dynamics, the criminal network's history, and the local police saga (Shahid *et al.* 2024). Sheikhpura, a district of Punjab province, is facing a multifaceted socio-economic problem that is leading to an increase in the crime rate. Unemployment and poverty among the population, urbanization, and slum development are adding to the problem. Social injustice, drug addiction, and improper access to education are also adding to the problem. Illegal gangs have developed from street crime to criminal gang outfits, victimizing susceptible businesses and individuals. Gangsterism has also expanded, with legal criminal gangs dominating the majority of criminal enterprises. Instability in the region is also caused by corruption claims against criminal gang activities and political actors. Online crime, like internet blackmail and scams, has also become prevalent because of improvements in technology. Sheikhpura police force is overwhelmed with fighting the incidence of crime, with limited resources and no exposure to modern policing techniques. Inefficiency and corruption have produced a lack of confidence in the police. Coordination and communication within society, enhanced public awareness programs, and application of technology to make the criminal justice system more efficient are needed to reverse this trend. The correctional system is redirected towards rehabilitation and social reinsertion (Shahid *et al.* 2024).

The intelligent policing strategy of Punjab relies on sparse crime records and socio-economic factors like unemployment, poverty, and urbanization. Emerging technologies like cybercrime also have an impact on the trends in crime. The strategy includes Safe City Projects, which utilize networks of CCTV surveillance, data processing, and communication systems for situational awareness, crime deterrence, and improved police response. The project command center, the PPIC3 center, addresses traffic management, crime detection, and public safety. The expected results of intelligent policing are crime prevention, enhanced detection, response time, and enhanced public

**Table 1.** The crime statistics from May 2024 to December 2024

Types of crime	May. 2024	Jun. 2024	Jul. 2024	Aug. 2024	Sep. 2024	Oct. 2024	Nov. 2024	Dec. 2024
Crime against property	271	377	322	100	94	93	84	62
Dacoity	8	7	5	1	2	0	0	1
Robbery/ snatching	122	120	92	37	28	40	32	19
Vehicle theft	106	125	109	55	49	39	39	31
Vehicle snatching	5	9	11	3	2	5	5	2
Burglary	24	34	37	4	13	9	8	9

**Fig. 1.** The crime statistics from May 2024 to December 2024. (A) dacoity, (B) robbery/snatching, (C) vehicle theft (D) vehicle snatching, (E) burglary, and (F) crime against property.

security. Shortcomings, however, are data confidentiality, algorithmic bias, infrastructure constraints, and socio-economic conditions. There must be systematic interrogation and examination in order to measure the efficacy of intelligent models of policing to solve the annoyance wrought (Cheema 2017).

Smart policing will bring down mugging, robbery, snatching, and street violence in Punjab. Some limitations of smart policing are also there, including high crime rates, a lack of proper monitoring, fear-based violence, and complexity in investigation. Smart policing can prevent opportunity crimes, enhance investigation, and optimize response time. Intelligent policing also needs to take care of the issue of gangs, drug deals, and the involvement of the community. It should have a comprehensive plan that

includes the gathering of intelligence, community liaison, and collaboration with law enforcement (Harding 2019). The Punjab Safe City projects in Pakistan have lowered rates of crime greatly, particularly in hotspots. The significant impacts are reduced property crime, such as robberies and motor vehicle theft, and better data analysis and monitoring, leading to the rapid arrest of criminals. Lowering emergency helpline calls regarding serious crimes and street crimes means a positive role of the Safe City projects. Live monitoring and control centers enable quick response by the police to incidents, enhancing crime deterrence and intervention. Mass installation of CCTV cameras and other monitoring devices gives a perception of increased surveillance, deterring crime hotspots, and enhancing public safety. Traffic management is also

included in Safe City projects, using technology like AI and Automatic Number Plate Recognition (ANPR) cameras to monitor traffic rules. Technology deployment, combined command centers, and data-based policing facilitate such an improvement. These, however, require due consideration of the reliability of crime data, privacy issues, and socio-economic conditions while quantifying the effect of such schemes. Crime displacement is not avoided but rather happens at other places where there is lower vigilance. In conclusion, while the Punjab Safe City initiatives have been successful, they require keen continuous oversight and ethical standards to ensure long-term success (Ashraf 2023). Crime displacement occurs when crime prevention initiatives within a specific location drive criminal activity to neighboring regions. It is also possible that it can be driven by technology, where Safe City initiatives can deter crime but may push it to less-watched locations. Integrative crime prevention focused on socio-economic conditions, community engagement, and police is important. Statistical evaluation and continuous monitoring are important in measuring whether there is potential displacement impact and setting unforeseen consequences (Repetto 1976).

Punjab Police has introduced an Electronic Challan System, which is linked with the Red-Light Monitoring System (RLMS) and Journey Time Monitoring System (JTMS) for serving traffic offense notices to defaulters. Defaulters are given a chance to pay fines online, while hand-held terminals enable officials to serve no-license or wrong-license notices. The system has enhanced service delivery by providing integrated services such as Rescue 1122, firefighting, and disaster. The scheme has minimized crimes such as rioting, destruction of properties of public and private properties, and motor vehicle-related crimes. Preventive technologies ensure real-time monitoring of processions and law and order situations, enabling effective resource deployment and efficient emergency response systems. Police operate through a dedicated LTE/4G network that facilitates secure communication, thereby strengthening coordination and delivery of public safety and emergency services. Enhanced policing of public spaces, better allocation of police personnel, and improved command and control facilities have collectively contributed to greater security for the capital city, its residents, and visitors (Ahmad 2021). The Data Protection Policy (DP3) is a policy that all users of PSCA data, including those gathered through PSCA infrastructure and communicated via social and electronic media. It requires that officers not intentionally record any act of a natural person violating rules unless there is reasonable cause to believe that the person is committing or attempting to commit an offense. Officers should remain vigilant while collecting information on women and children and should not use or retain information on personal devices without authorization from the competent authority. They should also refrain from monitoring citizens' private domains unless it is necessary and proportionate to legitimate needs. Officers must not

share objectionable photographs or videos that could infringe upon the rights of the parties involved. Any breach of DP3 shall constitute misconduct and will be dealt with against officers and employees. Operators' contracts will be terminated as a result of DP3 breaches, without prejudice to other legal liabilities.

## CONCLUSION AND FUTURE PROSPECTS

The Sheikhpura Safe City project has adopted technologies such as CCTV surveillance, data analysis, and real-time monitoring to improve the effectiveness of law enforcement. However, challenges remain, including crime displacement, socio-economic issues, technical and operational difficulties, public trust, data privacy, and the availability of integrated crime data. The success of the project relies on camera quality, network stability, and the efficiency of law enforcement. Future directions emphasize systems-based, evidence-driven assessment, citizen participation, timely technology updates, protection of privacy, and greater transparency. AI policing measures hold long-term potential, but it is the responsibility of Safe City Authorities to ensure strong data security, interoperability, public trust, openness, and responsiveness. AI-based policing can evolve from individual applications to a broader approach, but this requires a balance between high moral values, data protection, and citizen participation.

## DATA AVAILABILITY

Not applicable to this paper

## ETHICS APPROVAL

Not applicable to this paper.

## FUNDING SOURCE

Not applicable to this paper.

## REFERENCES

- Agha A (2016) A stakeholder-based assessment of developing country challenges and solutions in smart mobility within the smart city framework: A case of Lahore. Cambridge: University of Cambridge.
- Ahmad I, Bashir A (2021) Local governments and sustainable urban development: A case study of Punjab. *Journal of Evaluation* 13: 234–258.
- Araujo AC (2017) A predictive policing application to support patrol planning in smart cities. *2017 International Smart Cities Conference (ISC2)*: 1–6. IEEE. <https://doi.org/10.1109/ISC2.2017.8090817>.
- Ashraf SY (2023) Political implications of unplanned urbanization in Pakistan: A case of Punjab. *Journal of Development and Social Sciences* 4: 932–939. [https://doi.org/10.47205/jdss.2023\(4-II\)81](https://doi.org/10.47205/jdss.2023(4-II)81).
- Basthikodi MV (2024) AI-based automated framework for crime detection and crowd management. *Second International Conference on Advances in Information Technology (ICAIT)* 1: 1–6. IEEE. <https://doi.org/10.1109/ICAIT61638.2024.10690527>.



- Calder KE (2016) *Singapore: Smart City, Smart State*. Washington DC: Brookings Institution Press.
- Cheema AH (2017) Victimization, citizen engagement, and policing in Lahore. *Institute of Development and Economic Alternatives, Policy Report*, Lahore, Pakistan. Available at: [https://ideasdev.org/wp-content/uploads/2022/10/Victimization-Citizen-Engagement-and-Policing-in-Lahore-01.11.22\\_Report.pdf](https://ideasdev.org/wp-content/uploads/2022/10/Victimization-Citizen-Engagement-and-Policing-in-Lahore-01.11.22_Report.pdf).
- Cozens PM (2005) Crime prevention through environmental design (CPTED): A review and modern bibliography. *Property Management* 23: 328–356. <https://doi.org/10.1108/02637470510631483>.
- Dameri RP (2017) *Smart City Implementation*. Progress in IS. Genoa: Springer.
- Gautam VS (2025) AI-powered surveillance for smart cities. In: *Internet of Vehicles and Computer Vision Solutions for Smart City Transformations*. Cham: Springer Nature, 245–265. [https://doi.org/10.1007/978-3-031-72959-1\\_11](https://doi.org/10.1007/978-3-031-72959-1_11).
- Gilling D (2010) Crime control and due process in confidence-building strategies: A governmentality perspective. *The British Journal of Criminology* 50: 1136–1154. <https://doi.org/10.1093/bjc/azq051>.
- Haider A, Anwar S (2015) Socio-economic determinants of crimes: A cross-sectional study of Punjab districts. *International Journal of Economics and Empirical Research* 3: 550–560.
- Harding SD (2019) A typology of street robbery and gang organization: Insights from qualitative research in Scotland. *The British Journal of Criminology* 59: 879–897. <https://doi.org/10.1093/bjc/azy064>.
- Hong C (2022) Safe cities in Pakistan: Knowledge infrastructures, urban planning, and the security state. *Antipode* 54: 1476–1496. <https://doi.org/10.1111/anti.12799>.
- Joh EE (2019) Policing the smart city. *International Journal of Law in Context* 15: 177–182. <https://doi.org/10.1017/S1744552319000107>.
- Khan AA (2024) Reconceptualizing policing for cybercrime: Perspectives from Singapore. *Laws* 13: 44. <https://doi.org/10.3390/laws13040044>.
- Khan AF (2025) The role of technology in enhancing police efficiency in Pakistan's criminal justice system. *Sociology & Cultural Research Review* 3: 228–243.
- Maguire M (2012) Criminal investigation and crime control. In: *Handbook of Policing*. Uffculme: Willan, 430–464.
- Martin CJ (2018) Smart and sustainable? Five tensions in the visions and practices of the smart-sustainable city in Europe and North America. *Technological Forecasting and Social Change* 133: 269–278. <https://doi.org/10.1016/j.techfore.2018.01.005>.
- Montasari R (2023) The application of big data predictive analytics and surveillance technologies in the field of policing. In: *Countering cyberterrorism: The Confluence of Artificial Intelligence, Cyber Forensics and Digital Policing in US and UK National Cybersecurity*. Cham: Springer.
- Rege A (2014) A criminological perspective on power grid cyber attacks: Using routine activities theory to rational choice perspective to explore adversarial decision-making. *Journal of Homeland Security and Emergency Management* 11: 463–487. <https://doi.org/10.1515/jhsem-2013-0061>.
- Reporter OS (2024, October 25) CM Maryam inaugurates safe city project in Sheikhupura. *The Nation*. Available at: <https://www.nation.com.pk/25-Oct-2024/cm-maryam-inaugurates-safe-city-project-in-sheikhupura>.
- Repetto TA (1976) Crime prevention and the displacement phenomenon. *Crime & Delinquency* 22: 166–177. <https://doi.org/10.1177/001112877602200204>.
- Ristvej JM (2020) On smart city and safe city concepts. *Mobile Networks and Applications* 25: 836–845. <https://doi.org/10.1007/s11036-020-01524-4>.
- Schiff KJ (2025) Institutional factors driving citizen perceptions of AI in government: Evidence from a survey experiment on policing. *Public Administration Review* 85: 451–467. <https://doi.org/10.1111/puar.13754>.
- Shah S, Bukhari M (2021) Understanding crime dynamics: Social and demographic influences in Punjab, Pakistan. *Journal of Business and Economic Options* 4: 30–38.
- Shahid M, Ahmad K, Inayat MA, Bhatti MK (2024) Socio-economic determinants of property crime across the districts of Punjab: Highlighting the role of law enforcement agencies of Pakistan. *Bulletin of Business and Economics* 13: 22–36. <https://doi.org/10.61506/01.00289>.
- Springs D (2024) Smart city planning focused on US cities in need of policing innovations and public health safety technologies and strategies. *Health Economics and Management Review* 5: 117–128. <https://doi.org/10.61093/hem.2024.1-09>.
- Tariq HA (2024) Unveiling the efficacy: Examining the influence of public surveillance cameras on crime rates. *Journal of Excellence in Social Sciences* 3: 33–46. <https://doi.org/10.69565/jess.v2024i3.185>.
- Tutak M, Brożek M (2023) A smart city is a safe city: Analysis and evaluation of the state of crime and safety in Polish cities. *Smart Cities* 6: 3359–3392. <https://doi.org/10.3390/smartcities6060149>.
- Yamin MM (2020) Smart policing for a smart world: Opportunities, challenges and way forward. In: *Advances in information and communication: Proceedings of the 2020 Future of Information and Communication Conference (FICC), Volume 1*. Cham: Springer International Publishing: 532–549. [https://doi.org/10.1007/978-3-030-39445-5\\_39](https://doi.org/10.1007/978-3-030-39445-5_39).



## Morphological Note on Sweet Orange at Different Developmental Stages

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### METADATA

#### Paper history

Received: 15 April 2025

Revised: 25 July 2025

Accepted: 18 August 2025

Published online: 28 October 2025

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#### Keywords

Growth

Stomata

Flavedo

Albedo

Developmental stages

#### Citation

Nazeer S, Bashir S (2025). Morphological note on citrus. *Innovations in STEAM: Research & Education* 3: 25030202.

<https://doi.org/10.63793/ISRE/0029>

### ABSTRACT

**Background:** *Citrus sinensis* (L. Osbeck) is commonly known as sweet orange and widely distributed as an excellent source of antioxidants and vitamin C, which play a pivotal role in strengthening the immune system. The peel of sweet orange is a major source of various bioactive compounds that are utilized in different medicines.

**Objective:** This study aimed to explore the anatomical changes occurring at different developmental stages of sweet orange fruit.

**Methodology:** Orange fruits at different developmental stages were collected from the Botanical Garden of the University of Agriculture, Faisalabad. For this purpose, 16 developmental stages of sweet orange were selected on the basis of their size, growth, and development. Data were obtained for stomatal density, number of cells, and cell size. Furthermore, the thickness of albedo and flavedo was also recorded at each developmental stage.

**Results:** Results revealed that fruit diameter, cell size, stomatal number, number of hesperidia, and the thickness of albedo and flavedo increased progressively with fruit development and maturation. The maximum increase in all the recorded parameters was noted at stage 16, followed by stage 15. Moreover, significant variations in anatomical structures were observed across different developmental stages.

**Conclusion:** This study highlights the growth dynamics and structural modifications of sweet orange across different developmental stages and explores the progressive enlargement of cell size, albedo, and flavedo thickness with developmental stages.

## INTRODUCTION

Citrus is one of the most popular fruits worldwide, grown in over 130 countries, including Brazil, China, and the USA (Ladaniya 2008; Spreen *et al.* 2020). It has major nutritional and economic importance (Liu *et al.* 2012) and plays a key role in the fresh juice market (Cuenca *et al.* 2018). In the early 20<sup>th</sup> century, the words citrus production surpassed 105 million metric tons per year (FAOSTAT 2019). However, biotic and abiotic stress hindered its growth during the last two decades (Febres *et al.* 2011; Luckstead and Devadoss 2021). These problems significantly affect fruit development and quality, leading to a yield penalty (Gong and Liu 2013; Gottwald 2007). Sweet orange (*Citrus sinensis* (L.) Osbeck), widely regarded as a cornerstone of global agriculture, accounts for nearly half of

total citrus production. The crop maintained an estimated yield of 47.4 million tons for 2023–2024, with major contributions from Brazil, the United States, and China (Gabash *et al.* 2023). Sweet orange grows well in subtropical and tropical regions, representing both natural adaptation and human cultivation, and holds immense economic value. True citrus species are characterized by distinct morphological traits such as pulp vesicles, which make them among the most advanced within this genus (Penjor *et al.* 2014).

Morphological and biochemical analyses have long played an important role in clarifying citrus phylogeny, but these methods are often limited by environmental variability (Martasari *et al.* 2013). Other studies have described phylogenetic relationships based on the origin of oil glands in citrus, which arise through schizogenous and lysigenous



processes (Thomson *et al.* 1976; Bosabalidis and Tsekos 1982; Turner *et al.* 1998). Sweet orange fruit formation occurs in three layers: the exocarp (flavedo), mesocarp (albedo), and endocarp. The flavedo layer consists of secretory cavities of volatile compounds that are an enriched source of monoterpenes, responsible for botanical and economic values (Bishnoi *et al.* 2025). Moreover, orange peel is a great source of bioactive compounds, including monosaccharides, pectin, minerals, fibers, polyphenols, and essential oils (Brezo-Borjan *et al.* 2023). The essential oil fraction is characterized by terpenoid compounds dominated by limonene. These are oxygenated derivatives and include ester forms, aldehyde, and alcohol (Senit *et al.* 2019). Polyphenolic compounds are another major group of biomolecules that are present in orange peel, which includes flavonoids, phenolic acid, and their derivatives (Senit *et al.* 2023; Rathod *et al.* 2023). The major carbohydrates include hemicellulose, cellulose, glucose, monosaccharides, disaccharides, and pectin (Brezo-Borjan *et al.* 2023). However, orange peel is often discarded as waste, but it can be utilized for the treatment of diseases (Grover *et al.* 2024; Odetayo *et al.* 2025). The present research was conducted to examine anatomical modifications at different growth stages of fruit development to explore its structural changes and growth patterns.

## MATERIALS AND METHODS

An experiment was conducted to study the anatomical changes at different developmental stages of the sweet orange. Samples were collected from the Botanical Garden

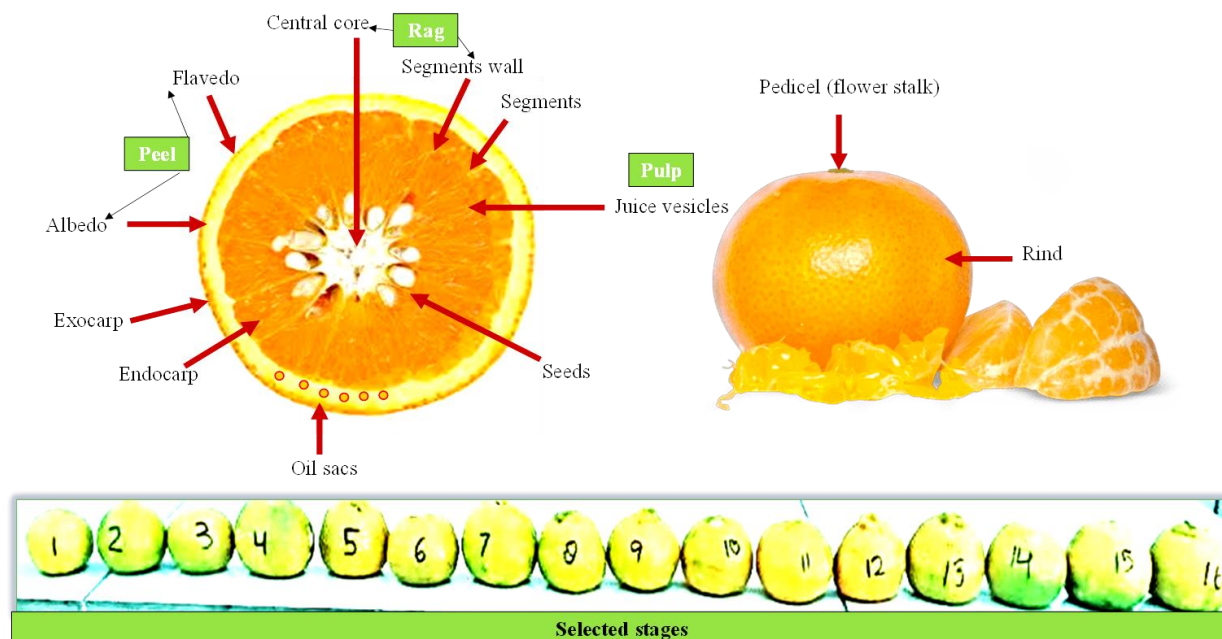
of the University of Agriculture, Faisalabad. Sixteen orange stages were selected based on their growth stages, and the fruit diameter of each was measured using a vernier caliper. The samples were washed with water, dried, and coated with transparent nail polish on the fruit surface. Three replicates of similarly sized oranges were taken to minimize the experimental error. After drying, the nail polish layer was carefully peeled away. The replicas of stomata were placed on glass slides, examined under a microscope, and stomatal density was calculated. Cell size and the number of cells on the orange surface were also recorded. Finally, the thickness of the flavedo and albedo was measured at each developmental stage (Fig. 1).

## RESULTS

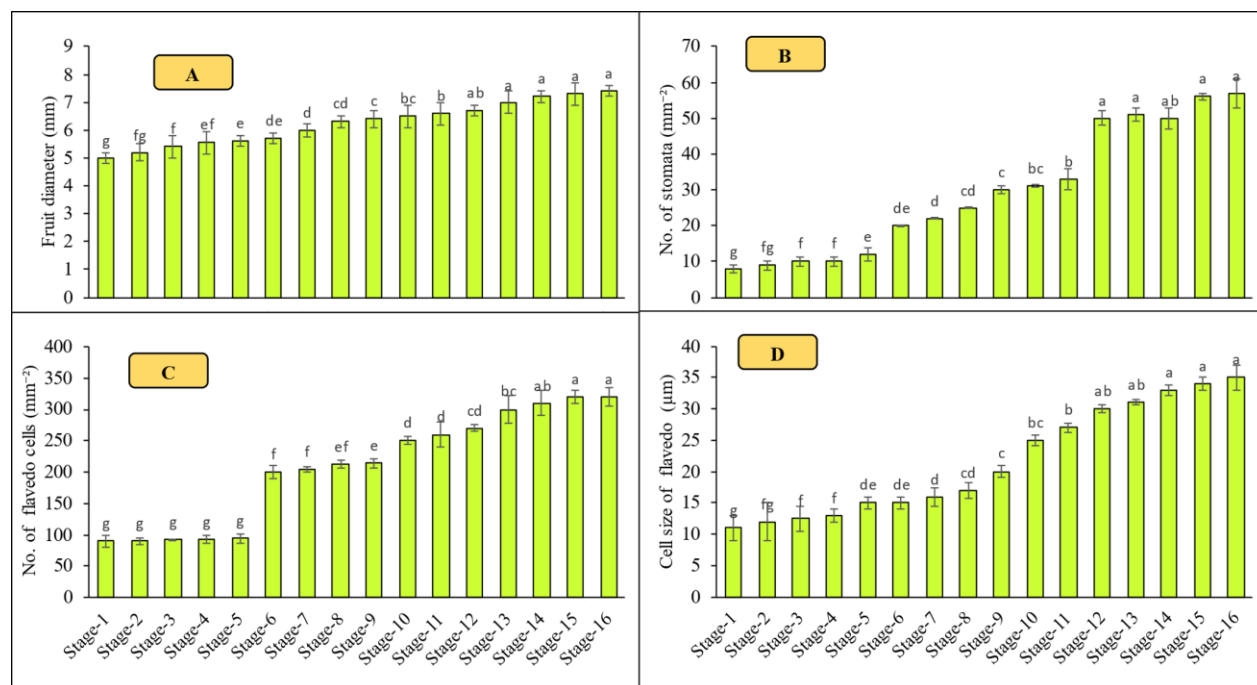
### Fruit diameter

Graphical data indicated that fruit diameter increased progressively with developmental stages. The diameter at stage 16 was larger than at other stages. Overall, the results showed that fruit size increased with development, while non-significant differences were observed between stages 15 and 16. The trend of improvement for this attribute was; Stage-16 > Stage-15 > Stage-14 > Stage-13 > Stage-12 > Stage-11 > Stage-10 > Stage-9 > Stage-8 > Stage-7 > Stage-6 > Stage-5 > Stage-4 > Stage-3 > Stage-2 > Stage 1 (Fig. 2 A).

### Number of stomata



**Fig.1:** Depiction of different parts of the sweet orange peel and the selection of various developmental stages of citrus fruit



**Fig. 2:** Variations in the fruit diameter (A), number of stomata (B), number of flavedo cells (C), and cell size of flavedo (D) of sweet orange at different developmental stages

Results revealed that the number of stomata increased with developmental stages. The lowest stomatal count was recorded during the early stages; however, the number increased during fruit ripening. Stomatal density increased progressively with fruit development: stage-16 > stage-15 > stage-14 > stage-13 > stage-12 > stage-11 > Stage-10 > stage-9 > stage-8 > Stage-7 > stage-6 > stage-5 > stage-3 = stage-4 > stage-2 > stage-1 (Fig. 2B).

#### *Number of cells in flavedo*

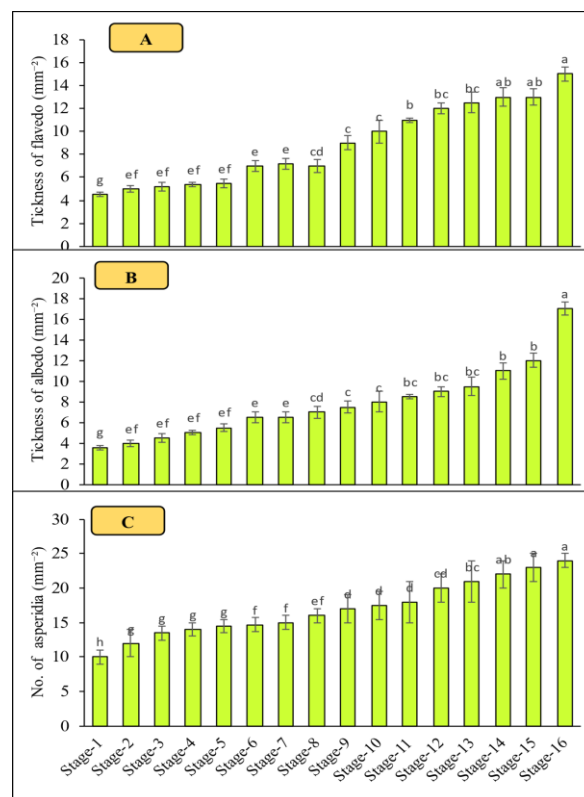
Graphical data demonstrated that the number of cells in the flavedo layer increased with developmental stages. The highest cell count was observed at stage-16, followed by stage-15. However minimum count was recorded at stage-1 (Fig. 2C).

#### *Size of cells in flavedo*

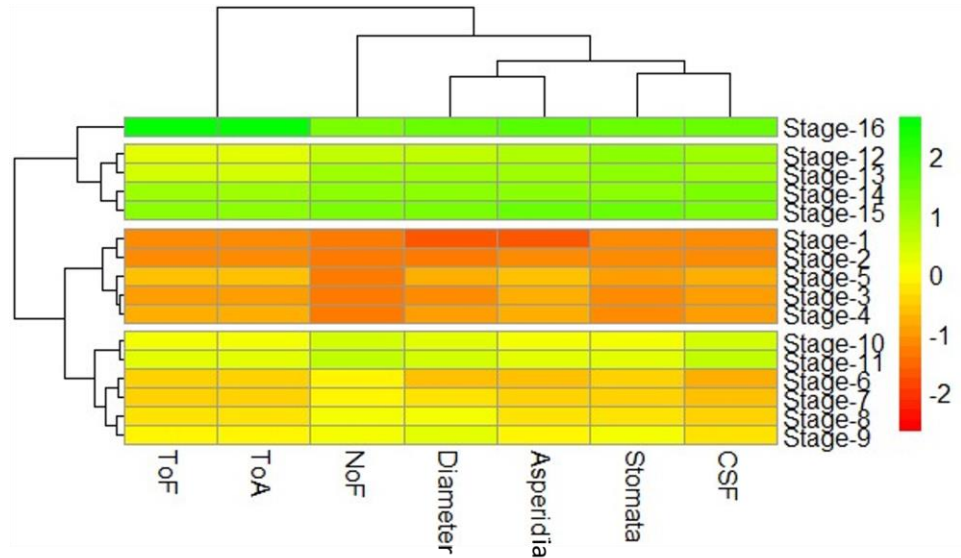
The data revealed that the cell size of the flavedo layer increased progressively with developmental stages. Cells at stage-16 were larger compared with other stages. Overall, results confirmed an increase in cell size with fruit development, and non-significant differences observed were recorded between stages-15 and 16 (Fig. 2D).

#### *Thickness of flavedo*

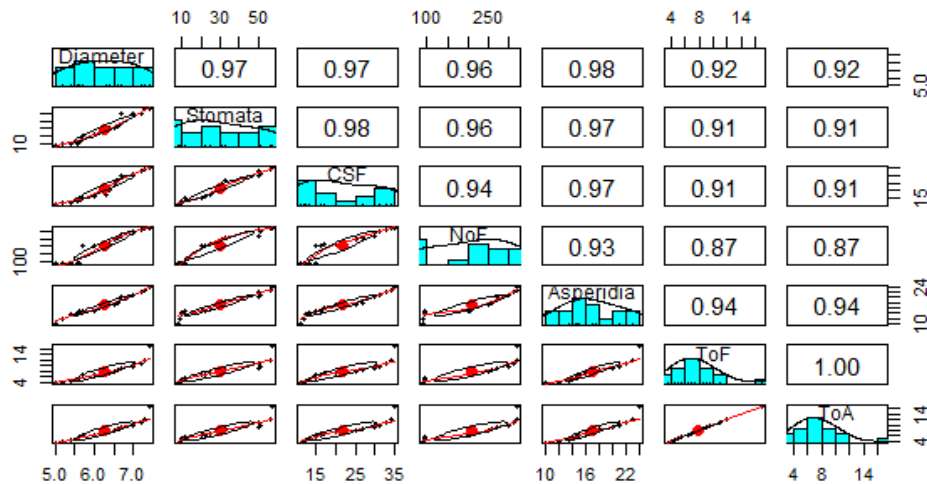
Results revealed that the thickness of the flavedo increased with developmental stages. The flavedo at stage-16 was



**Fig. 3:** Variations in the thickness of flavedo (A), thickness of albedo (B) and number of hesperidia (C), of sweet orange at different developmental stages



**Fig. 4:** The heatmap matrix on the variations in the thickness of flavedo; ToF, thickness of albedo (ToA), diameter of fruit; diameter, number of hesperidia, number of stomata; stomata and cell size of flavedo of sweet orange at different developmental stages



**Fig. 5:** Pearson correlation matrix on the variations in the thickness of flavedo; ToF, thickness of albedo (ToA), diameter of fruit; diameter, number of hesperidia, number of stomata; stomata and cell size of flavedo of sweet orange at different developmental stages

thicker compared with other stages. However slight reduction was observed at stage-15 as compared to stage-14, and again at stage-16 maximum length was recorded (Fig. 3A).

#### Thickness of albedo

Results indicated that the thickness of the albedo increased with developmental stages. The albedo at stage-16 was thicker compared with other stages. While minimum readings were recorded at stage-1. Overall, results showed that albedo thickness increased with fruit maturation. Intriguingly, abrupt increase in thickness was recorded at stage-16 (Fig. 3B).

#### Number of hesperidia

Data showed that the number of hesperidia increased with developmental stages. The lowest count was recorded at stage-1 compared with other stages, while the maximum count was observed at stage-16 (Fig. 3C).

#### Heatmap and Pearson correlation

The heatmap matrix showed a strong linear relationship of cell size of flavedo, number of stomata, hesperidia, diameter of fruit, no of cells in flavedo layer, thickness of flavedo, and



albedo with stages 12, 13, 14, and 15, while an opposite relation was recorded at stages 1, 2, 3, 4, and 5 (Fig. 4). Moreover, a non-significant relationship was observed at stages 6 to 10. Pearson correlation showed a strong positive relationship with all the studied parameters (Fig. 5).

## DISCUSSION

Citrus fruits rank among the top fruits not only in total production but also in economic value. Among them, oranges, specifically sweet oranges, are among the most widely cultivated citrus fruits in the world. The orange peel consists of a thin outer layer known as the flavedo and the thicker inner layer known as the albedo (Afifi *et al.* 2023). The flavedo is comprised of the carotenoids responsible for the typical fruit color (Kato *et al.* 2004), and vesicles (minute sacs/cavities) filled with peel oil. This peel oil is responsible for the fresh smell of the fruit. The white spongy inner albedo, on the other hand, is composed of various substances like flavonoids, d-limonene, limon, and pectin (Nieto *et al.* 2021). This experiment was conducted to explore anatomical changes at different developmental stages of the sweet orange. Samples were collected from the Botanical Garden of the University of Agriculture, Faisalabad. About 16 oranges were selected based on growth stages, and the fruit diameter of each orange was recorded using a vernier caliper. Graphical data indicated that flavedo cell size increased with developmental stages, so cells at stage-16 were larger in size compared to other stages (Fig. 2A–D). The observations of Rafiei and Rajaei (2007) also support these results. Cell number increased progressively with fruit development, reaching its maximum at stage-16, though variations between stages-15 and 16 were statistically non-significant. The number of stomata showed the same pattern, being minimal during initial development and rising significantly with ripening. Fruit diameter grew uniformly with stages of development, reaching the maximum value with stage-16, and showing no difference between stages-15 and 16. Flavedo thickness also rose steadily, and fruits with stage-16 had higher thickness compared to previous stages. Similarly, albedo thickness continued to increase with fruit development, and stage-16 fruits showed the maximum values (Fig. 3A–C).

Increase in albedo thickness with fruit ripening is reported (Oikeh *et al.* 2013). Our data showed that the number of hesperidia increased with developmental stages (Fig. 3). The lowest number of hesperidia was observed at stage 1 compared with other stages. The heatmap matrix revealed a strong linear relationship of cell size of flavedo, number of stomata, hesperidia, diameter of fruit, no of cells in flavedo layer, thickness of flavedo, and albedo with stages 12, 13, 14, and 15, while an opposite relation was recorded at stages 1, 2, 3, 4, and 5 (Fig. 4), indicating that maximum size and length were achieved at the end of the fruit maturity. Moreover, a non-significant relationship was observed at stages 6 to 10. Pearson correlation showed a strong positive relationship with all

the studied parameters (Fig. 5). Overall, the results affirmed that a progressive enlargement of cellular structures and tissue layers is a typical aspect of sweet orange fruit development and might be linked with the accretion of different bioactive compounds in this tissue.

## CONCLUSION

This study provides sufficient evidence that the anatomical features of sweet orange progressively transform during fruit maturation. It might be an adaptive strategy or an accretion and storage of bioactive compounds with the passage of time. The overall developmental trend indicated a persistent increase in tissue thickness that might be due to cell expansion and cell division. Maximum increase in flavedo, albedo thicknesses were noted between stages 15 and 16. These findings confirm structural changes in sweet orange peel with ripening. However, further studies on the composition of these layers at different growth stages are crucial, given the importance of medicinal and therapeutic uses.

## AUTHOR CONTRIBUTIONS

All authors contributed equally to the conception, design, and preparation of this manuscript.

## CONFLICT OF INTEREST

The authors declared no conflict of interest.

## ETHICS APPROVAL

Not applicable

## FUNDING SOURCE

No funding was acquired for this work.

## REFERENCES

- Afifi SM, Gök R, Eikenberg I, Krygier D, Rottmann E, Stübler AS, et al. (2023) Comparative flavonoid profile of orange (*Citrus sinensis*) flavedo and albedo extracted by conventional and emerging techniques using UPLC-IMS-MS, chemometrics and antioxidant effects. *Frontiers in Nutrition* 10: 1158473. <https://doi.org/10.3389/fnut.2023.1158473>.
- Bishnoi S, Singh G, Kalia A, Suneja Y, Rattanpal HS, Gupta M, Raina D (2025) Fruit micro-structural and biochemical traits diversity analysis of *Citrus × sinensis* (L.) Osbeck cultivars under Indian sub-tropics. *New Zealand Journal of Crop and Horticultural Science* 53: 2567–2589. <https://doi.org/10.1080/01140671.2025.2498444>.
- Bosabalidis A, Tsekos I (1982) Ultrastructural studies on the secretory cavities of *Citrus deliciosa* Ten. I. Early stages of the gland cells differentiation. *Protoplasma* 112: 55–62. <https://doi.org/10.1007/BF01280216>.
- Brezo-Borjan T, Švarc-Gajić J, Morais S, Delerue-Matos C, Rodrigues F, Lončarević I, et al. (2023) Chemical and biological characterisation of orange (*Citrus sinensis*) peel extracts obtained by subcritical water. *Processes* 11: 1766. <https://doi.org/10.3390/pr11061766>.

- Cuenca J, Garcia-Lor A, Navarro L, Aleza P (2018) Citrus genetics and breeding. In: Al-Khayri JM, Jain SM, Johnson DV (eds) *Advances in Plant Breeding Strategies: Fruits*. Cham: Springer, 403–436. [https://doi.org/10.1007/978-3-319-91944-7\\_11](https://doi.org/10.1007/978-3-319-91944-7_11).
- FAOSTAT, Trade and Markets Division (2019) *Citrus Fruit. Fresh and Processed. Statistical Bulletin 2016*. Available at: <https://openknowledge.fao.org/server/api/core/bitstreams/d90db7dd-3b56-45d3-a603-0b5e2613813a/content>.
- Febres V, Fisher L, Khalaf A, Moore GA (2011) Citrus transformation: Challenges and prospects. In: Alvarez M (ed) *Genetic Transformation*. London: IntechOpen. <https://doi.org/10.5772/24526>.
- Gabash HM, Rabea JARA, Mohammed KH (2023) Response of anatomical traits to environmental stresses in the leaves of local orange seedlings (*Citrus sinensis* L.). *Journal of Global Innovations in Agricultural Sciences* 11: 439–445. <https://doi.org/10.22194/JGIAS/23.1123>.
- Gong XQ, Liu JH (2013) Genetic transformation and genes for resistance to abiotic and biotic stresses in citrus and its related genera. *Plant Cell, Tissue and Organ Culture* 113: 137–147. <https://doi.org/10.1007/s11240-012-0267-x>.
- Gottwald TR (2007) Citrus canker and citrus Huanglongbing, two exotic bacterial diseases threatening the citrus industries of the Western Hemisphere. *Outlook on Pest Management* 18: 274–279. <https://doi.org/10.1564/18dec09>.
- Grover S, Aggarwal P, Kumar A, et al. (2025) Utilizing citrus peel waste: A review of essential oil extraction, characterization, and food-industry potential. *Biomass Conversion and Biorefinery* 15: 5043–5064. <https://doi.org/10.1007/s13399-024-05382-y>.
- Kato M, Ikoma Y, Matsumoto H, Sugiura M, Hyodo H, Yano M (2004) Accumulation of carotenoids and expression of carotenoid biosynthetic genes during maturation in citrus fruit. *Plant Physiology* 134: 824–837. <https://doi.org/10.1104/pp.103.031104>.
- Ladaniya MS (2008) Commercial fresh citrus cultivars and producing countries. In: *Citrus Fruit: Biology, Technology and Evaluation*. San Diego, CA: Academic Press, 13–65. <https://doi.org/10.1016/b978-012374130-1.50004-8>.
- Liu Y, Heying E, Tanumihardjo SA (2012) History, global distribution, and nutritional importance of citrus fruits. *Comprehensive Reviews in Food Science and Food Safety* 11: 530–545. <https://doi.org/10.1111/j.1541-4337.2012.00201.x>.
- Luckstead J, Devadoss S (2021) Trends and issues facing the U.S. citrus industry. *Choices* 36: 1–10. <https://www.jstor.org/stable/27098590>.
- Martasari C, Agisimanto D, Karsinah R (2013) Characterization of Indonesian tangerine cultivar by morphological and ISSR markers. *Acta Horticulturae* 975: 103–110. <https://doi.org/10.17660/ActaHortic.2013.975.10>.
- Nieto G, Fernández-López J, Pérez-Álvarez JA, Peñalver R, Ros-Berruazo G, Viuda-Martos M (2021) Valorization of citrus co-products: Recovery of bioactive compounds and application in meat and meat products. *Plants* 10: 1069. <https://doi.org/10.3390/plants10061069>.
- Odetayo A, Bassey GE, Adeyemi AL, Olayaki LA (2025) From waste to health: Exploring the male sexuality enhancing activities of the bioactive components of orange peel. *Frontiers in Endocrinology* 16: 1598545. <https://doi.org/10.3389/fendo.2025.1598545>.
- Penjor T, Mimura T, Matsumoto R, Yamamoto M, Nagano Y (2014) Characterization of limes (*Citrus aurantifolia*) grown in Bhutan and Indonesia using high-throughput sequencing. *Scientific Reports* 4: 4853. <https://doi.org/10.1038/srep04853>.
- Rafiei M, Rajaei H (2007) Structural and developmental studies on oil producing reproductive organs in lime (*Citrus aurantifolia* Swingle). *Journal of Biological Sciences* 7: 848–853.
- Rathod NB, Elabed N, Punia S, Ozogul F, Kim SK, Rocha JM (2023) Recent developments in polyphenol applications on human health: A review with current knowledge. *Plants* 12: 1217. <https://doi.org/10.3390/plants12061217>.
- Senit JJ, Velasco D, Manrique AG, Sanchez-Barba M, Toledo JM, Santos VE, et al. (2019) Orange peel waste upstream integrated processing to terpenes, phenolics, pectin and monosaccharides: Optimization approaches. *Industrial Crops and Products* 134: 370–381. <https://doi.org/10.1016/j.indcrop.2019.03.060>.
- Spreen TH, Gao Z, Fernandes W, Zansler ML (2020) Global economics and marketing of citrus products. In: Talon M, Caruso M, Gmitter FG (eds) *The Genus Citrus*. Amsterdam: Elsevier, 471–493. <https://doi.org/10.1016/B978-0-12-812163-4.00023-1>.
- Thomson WW, Platt-Aloia KA, Endress AG (1976) Ultrastructure of oil glands in the leaf of *Citrus sinensis* L. *Botanical Gazette* 137: 330–340. <https://doi.org/10.1086/336880>.
- Turner GW, Berry AM, Gifford EM (1998) Schizogenous secretory cavities of *Citrus limon* (L.) Burm. f. and a re-evaluation of the lysigenous gland concept. *International Journal of Plant Sciences* 159: 75–88. <https://doi.org/10.1086/297523>.





## Medicinal and Therapeutic Potential of *Withania somnifera* (Indian Ginseng)

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### METADATA

#### Paper history

Received: 12 May 2025

Revised: 22 June 2025

Accepted: 16 August 2025

Published online: 03 September 2025

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#### Keywords

Antioxidant

Anti-inflammatory

Hemolytic

Antidiabetic

Antimicrobial

#### Citation

Hafeez N, Saleem I, Hussain F (2025)

*Withania somnifera* (Indian Ginseng) – an overview of medicinal properties and uses.

*Innovations in STEAM: Research & Education* 3: 25030205.

<https://doi.org/10.63793/ISRE/0030>

### ABSTRACT

**Background:** The Solanaceae family includes the woody evergreen shrub, *Withania somnifera*, which is notable for its various medicinal properties and is used in folk, conventional, and natural therapies in India. It has been a part of traditional Indian medicine for around 3,000 years.

**Objective:** To critically highlight the therapeutic relevance of *W. somnifera* by evaluating its antioxidant, anti-inflammatory, antimicrobial, antidiabetic, and anticancer properties.

**Methodology:** Relevant literature from scientific databases like PubMed, Google Scholar, ScienceDirect, Web of Science, and ResearchGate was reviewed to evaluate the pharmacological potential of *W. somnifera*.

**Results:** *W. somnifera* exhibits significant antioxidant potential by neutralizing free radicals and reducing oxidative stress. It shows marked anti-inflammatory activity through inhibition of pro-inflammatory cytokines. It possesses considerable antimicrobial effects against multiple bacterial and fungal strains. *W. somnifera* effectively lowers blood glucose levels, demonstrating its antidiabetic properties. Moreover, it displays strong anticancer activity by inducing apoptosis and inhibiting proliferation in cancerous cells. It can limit the onset of particular diseases like insomnia, diabetes, Parkinson's and Alzheimer's diseases, epilepsy, hyperlipidemia, and heart attack etc.

**Conclusion:** *W. somnifera* is a medicinal herb known for its diverse therapeutic properties, including antioxidant, antidiabetic, antimicrobial, anti-inflammatory, and anticancer activities. Its bioactive compounds, particularly withanolides, contribute to the modulation of oxidative stress, immune function, and metabolic balance, making it a promising candidate for managing chronic diseases.

### INTRODUCTION

The use of plants for therapeutics and medicinal purposes to treat illnesses and enhance human health is known as herbal therapy or phytomedicine (Singirala *et al.* 2025). Medicinal plant parts that can be employed include leaves, seeds, roots, flowers, fruits, and even the entire plant. Phytochemicals are active compounds used as therapeutic agents. These active ingredients, such as secondary metabolites, phenols, vitamins of various types, essential lipids, flavonoids, alkaloids, and reducing sugars etc., are found in the majority of medicinal plants. Compounds originating from medicinal plants can significantly treat diseases like cancers of various types, such

as breast cancer, colon cancer, lung cancer, leukaemia, pancreatic cancer, and prostate cancer, etc., which are difficult to treat.

Medicinal plants can limit the onset of particular diseases like insomnia, diabetes, Parkinson's and Alzheimer's diseases, epilepsy, hyperlipidemia, and heart attack etc. (Jayakumari *et al.* 2020). Now-a-days, there is a growing demand and acceptance of medicinal herbs. The World Health Organization (WHO) estimated that the primary source of medical care for 3.5 billion people in poor nations is herbal remedies. There are more than 120 important polyphenols that come from plants, and about 90% of labelled plant medications come from natural resources.



Natural products make up around half of the medications being used in clinical settings (Singh *et al.* 2021). These herbs are very beneficial to human health, especially in areas where access to treatment is limited. Even while herbal therapy has gained popularity, concerns about its efficacy, safety, and quality still exist. Herbal therapy integrates emotional, spiritual, and mental aspects of health. Despite their widespread use and general assumption of safety, medicinal herbs have the potential to be poisonous (Aftab and Hakeem 2021). *Withania somnifera* L. is an evergreen, small, delicate, perennial shrub that reaches a height of roughly 2 meters and a width of approximately 1 meter. The stems are upright and have a brownish colour. It is grown as a medicinal crop in India because of its fleshy roots, which are rich in bioactive chemicals (Paul *et al.* 2021). It has been used for centuries by herbal healers. The plant is called "winter cherry" and is utilized in supplements and blends that are intended to provide a variety of effects. It has numerous medicinal applications in both conventional and contemporary medicine (Mahendran *et al.* 2024), such as Withanolide A, Withanolide D, Withasomnillide, Withanone, Withasomniferanolide, Somniferwithanolide, and Somniwithanolide. Ashwagandha that are used to treat thyroid problems, improve reproductive health, increase vitality, fight weariness, boost energy levels, and enhance general well-being, boost memory, lessen the ageing-related debility, rheumatism management, constipation relief; to treat goiter, carbuncles, ulcers, uncomfortable swellings, colds, and coughs. Its trunk cortex contains somniferanolide, Withasomnillide, somniferawithanolide, somniwithanolid, phenols, vitamins, essential lipids, flavonoids, alkaloids, and reducing sugars, etc. (Buchanan *et al.* 2021; Singirala *et al.* 2025). Major phytochemicals are shown in Fig. 1.

## MEDICINAL IMPORTANCE

A versatile therapeutic plant known as a member of the *Solanaceae* family. Folk medicine practitioners cure various ailments such as pyrexia, neoplastic conditions, hyperglycemia-related illness, metabolic disease, chronic airway inflammation, lesions, hepatic infection, vision-related problems, rheumatic conditions, piles, and swollen anal veins by using bioactive plant compounds such as natural steroidal lactones, psychotropic agents, hepatic safeguarding, inflammatory response modulator, fungal growth suppressor, free radical scavenger, heart-protecting, and physique-supporting agents (Saleem *et al.* 2020) (Fig. 2). *W. somnifera* protects several organs, regulates inflammation, suppresses pro-inflammatory cytokines, maintains immunological balance, and has antiviral, anti-stress, and antihypertensive qualities (Singh *et al.* 2022). Thyroid hormones thyroxine, thyroid-stimulating hormone, and T3 respond to stimulation from iodine. Withaferin A (WA) is the chemical that primarily increases thyroid function as well as cytotoxic properties. So, *W. somnifera* is used to treat hypothyroidism, diabetes, COVID-19, and many



**Fig. 1:** Role of AI in SMEs and challenges in its implementation



**Fig. 2:** Bioactivities of *W. somnifera*

other ailments. Witanoside V and somniferin may have the ability to block the main SARS-CoV-2M protease (Abdel-Wahhab *et al.* 2019).

## ANTIOXIDANTS ACTIVITY

The antioxidants restrict the oxidation of proteins, lipids, DNA, and other materials by stopping the progression steps of an oxidative chain reaction. Due to their higher iron and lipid content, which are thought to be significant contributors to the production of ROS, the brain and nervous system are comparatively more vulnerable to ROS damage than other tissues. The brain used 20% of the oxygen supply.

Neurological disorders like Alzheimer's, epilepsy, Parkinson's, schizophrenia, and others, as well as age-related cognitive decline, have been linked to ROS-impaired neurons. Antioxidants help neutralize ROS, thereby having the potential to protect the body from these diseases. The alkaloid content of the *W. somnifera* is shown to be primarily responsible for its total antioxidant and reducing activities, with flavonoids and withanolides ranking second and third, respectively. The fresh, dried leaves and tubers of this plant were reported to have more antioxidant compounds than the delicate roots and stems. Moreover, catechin is the primary flavonoid present at the highest concentrations in this plant (Chidambaram *et al.* 2024).

The effect of root aqueous extract was investigated in a study on endotoxin-induced stress in hares and rodents. Oxidative stress markers concentration in the blood increased upon endotoxin administration. A concurrent oral dose of 100 mg/kg of *W. somnifera* extract stopped the rise in lipid peroxidation. It reduced the elevated activity of cholinesterase and sodium, potassium, and adenyolphosphatase in the brain, striatum, and limbic structure, as well as a significant increase in the production of TNF-alpha. It also prevented the inhibition of nicotinic function through sustaining an appropriate acetylcholine effect. *W. somnifera* helps to improve memory because one of its compounds, WA, was found to increase the activity of a certain enzyme (ROS prostaglandin-endoperoxide synthase-2) in rabbit joint cells, which then promoted the production of type-2 collagen, depending on the dose and duration of use. The biological effect of *Withania* genus, especially their antioxidant properties, depends on how the plant is extracted. When compared to extracts made with water, acetone, or a mix of water and methanol (1:1), the extract made with methanol, chloroform, and water (1:1:1) showed stronger antioxidant and reduced activity. This was true for all tested plant compounds, except tannins (Balkrishna *et al.* 2022).

Lab-grown roots (in vitro sprouted) had higher levels of antioxidant activity than roots collected from the field. Methanol extracts of *W. somnifera* shoots, leaves, and roots contained high amounts of natural antioxidants like flavones and phenolic acids such as gallic, *p*-coumaric, syringic, and benzoic acids. These compounds are known for their ability to neutralize free radicals. Lighter plant compounds were best extracted using ethyl alcohol and *n*-hexane. The antioxidant capacity of *W. somnifera* extracts was measured using the phosphomolybdenum (PMo) method and expressed in vitamin C equivalents per gram. Among the different types of extracts tested, the one made with chloroform showed the strongest ability to scavenge free radicals. The total flavonoid content in each extract was measured using the aluminum chloride assay. Because of its antioxidant power, *W. somnifera* has been used to support brain health and manage neurological problems related to oxidative stress. It may also help in the recovery from neurodegenerative diseases (Paul *et al.* 2021).

## ANTI-DIABETIC ACTIVITY

Diabetes is an endocrine and metabolic condition in which the body does not use glucose properly. One aspect of pathophysiology is increased oxidative stress, which damages beta cells and alters the histology of the pancreas. Diabetes has several different complications, including organ infections, obesity, and both microvascular and macrovascular problems (Vesa *et al.* 2021). WA may cause changes in lipid profiles and glucose metabolism. It promoted weight loss and reduced inflammation in diabetic patients, which raised insulin sensitivity (Khalilpourfarshbafi *et al.* 2019). The WA reduced rat hepatic steatosis. Expression of genes for insulin signaling is downregulated in diabetes. WA therapy enhanced mRNA transcriptional activity that comprises the insulin receptor substrate-1, phosphatidylinositol 3-kinase. According to the investigations, WA lessens the effect of type-1 diabetes (Eguchi *et al.* 2021).

Additionally, diabetic patients' blood glucose levels were considerably reduced when *W. somnifera* root powder was taken orally. Lactonic steroids are responsible for their antidiabetic effects. Numerous withanolides that had been extracted from *W. coagulans* fruits had shown anti-diabetic activity, and many of their biological functions had been documented. Extracts from leaves and roots both enhanced the absorption of glucose in rat adipocytes (3T3-L1) and myotubes (L6) (Makhlouf *et al.* 2024). Furthermore, insulin production in insulin-producing cells was enhanced by more than fifty percent by leaf extract, despite root extract. It also stimulated cells that did not experience this effect. In patients with type 2 diabetes, raising basal insulin levels has been linked to decreased hepatic glucose synthesis, fasting glucose, and free fatty acid levels. Selected withanolides that had been extracted from *W. somnifera* were evaluated for their hyperglycemic properties. In diabetic mice, flavonols and polyphenols found in different plant parts effectively lowered blood glucose levels. While substantially greater levels of Alpha-lipoprotein were observed in diabetic rats, the phenolic content of root and leaf extracts helped lower blood sugar levels. The preclinical experimental outcomes were encouraging. Studies on animals have demonstrated their capacity to reduce blood glucose levels. Additionally, it was demonstrated that WA had great therapeutic promise since it can effectively regulate type-1 diabetes in rats that had been produced by modulating Nrf2/NFκB signaling. Using molecular docking, in silico studies have also validated the potential of steroidal lactone. It has a positive impact on the lipidemic profile. Both the antioxidant effect and the decrease in cholesterol levels are benefits of this plant observed in a white albino rat that had elevated cholesterol levels. Interesting outcomes were obtained in changing the lipidemic profile in the diabetic clinical trials, even though there was no effect on blood sugar level. There was a decrease in the patient's lipidemic profile evaluation using the DDS17 measure to gauge the patient's level of distress. A

standardized extract from *W. somnifera* under the brand name SENSORIL enhanced the lipidemic profile and antioxidant parameters while also proving the raw material's safety and tolerability. A change in the reflection index [RI] of 3.8% was shown and an impact on the lipidemic profile despite being safe and tolerable (Mikulska et al. 2023).

## ANTIMICROBIAL ACTIVITY

Drug resistance in microorganisms poses a significant and expanding problem. Hence, *W. somnifera* is a beneficial medicinal supplement in the management of various infections caused by bacteria. Even though most of the medications applied to treat infections induced by bacteria were effective, toxicity results in severe, harmful side effects. While *W. somnifera* has very few adverse effects. Research had demonstrated that it was an efficient source of suppressing, proliferation of methicillin resistant. *Enterococcus* species and *Staphylococcus aureus*, *Salmonella typhi*, *Citrobacter freundii*, *Proteus mirabilis*, *Pseudomonas aeruginosa*, and *E. coli*. Many of its characteristics were thought to represent the mechanism of its antimicrobial action. Studies related to animal models demonstrated that *W. somnifera* effectively reduced the progression of infection after contracting salmonellosis, indicating its efficacy as a treatment for the disease. It inhibited oral cavity bacterial growth, including *Streptococcus sobrinus* and *Streptococcus mutans*. Additionally, it prevented bacteria from producing acid, becoming acid-tolerant, and growing biofilms (Khanchandani et al. 2019).

It was especially effective in combating *S. typhi*. The withanolides that were extracted from *W. somnifera* cause *Leishmania donovani* promastigotes to die by triggering apoptosis. It causes reactive oxygen species to be released from mitochondria by altering mitochondrial membrane potential. According to studies, it also has important antifungal properties against a few different types of fungi, including *Candida albicans*. Glycoprotein has been isolated from its root tubers and has antibacterial and antifungal characteristics against *Clavibacter michiganensis* subsp. *Aspergillus flavus*, *Fusarium verticillioides* and *Fusarium oxysporum*. Root extract exhibited increased antibacterial activity against *P. aeruginosa*. Research on the action of root extract's antibacterial activity, utilizing membrane stabilization and morphological analysis revealed that it worked by rupturing the *P. aeruginosa* cell membrane. *W. somnifera* extracts, particularly in large quantities, in animal studies, were useful to treat malaria by considerably lowering parasitaemia. Flavonoids have demonstrated outstanding antibacterial properties against *E. coli*, *P. mirabilis*, *C. albicans*, and *S. aureus*, but are inefficient against *Aspergillus oryzae* var *flavus*. However, *W. somnifera* methanolic extract was found to have a minimum inhibitory concentration (MIC) against both *C. albicans* and *Neisseria gonorrhoeae*, but the aqueous extract was found to have MIC against *N. gonorrhoeae*. Its glycoconjugates exhibited

fungicidal activity for *Fusarium verticillioides* and *A. flavus*, as well as bactericidal potential for *Corynebacterium michiganense* (Mikulska et al. 2023). *W. somnifera* performed best against *B. thuringiensis* and *C. diphtheria*. Antibacterial agents such as steroids, anthroquinone, alkaloids, and Flavonols had all been detected in the plant leaves. The chloroform extract from *Withania* leaves had anti-*B. Therogenesis* and anti-*C. Diphtheria* properties. *W.* and *Calotropis procera* both possessed antibacterial qualities against pathogenic strains. The antibacterial properties were evaluated by phytochemical components in alcoholic and chloroform extracts of *W. somnifera* stems, leaves, and roots (Sandhiya et al. 2022). Human immunodeficiency virus (HIV) has claimed 40.1 million deaths, and there is no known cure, making it one of the most historically significant diseases. Alzheimer's disease (AD) patients have been linked to one of these HIV-related causes. Interestingly, WA demonstrated its effectiveness in treatment for HIV indirectly. Additionally, the study revealed that WA causes a higher means of CD4 cell count. Additionally, it was shown that WA inhibited the HIV strain by preventing the microbes' replication as well as transcription. *W. somnifera* lowered the progression of disease markers, on CD8+T lymphocytes and CD38, suggesting that it has anti-HIV properties (Ozeer et al. 2024).

It suppressed and controlled the coronavirus main protease and Membrane Receptor protein serine protease-10 and prevented severe acute respiratory syndrome coronavirus (SARS)-associated coronavirus entry by reducing the electrochemical element in the angiotensin-converting enzyme 2 (ACE2) complex and SARS-CoV-2 receptor binding domain. It is a powerful medicinal plant that fights COVID-19. It also helped to prevent infections. According to *in silico* research, *W. somnifera* suppressed the COVID-19 virus's ability to replicate via modifying T-cell separation and NK-cell cytotoxicity. Several withanolides downregulated nucleoplasmic sequences (N-gene) and viral envelope (E-gene) expression. It was discovered that withanolide P, mesoaniferine, withanolide O, bsitosterol, withanolide D, and somniwithanolide limited the coronavirus protease of SARS-CoV-2. At the same time, 3CLpro and PLpro were suppressed by tropine, choline, and withanisomniferol C. Apart from these, several potent substances might be effective in treating the sickness (Willett et al. 2022). In conclusion, WA exhibits protective effects against both bacterial and viral infections.

## ANTI-INFLAMMATORY ACTIVITIES

Because of above mentioned characteristics, *W. somnifera* was being studied to treat a wide range of inflammatory diseases, including diabetes, cancer, neurological disorders, and autoimmune, pulmonary, and cardiovascular conditions. Through the inhibition of inflammatory markers such as cytokines, nitric oxide, and reactive oxygen species, it can regulate mitochondrial activity, apoptosis and reduce

inflammation. Meanwhile, a possible inhibitory impact of powdered *W. somnifera* root was shown in a lupus-ridden mouse model in circumstances including nephritis and proteinuria. The effect of *W. somnifera* for rheumatic illnesses was also being studied. Rats were given powdered *W. somnifera* root orally for three days. Rats were fed phenylbutazone as part of the control group (positive control). A marked decrease in inflammation and altered quantities of several serum proteins, including pre-albumin, acute phase protein  $\alpha_1$  and  $\alpha_2$  glycoprotein, were observed after the use of *W. somnifera*. To find out how an aqueous extract from *W. somnifera* root inhibited the expression of pro-inflammatory cytokines like interleukin (IL)-8 and IL-6, a study was conducted using the human keratinocyte cell line (HaCaT). Ashwagandha aqueous extract (ASH-WEX) was found to have anti-neuroinflammatory effects against lipopolysaccharide-induced systemic neuroinflammation (Kanjilal *et al.* 2021).

In a preclinical study, ASH-WEX showed reduced expression of nitro-oxidative stress enzymes and inhibition of reactive gliosis in treated animals. The underlying molecular processes behind ASH-WEX's anti-inflammatory properties seem to entail blocking the P38, JNK/SAPK, MAPK, and NF $\kappa$ B pathways that were triggered by lipopolysaccharide (LPS). *W. somnifera* might be used to reduce nervous system inflammation linked to a variety of neurological conditions. It was demonstrated that treating patients' arthritic symptoms with *W. somnifera* extract administered for eight to twelve weeks could be helpful (Mikulska *et al.* 2023). ECM dysregulation and lung inflammation is caused by a variety of mechanisms, including loss of proteostasis, mitochondrial dysfunction, cellular senescence, stem cell exhaustion, genomic instability, epigenetic alteration, telomere attrition, incorrect intercellular communication, cellular senescence and unregulated nutrient-sensing. Ashwagandha showed a decrease in TNF- $\alpha$  and NF-KB and an increase in IL-10, which might be contributing factors to the development of lung inflammation. Because it made it easier to reduce skeletal muscle inflammation, decreased levels of IL-10 are intimately linked to lung inflammation (Kashyap *et al.* 2022).

### CARDIOPROTECTIVE ACTIVITY

Myocardial infarction (MI) is a leading cause of mortality globally, also an essential medical problem. Withanolide A 1 mg/kg stimulated the mitochondrial antiapoptotic pathway by reducing apoptotic cell death and upregulating the protein Bcl-2. According to the *in vivo* investigation, rats administered a modest quantity of WA showed protection against MI injury. WA demonstrated beneficial cardiac activity by inducing adenosine monophosphate kinase activation and inhibiting the intrinsic apoptotic pathway. WA can therefore be used therapeutically for cancer patients who also have cardiovascular system problems (Li *et al.* 2018).

*W. somnifera* treats heart diseases by reducing

oxidative stress, improving antioxidant enzyme activity, and reducing inflammation. Several heart conditions, including heart attack, hypertensive cardiomyopathy, chronic ventricular coronary artery disease, hypertrophic cardiomyopathy, and uncontrolled cardiomyopathy, were associated with cardiac collagen depositions and could be treated with *W. somnifera*. WA inhibited ferric chloride-induced platelet aggregation as well as thrombin-catalyzed fibrin polymerization, prolonged hemostasis, and suppressed tumour necrosis factor-alpha-induced inhibitor of plasminogen activator formation. These findings collectively demonstrated the cardioprotective potential of WA; however, given its safety and effectiveness, further clinical trials are required to substantiate its therapeutic function in heart disorders (Behl *et al.* 2020).

### OSTEOPOROSIS

An imbalance in bone growth and resorption was the hallmark of osteoporosis, a condition of the skeletal bones (Tit *et al.* 2018). *W. somnifera* promotes osteogenic cells' development and proliferation by regulating osteoblast-specific transcription factors' expression. WA prevented the synthesis of cytokines that cause inflammation. Moreover, WA inhibits the production of osteoclast acid phosphatase and osteoclast differentiation factor, receptor WA reduces the number of osteoclasts, also referred to as bone-resorbing cells (Saleem *et al.* 2020).

### ANTI-HEPATITIS ACTIVITY

*W. somnifera* shows strong potential against hepatitis-related liver damage, particularly in advanced conditions like NAFLD with hepatitis and nano-ALD, which increase the risk of chronic liver disease and cancer (Taylor *et al.* 2020). Excessive lipids lead to harmful fat accumulation, oxidative stress, inflammation, and ER stress – mainly due to ceramides. WA, a key compound in *W. somnifera*, reduces liver damage by lowering oxidative stress through oxygenase activity and activation of the NRF2 pathway, highlighting its role as a natural hepatoprotective agent (Kalluri *et al.* 2023).

### ANTISTRESS EFFECT

Stress is defined as a state of worry or mental tension that is brought on by a challenging situation. Antistress activity lowered the risk of most diseases. A widespread improvement in stress resilience was observed after using *W. somnifera*. Sitoindosides VII and VIII, two of its glycosides, had strong anti-stress effects in models including forced-swim immobility, stomach ulcers, auto-analgesia generated stress, altered thermic response to morphine, and morphine-induced toxicity in mice. *W. somnifera* exhibited noteworthy antistress efficacy, which was determined by swimming endurance tests (Speers *et al.* 2021).



## ANTICANCER/ CYTOTOXIC ACTIVITY

Unchecked cell division was a characteristic of a disease called cancer. Cancer was caused by modifications to proteins that encode genes that were part of the cellular division cycle, including prototypes of cancerous genes and non-cancerous genes. According to the diagnoses in the US alone in 2022, heart disease was the world's biggest cause of death, with cancer coming in second. Although *W. somnifera* did not affect healthy human cells, it was cytotoxic to a wide range of tumour cells, suggesting that it only affected cancer cells. It had been demonstrated that *W. somnifera* upregulated the expression of several conjugating enzymes, indicating that the phytochemicals worked either directly or through indirect means by regulating additional cell protective routes, including NFE2L2 (Kashyap *et al.* 2022). Researchers used a variety of molecular techniques, including global gene-expression sequencing, antibody-based protein detection assays (western blot), fluorescent immune-staining, real-time cDNA amplification, and siRNA-mediated gene silencing, to identify signalling cascades. It caused intrinsic apoptosis in Glioblastoma Multiforme (GBM) cells and markedly reduced GBM growth both in vivo and in vitro. Thr161 CDK1 was dephosphorylated, causing GBM cells to be stopped in the cell cycle's G2/M phase. This discovery holds significance for enhancing WA-based regimens intended for the multifactorial aggressive brain cancer reduction. Previous research identified that the extract reduced inflammation and damage from oxidative stress in the hepatic and splenic tissues, defending against the deleterious consequences of radiation exposure. These results indicate that *W. somnifera* root extract might have therapeutic uses in preventing damage to the liver and spleen, otherwise two important organs damaged by radiation (Mikulska *et al.* 2023).

### Prostate cancer

Prostate cancer is the 2<sup>nd</sup> leading type of cancer in men and accounts for 3.8% of men who die from cancer globally (Bray *et al.* 2018). Additionally, WA caused a weal to accumulate at the mitotic transition, which caused a dose-dependent decrease in cell survival. Through PAWR-mediated extrinsic signalling, downregulation of matrix gelatinase a by azido-modified WA limited cellular invasion. Furthermore, *in vivo* research showed that mice's angiogenesis was prevented, and p-ERK and p-Akt expression were reduced. By inducing ER stress and affecting the transition of prostate cancer cells from autophagy to apoptosis, 3-azido WA also demonstrated anticancer potential against prostate cancer. Metabolic reprogramming of lipids in cancer cells and an emerging method of triggering prostate cancer cell death might be revealed by recent reports (Hassannia *et al.* 2020).

### Colon cancer

Among all malignancies, colon cancer ranked second in

terms of mortality cases and third in terms of incidence worldwide. Ethanolic extracts of *W. somnifera* were found to exhibit azoxymethane-triggered immunomodulatory effects in Swiss strain mice with colorectal cancer (Mukherjee *et al.* 2021). Ashwagandha-derived steroidal lactone shows cytotoxic potential against anti-colorectal malignancy (Gharaibeh *et al.* 2020). Additionally, Balb/c nude mice with HCT116 xenograft tumours showed a significant decrease in tumour weight and volume after receiving steroidal lactone treatment. Mice treated with withanolids exhibited a significant reduction in tumour growth, volume, polyp size, and adenomas when compared to controls (Alnuqaydan *et al.* 2020).

### Ovarian cancer

*W. somnifera* treated ovarian cancer in several ways. WA stopped the G2/M phase cell cycle in human ovarian cancer cell lines (SKOV3 and CaOV3) (Davis *et al.* 2024). By suppressing the cell signalling and apoptosis regulation, WA triggers apoptotic protease activation, which results in cell death. At suboptimal doses, withanolids, cisplatin, and doxorubicin produce ROS and kill cells (Atteeq *et al.* 2022). In xenografted tumors, WA lowered the levels of phospho-p65 cytokines linked to NF- $\kappa$ B both in the cytosol and the nucleus (Kelm *et al.* 2020).

### Leukemia

*W. somnifera* is a potent medicinal herb fighting against leukaemia. When *W. somnifera* was applied to solid tumours, withaferin-A (an essential phytochemical of *W. somnifera*) showed strong anticancer properties. Its effectiveness in preventing haematological malignancies has shown significant results, WA inducing apoptosis through the p38/MAPK signalling pathway, inhibiting cell growth in several leukemic lymphocytes as well as cancerous blood cells, and cytotoxicity. Additionally, *W. somnifera* shows many anti-leukemic properties, including the capacity of phytochemical extracts to enhance superoxide production, trigger cell cycle arrest, deliver Ca<sup>2+</sup> ions homeostasis, also weaken the T-lymphoblastoid cell lines and DNA structure (Dutta *et al.* 2019).

### Lung cancer

The leading cause of cancer-related deaths worldwide is lung cancer (Siegel *et al.* 2021). Swiss albino mice with benzopyrene-induced lung carcinogenesis were protected against oxidative impairment by the antioxidant activity of *W. somnifera* (Singh *et al.* 2021). Its withanolides also reduced the attachment of human leukemia monocytes to lung adenocarcinoma cells by blocking Akt phosphorylation, inhibiting NF- $\kappa$ B activity, and lowering the expression of VCAM-1 and ICAM-1 (Mandlik *et al.* 2021). Moreover, in lung adenocarcinoma cells, withanolides counteracted

cachexin-induced changes, disrupted cytokine signaling, and promoted cancer cell death (Dutta *et al.* 2019). These results supported the investigation of *W. somnifera*'s effectiveness in treating pulmonary malignancy (Kumar *et al.* 2023).

#### Breast cancer

The most common type of cancer among women is breast cancer (Siegel *et al.* 2021). Aggressiveness and the spontaneous metastasis of breast cancers were strongly affected by their structural distinctions (Al-Mahmood *et al.* 2018). Research employing fluorescence microscopy demonstrated that WA was useful in phosphorylating the H3 histone at the Ser10 position and inducing a mitotic stop in MDA-MB-2 and MCF-7 cell lines in breast cancer cells (Kumar *et al.* 2023). The same cells also underwent FOXO3a-induced apoptosis. WA demonstrated a unique mechanism for inducing apoptosis. It had been demonstrated that WA inhibited oxidative phosphorylation in breast cancers and triggered ROS to cause cell death and improved anti-metastatic and anti-invasive behaviors (Paul *et al.* 2021). WA methylates or demethylates a large number of genes linked to basal-like breast carcinoma, blocks the unique characteristics of slightly vigorous luminal breast cancer, and improves healing efficacy (Vel Szic *et al.* 2019).

### CONCLUSION

*W. somnifera* exhibits a wide spectrum of pharmacological effects, including antioxidant, antidiabetic, antimicrobial, anti-inflammatory and anticancer activities. These effects are largely attributed to its diverse phytoconstituents, particularly withanolides. Extensive *in vitro* and *in vivo* studies have confirmed its ability to scavenge free radicals, modulate immune responses, inhibit microbial growth, regulate blood glucose levels and induce apoptosis in cancer cells without harming normal tissues. Furthermore, emerging clinical investigations support its therapeutic potential in managing chronic diseases, highlighting its role as a promising natural candidate for integrative medicine. Continued exploration through advanced research and clinical trials is essential to validate its efficacy, safety and mechanistic pathways in human health. These multifaceted benefits underscore the plant's therapeutic versatility and justify further exploration in clinical settings. Yet, extensive research is still needed to validate its medicinal claims and understand its active compounds. Advances in biotechnology and sustainable cultivation can further support clinical and pharmaceutical applications.

### AUTHOR CONTRIBUTIONS

NH: Conceptualized and drafted the manuscript; IS: Conducted literature review and editing; FH: Critically revised the manuscript. All authors approved the final manuscript.

### CONFLICT OF INTEREST

The authors affirm that they possess no conflicts of interest.

### DATA AVAILABILITY

Not applicable

### ETHICS APPROVAL

Not applicable

### FUNDING SOURCE

No funding was acquired for this work.

### REFERENCES

- Abdel-Wahhab KG, Mourad HH, Mannaa FA, Morsy FA, Hassan LK, Taher RF (2019) Role of *ashwagandha* methanolic extract in the regulation of thyroid profile in hypothyroidism modeled rats. *Molecular Biology Reports* 46: 3637–3649. <https://doi.org/10.1007/s11033-019-04721-x>.
- Aftab T, Hakeem KR (eds) (2020) *Medicinal and Aromatic Plants: Expanding their Horizons through Omics*. London: Academic Press. <https://doi.org/10.1016/C2019-0-00313-5>.
- Al-Mahmood S, Sapiezynski J, Garbuzenko OB, Minko T (2018) Metastatic and triple-negative breast cancer: Challenges and treatment options. *Drug Delivery and Translational Research* 8: 1483–1507. <https://doi.org/10.1007/s13346-018-0551-3>.
- Alnuqaydan AM, Rah B, Almutary AG, Chauhan SS (2020) Synergistic antitumor effect of 5-fluorouracil and withaferin A induces endoplasmic reticulum stress-mediated autophagy and apoptosis in colorectal cancer cells. *American Journal of Cancer Research* 10: 799–815.
- Atteeq M (2022) Evaluating anticancer properties of withaferin A—a potent phytochemical. *Frontiers in Pharmacology* 13: 975320. <https://doi.org/10.3389/fphar.2022.975320>.
- Balkrishna A, Solleti SK, Singh H, Sharma N, Varshney A (2022) Withanolides from *Withania somnifera* ameliorate neutrophil infiltration in endotoxin-induced peritonitis by regulating oxidative stress and inflammatory cytokines. *Planta Medica* 88: 466–478. <https://doi.org/10.1055/a-1438-2816>.
- Behl T, Sharma A, Sharma L, Sehgal A, Zengin G, Brata R, Fratila O, Bungau S (2020) Exploring the multifaceted therapeutic potential of withaferin A and its derivatives. *Biomedicines* 8: 571. <https://doi.org/10.3390/biomedicines8120571>.
- Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A (2018) Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA: A Cancer Journal for Clinicians* 68: 394–424. <https://doi.org/10.3322/caac.21492>.
- Buchanan RW (2021) *Withania somnifera*-characteristics, uses and side effects. *Natural Products Chemistry and Research* 9: 413.
- Chidambaram SB, Anand N, Varma SR, Ramamurthy S, Vichitra C, Sharma A, Mahalakshmi AM, Essa MM (2024) Superoxide dismutase and neurological disorders. *IBRO Neuroscience Reports* 16: 373. <https://doi.org/10.1016/j.ibneur.2023.11.007>.
- Davis CC, Choisy P (2024) Medicinal plants meet modern biodiversity science. *Current Biology* 34: 158–173. <https://doi.org/10.1016/j.cub.2023.12.038>.
- Dutta R, Khalil R, Green R, Mohapatra SS, Mohapatra S (2019) *Withania somnifera* (Ashwagandha) and withaferin A: Potential in integrative oncology. *International Journal of Molecular Sciences* 20: 5310. <https://doi.org/10.3390/ijms20215310>.

- Eguchi N, Vaziri ND, Dafoe DC, Ichii H (2021) The role of oxidative stress in pancreatic  $\beta$  cell dysfunction in diabetes. *International Journal of Molecular Sciences* 22: 1509. <https://doi.org/10.3390/ijms22041509>.
- Gharaibeh L, Elmadany N, Alwosaibai K, Alshaer W (2020) Notch1 in cancer therapy: Possible clinical implications and challenges. *Molecular Pharmacology* 98: 559–576. <https://doi.org/10.1124/molpharm.120.000006>.
- Hassannia B, Logie E, Vandenabeele P, Vanden Berghe T, Vanden Berghe W (2020) Withaferin A: From Ayurvedic folk medicine to preclinical anti-cancer drug. *Biochemical Pharmacology* 173: 113602. <https://doi.org/10.1016/j.bcp.2019.08.004>.
- Jayakumari (2020) Phytochemicals and pharmaceutical: Overview. *Advances in Pharmaceutical Biotechnology: Recent Progress and Future Applications* 163–173. [https://doi.org/10.1007/978-981-15-2195-9\\_14](https://doi.org/10.1007/978-981-15-2195-9_14).
- Kalluri R, McAndrews KM (2023) The role of extracellular vesicles in cancer. *Cell* 186: 1610–1626. <https://doi.org/10.1016/bs.ctm.2024.06.010>.
- Kanjilal S, Gupta AK, Patnaik RS, Dey A (2021) Analysis of clinical trial registry of India for evidence of anti-arthritis properties of *Withania somnifera* (Ashwagandha). *Alternative Therapies in Health and Medicine* 27: 58–66. PMID: 34144529.
- Kelm NQ, Straughn AR, Kakar SS (2020) Withaferin A attenuates ovarian cancer-induced cardiac cachexia. *PLoS One* 15: e0236680. <https://doi.org/10.1371/journal.pone.0236680>.
- Khalilpourfarshbafi M, Devi Murugan D, Abdul Sattar MZ, Sucedaram Y, Abdullah NA (2019) Withaferin A inhibits adipogenesis in 3T3-F442A cell line, improves insulin sensitivity and promotes weight loss in high fat diet-induced obese mice. *PLoS One* 14: e0218792. <https://doi.org/10.1371/journal.pone.0218792>.
- Khanchandani N, Shah P, Kalwani T, Ardeshta A, Dharajiya D (2019) Antibacterial and antifungal activity of Ashwagandha (*Withania somnifera* L.): A review. *Journal of Drug Delivery and Therapeutics* 9: 154–161. <http://dx.doi.org/10.22270/jddt.v9i5.s3573>.
- Kumar S, Mathew SO, Aharwal RP, Tulli HS, Mohan CD, Sethi G, Ahn KS, Webber K, Sandhu SS, Bishayee A (2023) Withaferin A: a pleiotropic anticancer agent from the Indian medicinal plant *Withania somnifera* (L.) Dunal. *Pharmaceuticals* 16: 160. <https://doi.org/10.3390/ph16020160>.
- Li L, Zhao Q, Kong W (2018) Extracellular matrix remodeling and cardiac fibrosis. *Matrix Biology* 68: 490–506. <https://doi.org/10.1016/j.matbio.2018.01.013>.
- Mahendran G, Rahman LU (2024) A novel approach for direct shoot regeneration, anatomical characterization, and withanolides content in micropropagated plants of *Withania somnifera* (L.) Dunal—an important medicinal plant. *In Vitro Cellular & Developmental Biology—Plant* 60: 365–377. <https://doi.org/10.1007/s11627-024-10428-x>.
- Makhlouf EA, AlamElDeen YK, El-Shiekh RA, Okba MM (2024) Unveiling the antidiabetic potential of Ashwagandha (*Withania somnifera* L.) and its withanolides—a review. *Natural Product Research* 1–16. <https://doi.org/10.1080/14786419.2024.2439009>.
- Mandlik DS, Namdeo AG (2021) Pharmacological evaluation of Ashwagandha highlighting its healthcare claims, safety, and toxicity aspects. *Journal of Dietary Supplements* 18: 183–226. <https://doi.org/10.1080/19390211.2020.1741484>.
- Mikulska P, Malinowska M, Ignacyk M, Szustowski P, Nowak J, Pesta K, Szeląg M, Szklanny D, Judasz E, Kaczmarek G, Ejiohuo OP (2023) Ashwagandha (*Withania somnifera*)—current research on the health-promoting activities: A narrative review. *Pharmaceutics* 15: 1057. <https://doi.org/10.3390/pharmaceutics15041057>.
- Mukherjee PK, Banerjee S, Biswas S, Das B, Kar A, Katiyar CK (2021) *Withania somnifera* (L.) Dunal—modern perspectives of an ancient Rasayana from Ayurveda. *Journal of Ethnopharmacology* 264: 113157. <https://doi.org/10.1016/j.jep.2020.113157>.
- Ozeer FZ, Nagandran S, Wu YS, Wong LS, Stephen A, Lee MF, Kijssomporn J, Guad RM, Batumalaie K, Oyewusi HA, Verma A (2024) A comprehensive review of phytochemicals of *Withania somnifera* (L.) Dunal (Solanaceae) as antiviral therapeutics. *Discover Applied Sciences* 6: 187. <https://doi.org/10.1007/s42452-024-05845-x>.
- Paul S, Chakraborty S, Anand U, Dey S, Nandy S, Ghorai M, Saha SC, Patil MT, Kandimalla R, Proćków J, Dey A (2021) *Withania somnifera* (L.) Dunal (Ashwagandha): A comprehensive review on ethnopharmacology, pharmacotherapeutics, biomedicinal and toxicological aspects. *Biomedicine & Pharmacotherapy* 143: 112175. <https://doi.org/10.1016/j.biopha.2021.112175>.
- Saleem S, Muhammad G, Hussain MA, Altaf M, Bukhari SNA (2020) *Withania somnifera* L.: Insights into the phytochemical profile, therapeutic potential, clinical trials, and future prospective. *Iranian Journal of Basic Medical Sciences* 23: 1501. <https://doi.org/10.22038/IJBMS.2020.44254.10378>.
- Sandhiya K, Pandey A, Sharma R, Fatima K, Parveen R, Gaurav N (2022) Assessment of phytochemical and antimicrobial activity of *Withania somnifera* (Ashwagandha). *The Scientific Temper* 13: 376–383. <https://connectjournals.com/03960.2022.13.2.376>.
- Siegel RL, Miller KD, Fuchs HE, Jemal A (2022) Cancer statistics 2022. *CA: A Cancer Journal for Clinicians* 72: 1. <https://doi.org/10.3322/caac.21708>.
- Singh M, Jayant K, Singh D, Bhutani S, Poddar NK, Chaudhary AA, Khan SUD, Adnan M, Siddiqui AJ, Hassan MI, Khan FI (2022) *Withania somnifera* (L.) Dunal (Ashwagandha) for the possible therapeutics and clinical management of SARS-CoV-2 infection: Plant-based drug discovery and targeted therapy. *Frontiers in Cellular and Infection Microbiology* 12: 933824. <https://doi.org/10.3389/fcimb.2022.933824>.
- Singh N, Yadav SS, Rao AS, Nandal A, Kumar S, Ganaie SA, Narasimhan B (2021) Review on anticancerous therapeutic potential of *Withania somnifera* (L.) Dunal. *Journal of Ethnopharmacology* 270: 113704. <https://doi.org/10.1016/j.jep.2020.113704>.
- Singirala SK, Dubey PK, Roy S (2025) Extraction of bioactive compounds from *Withania somnifera*: The biological activities and potential application in the food industry — a review. *International Journal of Food Science* 2025: 9922626. <https://doi.org/10.1155/ijfo/9922626>.
- Speers AB, Cabey KA, Soumyanath A, Wright KM (2021) Effects of *Withania somnifera* (Ashwagandha) on stress and the stress-related neuropsychiatric disorders anxiety, depression, and insomnia. *Current Neuropharmacology* 19: 1468–1495. <https://doi.org/10.2174/1570159X19666210712151556>.
- Taylor RS, Taylor RJ, Bayliss S, Hagström H, Nasr P, Schattenberg JM, Ishigami M, Toyoda H, Wong VWS, Peleg N, Shlomai A (2020) Association between fibrosis stage and outcomes of patients with nonalcoholic fatty liver disease: A systematic review and meta-analysis. *Gastroenterology* 158: 1611–1625. <https://doi.org/10.1053/j.gastro.2020.01.043>.
- Tit DM, Bungau S, Iovan C, Nistor Cseppento DC, Endres L, Sava C, Sabau AM, Furau G, Furau C (2018) Effects of the hormone replacement therapy and of soy isoflavones on bone resorption in postmenopause. *Journal of Clinical Medicine* 7: 297. <https://doi.org/10.3390/jcm7100297>.
- Vel Szic KS, Declerck K, Crans RA, Diddens J, Scherf DB, Gerhäuser C, Vanden Berghe W (2017) Epigenetic silencing of triple-negative breast cancer hallmarks by withaferin A. *Oncotarget* 8: 40434–40453. <https://doi.org/10.18632/oncotarget.17107>.
- Vesa CM, Popa L, Popa AR, Rus M, Zaha AA, Bungau S, Tit DM, Corb Aron RA, Zaha DC (2020) Current data regarding the relationship between type 2 diabetes mellitus and cardiovascular risk factors. *Diagnostics* 10: 314. <https://doi.org/10.3390/diagnostics10050314>.
- Willett BJ, Grove J, MacLean OA, Wilkie C, De Lorenzo G, Fumon W, Cantoni D, Scott S, Logan N, Ashraf S, Manali M (2022) SARS-CoV-2 Omicron is an immune escape variant with an altered cell entry pathway. *Nature Microbiology* 7: 1161–1179. <https://doi.org/10.1038/s41564-022-01143-7>.

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