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Innovations in STEAM: Research & Education

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RESEARCH ARTICLE

Assessment of Stereopsis in Myopic and Hyperopic Anisometropia

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ABSTRACT

Background: Anisometropia, a condition where each eye has a different refractive power, can significantly affect binocular vision and depth perception. Myopic and hyperopic anisometropia may lead to disrupted stereopsis, impacting visual performance and quality of life.
Objective: The purpose of the study was to assess and compare the impact of myopic and hypermetropic anisometropia on stereopsis.
Methodology: This cross-sectional study was conducted from August 2021 to December 2021 at Madina Teaching Hospital, Faisalabad. About 30 individuals of both sexes were included, aged from 12 to 25 years. A non-probability convenient sampling technique was employed to access the data. Patients having refractive anisometropia were included, and those with any type of ocular pathology, infections, strabismus, and amblyopia were excluded. Stereopsis was assessed binocularly with best refractive correction by the TNO chart (a clinical test for evaluating stereopsis). An independent sample *t*-test was used with the help of IBM SPSS-20 to get a statistical result.
Results: The results of the study showed that the mean value of 120 ± 69.282 seconds of arc scoa red by myopic anisometropic and 276 ± 150.20 second of arc by hyperopic anisometropic. Although the normal values for stereopsis for an emmetropic person should score 60 seconds of arc, which means that minimum value for stereopsis reveal good and increased levels for stereopsis. As the mean values for stereopsis in both myopic and hyperopic anisometropia are greater than the normal value of stereopsis, this shows that both the myopic and hyperopic anisometropic persons have decreased levels of stereopsis.
Conclusion: The results of the study concluded that anisometropia reduces stereopsis; however, these reductions are more significant in hyperopic anisometropia as compared to the myopic anisometropia. Study recommends making stereopsis assessment and management as an integral part of routine examination to improve the qualities of life who are suffering.

INTRODUCTION

The term ametropia (refractive error) depicts any condition where light is inadequately focused on the retina of the eye, bringing about obscured vision. This is a typical eye issue and incorporates conditions, for example, myopia (near-sightedness), hypermetropia (far-sightedness), astigmatism, and presbyopia is an age-related decrease of vision. (Agarwal *et al*. 2002). Anisometropia is a condition in which both eyes of an individual have different refractive power (Khurana *et al*. 2014). In myopic anisometropia, the sharpness of distance vision in each eye is lower than normal, the more nearsighted eye having less clearance of vision. In any case, when the measure of nearsightedness in the less nearsighted eye is small (minus 0.25 or 0.50 D), the visual sharpness in that eye is adequately great with the goal that the patient may not know about the issue, regardless of whether the visual keenness in the more nearsighted eye is very poor.

In hypermetric anisometropia, the visual sharpness of the two eyes is moderately good as long as the patient has adequate accommodation (Khurana and Khurana 2015). Stereopsis is specifically referring to perception of depth in relation to binocular single vision (Fig. 1), which makes basics for seeing three dimensional images (Howard and

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Rogers 1995).

Coarse stereopsis and fine stereopsis are two main aspects of depth perception. Coarse stereopsis (also known as qualitative or gross stereopsis) is used to detect stereoscopic motion which is changes in binocular disparities in a real-life three-dimensional scene over time in ones surrounding (Barry 2009). Fine stereopsis (quantitative stereopsis) enables an individual to perceive the depth of an object in central visual area (pannum's fusional area). Fine stereopsis is essential to perform fine motor tasks (Barry 2012). TNO (Toegepast Natuurwetenschappelijk Onderzoek) test is used for assessment of stereopsis (Fig. 2)

Stereo acuity development in children with normal binocular single vision. The lower limits of stereo acuity compatible with normal binocular single vision were 3 1/2 years, 3,000 seconds; 5 years, 140 seconds: 5 1/2 years, 100 seconds; 6 years, 80 seconds; 7 years, 60 seconds; and 9 years, 40 seconds (Shah *et al*. 2009).

MATERIALS AND METHODS

A cross-sectional study was conducted from August 2021 to December 2021 in the Department of Ophthalmology, Madina Teaching hospital Faisalabad, Pakistan. All patients included in the study were selected through non-probability convenient sampling technique. Total number of patients included in the study was 30. Both genders were included, age ranged from 12 to 25 years. 15 patients with refractive hyperopic anisometropia (spherical) and 15 patients with refractive myopic anisometropia (spherical) were included. All the patients have anisometropia of greater than 1D without any ocular pathology. All cases with strabismus, media opacity, patients with history of any ocular surgery, ocular trauma, cataract, pseudophakia, aphakia, amblyopia, keratoconus, and ocular pathologies were excluded. After taking both verbal and written consent detailed history was taken.

Subjective plus objective refraction was done to confirm myopic and hyperopic anisometropia. Stereopsis was tested in both myopic and hyperopic anisometropia. TNO chart was used to measure the stereopsis with best corrected visual acuity of the anisometropic patient. After the collection of data, independent sample *t*-test was used with the help of IBM SPSS-20 to get statistical results.

RESULTS

To check the normality of data Shapiro-wilk test was applied. After analysis, it showed, that non-significant ($p>0.05$), so parametric test was used. The study included 30 anisometropic patients with age ranging from 12–25 years. Out of them 15 had myopic anisometropia and 15 had hyperopic anisometropia. The study showed that 12(40%) were male and 18(60 %) were female with mean age 16.93.

For qualitative assessment of stereopsis, among the myopic anisometropic group, gross stereopsis was present in

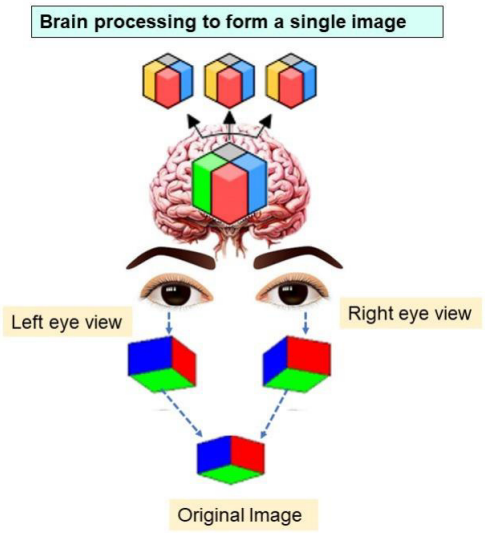


Fig. 1: Stereopsis view



Fig. 2: TNO test for stereopsis

13(86.6%) and remaining 2(13.3%) showed absence of gross stereopsis. Results obtained from the hyperopic anisometropic group showed that gross stereopsis was present 11(73.3%) and the remaining 4(26.6%) did not had gross stereopsis and showed absence of stereopsis. More subjects from myopic anisometropic group showed presence of stereopsis as compared to the hyperopic anisometropic group By analyzing both of the results, presence of gross stereopsis in myopic anisometropic was more significant than that of hyperopic anisometropic.

Quantitative assessment of stereopsis for myopic and hyperopic anisometropia was done which resulted means value of 120 ± 69.282 sec of arc scored by myopic anisometropic and 276 ± 150.20 sec of arc by hyperopic anisometropic subjects included in this study. Although the normal values for stereopsis for an emmetropic person should score 60 sec of arc which means that minimum value for stereopsis reveals good and increased levels for stereopsis. As

Table 1: Comparison of stereopsis of myopic anisometropia and hyperopic anisometropia

Levense's test for equality of variance			*t*-test for equality of means						
Parameters	F test	Significance	*t* test	df	Significance (2-tailed)	Mean difference	Standard error difference	95% confidence interval of difference	
								Lower	Upper
Equal variance assumed	6.789	0.018*	-2.982	18	0.008**	-156.00	52.307	-265.892	-46.108
Equal variance not assumed			-2.982	12664	0.011*	-156.00	52.307	-269.308	-42.692

* P<0.05; ** P<0.01

the mean values for stereopsis in both myopic and hyperopic anisometropia are greater than the normal value of stereopsis, shows that both the myopic and hyperopic anisometropic persons have decreased levels of stereopsis.

On comparison, we find that as myopic anisometropic persons have scored mean value of 120 sec of arc, which is closer to the normal value that is 60 sec of arc as compared to the mean value for stereopsis scored by hyperopic anisometropic subjects that is 276 sec of arc, which is less close to the normal value for stereopsis (Table 1). In this table *P*-value of being less than 0.05 i.e., 0.008 shows that there is difference between the mean effects of the myopic and hyperopic anisometropes which makes it statistically significant. Our results showed that both the myopic and hyperopic anisometropic persons have decreased levels of stereopsis than that of normal value, but this reduction is more obvious among hyperopic anisometropes as compared to the myopic anisometropes.

DISCUSSION

The aim of our study was assessment of stereopsis in myopic and hyperopic anisometropia. Our main objectives are to compare stereopsis in myopic anisometropes with hyperopic anisometropes. Results obtained in the previous research study completely agree to our study that anisometropia causes reduction of stereopsis (Habiba and Hussain 2017).

Another study which agrees to our results that spherical hyperopic anisometropia had much adverse effects on binocular visual functions and stereopsis than that of myopic anisometropia (Weakley 2001). Previous research suggested that anisometropia causes reduction of stereopsis in myopic and hyperopic forms of anisometropes, which makes an agreement to the results of our study but disagrees when his results says that these reduced changes are more significant among myopic type of anisometropia. This opposition is due to the fact that previous study experimentally induced anisometropia on emmetropes to carry out his study, but we have assessed stereopsis on subjects having refractive anisometropia and our results disagree to that research results because our results show that reduction of stereopsis is more obvious among hyperopic form of anisometropia as compared to the myopic form of anisometropia (Nabie *et al*. 2017).

According to past research, increasing degree of anisometropia causes decrease in the levels of stereopsis which supports the results of our study. Likewise, it has been found in the study that anisometropia has adverse effects on stereopsis which also agree to our study (Tarczy-hornoch *et al*. 2011). Lee *et al*. (2013) reported that wearing glasses was better in myopic anisometropia as compared to the hyperopic anisometropia that agree to our study but they also concluded that the stereopsis was clinically normal in anisometric patients wearing their subjective corrections despite of the extent of anisometropia, that contradicts our findings, our study found that there is decrease in stereoacuity with increasing degree of anisometropia.

CONCLUSION

Our results showed that Anisometropia has worse effects on stereopsis in both myopic and hyperopic forms of anisometropia. Loss of stereopsis is more significant in hyperopic anisometropia as compared to myopic anisometropia. anisometropia causes a reduction of stereopsis, which in turn affects the quality of life of patients. This study suggests making stereopsis an integral part of routine examination. Patients, especially those of a younger age, should be carefully treated as their chances of developing amblyopia is common with anisometropia.

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AUTHOR CONTRIBUTIONS

SA: Conceptualization of study design, data analysis, data interpretation; AK: Data collection, data analysis; QA: Data collection, write-up; ZR: Literature search; RFN: Literature search, write-up

CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest

DATA AVAILABILITY

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

ETHICS APPROVAL

Not applicable

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RESEARCH ARTICLE

Solubility Enhancement of Lipophilic Drugs via Novel Vesicular System

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ABSTRACT

Background: Pharmaceutical development of lipophilic drugs is deficient for poor aqueous solubility which causes decreased bioavailability and therapeutic efficiency.
Objective: The aim of this study was to utilize a new vesicular system to increase solubility and bioavailability of poorly water-soluble drugs.
Methodology: In order to enhance the solubility and release into the digestive tract of diacerein (DCT), phospholipid base, polyethylene glycol 400, and tween 80 were used to synthesize liquid proliposomes. Water has a low solubility for dialerase, but organic solvents make it soluble.
Results: Based on previous results, a vesicular self-assembled carrier was developed, optimized and evaluated with respect to the drug loading, entrapment efficiency and *in vitro* drug release. The formulation and particle size was prepared by modifying a thin film hydration method and a uniform distribution of nano sized particles was obtained by particle size analysis. Solubility studies resulted in a 3–5 fold higher solubility than pure drug. It was verified that *in vitro* drug release lasted more than 24 h in the case of controlled drug delivery. Optimized formulation was highly stable under physiological conditions itself. Additionally, *ex vivo* permeation showed more possibility of drug absorption and thus bioavailability. The study shows potential of vesicular carriers to resolve the solubility problem of lipophilic drugs. This approach, therefore, has opened doors towards new avenues for potentiating the therapeutic efficacy and patient compliance using novel approaches.
Conclusion: This novel system proved to be effective and therefore future research should focus on this system's *in vivo* evaluation and clinical translational approach to prove this system's application in pharmaceuticals.

INTRODUCTION

Vesicular systems have shown to be very beneficial carrier systems in several scientific contexts. A vesicular system is a bilayer of concentrated lipids that is highly organized and may consist of a single or several assemblies. Flexibility, safety, patient consistency, and the ability to identify medication at specified location are desirable aspects of the oral route for liposome. The usage of liposomes has been restricted due to their physicochemical features, which include sedimentation, hydrolysis, oxidation, and storage conditions. So, to address these problems with liposomes, proliposomes were developed. To improve the bioavailability and solubility of medications that are not very soluble, the proliposomal formulation was developed. The original definition of a proliposome was a dry, free-flowing particle that, when hydrated, transformed into a liposomal suspension. Liposomes can be more reliably formed at the location of delivery, making them more suited for sterilisation and long-term storage (Ren *et al*. 2022).

The most common kind of arthritis, osteoarthritis, affects both sexes equally and is a global health concern. Women are more likely to have joint inflammation. Bone fractures, brought on by injuries, may aggravate ligament injury. Overweight, family history, age and prior injury are risk factors. Inflammation, stiffness, gradual degradation of cartilages, and deterioration of joints all worsen with age, making it the most prevalent cause. When joint inflammation is mild to severe, pain management and joint replacement are effective treatments.

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The aim of this study was to utilize a new vesicular system to increase solubility and bioavailability of poorly water-soluble drugs. To enhance the solubility and release into the digestive tract of diacerein (DCT), phospholipid base, polyethylene glycol 400, and tween 80 were used to synthesize liquid proliposomes. Water has a low solubility for dialerase, but organic solvents make it soluble. The material seems like a powder with a yellowish hue to it. It helps with osteoarthritis. Instead of histamine or serotonin, the colon metabolizes the unabsorbed diacerein to Rhein, which causes chloride secretion to be activated by stimulating submucosal neurons and the release of endogenous prostaglandin and acetylcholine (Lee 2020).

MATERIALS AND METHODS

A free sample of Diacerein was kindly provided by Pacific Laboratories (Pvt.) Ltd of Multan Road, Lahore. The soy lecithin phospholipid was sourced from ELMA in Belgium. Hydrogen, polysorbate-80, and polyethylene glycol-400 (PEG-400). The University of Faisalabad's Research Lab, Department of Pharmacy, newly manufactured double distilled water. The chemicals used were all of analytical quality and could be used as-is. Soft gelatin capsules containing DCT proliposomes were prepared using the film deposition on carrier technique. The following steps were taken to make the solution: dissolve phospholipids, DCT, PEG-400, and polysorbate-80 in absolute ethanol. The liquid was then mixed with a magnetic stirrer for 15 min at 2000 rpm until it became clear and white. Using a syringe (BD, Malaysia) for precise weighing of the proliposomes, the capsules were sealed with a heated metal spatula. The finished capsules were transferred to glass vials and let dry at room temperature. Six distinct formulations were created, each with a unique concentration of DCT and phospholipids (Table 1).

Determination of diacerein contents

Using a UV/VIS Spectrophotometer set at 256 nm, the DCT contents in the proliposomes were measured. Using a standard calibration curve, we assessed the DCT contents of several DCT proliposomal formulations, each of which included 80 mg of DCT.

Measurement of zeta potential, particle size and polydispersity index

The hydrated liposomes were measured for size, zeta potential, and polydispersity index (PDI) using the Zeta Sizer, which makes use of the dynamic light scattering approach. The size of the samples was determined by hydrating them with double distilled water. In order to hydrate the proliposomes to the proper concentration for the aforementioned apparatus, distilled water and 0.1 M HCl were mixed after the required dilution. Romana *et al*. (2020) reported that for every batch, three distinct formulations were used to get the findings, which were expressed as the mean ± standard deviation.

Table 1: DCT proliposomal formulation contents

Formulation code	DCT (mg)	Phospho-lipid (mg)	PEG-400 (mL)	Tween 80 (mL)	Ethanol (mL)
DCT-1	80	80	1	0	9
DCT-2	80	200	1	0	9
DCT-3	80	400	1	0	9
DCT-4	80	800	1	0	9
DCT-5	80	800	1	2	9
DCT-6	80	800	1	4	9

Rate of conversion from proliposomes to liposomes

The transformation of proliposomes into liposomes was monitored by measuring their absorbance. In order to prepare the sample, a quartz cuvette with a 1 cm path length was used to mix a pre-weighed quantity of DCT proliposomes with 0.1 M HCl. The source filling the cuvette with 0.1 M HCl instantly nullified the buffer absorbance. A Shimadzu UV/VIS Spectrophotometer from Germany was used to measure absorbance.

RESULTS

Particle size, zeta potential and polydispersity index

Table 2 provides information on the formulations, including their particle size, PDI, and zeta potential. After the proliposomal formulations were hydrated in 0.1 M HCl to mimic stomach fluid conditions, the particle size ranged from 212 ± 12 to 414 ± 18.3 nm. Every formulation had a PDI below 0.5, suggesting that it was monodisperse, and all of the sizes were in the nanometer range. An essential element associated with the liposomes' stability and surface characteristics is their zeta potential. The formulations are more stable when the zeta potential values of the nanoparticles are high, which indicates that they have a strong repelling activity among themselves. Indicative of the formulations' excellent stability, the zeta potential values for DCT ranged from 27.5 ± 1.9 mV to 33.6 ± 5.2 mV. A negative zeta potential value, greater than 20 mV, prevents the coalescence among the nanovesicles, which in turn reduces the likelihood of aggregation and increases the size of the particles. Additionally, adsorption of proteins during blood circulation and suppression of sediments generation are both linked to higher zeta potential values.

Scanning electron microscopy

The improved formulations are given in Fig. 1 as electron micrograph. Since the two approaches use distinct sample preparation techniques, the Fig. 1 shows that particle size was varied with light scattering. In most instances, the particles had a spherical shape, and there was undeniable proof of drug loading inside the vesicles. The development of liposomes from the proliposomal formulation was confirmed by the particles' distinct borders (Ameta, Soni and Bhattarai 2023).

Table 2: Particle size, zeta potential and PDI of DCT formulations

Formulation code	Particle size (nm)	Zeta potential (mV)	PDI
DCT-1	212 ± 12	27.5 ± 1.9	0.39 ± 0.01
DCT-2	236 ± 16.3	29.3 ± 1.5	0.28 ± 0.01
DCT-3	315 ± 22.5	31.2 ± 2.6	0.47 ± 0.01
DCT-4	405 ± 13.5	30.6 ± 2.1	0.39 ± 0.02
DCT-5	412 ± 8.4	33.6 ± 5.2	0.44 ± 0.01
DCT-6	414 ± 18.3	33.4 ± 2.9	0.24 ± 0.02

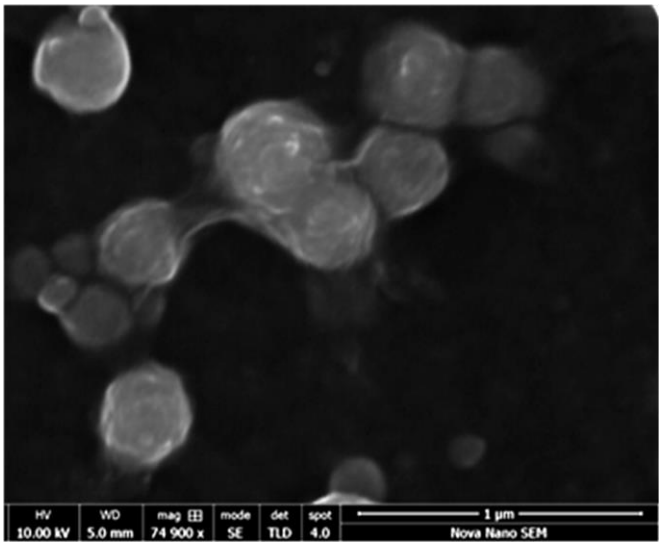


Fig. 1: SEM image of DCT formulation

Entrapment efficiency

The kind of phospholipid is causally connected to EE. In the case of 0.1 M HCl, there was a little reduction in the EE % of DCT liposomes, which ranged from 76.3 ± 2.2 to 80.2 ± 4.9%, whereas in distilled water, it varied from 84.8 ± 4.2 to 89.6 ± 3.5%. A drug's lyophobic nature is likely linked to a higher EE % score. With 0.1 M HCl, the EE% was lower, but it was still more than 75% for all of the produced formulations. When exposed to an acidic media with a low pH, certain liposomes may be disrupted, leading to medication leakage. The inflexibility of the liposomal membranes is another mechanism by which phospholipids are known to raise the EE percentage. Table 3 provides the EE % for DCT formulations.

Conversion rate of liposomes from proliposomal formulations

Prior to hydration, the DCT liposomes that were produced were a clear liquid. A noticeable shift in the turbidity was seen with the introduction of distilled water. The data indicated a gradual and quick conversion to liposomes, because the maximum absorption occurred at 30 seconds and there was no further rise thereafter. When given orally, the manufactured proliposomes should undergo a quick conversion into liposomes once they come into touch with the body's physiological fluids.

Diacerein contents in the proliposomal formulations

More than 97% of the DCT was shown to be integrated into the proliposomes, as shown in Table 4. Every formulation had a consistent distribution of the medication. Between 97.7 ± 0.4 and 99.4 ± 1.2% of the medication was present.

Release kinetics

A biphasic release pattern is typical for liposomes. Phase one is characterized by a fast release, while phase two is characterized by a slower, more gradual release, often of the sustained variety, which may last for 12 h or more. The degradation of the outer surface, caused by the absence of any entrapped medication, may be linked to the first quick release. As the phospholipid concentration increased, the release rate decreased because the lipid bilayers were more stabilized, suggesting a more likely depot action. The key was to use proliposomes to boost the drug's solubility, which was previously insoluble. Phospholipids are responsible for this because they make DCT more soluble. Additionally, the drug's stability and its gradual release at the site of necessity show that the generated proliposomes were appropriate for maintaining the drug's DCT release. The release of DCT1 was around 96.4% after 12 h of disintegration.

Table 3: EE % of DCT formulations

Formulation code	EE (%) in distilled water	EE (%) in 0.1 M HCl
DCT-1	84.8 ± 4.2	76.3 ± 2.2
DCT-2	85.3 ± 3.7	76.8 ± 4.1
DCT-3	86.4 ± 2.9	79.3 ± 2.6
DCT-4	88.4 ± 3.8	78.9 ± 2.9
DCT-5	85.1 ± 3.1	79.4 ± 4.5
DCT-6	89.6 ± 3.5	80.2 ± 4.9

Table 4: Contents (%) of DCT in formulations

Formulation code	DCT (%)
DCT-1	98.7 ± 0.4
DCT-2	98.9 ± 0.3
DCT-3	97.7 ± 0.9
DCT-4	99.4 ± 1.2
DCT-5	98.4 ± 0.6
DCT-6	99.1 ± 1.3

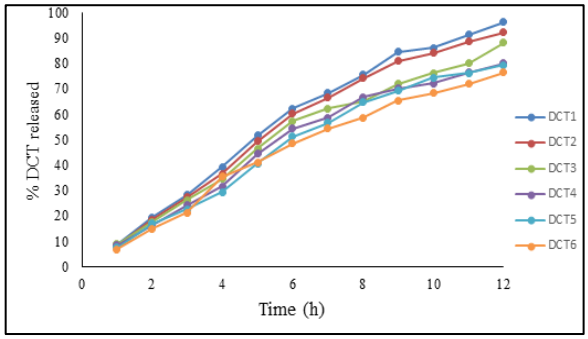


Fig. 2: Dissolution data of DCT formulations following 12 h of release

The statistics also clearly show that the drug's absorption is much improved when the DCT is encapsulated in proliposomes. The absorption of the liposomal formulation from the gastrointestinal tract, where particle size is a key component, is one of many potential game-changers in this context. The small intestine is an excellent uptake site for liposomes larger than 300 nm, particularly in lymphoids tissue; this may be the outcome of the liver's escape from the first pass effect. Formulations DCT5 and DCT6, which include Tween 80 as a surfactant, have a clear correlation between solubility and membrane permeability. Additionally, the medicine may be protected against bacterial and enzymatic breakdown during absorption by integrating it into the lipid bilayer of vesicles. Since DCT encapsulation in proliposomes increases circulation time in the blood, it follows that it may also increase the drug's bioavailability.

Stability testing of DCT proliposomal formulations

The dissolving statistics of several DCT formulations are shown in Fig. 2. With regression coefficient values of 0.99 for nearly all DCT formulations, Korsmeyer Peppas stands out as the best-fitting mathematical model (Table 5). All of the proliposomal formulations have "n" values greater than 0.45, which means that swelling and diffusion are both involved in the drug release process. In less than a minute after adding distilled water, proliposomes were successfully converted to liposomes. This causes formulations to swell and, depending on the amount of phospholipids in the formulations, the drug to leak out of the liposomes. Table 6 displays the results of the stability tests conducted under both situations. Translucent DCT proliposomes DCT was close to 76.4%.

The reconstituted liposomes' EE% and particle size were unchanged following storage for the aforementioned duration. It was also shown that DCT proliposomal formulations, which are typically very vulnerable to phenomena like hydrolysis and oxidation, were more stable when housed in soft gelatin capsules. Also, the fact that the % DCT contents don't change much during the storage time suggests that the proliposomes aren't leaking any medication. Xu *et al.* (2022) found that zeta potential and PDI values made it clear that the nanospheres did not aggregate when stored at room temperature, preserving both the particle size and the formulations' integrity.

DISCUSSION

In the findings of this study, it is revealed that lipophilic drugs have been demonstrated to readily dissolve and be absorbed by the use of proliposomal formulations. The fate of a poorly water soluble drug (diacerein) was established within a proliposomal system represented by significant enhancement in dissolution, controlled release, and formulation stability of the drug. These results are consistent with a body of literature that suggested that vesicular drug delivery systems can improve hydrophobic drug pharmacokinetics by increasing drug solubility and absorption (Khan *et al.* 2016). Particle size analysis confirmed the formation of nano sized vesicles which were found to have an average size between 212 and 414 nm and thus it would provide better absorption in the gastrointestinal tract. Moreover, studies have shown that nanoparticles less than 500 nm are better permeable and bioavailable because they are efficiently taken up by the intestinal lymphatic system and avoid first pass metabolism (Singh *et al.* 2011). The fact that PDI values were always below 0.5 indicates a monodisperse system important for uniform distribution of drug and precise pharmacokinetics. The high stability of formulation due to values of the zeta potential in the range of 27.5–33.6 mV limits aggregation and increases shelf life. This is in agreement with previous literature that zeta potential in excess of ±20 mV inhibits nanoparticle coalescence and stabilizes formulation The entrapment efficiency (EE%) of the proliposomal formulations was found to be highly consistent (76.3–89.6%) which supports the hypothesis that phospholipid carriers provide increased drug loading yet decrease premature degradation. As with previous reports that lipid based vesicular systems have an excellent microenvironment for hydrophobic drugs, this finding indicates that using these systems can enhance drug's stability and the retention within the carrier system. EE% was slightly decreased under acidic conditions, which may confer stability problems during gastric transit. Nevertheless, under hydration drug was rapidly converted from proliposome to liposome that caused

Table 5: Mathematical models for *in vitro* drug release of DCT proliposomal formulations

Formulation code	Zero order		First order		Higuchi		Hixon crowell		Korsmeyer peppas		
	K_0	R^2	K_1	R^2	K_h	R^2	K_{hc}	R^2	K_{kp}	R^2	N
DCT1	9.15	0.845	0.236	0.92	28.63	0.765	0.036	0.96	14.26	0.99	0.843
DCT2	8.98	0.89	0.214	0.91	26.54	0.774	0.038	0.95	13.4	0.99	0.862
CT3	8.64	0.84	0.168	0.94	24.48	0.832	0.034	0.93	12.44	0.98	0.834
DCT4	7.632	0.88	0.132	0.93	21.23	0.841	0.028	0.92	8.32	0.99	0.792
DCT5	6.235	0.79	0.198	0.90	19.25	0.792	0.021	0.91	8.56	0.98	0.816
DCT6	5.32	0.86	0.145	0.94	16.32	0.745	0.031	0.94	6.65	0.99	0.824

Table 6: Results of DCT formulations in stability testing at refrigerated temperature

Formulation code	EE (%) in distilled water	EE (%) in 0.1 M HCl	Particle size	Zeta potential	PDI	DCT contents (%)
DCT-1	82.1 ± 2.9	75.3 ± 2.8	226	28.1	0.34 ±0.01	96.4
DCT-2	83.2 ± 2.1	76.2 ± 4.1	262	30.2	0.41 ±0.01	97.4
DCT-3	80.2 ± 2.2	77.3 ± 2.9	313	28.6	0.39 ±0.01	94.2
DCT-4	83.3 ± 4.1	79.6 ± 5.2	342	30.2	0.35 ±0.02	96.9
DCT-5	84.2 ± 3.1	76.3 ± 3.9	368	31.2	0.24 ±0.01	95.5
DCT-6	81.2 ± 2.6	79.4 ± 4.2	426	34.2	0.39 ±0.01	97.1

drug release at the appropriate site of drug absorption.

Dissolution studies demonstrated a biphasic release wherein there was an initial rapid release proportion and a gradual release from 12 h. Phospholipids possess the property of stabilizing; they form depot effect and its delayed release and improved characteristics of the drug. It was found that the release kinetics can be fitted using the Korsmeyer Peppas model, indicating that the drug release was diffusion controlled. These results are also in agreement with other studies which have demonstrated that vesicular drug carriers enhance dissolution profiles through modulation of release kinetics and retard the elimination of a drug from the system (Glyn-Jones *et al*. 2015).

Furthermore, stability test of proliposomal formulation confirmed its stability under long term storage period up to 12 months by showing marginal change in particle size, EE%, and zeta potential. This shows that proliposomal formulations are stable to physiological pH and therefore have a potential value in making good pharmaceuticals. Soft gelatin capsules were used to provide another protection from outside environmental factors that resistance to oxidation and hydrolysis. This aligns with the potential of proliposomal carriers as a promising alternative to conventional solubility enhancement methods, which can be suffered by faces of instability (Garg *et al*. 2021). Proliposome offers several advantages over conventional drug delivery systems like higher loading capacity of drug, better gastrointestinal stability and improved drug permeability along with controlled drug release. This is unlike traditional solubility enhancement techniques which require means.

CONCLUSIONS

The film deposition on carrier approach was used to effectively generate proliposomes containing DCT by adjusting the ratios of phospholipid and surfactant. The zeta potential, particle size, and PDI were all determined to be within acceptable limits. The production and trapping of the likely spherical-shaped DCT proliposomal structures were verified by scanning electron microscopy. In terms of storage stability, the formulations' zeta potential, particle size, EE%, PDI, and DCT test had almost no changes. The formulations with sustained drug release for 12 h were best modeled by the Korsmeyer-Peppas model, and *in vitro* drug release demonstrated Fickian drug release with n values > 0.45 for all formulations, indicating both swelling and diffusion as potential release mechanisms (Gidde *et al.* 2021).

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AUTHOR CONTRIBUTIONS

SS: original draft, methodology, formal analysis; SJ: review and editing, conception & design; AA: investigation and research; BF: editing and data analysis; SR: data validation and review; FR: technical and software support

CONFLICT OF INTEREST

The authors affirm that they possess no conflicts of interest.

DATA AVAILABILITY

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

ETHICS APPROVAL

Not applicable to this paper

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REVIEW ARTICLE

Influence of Gut Microbiota on Progression, Maintenance, and Control of Sepsis: A Comprehensive Review

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ABSTRACT

Background: Sepsis is a medical condition that is characterized by an unbalanced immune response to an infection, leading to organ damage. The dysfunction of gut microbes due to sepsis has significant impact on body organs and tissues. Several therapies include fecal microbiota transplantation, dietary fiber intake and antibiotic scavengers which reduce the impact of antimicrobial agents on gut microbiota while enhancing their presence at main infection sites.
Objective: The aim of this review is to explore recent development in the field, evaluate microbiota based therapeutic options, and highlights the need for further research to evaluate their role in sepsis management.
Methodology: A comprehensive search of databases, including PubMed, Scopus, and Web Science was conducted to identify relevant studies published up to 2023. The inclusion criteria covered clinical trials and observational studies assessing the effect of fecal microbiota transplantation, probiotics and prebiotics, postbiotics, synbiotics and antibiotics in sepsis management.
Results: The review included studies that have beneficial impact of prebiotics, probiotics, fecal microbiota transplantation on sepsis. Specifically, these interventions were found to improve intestine barrier characteristics, restore intestine microbial variety and decrease infection.
Conclusion: The gut microbiota plays a crucial role in sepsis pathogenesis, affecting immune responses, gut barrier function, and bacterial translocation. Modulating the gut microbiota through various therapeutic interventions holds promise in sepsis management. However, further research is required to fully understand the underlying mechanisms and optimize treatment strategies for personalized care.

INTRODUCTION

Sepsis is a fatal syndrome resulting in abnormal functioning of body organs due to host systemic inflammatory reaction to infection. This disorder is a serious, global health concern leading to both morbidity and death (20%) in worldwide (Singer *et al.* 2016). It is the major cause of health expenses in United States that leads to the public health problem (Rudd *et al.* 2020). Therefore, to understand the cause of dysregulation, research of sepsis condition has target point for researchers on the host immune' response (Rhee *et al.* 2017). The vital role of the gut microbiota in both the progression as well as persistence of sepsis especially in predispose sepsis in adults and post-operative sepsis (MacFie *et al.* 1999).

When an individual has sepsis, their microbial community generally experiences a drop in variety, loss of helpful bacteria, and a rise in the development of dangerous bacteria such as Enterococcus and Staphylococcus (Dickson *et al.* 2016). The host body's inflammatory reactions and increased intestinal microbiota permeability that result from gut microbiota dysbiosis cause infections to spread to organs like the liver. (Fox *et al.* 2012). As a result, changed microbiota compositions in the sepsis state, the precise root cause for the beneficial function of gut microorganisms in sepsis, and the lack of diagnostic procedures or treatments targeting the gut microbiome in the treatment of sepsis are all unresolved issues (Knoop *et al.* 2016).

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ROLE OF GUT MICROBIOTA IN THE MANAGEMENT OF SEPSIS

In gastrointestinal tract, microbiota refers to the diverse group of microorganisms that ultimately inhabit the gastrointestinal tract. Microorganisms such as bacteria, viruses, fungus, and archaea are all part of the microbiome. Numerous facets of host physiology, metabolism, and immune response are significantly influenced by this intricate ecosystem. The microbiome, a varied group of microorganisms that live in both internal and external body parts, is found in the human body (Gilbert *et al.* 2018).

Metabolites produced by commensal gut bacteria are considered important for the characteristics of immune cells and contribute to the various systemic effects that intestinal microbes have on host defense (Fox *et al.* 2017). Recent research has focused on the key immunological pathways influenced by these metabolites. One example is the role of Kupffer cells, the macrophages in the liver, which capture and eliminate circulating pathogens. Gut commensal bacteria produce D-lactate, which is transported to the liver via the portal vein and helps maintain the integrity of the intravascular barrier regulated by Kupffer cells (Schlechte *et al.* 2022). Butyrate, a short-chain fatty acid (SCFA) produced through anaerobic bacterial fermentation, plays a role in the differentiation of monocytes into macrophages. Butyrate promotes the production of antimicrobial peptides and enhances antimicrobial activity in both mice and in vitro (McDonald *et al.* 2020).

Oral administration of short-chain fatty acids (SCFAs) has been shown to boost macrophage phagocytic activity against Klebsiella pneumoniae. This effect is triggered by the activation of G protein-coupled receptor 43. SCFAs also significantly enhance the macrophage-driven removal of the bacteria during infection. The antimicrobial effector LAMTOR2 is overexpressed, which leads to pneumonia infection. The LAMTOR2 receptor activates extracellular signal-regulated kinase, facilitating phagosome-lysosome fusion (Schulthess *et al.* 2019). Metabolites produced by the gut microbiota have a multifaceted effect on the host immune response. In addition to influencing the host's immune system, commensal bacteria can alter their metabolic activity in response to immune stimuli. For example, when mice colonized with four anaerobic commensal bacteria were exposed to acute immune stimulation via flagellin or anti-CD3 antibody, rapid transcriptional changes were observed. Although the overall abundance of the bacteria remained relatively unchanged, this reprogramming led to an increase in the expression of strain-specific reaction mediators. Intestinal metabolite production was observed to change within six hours of immune activation, with a marked reduction in the levels of short-chain fatty acids (SCFAs) like acetate and propionate (Wu *et al.* 2020)

SEPSIS AND ITS TREATMENT AFFECTS THE GUT MICROBIOME

There are several factors that affect sepsis (Miller et al. 2020; Fig. 1). The unclear underlying cause of the beneficial role of gut microorganisms in sepsis, and the lack of diagnostic approaches or therapies targeting the gut microbiota that are effective in sepsis treatment, are still unresolved issues involve the changes in microbiota composition during sepsis (Ubeda *et al*. 2010; Taur *et al*. 2012). One finding is that stress by itself can change the makeup of the gut microbiota. Artificial nutrition feeding is one of the many necessary strategies to help septic patients. Given that food composition is one of the most well researched elements that might alter the makeup and function of the intestinal microbiota, careful study is warranted here (David *et al*. 2014). An instance of this phenomenon involves human participants who were given a meal heavy in animal protein, fat, and low in fiber. Remarkable alterations in the makeup of their gut microbiota were seen during a just 24-hour period. In contrast to those who consume a diet mostly based on plants and rich in fiber, those who follow a different dietary pattern exhibit reduced levels of short-chain fatty acids (SCFAs) and increased levels of secondary bile acids. These secondary bile acids possess the ability to impede the proliferation of beneficial bacteria such as Firmicutes and Bacteroidetes, which are known to contribute to overall health. However, it is common for sepsis patients who are hospitalized to receive casein-based, sterile, chemically specified meals via an enteric tube, which are devoid of dietary fiber (Reis *et al*. 2018).

Gut barrier integrity and bacterial translocation

An initial line of defense in case of infections and dietary antigens is the gut barrier (Paone *et al.* 2020). The intestinal barrier is selectively permeable in a healthy organism, which implies that while it is impenetrable to macromolecules, poisons, food allergies, and infections, it is permeable to ions, water, and low-molecular compounds (Thoo *et al.* 2019). When these compounds leave the colon, the immune system becomes hyperactive, leading to inflammation. Prolonged inflammation can significantly impact health (Paone and Cani et al. 2020). The intestinal barrier is composed of the mucus layer, intestinal microbiota, intestinal epithelial cells (IECs), and lamina propria (Takiishi *et al.* 2017). Intestinal epithelial cells and bacteria are essential for maintaining the integrity of this barrier. The IECs act as a physical barrier, preventing harmful substances from leaking out of the intestinal lumen (Vancamelbeke and Vermeire 2017).

Role of gut microbiota in bacterial translocation

Inflammatory illnesses that can impact the gut and distant organs can be brought on by microorganisms, bacterial

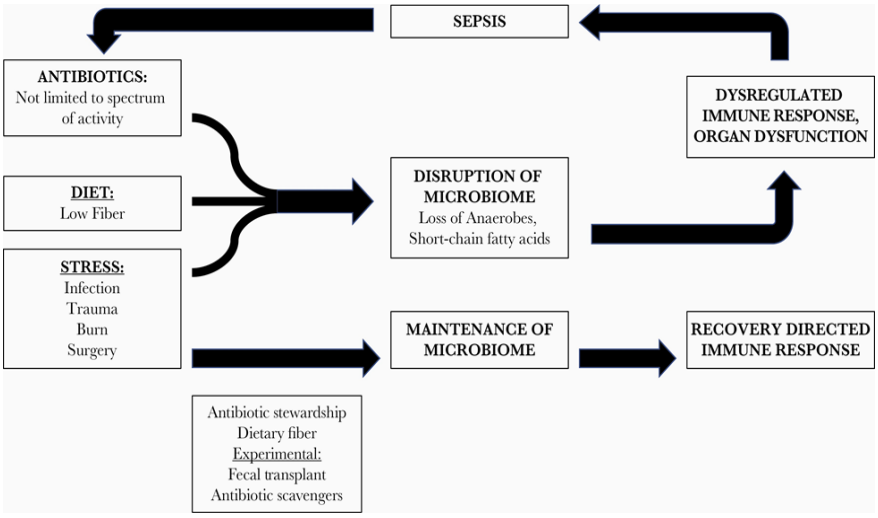


Fig. 1: Factors affecting microbiome in sepsis (derived from Miller *et al.* 2020)

chemicals, or toxins that are unable to pass through the epithelium due to anomalies in the gut barrier (Sorini *et al*., 2019). Mucins comprise the majority of intestinal mucus and are complex clusters of glycoproteins with distinct O-linked glycan's that are produced by goblet cells (Sicard *et al.* 2017). There are several sources of verification that show functional and structural changes to the intestinal barrier are connected to dysbiosis and occur in both human and animal models (Camara-Lemarroy *et al*. 2018). Chronic exposures to molecules from microbial translocation and ongoing dysbiosis, which are to responsible for the rise of bacterial species that are harmful, are to fault. (Mirza *et al*. 2017). In mice, changes in the microbiota of the gut brought on by antibiotic therapy can cause enteric bacteria to move across the epithelium (Knoop *et al*. 2016). Due to changes in the gut microbiota, this relationship may become pathogenic in critical disease, resulting in bacterial translocation, gut-derived sepsis, intestinal homeostasis disruption, and harmful clinical consequences (Wischmeyer *et al.* 2016).

Alteration in gut barrier function in sepsis

Sepsis is a life threatening medical emergency. As our body shows extreme response towards a particular infection. Organ not performing their particular function properly are linked with high risk of death and disease, as the disease turns sever, several abnormalities begin to appear across multiple organs. Sepsis and septic shock frequently result in damage to the digestive system (Fig. 2).

The very first signs are increased permeability, passage of viable bacteria from GI tract to GI sites, and difficulty in absorption are the primary signs of shock (Longhitano *et al.* 2020). Three main lines of defense make up the gut barrier function. the typical intestinal flora (gut microbiota), which makes up the intestinal barrier. Important metabolic, immune, and gut-protective functions are carried out by the

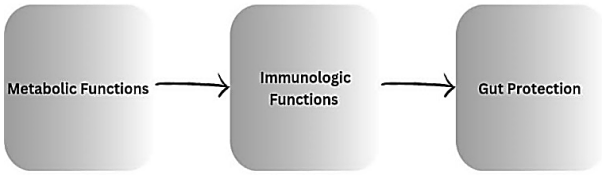


Fig. 2: Major functions of microbiota

microbiota (Assimakopoulos *et al.* 2007). Multiple organ dysfunction syndrome in sepsis is hypothesized to be influenced by intestinal barrier disruption. Although there are some commonalities in the clinical course of sepsis, the host response varies significantly depending on the initiating organism, disease time course, and pathways of gut injury in several preclinical models of sepsis (Yoseph *et al*. 2016). Critically sick individuals have leaked gut malabsorption which leads to the emergence of multiple organ dysfunction syndrome (MODS) and various health issues like systemic inflammatory response syndrome (Assimakopoulos *et al.* 2018). Strange assumptions of gut related sever issues and MODS awaited clinical validation.

In 1991, a researcher took 20 seriously injured individuals in an effort to gather information about bacterial translocation in critically ill patients. 60% added prevention of patient shock 30% of patients develop MODS, 2% test positive, and no one develops systemic endotoxemia (Moore *et al.* 1991). The investigation shows fair doubts about the validity of the gut hypothesis of sepsis, but it was challenging to conduct similar trials with badly damaged individuals (Assimakopoulos *et al.* 2018). Group randomized multicenter trial with surgical 2762 and non-surgical patients 3165 showed that the SDD patients had a fair chance of surviving. An extensive clinical trial in which researchers and patients knows the given drug or treatment, clustered group-randomized crossover study in 13 intensive care units in the

Netherlands with 5927 patients revealed comparable effects of the two treatments in terms of infection rate, as well as low levels of colonization with antibiotic-resistant pathogens. It is similar to the comparison of SDD and SOD strategies (Oostdijk *et al*. 2013).

Sepsis's systemic effects have been deeply studied, and proof of local changes and effects in the intestinal mucosal compartment is increasingly defining changes related to the gut during sepsis (Neish 2014). Six pertinent studies conducted between 1998 and 2006 on 2125 patients found that 5% of patients had post-operative infections, which escalated to 45% of infections overall, whereas 19% of patients were in perfect health. A bigger randomized experiment is being conducted to examine the impact of immune-nutrients, and 1223 critically ill adults with multiple organ failure who are hospitalized to 40 ICUs across different nations are included. To increase mortality, glutamine is administered to them (Goodrich *et al.* 2014). The risk factor of sepsis is due to the disturbance of gut microbiome as shown by circumstantial confirmations, according to two recent large epidemiologic studies (Gergianaki *et al*. 2018).

A meta-analysis of all randomized clinical trials was conducted in 2012 to upshot the effects of micronutrients and antioxidants. Because selenium is highly effective and has shown the survival by 28 days in these patients, a high dose of selenium was given in this study (David *et al.* 2018). A test is carried out in 2004 to determine the overall impact of beneficial bacteria on gut health. After one week of therapy, occurrences of pathogen diminish pathogenic bacteria 43% and multi-organism 39%, there is no discernible difference among the 90 patients admitted to the ICU who received the RCT, received a placebo effect, and experienced the symbiotic effect. Prebiotics and probiotics are employed in two major clinical therapies for intestinal bacterial over-growth, and 5393 individuals are treated using this approach. As a result, there is a 50% reduction in incidence and an 11% reduction in mortality. SDD in 28 days, SOD reduces mortality by 2.9% while reducing motility by 3.5%. 10,000 beneficiaries in this cohort study were studied in 2015 (Sori *et al*. 1988)

GUT MICROBIOTA BASED THERAPEUTIC INTERVENTIONS IN SEPSIS

Gut microbiota, a set of microorganisms dwelling with inside the GI tract that has been diagnosed as the critical factor for human fitness. Recent studies have shed mild at the massive position of intestine microbiota in sepsis, a life-threatening situation characterized through systemic inflammatory reaction syndrome (SIRS) as a result of excessive contamination. Therapeutic interventions concentrated on intestine microbiota in the sepsis that have emerged as a promising location of investigation, aiming to modulate the microbial composition and feature to enhance affected person consequences (Wang *et al*. 2022). Fig. 3 explains some of the strategies used for the treatment of sepsis.

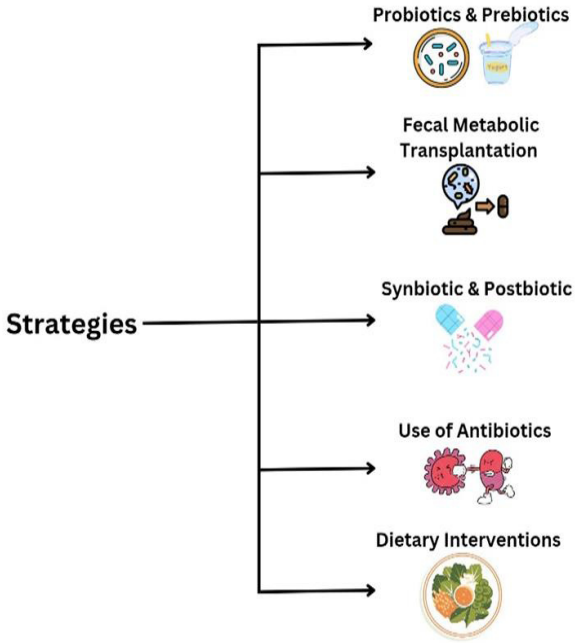


Fig. 3: Strategies used for treatment of sepsis

Probiotics and prebiotics

Probiotics, stay microorganisms that confer fitness advantages whilst administered in ok amounts, have received interest as an ability healing choice for sepsis. Lactobacillus and Bifidobacterium species, generally used as probiotics, have proven promise in restoring intestine microbial variety, improving intestine barrier characteristic, and modulating immune responses in animal fashions of sepsis (Shimazu *et al.* 2012). Clinical trials have proven that probiotic management in septic sufferers improves intestine barrier characteristic, reduces infection markers, and reduces mortality charges (Petrof *et al.* 2012). However, in addition studies is important to validate the efficacy and protection of probiotics in sepsis. Prebiotics, indigestible compounds that selectively stimulate the boom and pastime of useful intestine microorganisms, have additionally emerged as capability interventions for sepsis. Fructo-oligosaccharides and galacto-oligosaccharides, not unusual place prebiotics, were proven to enhance intestine barrier feature, sell the boom of useful bacteria, and decrease intestine-derived irritation in sepsis animal fashions (Ciorba 2012). Although medical research investigating using prebiotics in septic sufferers are limited, initial proof shows that prebiotic management may also lessen contamination costs and enhance medical results (Schulthess *et al.* 2019).

Fecal microbiota transplantation (FMT)

The FMT includes shifting wholesome donor fecal fabric to a recipient's gastrointestinal tract, aiming to repair a balanced

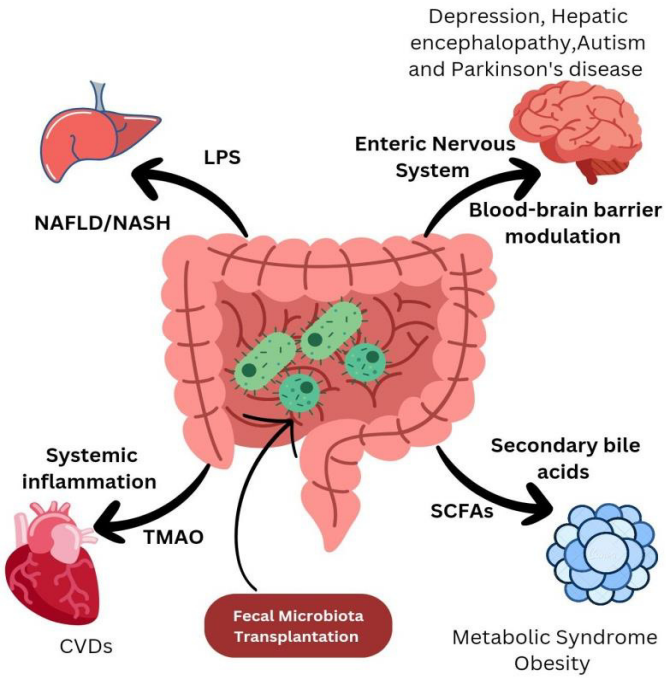


Fig. 4: Illustration of proposed methods via which FMT might influence the development and progression of particular diseases

microbial community. Several research has explored the capacity of FMT in modulating intestine microbiota in sepsis (Fig. 4). Animal fashions have proven that FMT restores microbial variety, improves intestine barrier feature, and decreases infection (Li *et al.* 2016). While medical proof concerning FMT in septic sufferers is limited, early research has said high quality results in phrases of decreased contamination costs and advanced medical parameters (Zuo *et al.* 2018). However, similarly studies and scientific trials are had to set up the protection and efficacy of FMT in sepsis management.

The manipulation of the blood-brain barrier and signaling via the enteric nervous system by microbial metabolites has been shown to have an impact on several mental and neurological illnesses. These disorders include autism spectrum disorders, depressive disorder, hepatic encephalopathy, and Parkinson's disease. The modulation of the metabolic disorder and obesity seems to be influenced by the presence of short-chain fatty acids (SCFA) and secondary bile acids, which are generated by bacterial activity. The introduction of lipopolysaccharides (LPS) together with other structural molecules derived from bacteria into the portal circulation has an impact on the overall health of the liver. The regulation of cardiovascular health has been seen to be influenced by the production of Trimethylamine-N-oxide (TMAO) by bacteria, as well as the induction of systemic inflammation caused by the existence of circulatory bacteria and their byproducts. Fecal microbiota transplantation (FMT), short chain fatty acids (SCFA), lipopolysaccharide (LPS), and Trimethylamine-N-oxide (TMAO) are the respective acronyms for the following terms (Baggs *et al.* 2018).

Postbiotics and synbiotics

Postbiotics are non-feasible microbial mobileular additives or metabolites which have proven capacity blessings in sepsis interventions. These additives, which includes mobileular wall fragments, secreted proteins, and short-chain fatty acids, exert anti-inflammatory and immunomodulatory effects, thereby mitigating sepsis-related complications. In a current observe via way of means of, postbiotics derived from Lactobacillus traces have been discovered to lessen organ dysfunction, decorate microbial diversity, and enhance survival costs in sepsis models. Synbiotics, the mixture of prebiotics and probiotics, have additionally proven promise in sepsis intervention (Meszaros *et al.* 2021). The state-of-the-art studies through proven that synbiotics intervention stepped forward intestine barrier integrity, attenuated systemic inflammation, and ameliorated sepsis-caused lung injury. These findings underscore the capability of postbiotics and synbiotics as novel healing procedures for sepsis management (Shimizu *et al.* 2021).

Antibiotics and gut microbiota

Antibiotics play a critical position in sepsis interventions through focused on and killing the infectious pathogens. However, they also can disrupt the sensitive stability of the intestine microbiota, main to dysbiosis and impaired immune

response (Hutchings *et al.* 2019). Strategies together with antibiotic stewardship and aggregate treatment options with probiotics or fecal microbiota transplantation may also preserve promise in mitigating the negative outcomes of antibiotics on sepsis results (Arora and Backhed 2016).

Dietary modifications

Dietary changes constitute any other road to modulate intestine microbiota in sepsis. High-fiber diets, wealthy in fruits, vegetables, and entire grains, were related to elevated microbial variety and progressed intestine barrier characteristic, probably lowering the danger of sepsis (Jandhyala *et al.* 2015). Conversely, diets excessive in saturated fat and occasional in fiber content material had been related to dysbiosis and elevated susceptibility to infections (Gutierrez *et al.* 2015). However, in addition studies is needed to set up top-rated nutritional techniques for sepsis prevention and management.

Visual acuity for all participants was measured, and those with clinically significant macular edema (CMO) or pre-proliferative/proliferative retinopathy were excluded from the study. Color vision impairment was most pronounced along the tritan axis, particularly in diabetic pseudophakes with background retinopathy. A significant association was found between color vision defects and the presence of background retinopathy ($p = 0.05$). The distribution of color vision defects in the study population was as follows: 16.9% with normal color vision, 10.2% with red-green defects, and 61% with tritan defects. Tritan discrimination sensitivity changes were determinant for figuring out patients liable to excessive retinopathy. The poorer color changes following cataract surgical operation in diabetic pseudophakes can be due to extended short-wavelength transmission thru the intraocular lenses, that could lead to retinal damage. This is mainly true inside the case of phacoemulsification, in which there's a shorter period of exposure to radiation all through lens removal, probably aggravating retinal harm.

FUTURE PERSPECTIVES AND CHALLENGE

- Provision of proper approaches of treatment as well as carefully selected probiotics to the identified patients well helpful for selecting future therapies for gut microbiota for the treatment of sepsis. It will be helpful in the upcoming years in targeting and curing sepsis disease.
- Mostly in practice setting, clinical trials are needed for benefiting septic patients and among them, fecal microbiota transplant (FMT) is helpful in the treatment of sepsis but may be its only beneficial for a fixed number of patients and there's possibility of occurrence of different factors that can cause alteration in the treatment of sepsis via using FMT therapy. These factors included selection of patient, proper rout for administration, timing etc. It is expected that by improving mechanistic insights into the interaction between gut microbiome and sepsis will allow the development of microbiome-based therapeutics for mitigation of sepsis morbidity and mortality.
- Probiotics are helpful in treating gastrointestinal disorders through changes in the gut microbiota but there are still various aspects remaining that will have to address better results such as safety issues, evaluation models, stress resistance etc.
- Hopefully in the future research, there must be some mechanisms or ways to use gut microbiome composition as biomarkers for getting better results of sepsis and this is all possible when all the aspects of the gut microbiome are fully studied. This may be effective in changing the level of mortality and morbidity of sepsis.
- As the world is becoming more advanced day by day, still a lot of things are hidden and need more research to find out the main reasons behind the alterations of gut microbiota normal mechanism and will need more research regarding the factors affecting the normal mechanism of gut bacteria which play an important role in the growth and maintenance of gut

CONCLUSIONS

The gut microbiota plays a crucial role in sepsis pathogenesis, affecting immune responses, gut barrier function, and bacterial translocation. Modulating the gut microbiota through various therapeutic interventions holds promise in sepsis management. However, further research is required to fully understand the underlying mechanisms and optimize treatment strategies for personalized care. Gut microbiota healing interventions maintain enormous ability for enhancing sepsis consequences. Probiotics, FMT, prebiotics, and nutritional changes have proven promise in restoring intestine microbial balance, improving intestine barrier feature, and modulating immune responses in septic sufferers. Nevertheless, the efficacy, protection, and finest dosage of those interventions want to be in addition elucidated thru large-scale scientific trials. Manipulating intestine microbiota represents a promising approach to lessen mortality and enhance affected person effects in sepsis.

AUTHOR CONTRIBUTIONS

Both the authors contributed equally to the write up.

CONFLICTS OF INTEREST

The authors affirm that they possess no conflicts of interest.

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Not applicable

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RESEARCH ARTICLE

Attitude and Consumer Behavior Towards Ecofriendly Products in Punjab, Pakistan: A Way Forward for Sustainable Consumer Behavior

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ABSTRACT

Background: Sustainable consumption patterns are need of hour to achieve the sustainable development goals especially in developing world where the population is increasing at fast pace. In developing countries, environmental consideration while consumption is very important. Awareness is still at the beginning stages in most of the developing countries like Pakistan. Now both marketers and consumers are diverting to the ecofriendly products because of awareness about imputation of global warming, non-biodegradable solid waste, harmful import of pollutant etc. These environmental issues lead to further investigation in order to acquire more knowledge in handling this matter.
Objective: This study examined the factors that trigger consumer to buy ecofriendly products. The present study was conducted to determine the consumer purchase intention (as dependent variable) among the consumers in study area, by using product price, environmental awareness and concerns, green promotion and availability (as independent variables).
Methodology: A well-structured questionnaire was developed to collect primary data from 220 respondents. Simple random sampling technique was used to collect data.
Results: Advertisement and the significance of environmental knowledge prompt to believe consumer on green claim products. Pay an extra price for ecofriendly products also showed a positive attitude towards purchase intention. Furthermore, positive attitude does not lead to action i.e. purchase of these products.
Conclusion: With the growing awareness, many businesses have accepted their responsibility not to harm the environment and not to waste the natural resources. Consumer buying choices can make a difference to the environment just because of awareness and could be accelerate the consumption of ecofriendly products. The most powerful element that influence purchase of green products was environmental concerns that will help to reduce in production of unsustainable products.

INTRODUCTION

Sustainable development requires the development and use of sustainable and ecofriendly products (Majeed *et al*. 2022). The developing world is facing serious concerns to sustain production and consumption in the presence of rising population, externalities and market inefficiencies (Gilal *et al*. 2020; Waqas *et al*. 2020). Nature blessed humans with a beautiful and clean environment, but the humans destroy the environment with their activities. Overpopulation, polluted air, land, water, loss of public spaces, disappearance of biological diversity and all environmental problems that whole world is currently facing environmental sustainability issues due to which environmental issues have effects and changed the ornament of human life activates (Batool *et al*. 2023; Durrani *et al*.

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2023). The use of worldwide electronic products has increased due to the accelerated growth in technology that creates environmental deterioration. Human resource management, economic, financial and technical advancement affected the environmental issues at large in many ways (Ionescu *et al.* 2021). The utilization of resources depends upon the decisions taken by human resources in economic cycle from farm to fork in whole value chain. Technological innovation can deteriorate and save the environment according to human requirements and choices. Marketing at large creates such an environment that can influence the human behavior, attitude and decisions towards any product and its credentials. There are different marketing terms used to represent ecofriendly products like green, nature friendly etc. to promote good and services, sometimes with extra, more specific certifications such as ECO labels. The International Organization Standardization has developed ISO 14024 to demonstrate principle and procedure that Eco labelers should follow. Worldwide big industrialization is just the result of fast economic growth and increasing consumption of natural resources. This in turn has resulted in eruption of environment due to explosion of natural resources (Nguyen 2020).

Demand for ecofriendly products across the globe has just grown by rise in buyer education. Therefore, the ideology of understanding the environmental attitude of the consumers come out from eco-friendly marketing and green marketing (Ud Din *et al.* 2023). Pattie defined Green Marketing as "the holistic management practice responsible for identifying, anticipating and satisfying the requirements of clients and society, in a profitable and sustainable way" (Peattie 2016). Due to the energy crisis many developing countries are adopting energy-efficient appliances (EFAs), this is s best way out about sustainable consumption and energy related issues for consumers. EFAs are environmentally friendly too (Ahmed *et al*. 2023; Bhutto *et al*. 2021). There are a lot of factors that affect consumer interest in adopting ecofriendly friendly products including inadequate information about products, lack of finance for firms, cost of output and the trust that individual actions alone cannot impact the tendency to free ride. In this regard marketing can do wonders, they identify the needs and wants of consumers and urge them to buy eco-friendly products (Hayyat *et al*. 2023; Sharma and Trivedi 2016). In these days, educated peoples are aware of ecofriendly products and have knowledge about environment related issues. Altruistic movies were more important for buyers. Purchase of green products could be affected by false assumptions about word "ecofriendly" (Barbarossa and De Pelsmacker 2016; Saeed *et al.* 2023). Customer awareness about the product is the constitutional right of consumer is called consumer awareness. Brand awareness is to the extent to which consumer insight into the particular products or services and familiarize the customer with unique and new designs (Prakash and Thakur 2023). Consumers are becoming more bionomical responsive and desire to purchase green products (Suganya and Kavitha 2017). Level of knowledge, attitudes, values and practice of consumers are depending by the serious quality of environment (Pillai 2016).

The aim of this study was to find out consumer buying behavior towards ecofriendly products in concerns of environmental issues and whether they would be able or not to pay an extra price for such products. This research is based on five ecofriendly products: cloth or cotton bag, recycled fabric cloth, paper packaging and disposable plates, solar energy sources and LED blubs. The main reason why cloths bags are better than plastic bags are: cloths bags are reusable, decreasing the need to buy them again and again. Therefore, it decreases the use of plastic and plastic pollution. There are some fabrics that are ecofriendly due to availability in nature, clean from any chemical or toxic substance and no harm to others like hemp, wool, organic cotton, soy silk, bamboo fabric, jute, corn fiber etc. Conventional or organic cotton require more resources as compared to recycled cotton that prevents additional textile waste. The objective of this study was to analyze the consumer behavior towards ecofriendly products, the demand of ecofriendly products and determinants of ecofriendly products adoption.

MATERIALS AND METHODS

For getting better understanding of the theoretical framework and including the factors that affect purchase of ecofriendly products, a conceptual framework is shown in Fig. 1 to extract the ideas from the theoretical framework discussed here. The main population targeted in this study was basically from Faisalabad, which is the third most populous city in Pakistan. A well-structured and pre-tested questionnaire was used to collect primary data. The data was collected through planned interviews. Simple random sampling was used to pick respondents, and 220 participants were selected from study area.

The method used to separate systematic variation from noise is being a multivariate exploratory analysis that's called principal component analysis. It allows envision of objects and variables and preserve the relevant information of the original data that allowed to define a space of reduced dimensions (Geladi and Linderholm, 2020). A statistical process that allows you to summarize the information by mean of smaller set of "summary indices" from information content in large data tables is called Principal component analysis. PCA is very helpful tool to find out the analysis of dataset that may contain multicollinearity, missing value, categorical data and vague measurements due to this property it is flexible tool. The main object is to express information as a set of précises and draw out the important information from data is called principal components.

The most common application of principal component analysis (PCA) is to reduce the dimensionality of data in order to obtain lower-dimensional data. This is



Fig. 1: Conceptual framework

accomplished by projecting each data point onto only the first few principal components, all the while attempting to preserve as much of the variation in the data as possible. In addition to that, it is utilised in the process of predictive modelling and the examination of explanatory data. A correlation matrix with ones on the diagonal is affected negatively by this. The value of variance is responsible for determining whether the number of observed values, the sum of diagonals, or the trace of the matrix are correct. In this study, the number of observed variables has been shown to be either fewer than or equal to the number of major components. The first major component has a significant proportion of the data's variation in comparison to the second component, which also has a great amount of the data's variance and is completely uncorrelated (orthogonal) with the first one, and so on. Due to the fact that they are orthogonal, which means they are symmetric, the primary components are the eigenvectors of the covariance matrix. Eigenvectors are weights that are utilised in the process of estimating elemental score.

To evaluate the consumer's response towards ecofriendly products was being used a contingent valuation (CV) approach. A direct estimation of WTP just allowed by the CV approach by means of different (direct) extract technique. A type of regression where dependent variable just has two values is called Probit model. The dependent variable Y is a distinct variable that represent a choice, or category, from a set of mutually exclusive choices or categories. In this study probit model is used to estimate the willingness to pay for ecofriendly products and recognized the factors affecting the willingness to pay (Gujarati and Porter 2009). There are some factors that explain positively affect while other factors negatively affect willingness to pay for ecofriendly products. Probit model is basically applied for those variables that are qualitative in nature and have answers in two types like "yes or no" and "purchase or no purchase"

In probit regression, when dependent variable is binary is being used to model the regression function that's called increasing standard normal distribution Φ, that is we assume:

$$E(Y|X)=P(Y=1|X) = \Phi(\beta 0+\beta 1X).$$

Assume that Y is a binary model. The model

$$Y = \beta 0 + \beta 1 * X1 + \beta 2 * X2 + \beta 3 * X3 + \cdots \cdots \beta n * Xn$$

With

$P(Y=1 \mid X_1, X_2,\ldots\ldots..X_K) = \Phi\ (\beta_0+\beta_1X_1+ \beta_2X_2+\ldots\ldots B_kX_k)$ is the population probit model Φ is the cumulative standard normal distribution function with multiple repressor model X_1, X_2 …..X_k (Kantar 2021).

RESULTS AND DISCUSSION

Ranking of constraints

The collected data of 220 respondents were interviewed from different colonies of Faisalabad and mostly people are employed. The mean ranking of constraints that play a great role to purchase ecofriendly products (Table 1).

Principal component analysis

In this particular research, a Bartlett test of sphericity (BTS) and a Kaiser-Meyer Olkin (KMO) were utilised in order to establish that principal component analysis is an effective method to use. The value of BTS in Table 2 is displayed as 3183.391, and its degree of significance is shown to be significant, which suggests that the data were suitable for PCA analysis (Table 3). Because KMO has a value of 0.926, it may be deduced that there are adequate items for each factor. Throughout the course of our inquiry, we settled on a total of seventeen important aspects to look at.

Finding common elements, or principal components, in the form of linear combinations of the studied constraints is the goal of PCA, and these factors are then ranked according to their significance. Three components have eigenvalues greater than one, and these three components are responsible for 71.58% of the variance. It is important to note that only the factors with eigenvalues greater than one are kept. Only three elements are above the one eigenvalue, according to the eigenvalue plot (benchmark). The variance of 50.44% is accounted for by the first component's eigenvalue of 8.575. The first part is made up of eight components. Quality, performance, flavor, the environment, being healthy, being promoted, being available, and a reasonable price are the limitations that apply to this component. Performance (0.900), environment (0.894), flavor (0.866), health (0.875), promoted (0.803), availability (0.792), and fair costs are also factors, with quality having the greatest factor loading at 0.912. (0.784).

Rotated component matrix

The factor loading of component 1 as a whole is 6.846 (Table 4; Fig. 2). The variance for the second component is 14.88% with an Eigen value of 2.530. This component consists of eight items, as follow: packaging design (0.808), advertisement (0.780), satisfaction (0.762), environmental concern (0.745), environmental knowledge (0.742),

Table 1: The mean ranking of constraints playing a great role to purchase ecofriendly products

S. No.	Variables	Mean	SD	Minimum	Maximum
1	Higher willingness to pay for ecofriendly products.	1.21	1.017	1	5
2	Eco-friendly products have reasonable price.	3.18	1.339	1	5
3	Price of eco-friendly products is assuming to be higher as compare of ecological-non friendly products	3.25	1.237	1	5
4	Green products have a better quality/performance.	3.35	1.374	1	5
5	Eco-friendly products have superior quality/performance than non-ecofriendly products.	3.31	1.329	1	5
6	Eco-friendly products have a good taste and/or pleasant smell.	3.21	1.386	1	5
7	Before making a purchase, you take into account whether your goods and its packaging are made to be recycled.	3.61	1.167	1	5
8	Eco-friendly products are well promoted.	3.16	1.372	1	5
9	When you read a product's eco-friendly label, your decision to buy alters.	3.37	1.203	1	5
10	Advertisement for ecofriendly products are effective in creating awareness of problem facing the environment.	3.67	1.179	1	5
11	Eco-friendly items are beneficial.	3.54	1.494	1	5
12	Eco-friendly products are healthy.	3.44	1.453	1	5
13	As a customer, you think about a product's impact on the environment before buying it.	3.51	1.157	1	5
14	You consider your purchase eco-friendly product is correct from the environmental point of view.	3.69	1.104	1	5
15	You willing to go out your way to obtain ecofriendly products.	3.60	1.079	1	5
16	In purchasing the products, you believe there is enough information available about their eco-friendly aspects.	3.37	1.233	1	5
17	Eco-friendly products are accessible /available in the supermarket.	3.20	1.404	1	5

Source: Author's own calculations

Table 2: Bartlett test of Sphericity (BTS) and Kaiser–Meyer–Olkin (KMO) tests

Bartlett Test of Sphericity Approx. (Chi-Square)	0.926
Kaiser–Meyer–Olkin test for sampling adequacy	3183.391
Degree of Freedom	136.000
Significance	0.000

Source: Author's own calculations

Table 3: Total variance explained by PCA for ecofriendly products

Component	Eigenvalue	Difference	Proportion	Cumulative
1	8.575	6.045	0.50443	0.50443
2	2.530	1.467	0.14885	0.65328
3	1.063	0.308	0.06256	0.71584
4	.755	0.061	0.04442	0.76026
5	.694	0.071	0.04083	0.80109
6	.623	0.123	0.03664	0.83773
7	.500	0.075	0.02942	0.86715
8	.425	0.056	0.02499	0.89214
9	.369	0.037	0.02173	0.91387
10	.332	0.039	0.01952	0.93339
11	.293	0.072	0.01725	0.95064
12	.221	0.051	0.01299	0.96363
13	.170	0.032	0.00998	0.97361
14	.138	0.008	0.00812	0.98173
15	.130	0.03	0.00763	0.98936
16	.100	0.02	0.00591	0.99527
17	.080	0.08	0.00473	0.10000

Source: Author's own calculations

information (0.729), labelling (0.701) and high premium price (0.589). The sum of factor loading of this component is 5.856. The third component's Eigen value is 1.063, which explains a variance of 6.216%. There is only one thing in this component. This component's constraint is more willing to spend for environmentally friendly goods. The factor loading with the highest value is the greater willingness to pay (0.935).

The combined variance of the three components is 71.58, which indicates that these three components'

Table 4: Rotated component matrix

Construct	Items	Constraints	Component		
			1	2	3
Personal capabilities	PC-1	Eco-friendly products have a better quality/performance than conventional products.	0.912	-	-
	PC-2	Eco-friendly products have a good quality/performance.	0.900	-	-
	PC-3	Eco-friendly products are good for the environment.	0.894	-	-
	PC-4	Eco-friendly products have a good taste and/or good smell.	0.886	-	-
	PC-5	Eco-friendly products are healthy.	0.875	-	-
	PC-6	Eco-friendly products are well promoted.	0.803	-	-
	PC-7	Eco-friendly products are accessible/available in the supermarket.	0.792	-	-
	PC-8	Eco-friendly products have reasonable price.	0.784	-	-
Social norms	SN-1	You consider your product, and its package are designed to be recycled before making a purchase.	-	0.808	-
	SN-2	You consider advertisement for ecofriendly products are effective in creating awareness of problem facing the environment.	-	0.780	-
	SN-3	You willing to go out your way to obtain ecofriendly products.	-	0.762	-
	SN-4	You consider your purchase eco-friendly product is correct from the environmental point of view.	-	0.745	-
	SN-5	As a consumer while purchasing a product you consider its effect on the environment.	-	0.742	-
	SN-6	You feel there is enough information about eco-friendly product features while buying the products.	-	0.729	-
	SN-7	Your purchase decision changes when you see the label of a product eco-friendly.	-	0.701	-
	SN-8	You 4 that the price of eco-friendly products is supposed to be higher.	-	0.589	-
Intention	INT-1	You are willing to pay more for ecofriendly products.	-	-	0.935

Source: Author's own calculations

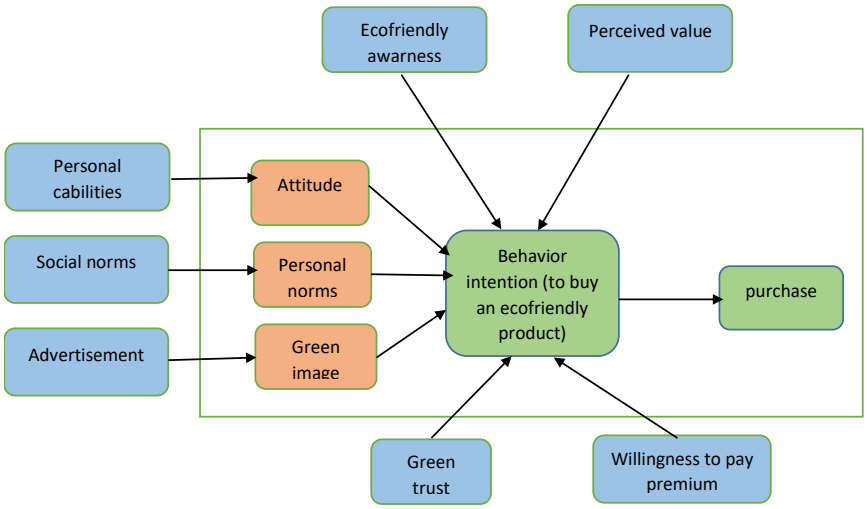


Fig. 2: Probit Model regression results for ecofriendly products

underlying restrictions account for 72% of the available data. Based on the aforementioned empirical data, the main restraint is consumers' increased willingness to pay for environmentally friendly goods.

Probit model

Probit model regression was done for ecofriendly products and willingness to pay as a dependent variable. And also evaluate those factors that affects consumer's willingness to pay.

The age coefficient was positive, indicating a favorable correlation between age and consumer purchasing intentions. Value of this coefficient indicate that when one-year increase in age of consumer than purchase of ecofriendly product is increased by 0.15% keeping all other factors constant. Furthermore, positive sign of education shows the as the number of schooling years increase of the people, they were showed more willing to pay for ecofriendly product that may be due to higher awareness level for ecofriendly products. Value of this coefficient indicate that when one-year increase in education of consumer than purchase of ecofriendly product is increased by 0.75 percent, keeping all other factors constant (Table 5).

Table 5: Probit Model regression results for ecofriendly products

Variable	B	S.E.	Sig	Exp (B)
Intercept	-2.015	.8494	.018**.	.133
Age	.015	.0144	.297.	1.015
Education	.075	.0332	.023**.	1.078
Family size	.002	.0401	.958	1.002
Household income	.228	.1316	.091***.	1.000
Green promotion	.285	.1296	.028**.	1.329
Environmental knowledge	.238	.1133	.035**.	.788
Environmental concern	.259	.1476	.050**.	1.296
Availability of ecofriendly product	.370	.1242	.001*.	1.447
Product Price	-.550	.1130	.000*.	.577
Likelihood ratio Chi square	(df=9) significance test result 83.77 (p-value=0.000)			

Significant at: * 1%, ** 5% and *** 10% levels of probability

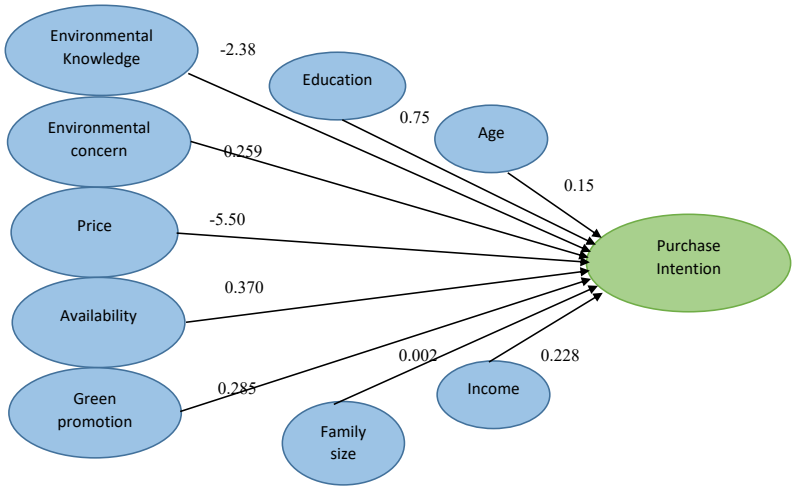


Fig. 3: Probit Model regression results for ecofriendly products

Family size and purchasing intention are positively correlated, as seen by the sign of the coefficient for the independent variable household. Despite the fact that this variable's magnitude is 0.002, which suggests that chances of household purchase intention rise by 0.002 percent for every additional household member, the results are inconsequential. The total monthly income of a household includes all sources of revenue for all family members. When income increases by one rupee, the purchase of environmentally friendly products increases by 0.228 percent, all other variables remaining constant. This is indicated by the positive sign of the variable's coefficient. Green promotion affects consumer purchase intention. Better the green promotion better will be the consumer buying behavior (Kotler and Keller 2009).

The environmental knowledge coefficient, which had a negative sign and was significant, was -0.238 (Fig. 3). According to the coefficient of variation, consumer purchase intention will fall by 0.238 percent for every unit increase in environmental knowledge, assuming all other variables remain constant. Consumer buying intent may also diminish when environmental understanding declines (Iftikhar *et al.* 2022). The coefficient of environmental concern, which was substantial at 0.259, displayed a positive sign. When all other factors are held constant, the coefficient of variable showed that for every unit rise in environmental concern, there may be an increase in consumer purchase intention of 0.259%. Consumer buying intent can increase as environmental worries do (Peattie 2010). As prices increase, consumer purchase intention will be decrease due to higher prices (Yoshida and Gordon 2012). The coefficient of this price is -0.550 showed a negative sign and was significant.

The Availability of ecofriendly product shows the positive sign and significant. Positive sign shows that 1 unit increase in the availability of the ecofriendly products 0.370 percent increase willing to pay for ecofriendly products keeping all other variables constant. All these variables play a significant role to determine the factors that affect ecofriendly purchase intention (Zhang *et al.* 2022; Chen *et al.* 2018; Hur *et al.* 2013; Suki 2016).

CONCLUSIONS AND POLICY RECOMMENDATIONS

The ecosystem is dynamic by nature; thus, we have a duty to

preserve it. Environmentally friendly items are in demand as consumers have increased their environmental awareness. On one hand, the environment and economy might benefit from a measure like the Green Tax System levied on textile manufacturers for their carbon footprint. If manufacturers must utilize ecologically friendly practices, then consumers must also do their part to protect the environment.

The study findings recommend that identifying these consumer groups will help green marketers build and promote products at reasonable pricing points. It's crucial for businesses looking to create innovative eco-friendly items to make sure their goods operate well. Environmental education is a crucial component that doesn't seem to be included in popular books. Consumers can be made aware of the environmental damage caused by hazardous items through appropriate counselling and environmental education. The majority of consumers are unaware that the products are sold in markets. The products will eventually enter the consideration stage once they are accessible to a significant percentage of consumers.

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AUTHOR CONTRIBUTIONS

JN, RK and SS contributed to the development of research concept, design of the study and data collection; BN, ZU and TU supported the formatting and proofreading and execution of the research.

CONFLICTS OF INTEREST

Authors affirm that they possess no conflicts of interest.

DATA AVAILABILITY

The data will be made available on a fair request to the corresponding author.

ETHICS APPROVAL

Not applicable.

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RESEARCH ARTICLE

Biological Significance of Changes in Stomatal Density and Stomatal Index of *Aloe* Species

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ABSTRACT

Background: *Aloe* is a genus containing over 500 species of flowering succulent plants with enormous medicinal potential. Stomatal density and index vary greatly in the different species of this genus.
Objective: *Aloe* species were collected from the new botanical garden, University of Agriculture, Faisalabad. The size of stomata, the size of epidermal cells, the number of epidermal cells, and the number of stomata per ocular were measured to evaluate the anatomical changes in *Aloe* species.
Methodology: The experiment was conducted to explore stomata modifications in upper and lower leaf lamina of different *Aloe* species that are *A. vera*, *A. karabergensis*, *A. striata*, *A. spinosissima*, *A. pachygaster*, *A. microstigma*, *A. hemingii*, *A. globuligemma*, *A. eru* and *A. conifera* in Nov, Dec and Jan.
Results: Significant interspecific variation was observed in both stomatal index and stomatal density among the studied *Aloe* species, including *A. vera*, *A. pachygaster*, *A. eru*, *A. striata*, *A. karabergensis*, *A. hemingii*, *A. conifera*, *A. microstigma*, and *A. globuligemma*. Among these, *A. pachygaster* exhibited the highest stomatal density, whereas *A. microstigma* demonstrated the lowest.Analysis of epidermal characteristics revealed that *A. hemingii* had the greatest number of epidermal cells, while *A. microstigma* had the fewest. Furthermore, stomatal size was markedly larger in *A. pachygaster* and *A. striata*, whereas reduced stomatal size was observed in *A. vera* and *A. conifera* across both adaxial and abaxial surfaces. These findings suggest species-specific adaptive responses likely linked to ecological or physiological traits, offering insights into taxonomic differentiation and potential drought resilience mechanisms in *Aloe* species.
Conclusion: Overall results showed great variations in all parameters in different *Aloe* species. The information obtained from this study can be used to assess the photosynthetic performance and dry matter yield of these species.

INTRODUCTION

Aloe vera (L.) Burm. f. is a well-known medicinal succulent appreciated for its pharmaceutical, nutraceutical, and cosmetic uses globally (Manye *et al*. 2023). Its inherent adaptation to dry and semi-dry habitats is primarily due to its CAM photosynthetic process, which improves water-use efficiency by enabling carbon fixation at night and reducing transpiration during the day (Males 2017). The gel from *A. vera* leaves is a complex mixture abundant in bioactive substances like acemannan, anthraquinones, and aloins, which have shown anti-inflammatory, antioxidant, and wound-healing effects (Eshun and He, 2004). Even with extensive research on the chemical makeup of *A. vera* gel, the specific anatomical and physiological processes that contribute to its drought resistance and metabolite production are still not thoroughly investigated. The majority of studies conducted so far have concentrated on the biochemical analysis of leaf extracts but have neglected the structural adaptations that enable survival in water-scarce habitats

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(Hamman 2008). The fleshy leaf tissues, comprising hydrenchyma for storing water and chlorenchyma for photosynthetic activity, are essential for CAM functionality and the buildup of secondary metabolites, but quantitative anatomical studies of these tissues in *A. vera* are limited.

Comprehending these anatomical characteristics is crucial for clarifying how *A. vera* maintains water conservation while producing bioactive substances, particularly during environmental stress. Additionally, the distribution of bioactive metabolites in particular tissue compartments and their connection to anatomical characteristics have not been thoroughly examined. This gap hinders our capacity to optimize *A. vera* farming for improved therapeutic effectiveness and stress resistance. Comparative anatomical research among various *Aloe* species has revealed considerable variability associated with ecological adaptation (Pérez-López *et al*. 2023). Tackling this gap could enhance breeding and biotechnological strategies focused on boosting crop performance and metabolite production under different ecological conditions. This research aims to fill these gaps through a comprehensive examination of leaf anatomical structures in different *A. vera* species.

Coarse stereopsis and fine stereopsis are two main aspects of depth perception. Coarse stereopsis (also known as qualitative or gross stereopsis) is used to detect stereoscopic motion which is changes in binocular disparities in a real-life three-dimensional scene over time in ones surrounding (Barry 2009). Fine stereopsis (quantitative stereopsis) enables an individual to perceive the depth of an object in central visual area (pannum's fusional area). Fine stereopsis is essential to perform fine motor tasks (Barry 2012). TNO (Toegepast Natuurwetenschappelijk Onderzoek) test is used for assessment of stereopsis (Fig. 2)

Stereo acuity development in children with normal binocular single vision. The lower limits of stereo acuity compatible with normal binocular single vision were 3 1/2 years, 3,000 seconds; 5 years, 140 seconds: 5 1/2 years, 100 seconds; 6 years, 80 seconds; 7 years, 60 seconds; and 9 years, 40 seconds (Shah *et al*. 2009).

MATERIALS AND METHODS

An experiment was conducted to study the stomatal changes on the adaxial and abaxial surfaces of the leaf laminae of ten *Aloe* species: *A. vera*, *A. karabergensis*, *A. striata*, *A. spinosissima*, *A. pachygaster*, *A. microstigma*, *A. hemingii*, *A. globuligemma*, *A. eru* and *A. conifera*. Leaf samples were collected in November, December and January at the Old Botanical Garden of the University of Agriculture, Faisalabad. The samples were first fixed in FAA (formalin acetic acid alcohol) for 24 hours and then transferred to 70% ethanol for preservation. The leaf lamellae were cut by hand to produce anatomical preparations. The sections were then subjected to the following staining procedure.

The leaf lamellae were carefully peeled from selected specimens and immersed in 30% ethanol for 10–15 minutes. The tissues were then successively placed in 50% ethanol for 10–15 minutes and then in 70 ethanol. After dehydration with ethanol, the samples were stained with a few drops of safranin for 5 minutes. Excess safranin was removed by washing the tissue three times in 90% ethanol for 5 minutes. The tissues were then washed 2–3 times in 100% ethanol and cleared with a graded series of xylene (25%, 50% and 100%). Each section was permanently embedded in a drop of Canada balsam. Microscopic images of the samples were taken using a compound light microscope. Stomatal density was determined according to the method described by Salisbury. The stomatal index was calculated according to the formula of Paul *et al*. (2017).

RESULTS

Number of stomata

Graphical analysis revealed that *A. pachygaster* exhibited the highest number of stomata on both the adaxial and abaxial surfaces. In contrast, the lowest stomatal density was recorded in *A. microstigma* on both surfaces. Overall, significant interspecific variation was observed in stomatal number across the studied *Aloe* species (Fig.1A).

Number of epidermal cells

The highest number of epidermal cells was observed in *A. hemingii* on both leaf surfaces, whereas *A. microstigma* exhibited the lowest count. The data demonstrated statistically significant differences in epidermal cell number among the different *Aloe* species (Fig.1B).

Length of guard cells

The longest guard cells were measured in *A. pachygaster* at both upper and lower epidermal layers. Conversely, the shortest guard cells were found in *A. globuligemma*. The results indicated substantial variation in guard cell length across the *Aloe* species (Fig.1C).

Width of guard cells

The maximum guard cell width was recorded in *A. striata* and *A. hemingii* on both leaf surfaces, while *A. vera* exhibited the minimum width. Significant differences in guard cell width were noted among the evaluated *Aloe* species (Fig.1D).

Length of epidermal cells

The greatest epidermal cell length was observed in *A. conifera* on both the adaxial and abaxial surfaces. In contrast,



Fig. 1: Number of stomata (A), number of epidermal cells (B), length of guard cells (C), width of guard cells (D), length of epidermal cells (E), width of epidermal cells (F), length of stomatal pore (G), width of stomatal pore (H) and stomatal index (I) of different *Aloe* species

A. pachygaster showed the shortest epidermal cells. Overall, significant differences were detected in epidermal cell length among the species (Fig.1E).

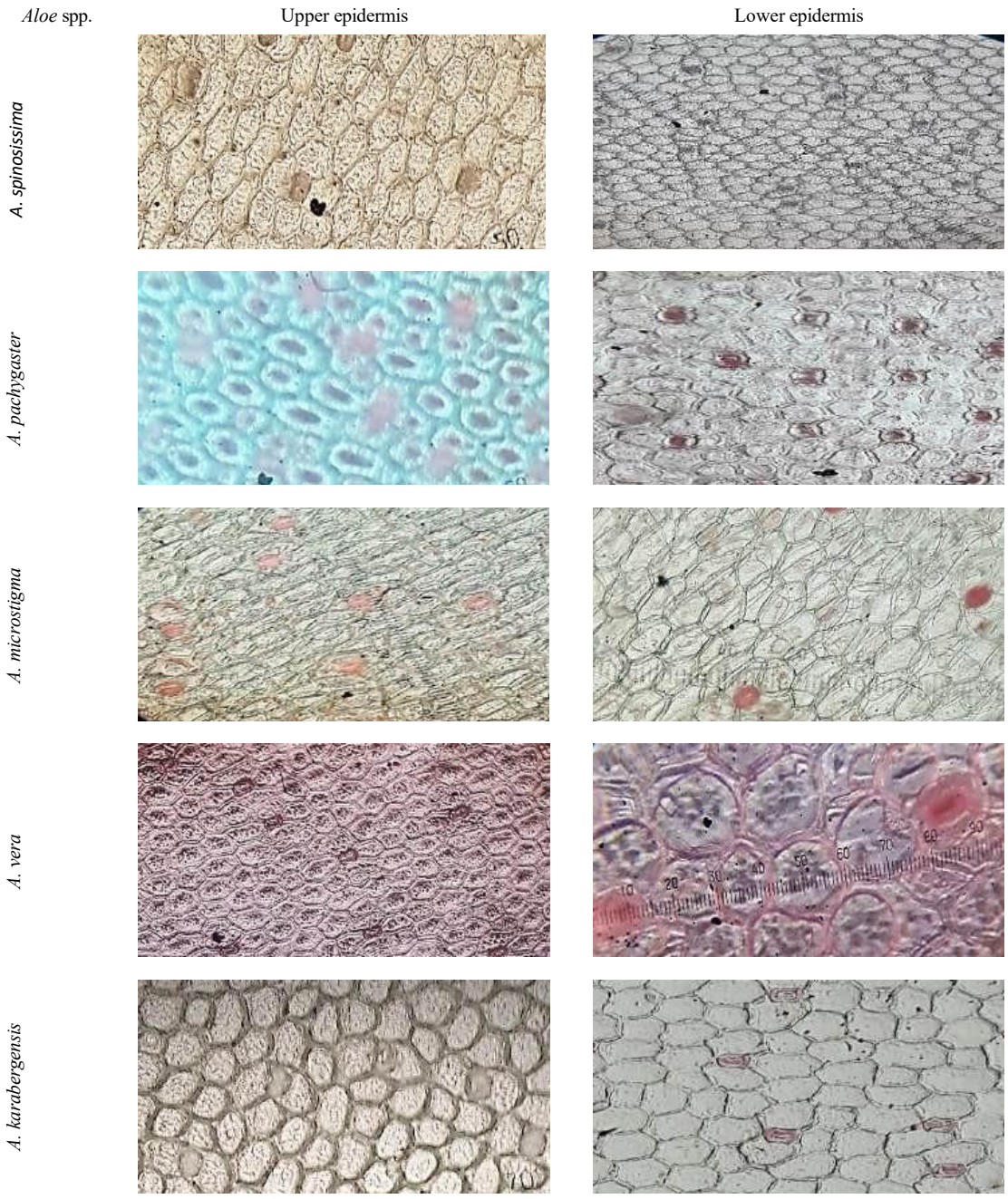


Fig. 2: Anatomical variations in stomatal density and index of different *Aloe* species (*A. spinosissima, A. pachygaster* and *A. microstigma, A. vera, A. karabergensis and A. stirata)*

Width of epidermal cells

The width of the epidermal cells varied significantly between the *Aloe* species studied, with clear differences observed between the upper and lower epidermal layers. *A. karasbergensis* and *A. hemmigii* had the widest upper epidermal cells, while their lower epidermal cells were comparatively narrower. Similarly, *A. spinosissima* and *A. conifera* had relatively wide upper epidermal cells, with slight differences compared to the lower epidermis. *A. microstigma* showed almost equal widths in both layers, indicating a uniform epidermal thickness (Fig. 1F). These results highlight significant interspecific variation in epidermal cell width.

Length of the stomatal pore

Results revealed that *A. spinosissima* exhibited the greatest



Fig. 3: Anatomical variations in stomatal density and index of different *Aloe* species (*A. hemengii, A. globuligema*, *A. eru* and *A. conifera*)

stomatal pore length on both epidermal surfaces, whereas the shortest pores were observed in *A. conifera*. The results revealed statistically significant differences in stomatal pore revealed statistically significant differences in stomatal pore length among the studied species (Fig.1G).

Width of stomatal pore

The highest stomatal pore width was found in *A. spinosissima*, while *A. conifera* exhibited the minimum pore width at both the adaxial and abaxial surfaces. The data indicate significant interspecific differences in pore width (Fig. 1H).

Stomatal index

The highest stomatal index was calculated in *A. pachygaster*, while the lowest was observed in *A. microstigma* on both

leaf surfaces. Overall, the stomatal index showed significant variation among the *Aloe* species analyzed (Fig. 1–3).

DISCUSSION

Least number of stomata was observed in *A. microstigma* at both lower and upper epidermis while it is observed that maximum number of epidermal cells was observed in *A. hemingii* at both lower and upper epidermis. However least number of epidermal cells was present in *A. microstigma* at both lower and upper epidermis. Observations showed that the maximum length of guard cells was present in *A. pachygaster* at both lower and upper epidermis. However, the minimum length of guard cells is present in *A. globuligemma* at both lower and upper epidermis. Oyeleke *et al*. (2004) also reported that the maximum width of guard cells was present in *A. striata* and *A. hemengii* at upper and lower epidermis. However, the minimum width of guard cells was noted in *A. vera* at both lower and upper epidermis. Camargo and Marenco (2011) confirmed that maximum number of stomata was observed in *A. pachygaster* at both lower and upper epidermis (Fig. 1-3).

However, the minimum length of epidermal cells is present in *A. pachygaster* at both lower and upper epidermis. Coopoosamy *et al*. (2011) also confirmed that the maximum width of epidermal cells was seen in *A. karabergensis* and *A. spinosissima* at upper and lower epidermis. However, the minimum width of epidermal cells was present in *A. vera* at both lower and upper epidermis while the maximum length of pore was found in *A. spinosissima* at upper and lower epidermis. However, the minimum length of pore was measured in *A. conifera* at both lower and upper epidermis. Results showed that the maximum width of pore was present in *A. spinosissima* at upper and lower epidermis. Lake *et al*. (2000) observed that the maximum stomatal index was present in *A. pachygaster* at upper and lower epidermis. Moreover, the minimum stomatal index was measured in *A. microstigma* at both lower and upper epidermis. Overall result showed significant differences in stomatal index in different *Aloe* species. In short, *Aloe* species indicated great variations in the leaf stomatal number, distribution, size and frequency. This information can be used to assess the photosynthetic performance and biomass yield of these species, which may carry implications for their commercial and medicinal importance (Tinti *et al*. 2023).

CONCLUSION

It was observed that stomatal index and density show significant differences among different *Aloe* species. Results revealed that maximum number of stomata was seen in *A. pachygaster* while the minimum number is observed in *A. microstigma*. Further studies shows that number of epidermal cells was higher in *A. hemingii* while least number of epidermal cells are present in *A. microstigma*. However, stomatal size was larger in *A. pachygaster* and *A. striata* while it was reduced in *A. vera* and *A. conifera* at both upper and lower epidermis. The information obtained from this study can be used to assess the photosynthetic performance and dry matter yield of these species.

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DATA AVAILABILITY

The data will be made available on a fair request.

ETHICS APPROVAL

Not applicable to this paper.

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