Original article

Effect of Different Doses of Sea Buckthorn (*Hippophae rhamnoides* L.) Seed Extract (Oil) on Histology of Islets of Langerhan of Pancreas in Alloxan Induced Diabetic Male Wistar Rats

Mehreen Salahuddin[1], Godfrey Paul William[1], Saira Munawar[2], Arooj Nawaz[3], Shagufta Nasreen[4], Fozia Farzana[5]

[1]Department of Anatomy, FMH College of Medicine and Dentistry. Lahore, [2]Department of Anatomy Fatima Jinnah Medical University Lahore, [3]Department of Anatomy, University of Lahore, [4]Department of Anatomy, Postgraduate Medical Institute Lahore, [5]Department of Anatomy, Continental Medical College Lahore

Correspondence to: Mehreen Salahuddin, Email: dr.mehreensalahuddin@gmail.com

ABSTRACT

Background: Sea buckthorn seed oil extract contains saturated and unsaturated fatty acids. Alloxan monohydrate is oxygenated pyrimidine derivative, it produces diabetes in experimental animals. The objectives of this study was to determine the histological changes of sea buckthorn extract (seed oil) on Alloxan induced diabetic β-cells of pancreatic tissue in male Wistar rats.

Method: 32 male Wistar rats were divided into 4 groups: A, B, C and D. Alloxan monohydrate in dose of 130mg/kg body weight was given as a single dose to experimental groups B, C and D. From each group half of the group the rats were sacrificed at day 4 of study and the rest on 25th day after administration of Sea buckthorn seed oil extract.

Results: In comparison with the control group on the 4th day of the sacrifice all the rats in the experimental groups showed a decrease in diameter of islets of Langerhans, number of beta cells and an increase in number of beta cells pyknosis and vacuolization. On the 25th sacrificial day, only high dose Sea buckthorn (4ml) treated group D showed an increased in diameter of islets of Langerhans very close to that of controls while low dose (2ml) treated group C showed late recovery as compared to control group.

Conclusion: The study assesses Sea buckthorn's (seed oil) effects on serum glucose levels by using glucometer and histological parameters (diameter of islet, beta cell pyknosis, number of inflammatory cells) of islets of Langerhans of pancreas. It focuses on animal models, but human clinical trials are required to validate its efficacy and safety.

Keywords:

Diabetes, Sea buckthorn seed oil, Alloxan monohydrate.

INTRODUCTION

Diabetes mellitus is a growing health concern in the 21st century, associated with macrovascular complications (heart disease, stroke, peripheral arterial disease) and microvascular complications (kidney disease, retinopathy, neuropathy).[1] According to the IDF Diabetes Atlas 2021, Pakistan has the third-largest diabetic population globally. The Second National Diabetes Survey of Pakistan (NDSP 2016–2017) reported a prevalence rate of 26.3%—28.3% in urban areas and 25.3% in rural areas—while 14.4% of the population was classified as pre-diabetic. This shift is attributed to the growing incidence of diabetes-related factors in the country, including a rise in obesity, sedentary lifestyles, population growth, and aging population[2].

The chemical agent used in this experiment is alloxan monohydrate which is a drug used to induce diabetes in experimental animals. This process leads to the formation of antioxidants such as superoxide radicals, hydrogen peroxide and eventually hydroxyl radicals. These hydroxyl radicals are identified as the primary source of necrosis of β-cells leading to apoptosis through a series of cellular changes[3-6].

Sea buckthorn (Hippophae L., Elaeagnaceae) generic name "Hippophae" means shining horse and it came from its traditional use as fodder for horses since long[7-8]. It thrives in high mountainous and semi-arid ecosystems across countries such as China, Pakistan, Russia, Nepal, India, Bhutan, Mongolia, Afghanistan, Kazakhstan,

ARTICLE INFO

Article History

Received: 23.12.2024 | Accepted: 27.06.2025

Conflict of Interest: The authors declare no conflict of interest.

Funding: None.

Citation: Salahuddin M, William GP, Munawar S, Nawaz A, Nasreen S, Farzana F. Effect of different doses of sea buckthorn (*Hippophae rhamnoides* L.) seed extract (oil) on histology of islets of Langerhans of pancreas in alloxan induced diabetic male wistar rats. J Fatima Jinnah Med Univ. 2025; 19(2): 62-68.

DOI: https://doi.org/10.37018/TSYP7935

Hungary, Switzerland, Romania, Germany, France, Britain, Finland, Sweden, and Norway[9-10]. Chinese Ministry of Public Health declared Sea buckthorn as a medicinal drug and officially included in their pharmacopoeia for the first time in 1977[11]. Ancient Chinese medicinal literature documented the use of Sea buckthorn berries for inflammation, circulatory disorders, cough, cold, hepatic disease, abscesses, tumours, ischemic heart diseases, digestive disorders, metabolic disorders, blood purification and gynaecological diseases[12]. Several studies on Sea buckthorn show that it is a potentially hypoglycemic medicinal plant. It is found to have an antidiabetic and antioxidant effect along with tissue regeneration properties in pancreas of diabetes induced rats[13-15].

MATERIALS AND METHODS

This experimental study was conducted at Postgraduate Medical Institute (PGMI) Lahore and in association with advance board of research Studies University of Health Science Lahore. Thirty-two adult male Wistar rats were randomly selected for this study. The average weight of the animals varied between 180-220 g. The rats were kept in the animal house PGMI at room temperature 26°C with 12-hour light and dark cycle. A normal diet was given to all the animals with water supply ad libitum. The rats were divided into four group A, B, C and D. Each group had 8 rats.

Sea buckthorn seeds were obtained from Skardu a northern province of Pakistan. The seeds were given to Pakistan council of scientific and industrial research (PCSIR) Laboratory, for extraction of oil by soxhlet extraction technique. However, Alloxan monohydrate was used in crystalline powder form. The group were arranged as follow:

1. Control Group A: Eight rats as a control group received distilled water 5ml/kg through oral gavage tube and were given normal saline 0.9% w/v 5ml/kg intraperitoneally for initial 3 days.
2. Experimental Group B: Eight rats of this experimental group received distilled water 5ml/kg orally through gavage. A single dose of Alloxan 130mg/kg (Dar et al., 2017) dissolved in normal saline 0.9% w/v was given intraperitoneally in rats after overnight fasting for 12 hours to induce diabetes. Half of the rats were sacrificed on 4th and half on 25th day.
3. Experimental Group C: Total Eight rats of this experimental group were injected with single intraperitoneal dose of Alloxan to induce diabetes after which half of the rats were sacrificed at 4th day and half of the rats from 4th day were given Sea buckthorn extract seed oil 2ml/kg (Sharma et al., 2011) by oral gavage for 21 days. These rats were sacrificed on day 25 of the study.
4. Experimental Group D: Total Eight rats of this experimental group were injected with single intraperitoneal dose of Alloxan to induce diabetes in them, after which half of the rats were sacrificed at 4th day and half of the rats from the 4th day were given Sea Buckthorn extract seed oil 4ml/kg by oral gavage for 21 days[12]. These rats were sacrificed on day 25 of the experiment.

All the data was collected on Performa. Qualitative data beta cell pyknosis, beta cell vacuolization was presented in the form of frequency and percentages. The quantitative data (test of normality) like diameter of Langerhans cells number of beta cells and number of inflammatory cells, was expressed in the form of Mean ± S (standard deviation). The quantitative data was analysed using ANOVA (Analysis of Variance) to determine the mean difference in quantitative variables among the groups followed by Post hoc analysis (Tukey's test). Statistical Pearson's Chi Square test was applied to observe the association of categorical variables with the groups. Analysis was done by SPSS version 22.0. A p-value of ≤ 0.05 was considered as statistically significant.

RESULTS

Diameter of Langerhans Islet (µm): The diameter of the experimental groups B, C and D were compared with the control group A using ANOVA. At day 4 the diameter was decreased significantly in group D (Figure 2 a, b, c) as compared to control group A (Figure 1, Table 1). However, on day 25 the diameter of the islet of Langerhans showed an increase in size reaching a maximum of 17.2 ± 1.6in group D (Figure 5, Table 1).

Pairwise comparison between control and experimental groups was done using post hoc-Tukey's test which showed that in group D (Figure 5) diameter of Langerhans islets at day 25 was substantially higher than that of groups A and C (Figure 4, Table 2). The diameter of the Langerhan islet did not significantly differ among the other two experimental groups B and D.

On day 25th in group B alloxan treated showed slight increase in diameter of islet of Langerhans as compare to experimental group C and D (Figure 3).

Number of beta cells per mm^2: At day 4, the number of beta-cells were decreased in the experimental group B, C, and D as compared to the control group A (p-value 0.001) (Table 3). However, no significant difference was seen between groups B, C, and D. Likewise at day 25, there was a substantial difference in the number of beta cells across the experimental groups especially between group B and D (Table 3). (p -value = 0.011). Group B showed the least difference even at day 25 as compared to the control group A and other two experimental group C and D.

Reason being that no Sea Buckthorn was given in this experimental group (Table 3).

Number of Inflammatory Cells: The number of inflammatory cells was compared using the Fisher's extract test. As compared to the control group A. The experimental group B, C and D showed mild to moderate infiltration (Table 4) at day 4 (Figure 6 and 7). However, at day 25 group D showed the least infiltration as compared to the other two experimental group B and C (Table 4).

Beta Cell Pyknosis: At day 25, in group A, beta cell pyknosis were not seen in all four rats. In group B beta cell pyknosis was seen in all four rats. However, in group C, beta cell pyknosis was seen in 2 (50%) rats and in group D, beta cell pyknosis was seen in1 (25%) of rats. Beta cell pyknosis was seen in all the 3 experimental groups B, C and D (Fig 8) as compared to the control group A at day 4 (Table 5).

Table 1: Showing mean diameter of Langerhans islets at day 4 and 25 among group

Parameters	Groups				p-value#
	A	B	C	D	
Diameter of Langerhans islets at day 4	13.8 ± 1.1	11.6 ± 0.4	10.3 ± 1.4	9.6 ± 0.9	< 0.001*
Diameter of Langerhans islets at day 25	11.9 ± 1.6	15.3 ± 3.1	12.7 ± 0.7	17.2 ± 1.6	0.009*

#One-way ANOVA *p value ≤ 0.05 is considered statistically significant.

Table 2: Pair wise comparison of diameter of Langerhans islets among study groups.

Variable	Group	Group	Mean Difference	Std. Error	p-value
Diameter of Langerhans Islets at day 4	A	B	2.25500*	0.72507	0.039
		C	3.52000*	0.72507	0.002
		D	4.24000*	0.72507	0.000
	B	C	1.26500	0.72507	0.344
		D	1.98500	0.72507	0.074
	C	D	0.72000	0.72507	0.756
Diameter of Langerhans Islets at day 25	A	B	-3.36000	1.36151	0.116
		C	-0.85125	1.36151	0.922
		D	-5.26500*	1.36151	0.010
	B	C	2.50875	1.36151	0.302
		D	-1.90500	1.36151	0.523
	C	D	-4.41375*	1.36151	0.031

*p value ≤ 0.05 is considered statistically significant

Table 3: Showing mean number of beta cells at day 4 and 25 among groups.

Parameters	Groups				p-value#
	A	B	C	D	
Number of beta cells per mm^2 at day 4	41.3 ± 8.1	12.3 ± 3.3	8.0 ± 3.9	6.8 ± 3.3	< 0.001*
Number of beta cells per mm^2 at day 25	38.2 ± 5.6	8.0 ± 2.9	12.8 ± 5.4	15.0 ± 4.7	0.011*

#One-way ANOVA *p value ≤ 0.05 is considered statistically significant.

Table 4: Distribution of inflammatory cell counts within several groups.

Day	Number of Inflammatory Cells	Groups				p-value
		A n (%)	B n (%)	C n (%)	D n (%)	
4	Absent	4	0	0	0	0.002*
		100.0%	0.0%	0.0%	0.0%	
	Mild	0	2	1	0	
		0.0%	50.0%	25.0%	0.0%	
	Moderate	0	2	3	4	
		0.0%	50.0%	75.0%	100.0%	
25	Absent	4	0	0	2	0.010*
		100.0%	0.0%	0.0%	50.0%	
	Mild	0	1	3	2	
		0.0%	25.0%	75.0%	50.0%	
	Moderate	0	3	1	0	
		0.0%	75.0%	25.0%	0.0%	

Fisher's exact test *p value ≤ 0.05 is considered statistically significant

Table 5: Distribution of beta cell pyknosis among groups.

Day	Beta cell Pyknosis	Groups				p-value
		A n (%)	B n (%)	C n (%)	D n (%)	
4	Absent	4	0	0	0	0.002*
		100.0%	0.0%	0.0%	0.0%	
	Present	0	4	4	4	
		0.0%	100.0%	100.0%	100.0%	
25	Absent	4	0	2	3	0.032*
		100.0%	0.0%	50.0%	75.0%	
	Present	0	4	2	4	
		0.0%	100.0%	50.0%	25.0%	

Fisher's exact test *p value ≤ 0.05 is considered statistically significant

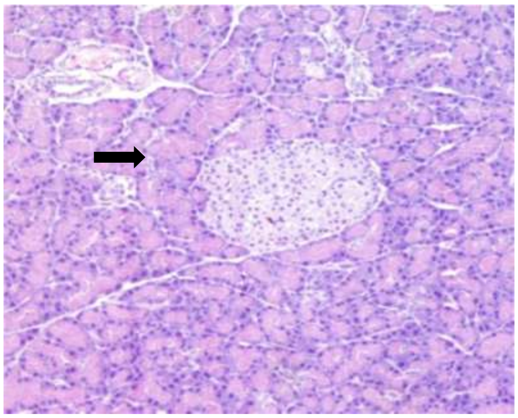

Figure 1: Photomicrograph of Control Group A rat pancreas showing islets of Langerhans (black arrow) with aldehyde fuchsin stain, 10 X magnification.

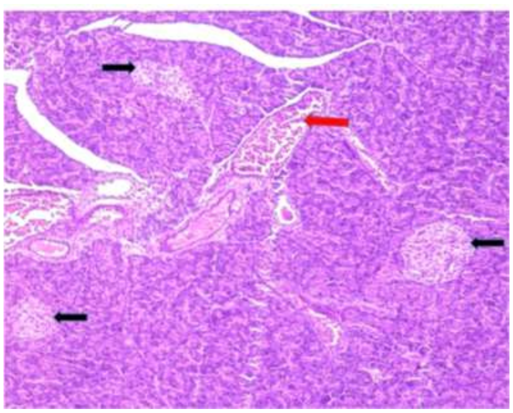

Figure 2(a): Photomicrograph of group B rat pancreas treated with alloxan monohydrate at day 4 with low magnification 10X showing degradation of pancreas islets of Langerhans with decrease in the size of islets of Langerhans (Black arrows) and congestion of blood vessels (Red arrow)

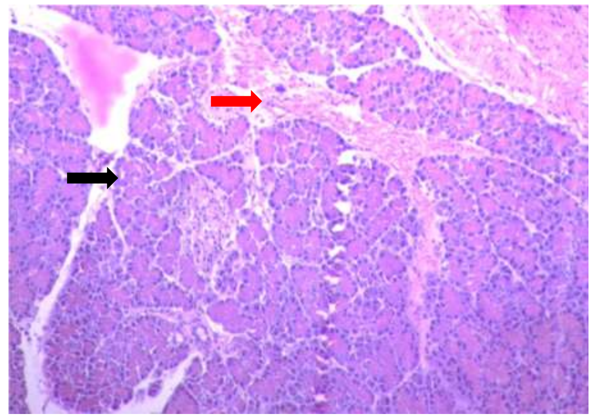

Figure 2(b): Photomicrograph of group C rat pancreas treated with alloxan monohydrate at day 4 with low magnification 10X showing degradation of pancreas islets of Langerhans with decrease in the size of islets of Langerhans (Black arrows) and congestion of blood vessels (Red arrow)

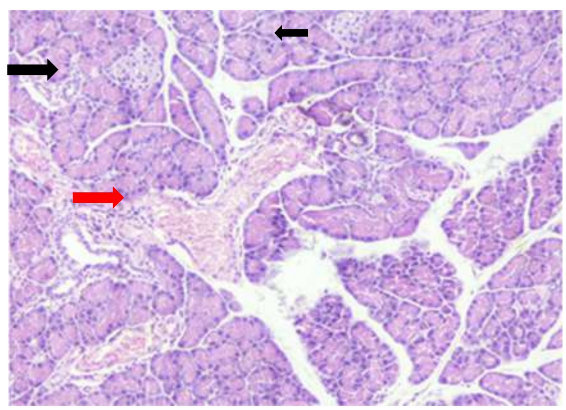

Figure 2(C): Photomicrograph of group D rat pancreas treated with alloxan monohydrate at day 4 with low magnification 10X showing degradation of pancreas islets of Langerhans with decrease in the size of islets of Langerhans (Black arrows) and congestion of blood vessels (Red arrow)

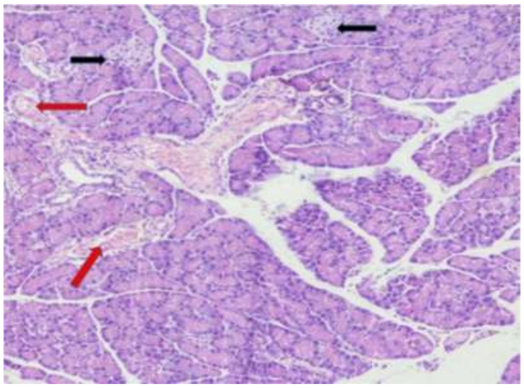

Figure 3: Photomicrograph of rat pancreas treated with alloxan showing group B at day 25, 10x showing variable increase in the size of islets of Langerhans (Black arrows), Red arrow showing congestion in blood vessels stain with aldehyde fuchsin.

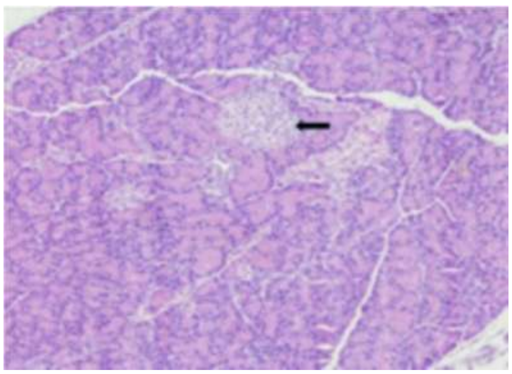

Figure 4: Photomicrograph of group C pancreas islets of Langerhans treated with alloxan monohydrate and Sea buckthorn seed oil extract (2ml) at day 25,10x stained with aldehyde fuchsin. Black arrow showing increase in size of islets of Langerhans post low dose of Sea buckthorn treatment.

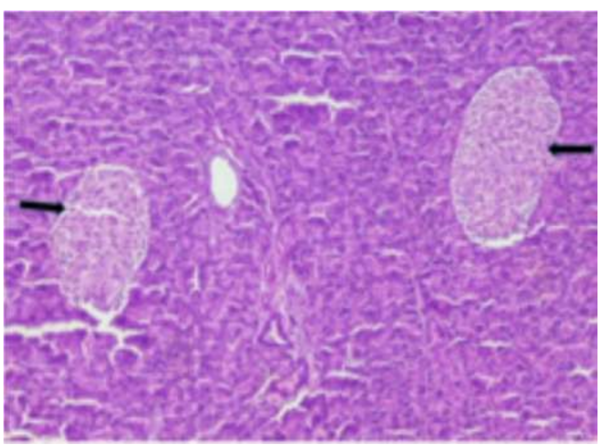

Figure 5: Photomicrograph of group D pancreas islets of Langerhans treated with alloxan monohydrate and Sea buckthorn seed oil extract (4ml) at day 25,10x stained with aldehyde fuchsin. Black arrow showing increase in size of islets of Langerhans post high dose of Sea buckthorn treatment.

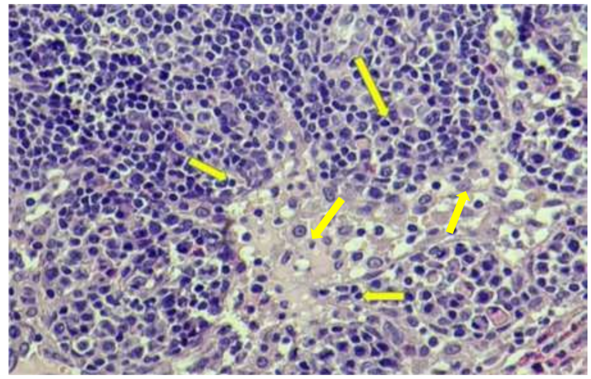

Figure 6: Photomicrograph of group B pancreas Langerhans treated with alloxan monohydrate showing lymphocytic infiltration around the islet of Langerhans (yellow arrow), with aldehyde fuchsin stain 40 X.

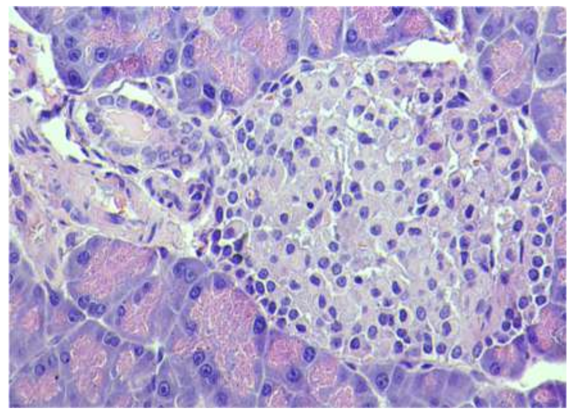

Figure 7: Photomicrograph of group D pancreas Langerhans treated with alloxan monohydrate showing lymphocytic infiltration around the islet of Langerhans (yellow arrow), with aldehyde fuchsin stain 40 X.

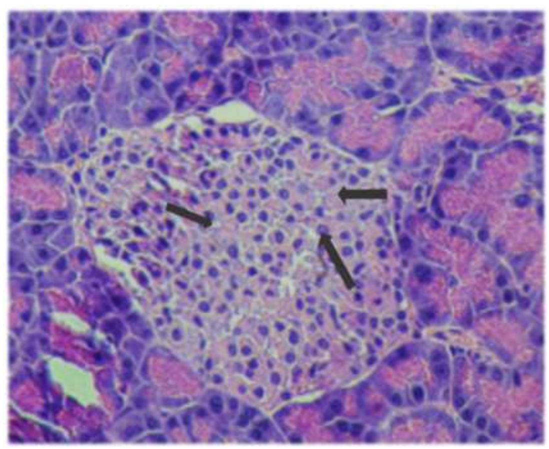

Figure 8: Photomicrograph of group B pancreas showing islet of Langerhans treated with alloxan monohydrate showing pyknosis (black arrow) stain with aldehyde fuchsin, 40X.

DISCUSSION

Sea buckthorn (*Hippophae rhamnoides* L.) is gaining recognition for its potential as a hypoglycemic medicinal plant[16]. As shown in multiple studies on pancreas[13, 17], skin damage[18], liver toxicity[19].

The effects of Alloxan on the pancreatic islet showed marked decrease in the diameter in alignment with previous studies by[13,20-22,24]. These changes were reflected in all experimental groups B, C, and D. These changes reinduce the effect of alloxan in inducing diabetes.

Our study highlighted that by day 25 significant difference in the diameter of islet of Langerhans were seen in the experimental group specially C and D who were given Sea buckthorn as compared to group B (Table 1; Figure 4.5). There finding are consistent with the study done by Sharma et al who also noted similar improvement in the islet diameter and pancreatic islet architecture with the use of Sea buckthorn fruit pulp in alloxan induced diabetes. By day 25, significant differences in islets diameter emerged between the control and experimental groups. The administration of Sea buckthorn seed oil extract to groups C and D resulted in a gradual increase in Langerhans diameter, the findings of this study was consistent with all these previous studies resulting in diabetes are a result of atrophy of islet of Langerhans. Sharma et al. (2011) also noted similar improvements in islet diameter and pancreatic tissue architecture with the use of Sea buckthorn fruit pulp[13].

The study also measured the number of beta cells per mm² at days 4 and 25 (Table 3). By day 4 a significant reduction in beta cell numbers was observed in experimental groups B, C, and D as compared to group A, which was consistent with the destructive effects of alloxan described by[3,25]. These effects include transient hypoglycemia, leading to morphological changes, and beta cell degranulation. However, by day 25, groups C and D showed significant increases in beta cell numbers, suggesting that Sea buckthorn seed oil extract facilitates beta cell regeneration (Table 3). Group D, receiving a higher dose, exhibited greater improvement compared to group C and B. These findings indicate a dose-dependent regenerative effect of Sea buckthorn on beta cells[13].

Inflammatory cell presence was another critical aspect observed. Moderate inflammatory cells were present in groups B, C, and D by day 4, but by day 25, groups C and D showed only mild inflammation, suggesting an anti-inflammatory effect of Sea buckthorn (Table 4; Figure 6.7). This reduction in inflammatory cells further supports the therapeutic potential of Sea buckthorn in mitigating the adverse effects of alloxan on pancreatic tissue. Histopathological observations revealed beta cell pyknosis and vacuolization in groups B, C, and D on day 4, with improvements noted by day 25 in groups C and D (Table 5, Figure 8). This aligns with findings from Ramadan et al. (2017) and Amin et al. (2017), who also observed alloxan-induced necrosis and atrophy in pancreatic tissue[24,22].

Additional studies reinforce these findings. Sharma et al. (2011) demonstrated antidiabetic and antioxidant effects of sea buckthorn fruit pulp in streptozotocin-nicotinamide-induced diabetic rats[13]in which results showed improved islet diameter and beta cell regeneration. Savici et al. (2016) reported enhanced pancreatic tissue organization and improved islet morphology in diabetic rats treated with Sea buckthorn extract[14]. This improvement was attributed to the antioxidant and cytoprotective properties of sea buckthorn, which mitigated oxidative stress and inflammatory damage induced by alloxan. In addition, treated animals showed better preservation of β-cell structure**,** suggesting a potential for regenerative stimulation or reduction of β-cell apoptosis**.** These findings are consistent with other studies demonstrating Seabuckthorn's ability to stabilize islet architecture**,** enhance insulin secretion, and support partial regeneration of islets, reinforcing its therapeutic promise in diabetes management. Yan et al., (2023) studied hypoglycemic activity of alcoholic extract of Sea buckthorn leaves in mice were its showed marked improved result. Its ability to restore islet diameter, increase beta cell numbers, and reduce inflammation highlights its potential as a valuable therapeutic agent in diabetes management[26]. Further research is warranted to explore the mechanisms underlying these effects and to confirm the therapeutic potential of Sea buckthorn in human studies.

CONCLUSION

This plant can have great benefits for human heath particularly in areas like diabetes. However further clinical trials are needed to verify its health benefits.

Author Contributions

Dr. Mehreen Salahuddin: Conception and design, analysis and interpretation of data, drafting the article, critical revision for important intellectual content, final approval.
Dr. Godfrey Paul William: Conception and design, analysis and interpretation of data.
Dr. Saira Munawar: Analysis and interpretation of data, drafting the article.
Dr. Arooj Nawaz: Acquisition of data, conception and design, analysis and interpretation.
Dr. Shagufta Nasreen: Analysis and interpretation of data, proofreading.
Dr. Fouzia Farzana: Head of Department of Anatomy: Conception and design, analysis and interpretation of data.

REFERENCES

1. Tomic D, Shaw JE, Magliano DJ. The burden and risks of emerging complications of diabetes mellitus. Nat Rev Endocrinol. 2022;18:525–539. doi: 10.1038/s41574-022-00690-7.
2. Din Z, Din ZU, et al. Evidence based prevalence of diabetes related depression in Kohat, Pakistan: a cross-sectional study. BMC Public Health. 2025;25:1938. doi: 10.1186/s12889-025-23151-y.

3. Lenzen S. The mechanisms of alloxan and streptozotocin-induced diabetes. Diabetologia. 2008;51(2):216–226. doi: 10.1007/s00125-007-0886-7.
4. Rohilla A, Ali S. Alloxan induced diabetes: mechanisms and effects. J Pharmacol Biomed Sci. 2012;3(2):819–823.
5. Ibrahim RM, Abdelhafez HM, El-Shamy SAEM, Eid FA, Mashaal A. Arabic gum ameliorates systemic modulation in alloxan monohydrate-induced diabetic rats. Sci Rep. 2023;13(1):5005. doi: 10.1038/s41598-023-31897-x.
6. Izuchukwu U, Peter I, Chisom O, Collins E. Dietary management of Vitex doniana (black plum) leaves in alloxan-induced diabetic albino rats. Asian J Res Biochem. 2024;14(6):1–9. doi: 10.9734/ajrb/2024/v14i6321.
7. Gâtlan AM, Gutt G. Sea buckthorn in plant based diets. An analytical approach of sea buckthorn fruits composition: nutritional value, applications, and health benefits. Int J Environ Res Public Health. 2021;18(17):8986. doi: 10.3390/ijerph18178986.
8. Chan LP, Yen TW, Tseng YP, Yuen T, Yuen M, Yuen H, et al. The impact of oral sea buckthorn oil on skin, blood markers, ocular, and vaginal health: a randomized controlled trial. J Funct Foods. 2024;112:105973. doi: 10.1016/j.jff.2023.105973.
9. Zhao P, Wang S, Liang C, Wang Y, Wen P, Wang F, et al. Acute and subchronic toxicity studies of sea buckthorn (*Hippophae rhamnoides* L.) oil in rodents. J Regul Toxicol Pharmacol. 2017;91:50–57. doi: 10.1016/j.yrtph.2017.10.002.
10. Nawaz MA, Khan AA, Khalid U, Buerkert A, Wiehle M. Superfruit in the niche—underutilized sea buckthorn in Gilgit-Baltistan, Pakistan. Sustainability. 2019;11(20):5840.
11. Mingyu X, Xiaoxuan S, Jinhua C. The medicinal research and development of seabuckthorn. J Water Soil Conser China. 1991;4:1–11.
12. Ballabh B, Chaurasia OP, Pande PC, Ahmed Z. Raw edible plants of cold desert Ladakh. Indian J Tradit Know. 2007;6(1):182–184.
13. Sharma M, Siddique MW, Shamim AM, Gyanesh S, Pillai KK. Evaluation of antidiabetic and antioxidant effects of sea buckthorn (*Hippophae rhamnoides* L.) in streptozotocin-nicotinamide induced diabetic rats. Con Prod J. 2011;2(1):53–58. doi: 10.2174/2210289201102010053.
14. Savici J, Brezovan D, Muselin F. The histological assessment of pancreas after milk thistle (Silybum marianum L.) and sea buckthorn (*Hippophae rhamnoides* L.) extract administration in diabetes induced rats. Metabolic Role Orexigenic Anorexigenic Compds. 2016;183.
15. Dupak R, Hrnkova J, Simonova N, Kovac J, Ivanisova E, Kalafova A, et al. The consumption of sea buckthorn (*Hippophae rhamnoides* L.) effectively alleviates type 2 diabetes symptoms in spontaneous diabetic rats. Res Vet Sci. 2022;152:261–269. doi: 10.1016/j.rvsc.2022.08.022.
16. Wang Z, Zhao F, Wei P, Chai X, Hou G, Meng Q. Phytochemistry, health benefits, and food applications of sea buckthorn (*Hippophae rhamnoides* L.): a comprehensive review. Front Nutr. 2022;9:1036295.
17. Veronique T, Muk Wing Y, Christian D. Sea buckthorn polyphenols: characterization, bioactivities and associated health benefits. Biochemistry [Internet]. IntechOpen; 2022. Available from: http://dx.doi.org/10.5772/intechopen.98706.
18. Wang Q, Cao B, Zhan J, Hu X, Yu Y, Li X, et al. Sea buckthorn oil promotes the PI3K-Akt-ERK signaling pathway and macrophage M2 polarization to reduce radiation-induced skin injury. Radiat Res. 2024;202(5):785–794. doi: 10.1667/RADE-23-00100.1.
19. Saeed GN, Ahsin S, Sarwar M. Hepatoprotective effect of sea buckthorn berry seed oil in cyclophosphamide-induced hepatic toxicity in BALB/c mice. Pak J Physiol. 2023;19(2):20–24. Available from: https://pjp.pps.org.pk/index.php/PJP/article/ view/1521.
20. Edo GI, Jikah AN, Onoharigho FO, Akpoghelie PO, Agbo JJ, Ekokotu HA, et al. The ameliorative effects of Vernonia amygdalina extract on superoxide dismutase and glutathione S-transferase in alloxan-induced diabetic male Wistar rats. Food Chem Adv. 2024;4:100620. doi: 10.1016/j.focha.2024.100620.
21. Mistry J, Biswas M, Sarkar S, Ghosh S. Antidiabetic activity of mango peel extract and mangiferin in alloxan-induced diabetic rats. Futur J Pharm Sci. 2023;9(1):22. doi: 10.1186/s43094-023-00472-6.
22. Amin A, Tahir M, Lone KP. Effect of *Citrullus colocynthis* aqueous seed extract on beta cell regeneration and intra-islet vasculature in alloxan-induced diabetic male albino rats. J Pak Med Assoc. 2017;67:715.
23. Qadori YT. Histological studies on pancreatic tissue in diabetic rats by using wild cherry. Iraqi Postgrad Med J. 2011;10(3):421–425.
24. Ramadan B, Basma K, et al. Hypoglycemic and pancreatic protective effects of *Portulaca oleracea* extract in alloxan-induced diabetic rats. BMC Complement Altern Med. 2017;17:37. doi: 10.1186/s12906-016-1530-1.
25. Ighodaro OM, Adeosun AM, Asejeje FO, Soetan GO, Kassim OO. Time course effects of 5,5-dihydroxyl pyrimidine-2,4,6-trione (alloxan) as a diabetogenic agent in animal model. Alexandria J Med. 2018;54(4):705–710. doi: 10.1016/j.ajme.2018.05.005.
26. Yan CY, Ding ZJ, Li XM, Mao XL, Yu ZS, Wang ZF, et al. The chemical constituents and hypoglycemic activity of alcoholic extract of sea buckthorn leaves. Acta Pharm Sin. 2023;58(2):396–404. doi: 10.16438/j.0513-4870.2022-0840.
27. Dar PA, Ali F, Sheikh IA, Ganie SA, Dar TA. Amelioration of hyperglycemia and modulation of antioxidant status by Alcea rosea seeds in alloxan-induced diabetic rats. Pharm Biol. 2017 Dec; 55(1):1849-1855.