GSC Advanced Research and Reviews

eISSN: 2582-4597 CODEN (USA): GARRC2

Cross Ref DOI: 10.30574/gscarr

Journal homepage: https://gsconlinepress.com/journals/gscarr/

(RESEARCH ARTICLE)

Pharmacotherapeutic effect of methanolic and ethanolic extract of *Spinacia oleracea* L. leaves on glycemic and lipidemic indexes of alloxan-induced diabetic mice

Shahanaz Khatun * and Mst. Khadiza Khatun

Department of Biochemistry and Molecular Biology, University of Rajshahi, Rajshahi-6205, Bangladesh.

GSC Advanced Research and Reviews, 2023, 16(03), 188–195

Publication history: Received on 08 August 2023; revised on 17 September 2023; accepted on 19 September 2023

Article DOI: https://doi.org/10.30574/gscarr.2023.16.3.0362

Abstract

With the various side effects associated with synthetic medicines for treating diabetes, there is a need to develop herbal remedies as an alternative. Although *Spinacia oleracea* L. leaves are used to treat various health conditions, including diabetes, there is little research to validate its antidiabetic properties. This study aimed to investigate the hypoglycemic and hypolipidemic effect of methanolic and ethanolic extracts of *Spinacia oleracea* L. leaves against alloxan-induced hyperglycemia in mice. Hyperglycemia was induced by an injection of alloxan monohydrate 80mg/kg bw. (i.p.). After 72 hours, mice with Blood Glucose Levels above 11.0 mmol/L were selected for the investigation. Both methanolic and ethanolic extracts at 200 mg/kg bw. doses were observed to have antidiabetic effect for 21 consecutive days. Blood Glucose Level was monitored after 3, 6, 9, 12, 15, 18, and 21 days and compared with Glibenclamide (0.5 mg/kg b.wt.). Serum lipid profile [total cholesterol, triglycerides, low-density lipoprotein, and high-density lipoprotein] and liver enzymes such as SGPT, and SGOT were also determined. Oral administration of both extracts showed significant ($P < 0.05$) antihyperglycemic activity in alloxan-induced diabetic mice. The diabetic mice had significant ($P < 0.01$) reduction in blood glucose; serum liver enzyme level (SGPT and SGOT) and lipid profile were compared with normal mice.

Keywords: Pharmacotherapeutic; *Spinacia oleracea* L; Glycemic; Lipidemic

1. Introduction

Diabetes mellitus is a serious and complex metabolic disorder that has proved to be a non-curable but controllable chronic burden, with its prevalence increasing worldwide. It is characterized by periods of hyperglycemia and glucose intolerance that impair the cell's ability to secrete insulin or cause the tissues to become very less sensitive to insulin. [1–3]. Lack of Insulin causes the illness to show signs and symptoms of osmotic diuresis, such as polydipsia, polyuria, calorie loss, weight loss, generalized weakness, and polyphagia [4,5]. Over long-term diabetes progresses to micro- and macrovascular complications, which damage both small and large blood vessels, respectively [6]. According to the American Diabetes Association, Type 1, Type 2, gestational and specific types of diabetes resulting from other causes are the four different types of diabetes mellitus [7]. According to the International Diabetes Federation (IDF), 415 million cases of diabetes were among individuals aged 20 to 79 in 220 countries in 2015. By the year 2045, the number of cases is predicted to rise to 693 million, from 642 million in 2040 G.C [8,9]. Dietary supplements are considered to be the main modifiable factor among the various risk factors that contribute to the incidence and progression of diabetes. Both experimental and epidemiological evidence suggest that consuming vegetables rich in phenolic compounds and having high antioxidant capacity may potentially have an inverse relationship with the incidence and prevalence of diabetes. [10].

Researchers are constantly searching for complementary and alternative medicine therapies to treat diabetes [11], including herbs and biologically based practices such as *Allium sativum* (garlic), *Coccinia cordifolia* (ivy gourd),

* Corresponding author: Shahanaz Khatun

Momordica charantia (bitter melon), *Opuntia streptacantha* (prickly pear cactus), *Panex ginseng* (ginseng) and *Trigonella foenum graecum* (fenugreek) used for diabetes [11]. *Vitex Nigundo* leaf [12], *Tamarindus indica* [13], *Vigna unguiculata* [14], *Artemisia herba alba* [15], *Aegle marmellos* leaf [16], *Moringa oleifera* [17], Carissa carandas [18], *Morus alba* fruit [19] and *Ortosiphon stamineus* leaf [20] were proven scientifically in diminution of fasting blood sugar level of diabetic animals.

Spinach (*Spinacia oleracea* L.) belongs to the family, Chenopodiaceae and is an annual plant (rarely biennial), which grows to a height of up to 30 cm. Spinach may survive over winter in temperate regions. The leaves are alternate, simple, and ovate to triangular-based, very variable in size from about 2–30 cm long and 1–15 cm broad, with larger leaves at the base of the plant and small leaves higher on the flowering stem. The flowers are inconspicuous, yellow-green, 3–4 mm in diameter, maturing into a small, hard, dry, lumpy fruit cluster 5–10 mm across containing several seeds.

It is the source of essential nutrients such as carotene (a precursor of vitamin A), ascorbic acid, and several types of minerals. According to the Agricultural Research Service (ARS) of the U.S. Department of Agriculture, 100 g of fresh spinach provides at least 20% or more of the recommended dietary intake of folate (vitamin B9), β-carotene (provitamin A), lutein, ascorbic acid (vitamin C) and α-tocopherol (vitamin E). Moreover, spinach leaves contain flavonoids [21] and phenolic acids such as ortho-coumaric, ferulic acid, and para-coumaric acids [22]. In 2009, a mixture of antioxidants defined NAO (natural antioxidant) isolated from spinach leaves that contain aromatic polyphenols, including the phenolic acids and the derivatives of glucuronic acid [23]. NAO can effectively counteract free radicals [24,25] resulting in an anti-inflammatory and antiproliferative potential, in vivo and in vitro [26]. The present study was conducted to evaluate the antidiabetic and hypolipidemic effects of *Spinacia oleracea* L. leaves on alloxan-mediated diabetes in mice.

2. Materials and methods

2.1. Chemicals and kits

Alloxan monohydrate, (Germany) methanol, ethanol (Merck, India), Glibenclamide (USV Ltd., India), Glucose, Plasma concentrations of triglyceride (TG), total cholesterol (TC), HDL-cholesterol (HDL-C), LDL-cholesterol (LDL-C), VLDL, SGPT and SGOT were measured using a quantification kit (Linear chemicals, Barcelona, Spain). All the chemicals used in this study were of analytical grade.

2.2. Collection and Sample Preparation

Spinach plants (*Spinacia oleracea* L.*)* were collected from Binodpur, Rajshahi, Bangladesh, in January 2017. Plant specimen was authenticated by Professor Dr. A.H.M. Mahbubur Rahman, Department of Botany, University of Rajshahi, Bangladesh. A voucher specimen number # 126 was deposited to the herbarium in the Department of Botany, University of Rajshahi. The plants were dried by sunlight. The dried samples were ground into coarse powder by a grinding machine. The powder of *Spinacia oleracea* L. leaves (200gm) was mixed with ethanol and methanol (95%) separately in a 1000 mL flask with mild shaking. The flask was closed with a cotton plug and aluminum foil at 48 hours at room temperature for 14 days. The extract was filtered through Whatman filter paper (No.1), and concentrated using a rotary evaporator at low temperatures (40-50°C). The extract was preserved in an airtight container and kept at 4°C until further use.

2.3. Test animal

Albino mice were selected as experimental animals to carry out this study. Mice weighing about 25-30g were collected from the Animal Resource Division of ICDDR'B Mohakhali, Dhaka.

2.4. Methods

2.4.1. Animals care

Test animals were collected from the International Centre for Diarrhoeal Disease Research, Bangladesh (ICDDR,B). Albino mice of both sexes weighting 25-30g were used for the study. They were individually housed in polypropylene cages in well-ventilated rooms under hygienic conditions. Feeding of animals was done and libitum, along with drinking water, and maintained at the natural day-night cycle. The institutional Animal Ethics Committee had approved this study.

2.4.2. Induction of diabetes

Diabetes was induced in overnight fasted mice by a single intraperitoneal injection of Alloxan (80 mg/kg body weight) in a 0.1M sodium citrate buffer (pH-4.5). The age-matched control mice received an equivalent amount of citrate buffer. Food and water intake were closely monitored daily after Alloxan administration. The development of hyperglycemia in mice was confirmed by fasting (16 hour) blood glucose measurement in the tail vein blood, 72 hours after Alloxan administration, with a Portable glucometer (Accu-Chek, Roche, Germany). The animals with fasting blood glucose levels≥ 11.0 mmol/L with other symptoms of diabetes mellitus such as polyphagia, polydipsia, polyuria, and weight loss were considered diabetic and included in the study.

2.5. Experimental animals grouping and treatment

After a one-week acclimatization period, the animals were divided into fiv red in all the groups. After 21 days of experiment, all mice were anaesthetized under chloroform vapor and the blood samples were collected directly from ventricle of heart. For coagulation, blood was kept about 20 minutes at room temperature. After centrifugation at 3000 g for 10 minutes at 4ºC, serum was drawn off and stored at -20°C until the experiments e groups with six animals in each.:

- **Group-1** (General control)**:** Non-diabetic control mice fed with a standard pellet diet and water.
- **Group-2** (Diabetic control): The mice were made diabetic by an intra-peritoneal injection of a single dose of 80 mg/kg body weight Alloxan. Animals whose blood glucose level exceeded 11.0 mmol/L at 72 h after treatment were considered diabetic. These animals served as untreated diabetic control.
- **Group-3** (Diabetic+MESO): The diabetic mice treated with methanolic extract of *Spinacia oleracea* L. (MESO) at a dose of 200 mg/kg body weight for 21 days.
- **Group-4** (Diabetic+EESO): The diabetic mice treated with ethanolic extract of *Spinacia oleracea* L. (EESO) at a dose of 200 mg/kg body weight for 21 days
- **Group-5** (Diabetic+ Glibenclamide): Diabetic mice were treated by Glibenclamide at a dose of 0.5 mg/kg b.wt.

2.6. Blood collection

Before giving the supplement of *Spinacia oleracea* L. extract, the basal blood glucose levels were measu were performed.

2.7. Measurement of biochemical parameters

Blood glucose concentration was estimated according to the glucose oxidase method using a reagent kit (Randox Laboratory Ltd., UK). Serum total cholesterol and HDL-cholesterol concentrations were measured according to CHOD-PAP method using a commercial kit. Serum LDL-cholesterol concentration was also estimated by CHOD-PAP method after precipitation with magnesium sulphate and phosphotunstic acid. Levels of SGPT and SGOT activity were estimated by using SGPT and SGOT assay kit, respectively. Triglyceride concentration was measured by GPO-PAP method using a commercial kit.

2.8. Statistical analysis

The assays were carried out in triplicate, and the results were expressed as mean values and standard deviation (SD). The statistical differences represented by letters were obtained through a one-way analysis of variance (ANOVA) followed by Tukey's honestly significant difference (HSD) post hoc test ($p < 0.05$). Correlations were established using Pearson's correlation coefficient (r) in bivariate linear correlations ($p < 0.001$). These were carried out using Microsoft Office Excel 2007 and SPSS version 16.0 program (IBM Corporation, New York, USA).

3. Result

The results were obtained with untreated diabetic control mice (Group-2) and diabetic mice treated with MESO (Group-3) and EESO (Group-4) at the dose of 200mg/kg body wt. on serum glucose, lipid profiles, and enzymes were compared with nondiabetic controls (General-control, Group-1), and glibenclamide was used as a reference drug.

Alloxan-induced diabetes resulted in a significant elevation in blood glucose levels in comparison to the non-diabetic control mice (table-1, Fig. 1.). After the administration of MESO and EESO extract to diabetic mice for 21 days, a significant reduction in blood glucose level was noticed and EESO administered group blood glucose level was found to

be very near to the glibenclamide-administered mice. In the 6th to 21st days, MESO and EESO supplementation groups glucose levels maintained 6.42 % - 44.93% and 11.02 % - 50.12% lower than the diabetic control group respectively whereas in the case of glibenclamide, it was 15.13% - 59.63% lower than diabetic control group.

The serum TG and TC increased significantly in diabetic mice as compared to non-diabetic mice (Table 2, Fig. 2). After 21 days, consumption EESO extracts at a dose of 200mg/kg body wt. brought the levels of blood lipids to near-normal values. Serum LDL level increased whereas HDL level decreased significantly in diabetic mice but after treatment with both extract LDL level decreased whereas HDL level increased significantly. It was seen that both MESO and EESO at the dose of 200 mg/kg body wt. the lipid profile was near to the values of both nondiabetic control mice and Glibenclamide treated mice. Administration of the MESO, EESO, and Glibenclamide demonstrated significant ($P<0.05$) reduction of TC by 19.02%, 21.99%, and 31.05% and TG by 20.75%, 25.28% and 35.84% (Fig. 2.), respectively. LDL level was also significantly reduced ($P<0.05$) by 21.59%, 24.62%, and 34.06% (Fig. 2.) in diabetic mice after MESO, EESO, and Glibenclamide treatment respectively. On the other hand, the HDL level was increased significantly ($P<0.05$) by 25%, 28.95%, and 30.77% (Fig. 2.) in diabetic mice after MESO, EESO, and Glibenclamide treatment, respectively.

Table 1 Effects of MESO and EESO on serum glucose level in Alloxan-induced diabetic mice

Groups	Plasma glucose concentration (mmol/L)							
	Initial day	**3 day**	**6 day**	**9 day**	**12 day**	**15 day**	**18 day**	**21 day**
General control	5.96±0.07	5.91±0.15	5.88±0.15	5.95±0.16	5.80±0.09	5.97±0.12	5.86±0.17	5.92±0.14
Diabetic Control	21.20±0.13[a]	22.04±0.16[a]	22.60±0.34[a]	22.01±0.21[a]	23.05±0.19[a]	23.48±0.13[a]	24.14±0.19[a]	24.08±0.21[a]
Diabetic+MESO	23.55±0.12[b]	22.55±0.34	21.15±0.29[b]	20.53±0.24[b]	18.36±1.13[b]	15.66±1.4[b]	14.01±0.27[b]	13.26±0.28[b]
Diabetic+EESO	23.61±0.07[b]	22.35±0.17	20.11±0.16[b]	18.05±1.4[b]	15.51±0.10[b]	14.21±0.17[b]	12.9±0.18[b]	11.01±0.36[b]
Diabetic+Glibencl amide	23.70±0.09[b]	23.15±0.29	19.18±0.28[b]	16.22±0.14[b]	13.80±0.10[b]	11.26±0.12[b]	10.33±0.17[b]	9.72±0.15[b]

Values were expressed as mean ± SD. In column wise comparison alphabet a indicated that the values are statistically significant from general control group at $p<0.001$. In column wise comparison alphabet b indicated that the values are statistically significant from Diabetic control group at $p<0.05$.

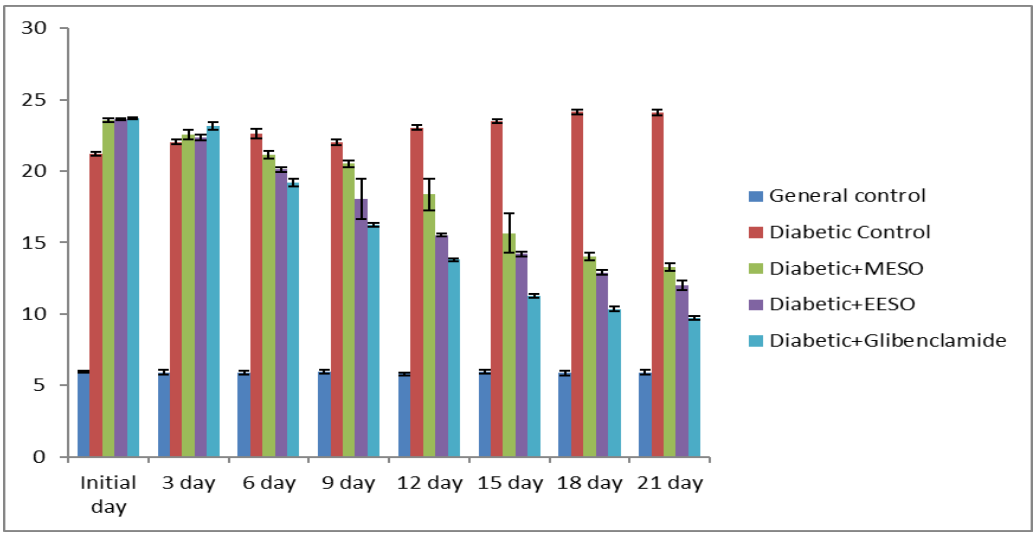

Figure 1 Changes in blood glucose level by the treatment of MESO, EESO and Glibenclamide.

Table 2 Effects of MESO and EESO on lipid profile in diabetic mice after 21 days treatment

Groups	Total Cholesterol (mmol/L)	Triglycerides (mmol/L)	LDL (mmol/L)	VLDL (mmol/L)	HDL (mmol/L)
Normal (Control)	5.30±0.13	1.65±0.06	4.45±0.16	0.75±0.07	0.39±0.03
Alloxan (Diabetic) control	6.73±0.24^{a*}	2.65±0.03^{a*}	5.93±0.16^{a*}	1.2±0.06^{a*}	0.27±0.04^{a*}
Diabetic+ MESO	5.45±0.17$^{b\#}$	2.1±0.03$^{b\#}$	4.65±0.30$^{b\#}$	0.95±0.05$^{b\#}$	0.36±0.03$^{b\#}$
Diabetic+ EESO	5.25±0.23$^{b\#}$	1.98±0.03$^{b\#}$	4.47±0.23$^{b\#}$	0.9±0.05$^{b\#}$	0.38±0.03$^{b\#}$
Diabetic+ Glibenclamide	4.64±0.15^{b*}	1.70±0.04^{b*}	3.91±0.18^{b*}	0.77±0.03^{b*}	0.39±0.05^{b*}

Values were expressed as mean ± SD. In column wise comparison symbol a* indicated that the values are statistically significant from general control group at *P<0.001.* In column wise comparison symbol b# indicated that the values are statistically significant from diabetic control group at *P< 0.05* and symbol b* indicated the values are statistically significant from diabetic control group at *P<0.05*

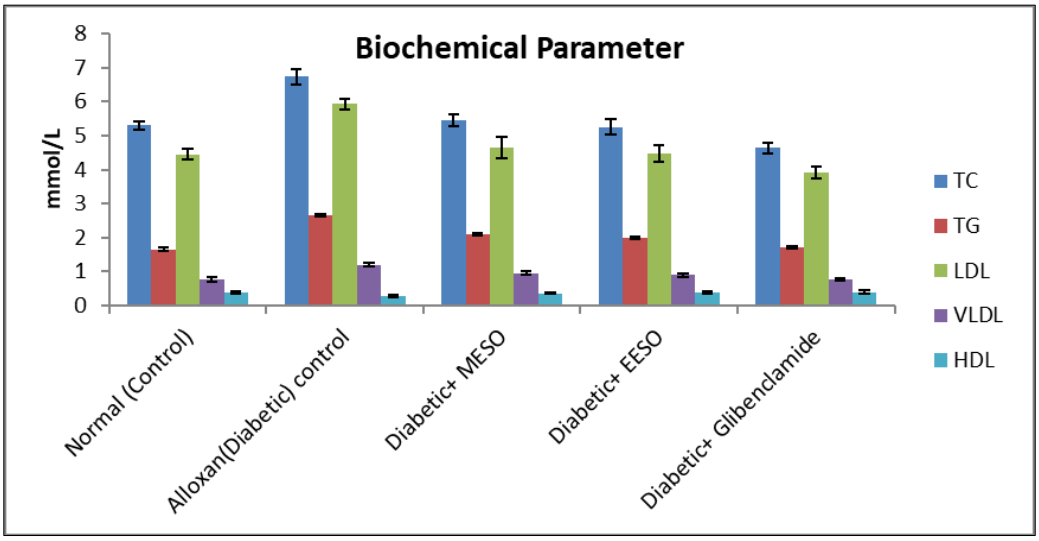

Figure 2 Changes in lipid profile by the treatment of the MESO, EESO and Glibenclamide in diabetic mice

Table 3 Effect of MESO and EESO on serum SGPT and SGOT of Alloxan induced diabetic mice

Group	SGPT(U/L)	SGOT(U/L)
General Control	60.12±3.6	45.30±4.0
Diabetic control	74.60±5.6*	97.45±3.4*
Diabetic+MESO	54.49±3.0**	78.34±5.4**
Diabetic+EESO	50.25±4.8**	70.12±4.4**
Diabetic +Glibenclamide	37.80±3.5**	61.33±4.1**

Serum SGPT and SGOT in the treated mice were significantly different from normal and diabetic control groups at P<0.05; * indicated the difference from normal group; whereas ** indicated the difference from Diabetic control group.

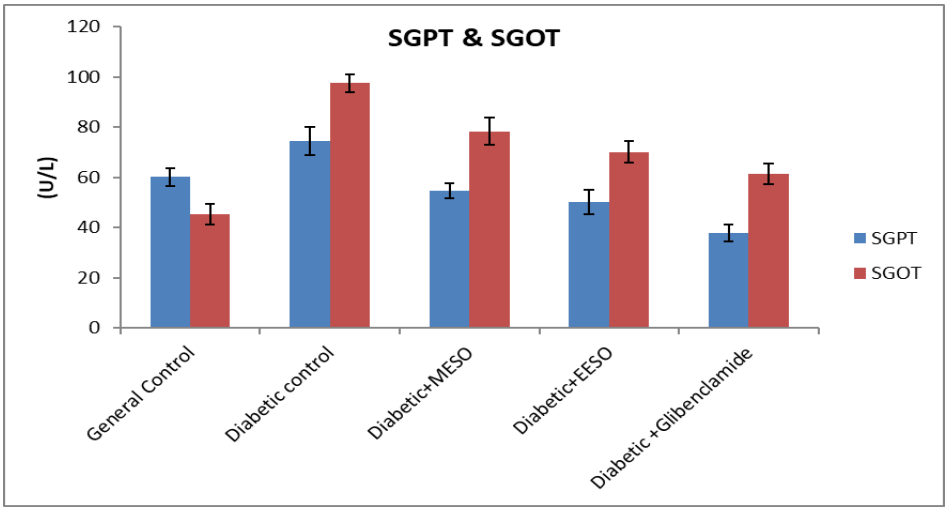

Figure 3 Changes in SGPT and SGOT levels after treatment of MESO, EESO and Glibenclamide

The serum enzymes SGPT and SGOT levels of diabetic mice also increased significantly ($P<0.05$) as compared to non-diabetic control mice (Table 3, Fig. 3.). After 21 days of MESO and EESO administration, the serum enzyme (SGPT and SGOT) levels of diabetic mice at a dose of 200mg/kg body wt. significantly ($P<0.05$). reduced as compared to the diabetic control group. The reduction of SGPT and SGOT by MESO, EESO, and Glibenclamide was 26.96%, 32.64%, 49.33% and 19.61%, 28.05% and 37.07%, respectively (Table 3, Fig. 3.).

4. Discussion

Alloxan monohydrate is a beta cytotoxin agent which destroys the β-cell of the islet of Langerhans in the pancreas of animals resulting in the reduction of the release of insulin which leads to the increase in blood glucose levels [27]. There was a significant decrease in blood glucose levels in alloxan-induced diabetic mice treated with *Spinacia oleracea* L. leaf extract for the entire period of the experiment. Both methanolic and ethanolic extracts of the plant were administered at a dose of 200 mg/kg bw. After 21 days of treatment with methanolic extract, the Blood Glucose Level (BGL) decreases from 23.55 to 13.26 mmol/L. The reduction in BGL by ethanolic extract at the same dose from 23.61 to 11.01 mmol/L was observed. The results in a decrease in BGL are comparable with the standard drug glibenclamide which decrease BGL from 23.70 to 9.72 mmol/L. However, the blood glucose levels of diabetic mice treated with the ethanol extract were similar to or slightly lower than those of the standard treated group mice, suggesting that hypoglycemic components in the plant are greater solubility in ethanol. The difference may be attributed to two reasons. One is the nature of biologically active components that are stable in ethanol. The second possible reason may be the stronger extraction capacity of ethanol which could have produced a greater number of active components responsible for blood glucose-lowering activity. In the present study, the methanolic and ethanolic extracts from *Spinacia oleracea* L leaf showed a significant hypoglycemic effect in alloxan-induced diabetic mice. It is possible that the mechanism of action of both extracts is insulin-independent. Alloxan monohydrate, which induces diabetes, causes damage to pancreatic cells by generating oxygen-free radicals. These radicals primarily target the DNA of pancreatic cells, leading to DNA fragmentation [28].

The study showed that when there is a deficiency in insulin or an increase in blood glucose level, it can lead to higher levels of cholesterol and triglycerides. This is because fat is stored in the liver in such conditions. The treatment with methanolic and ethanolic extract may improve insulin levels to an unknown mechanism which reduces the stored fat in the liver. Diabetes can have a significant impact on the liver, which is the major organ affected by this condition. An increase in liver enzyme activity can lead to liver damage, which could potentially worsen the overall condition [29]. Liver enzymes are known to be useful indicators of liver function. However, in diabetic conditions, the destruction of liver cells caused by a change in membrane structure may lead to the secretion and release of these enzymes into the blood circulation. This can result in alterations in liver function. [30]. Oral administration of methanolic and ethanolic extracts in 200 mg/kg doses significantly reduces the enzyme level in blood.

5. Conclusion

The current study shows that oral administration of *S. oleracea* L. extract produces significant hypoglycemic and hypolipidemic effects which lowers blood glucose as well as TG and TC, and increases HDL-cholesterol to near normal range in alloxan-induced diabetic mice. This investigation reveals that *S. oleracea* L. extract has potent antidiabetic and hypolipidemic effects in alloxan-induced diabetic mice.

Compliance with ethical standards

Acknowledgments

The authors are grateful to the Chairman of the Department of Biochemistry and Molecular Biology, University of Rajshahi, Rajshahi-6205, Bangladesh to give the lab facility to complete this research work.

Disclosure of conflict of interest

There is no conflict of interest related to this work.

Statement of ethical approval

This study with albino mice has been conducted with the approval of the laboratory ethical committee of Rajshahi University, Bangladesh.

References

[1] Sapra A, Bhandari P. Diabetes Mellitus – Stat Pearls - NCBI Bookshelf. Star Pearls Publication; 2022.

[2] Eberle C, Stichling S. Effect of telemetric interventions on glycated hemoglobin A1c and management of type 2 diabetes mellitus: systematic meta-review*. J Med Internet Res.* 2021; 23:1–14.

[3] Iatcu CO, Steen A, Covasa M. Gut microbiota and complications of type-2 diabetes. *Nutrients.* 2022;14. doi:10.3390/nu14010166

[4] Meresa A, Gemechu W, Basha H, et al. Herbal medicines for the management of diabetic mellitus in Ethiopia and Eritrea including their phytochemical constituents. *Am J Adv Drug Deliv.* 2017; 01:040–058.

[5] Piero MN. Diabetes mellitus – a devastating metabolic disorder. *Asian J Biomed Pharm Sci*. 2015; 4:1–7. doi:10.15272/ajbps.v4i40.645

[6] Saberzadeh-Ardestani B, Karamzadeh R, Basiri M, et al. Type 1 diabetes mellitus: cellular and molecular pathophysiology at a glance. *Cell J*. 2018; 20:294–301. doi:10.22074/cellj.2018.5513

[7] American Diabetes Association. 2. Classification and diagnosis of diabetes: standards of medical care in diabetes-2021. *Diabetes Care.* 2021; 44:S15–S33.

[8] Cho NH, Shaw JE, Karuranga S, et al. IDF Diabetes Atlas: global estimates of diabetes prevalence for 2017 and projections for 2045. *Diabetes Res Clin Pract.* 2018; 138:271–281. doi:10.1016/j.diabres.2018.02.023

[9] Ogurtsova K, da Rocha Fernandes JD, Huang Y, et al. IDF diabetes atlas: global estimates for the prevalence of diabetes for 2015 and 2040. *Diabetes Res Clin Pract.* 2017; 128:40–50. doi:10.1016/j.diabres.2017.03.024

[10] Bahadoran Z, Golzarand M, Mirmiran P, Saadati N, Azizi F (2013) The association of dietary phytochemical index and cardio-metabolic risk factors in adults: Tehran lipid and glucose study. *J Hum Nutr Diet.* 2013; 1: 145-153. 11.

[11] Birdee GS, Yeh G. Complementary and alternative medicine therapies for diabetes: a clinical review. *Clinical Diabetes.* 2010; **28**, 147-155.

[12] Falguni MFZ, Islam MA, Hasan MM, Mousun SMMMH, Ashraduzzaman M, Khatun S. Antioxidant and Antidiabetic Properties of *Vitex nigundo* L. Leaves, *American Journal of Life Sciences*. 2017; 5 (1): 21-26. doi: 10.11648/j.ajls.20170501.14

[13] Maiti R, Jana D, Das UK, Ghosh D. Antidiabetic effect of aqueous extract of seed of *Tamarindus indica* in streptozotocin-induced diabetic mice. *Journal of Ethnopharmacolgy.* 2004; **92**, 85-91.

[14] Ashraduzzaman M, Alam MA, Khatun S, Shabnam B and Absar N. *Vigna unguiculata* Linn. walp. seed oil exhibiting antidiabetic effects in alloxan induced diabetic mice. *Malaysian Journal of Pharmaceutical Sciences*. 2011; **9(1):** 13-23.

[15] Al-Shamaony L, Al-Khazraji SM, Twaij HAA. Hypoglycemic effect of *Artemisia herba alba.* on some blood parameters in diabetic animals. *Journal of Ethnopharmacology.* 1994; **43**, 167-171.

[16] Nasrin Ferdous, Md. Rabiul Karim and Khatun S. Antihyperglycemic and antihyperlipidemic effects of the alcoholic extracts of *Aegle marmellos* L. leaves. *International Journal of Biosciences*. 2014; **4(11):** 353-360.

[17] Jaiswal D, Rai PK, Kumar A, Mehta S, Watal G. Effect of *Moringa oleifera* Lam. leaves aqueous extract therapy on hyperglycemic mice. *Journal Ethnopharmacology.* 2009; **123**, 392-396.

[18] Khatun S. and Sultana S. Antidiabetic properties of methanolic extracts of *Carissa carandas* L. fruits in Alloxan-induced diabetic mice. *World Journal of Advance Healthcare Research*. 2023; 7(9): 79-85.

[19] Hasan MM, Begum MIA. and Khatun S. The effects of Mulberry Fruits (*Morus alba* L.) Extract on Alloxan Induced Diabetic mice. *World Journal of Advance Healthcare Research,* 2018; **2 (5):** 102-107.

[20] Han CJ, Hussain, AH, Ismail S. Effect of *Orthosiphon stamineus* leaf extracts on hepatic cytochrome P450, UGT and GST activity in STZ-induced diabetic mice. *Journal for the Advancement of Science and Arts.* 2009; **1**: 1-8.

[21] Gil M.I., Ferreres F., Tomas-Barberan F. Effect of postharvest storage and processing on the antioxidant constituents (flavonoids and vitamin C) of fresh-cut spinach*. J. Agric. Food Chem*. 1999; 47:2213–2217. doi: 10.1021/jf981200l. [PubMed] [CrossRef] [Google Scholar]

[22] Bunea A., Andjelkovic M., Socaciu C., Bobis O., Neacsu M., Verhé R., Van Camp J. Total and individual carotenoids and phenolic acids content in fresh, refrigerated and processed spinach (*Spinacia oleracea* L.) *Food Chem.* 2008;108:649–656. doi: 10.1016/j.foodchem.2007.11.056. [PubMed] [CrossRef] [Google Scholar]

[23] Hait-Darshan R., Grossman S., Bergman M., Deutsch M., Zurgil N. Synergistic activity between a spinach-derived natural antioxidant (NAO) and commercial antioxidants in a variety of oxidation systems. *Int. Food Res.* 2009; 42:246–253. doi: 10.1016/j.foodres.2008.11.006. [CrossRef] [Google Scholar]

[24] Bergman M., Varshavsky L., Gottlieb H.E., Grossman S. The antioxidant activity of aqueous spinach extract: Chemical identification of active fractions. *Phytochemistry.* 2001; 58:143–152. doi: 10.1016/S0031-9422(01)00137-6. [PubMed] [CrossRef] [Google Scholar]

[25] Lomnitski L., Bergman M., Nyska A., Ben-Shaul V., Grossman S. Composition, efficacy and safety of spinach extracts. *Nutr. Cancer*. 2003; 46:222–231. doi: 10.1207/S15327914NC4602_16. [PubMed] [CrossRef] [Google Scholar]

[26] Lomnitski L, Carbonatto M, Ben-Shaul V, Peano S, Conz A, Corradin L, Maronpot RR, Grossman S, Nyska A. The prophylactic effects of natural watersoluble antioxidant from spinach and apocynin in a rabbit model of lipopolysaccharide induced endotoxemia. *Toxicol. Pathol* .2000; 28: 588-600. doi:10.1177 /01926233000 28 00 413. [PubMed] [CrossRef]

[27] Dhanabal SP, Koate CK, Ramanathan M, Elango K, Suresh B. The hypoglycemic activity of *Coccinia indica Wight & Arn.* and its influence on certain biochemical parameters. *Indian Journal of Pharmacogy.* 2004;**36**, 244-250.

[28] Sarkar S, Pranava M, Marita RA. Demonstration of the hypoglycemic action of Momordica charantia in a validated animals model of diabetes. *Pharmacological Research.* 1996; **33**(1), 1-4.

[29] Amraie E, Farsani MK, Sadeghi L, Khan TN, Babadi VY, Adavi Z. The effects of aqueous extract of alfalfa on blood glucose and lipids in alloxan-induced diabetic mice. *Inter Med Appl Sci,* 2015;7(3):124–8.

[30] Udayakumar R, Kasthurirengan S, Mariashibu TS, Rajesh M, Anbazhagan VR, Kim SC, et al. Hypoglycemic and hypolipidaemic effects of *Withania somnifera* root and leaf extracts on alloxan-induced diabetic mice. *Int J Mol Sci* 2009; 10:2367–82.