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Research Article

EVALUATION OF ANTIHYPERGLYCEMIC AND ANTIHYPERLIPIDEMIC ACTIVITIES OF AN EDIBLE GASTROPOD (*ACHATINA FULICA*) IN ALLOXAN-INDUCED DIABETIC MICE

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ABSTRACT

Objective: The main purpose of this study was to evaluate antihyperglycemic and antihyperlipidemic activities of methanolic extracts of an edible gastropod (*Achatina fulica*) in alloxan-induced diabetic mice.

Methods: Alloxan (150 mg/kg, intraperitoneally [IP]) induced diabetic mice were treated with a methanolic extract of *A. fulica* (0.5 mg/dose/animal/IP) for a period of 10-day. The effect of the extract on body weight, organ indices, blood glucose levels, total cholesterol (TC), high-density lipoprotein-cholesterol (HDL-C), low-density lipoprotein (LDL) cholesterol, very LDL (VLDL) cholesterol, triglycerides (TGs), serum glutamic oxaloacetic transaminase (SGOT), serum glutamic pyruvic transaminase (SGPT), serum urea, and creatinine were assessed to divulge their activity in controlling diabetes-related metabolic alterations.

Results: Gas chromatography-mass spectrometry analysis of aqueous *A. fulica* extract lead to the identification of 7 bioactive compounds, and the major constituents of the extract were found to exert hypoglycemic activity. Diabetic animals with the treatment of *A. fulica* extract showed a significant decrease in fasting blood glucose concentration (222.0±0.9 mg/dl) and a decrease in the levels of TC (105±32.3 mg/dl), LDL (31.6±25.8 mg/dl), VLDL (22.76±0.27 mg/dl), and TG (113±3.3 mg/dl) and a potent elevation in the level of serum HDL-C (64.3±8.5 mg/dl) in the extract treated animals when compared with the untreated. Liver marker enzymes such as SGPT and SGOT levels were further reduced in the treated group (SGPT - 0.86 ±0.004 IU/L, SGOT - 0.04±0.004 IU/L) after the IP administration of the gastropod extract. The levels of serum urea (6.1±0.94 mg/dl) and creatinine (9.08±2.9 mg/dl) were also significantly decreased after treatment with the extract, respectively, compared to the mean values of the diabetic group (6.31±1.38 mg/dl, 11.4±2.9 mg/dl).

Conclusion: This confirms the antihyperglycemic and antihyperlipidemic activity of *A. fulica* extract in alloxan-induced diabetic animals. From the present study, it can be concluded that the gastropod extract seems promising for the management of diabetes mellitus.

Keywords: Diabetes, Antihyperglycemic, Antihyperlipidemic, Alloxan, *Achatina fulica*.

INTRODUCTION

Diabetes mellitus (DM) is a group of metabolic diseases characterized by hyperglycemia resulting from defects of insulin secretion and increased cellular resistance to insulin [1]. DM is ranked seventh among the leading causes of death and is considered third when its fatal complications are taken into account. In people with diabetes, either not enough insulin is produced or it is not working properly, and therefore, glucose builds up in the blood [2]. Statistical projections about India suggest that Indians are genetically more susceptible to diabetes, and the World Health Organization predicts the number of diabetic persons in India would go up to 40 million by 2010 and to 74 million by 2025 [3].

Experimental induction of DM in an animal model is essential for the advancement of our knowledge and understanding of the various aspects of its pathogenesis and ultimately finding new therapies and cure. Alloxan produces diabetes in a large number of species of animals by a selective destruction of the beta cells of islets of Langerhans [4]. Alloxan is selectively toxic to insulin-producing pancreatic beta cells because it preferentially accumulates in beta cells through uptake via the glucose transporter. Alloxan diabetes has been commonly utilized as an animal model of insulin-dependent DM, i.e. Type I. Typical DM will develop about 24-48 hrs after the injection of a diabetogenic dose of alloxan when it is administered intraperitoneally (IP) [5]. The IP dose of 150 mg/kg body weight may be sufficient for inducing diabetes in the mice [6].

Diabetes treatment comprises one or more of the following modalities: Medical nutrition therapy, exercise, insulin, and non-insulin agents, including oral medications and the non-insulin injectable drug exenatide [7]. The modern oral hypoglycemic agents produce undesirable side effects, and so the management of diabetes is a global problem until now, and successful treatment is not yet discovered. Thus, alternative therapy is required [8]. More than 400 plant species were reported to have hypoglycemic activity, and most of the antidiabetic natural products were, so far, isolated from plants. In contrast, marine bacteria and fungi are poorly investigated for antidiabetic activity but may be of great promise in the search for new antidiabetic drugs for the future [9]. "Bioactive compounds" are extra nutritional constituents that typically occur in small quantities in foods. Among the marine invertebrates, the molluscs are a potential source of bioactive substances. Many studies on bioactive compounds from gastropods have been reported worldwide [10]. Many studies on bioactive compounds from molluscs exhibiting antitumor, antileukemic, antibacterial, and antiviral activities have been reported worldwide [11].

It has been noted that snail meat contains a low level of sodium, cholesterol, and high level of potassium; hence, it is used in the treatment of arteriosclerosis, anemia, high blood pressure, and other fat-related ailments. Snail is also said to be rich in mineral salt, e.g., calcium, phosphorus, iron, and copper which are very helpful for the body of its consumers. Hypertension can also be cured by fluid produced by snails.

No works have been done on determining the antidiabetic activity of the edible snail. Therefore, the present study was undertaken to evaluate the effectiveness of edible natural product of *Achatina fulica* in alloxan-induced diabetic mice for its antihyperglycemic and antihyperlipidemic activity.

METHODS

Gastropod collection

The gastropod species *A. fulica* (Ferussac, 1821) a giant African snail was collected from the Karunya University Campus and identified as *A. fulica* by Dr. V. Deepak Samuel (Project Support Associate, UNDP-GEF, Energy and Environment Unit, United Nations Development Programme, India) who is a mollusc expert and also a member of the Tamil Nadu Forest Department endangered animals group.

Preparation of gastropod extract

The edible gastropod meat was separated and washed in distilled water and dried in hot air oven at 50-55°C. The dried meat was then powdered and extracted overnight by stirring with 10 volumes of 75% methanol. The supernatant was collected after centrifuging at 3000 rpm for 10 minutes. Then, the solvent was allowed to evaporate using rotary evaporator at 45°C. The yield of the extract was 10%.

Experimental animals

Inbred BALB/C (6-8 weeks) mice, weighing 23-28 g, were obtained from Pasteur Institute, Breeding Section, Coonoor. The animals were housed in ventilated plastic cages at 37±1°C, 40±10% humidity, and 12-12-hrs light-dark cycles during the experimental period. The animals were fed with normal mouse chow (Sai Feeds, Mumbai, India) and given water *ad-libitum.* All animal experiments were conducted according to the rules and regulations of Animal Ethics Committee, Government of India.

Experimental induction of diabetes

Experimental diabetes was induced in overnight fasted mice by single IP injection of alloxan monohydrate (Sigma-Aldrich Chemicals, Pvt., Ltd., Bengaluru) dissolved in sterile normal saline at a dose of 150 mg/kg body weight. After 72 hrs of alloxan injection, the mice with persistent hyperglycemia the blood glucose range of above 250 mg/dl were included in the study. All other chemicals and reagents used were of analytical grade.

Experimental design

The animals were divided into four groups of six animals each as follows:

- Group 1: Control animals, received normal feed without any treatment (normal healthy control)
- Group 2: Animals were induced with alloxan monohydrate (150 mg/kg b.w) (untreated diabetic control)
- Group 3: Animals received *A. fulica* (0.5 mg) methanolic extract dissolved in 1% gum acacia IP for 10 consecutive days alone (negative control)
- Group 4: Diabetic mice received *A. fulica* (0.5 mg) methanolic extract dissolved in 1% gum acacia IP for 10 consecutive days (treated group).

Experimental procedure

At the end of the experiment, animals from each group were sacrificed by cervical dislocation for biochemical and histopathological studies. Blood was collected on decapitation and serum was separated by centrifugation at 3500 rpm for 10 minutes. Tissues such as liver, kidney, and pancreas were excised immediately and thoroughly washed in ice-cold saline. The liver was collected and used for histological studies.

Biochemical assays

At the end of 0^{th}, 2^{nd}, 4^{th}, 6^{th}, 8^{th}, and 10^{th} day, blood samples were collected in tubes containing potassium oxalate and sodium fluoride solution for the estimation of blood glucose according to the ortho-toluidine method described by Sasaki and Matsui [12]; serum total cholesterol (TC) and high-density lipoprotein-cholesterol (HDL-C) levels described by the method of Pierre *et al.* [13]; low-density lipoprotein-cholesterol (LDL-C) and very LDL-C (VLDL-C) determined by the method of Friedewald *et al.* [14]; triglyceride (TG) assay using glycerol phosphate oxidase-phenol + aminophenazone method described by McGowan *et al.* [15]; serum glutamic pyruvic transaminase (SGPT) and serum glutamic oxaloacetic transaminase (SGOT) levels estimated by Reitman and Frankel [16]. Levels of serum urea and creatinine were determined by Marsh *et al.* method and Brod and Sirota, respectively [17-19].

Statistical analysis

The experimental data were expressed as mean ± standard deviation. The significance of difference among the various treated groups and control groups were analyzed by means of one-way ANOVA followed by Dunnett's multiple comparison test using GraphPad InStat 3.0 Software (San Diego, CA, US). Changes were considered to be statistically significant if the $p<0.05$.

RESULTS AND DISCUSSION

Bioactive components in methanolic extract of *A. fulica* were identified by gas chromatography-mass spectrometry (GC-MS) analysis (Fig. 1). GC-MS analysis revealed the presence of 1,2-benzene dicarboxylic acid, dibutyl ester, linoleic acid, methyl arachidonate, eicosatetraenoic acid, octadecadienoic acid, 13-methyltetradecanoate, and tridecanal indicating the role of these compounds in the observed effect. The active principles with their retention time, molecular formula, molecular weight, and reverse fit factor are shown in Table 1.

Studies have shown that conjugated linoleic acids (CLA) exert a protective effect in animal models of DM. The supplementation significantly resulted in increased fasting glucose concentration and reduced insulin sensitivity [20]. CLAs alters lipid metabolism and reduced the TC, TGs, LDL-C, and increased HDL-C [21]. Suresh and Das showed that alloxan-induced *in vitro* cytotoxicity can be prevented by oral administration of arachidonic acid in experimental animal models suggesting that polyunsaturated fatty acids may be useful to prevent diabetes [22]. The two major long-chain ω3 polyunsaturated fatty acids, eicosapentaenoic acid and docosahexaenoic acid, found in the gastropod have been proved to be essential for many functions in the humans [23].

The emerging insulinopenia causes a state of experimental DM called "alloxan diabetes." Alloxan diabetes is a form insulin-dependent diabetes the endocrine pancreas; occlusion of the arterial blood supply to the pancreas prevents access of the injected alloxan to the beta cells [24].

Effect of *A. fulica* extract on body weight and organ weight of alloxan-induced mice

The effect of *A. fulica* extract on body weight and organ weight of alloxan-induced mice was depicted in Tables 2 and 3, respectively. Normal control animals were found to be stable in their body weight

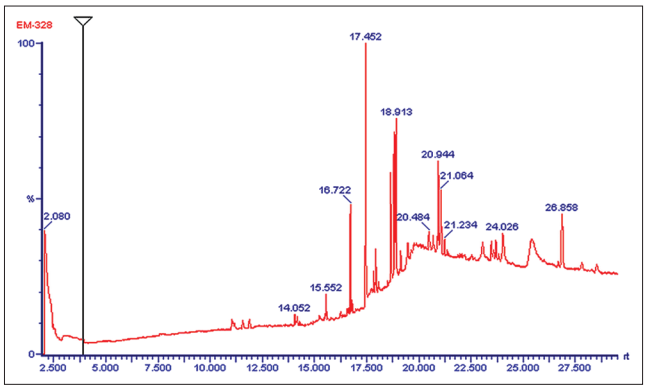

Fig. 1: Total ionic chromatogram (gas chromatography-mass spectrometry) of methanolic extract of *Achatina fulica*

Table 1: Bioactive components identified in methanolic extract of *A. fulica* by GC-MS analysis

S.No.	RT	Rf value	Name of the compound	Molecular formula	REV	MW
1	17.452	63.000	1,2-Benzene dicarboxylic acid, Dibutyl ester	$C_{16}H_{22}O_4$	966	278
2	18.913	63.000	Ethyl linoleate, Linoleic acid	$C_{20}H_{36}O_2$	865	308
3	20.944	63.000	Methyl arachidonate, Eicosatetraenoic acid	$C_{21}H_{34}O_2$	820	318
4	21.064	63.000	Octadecadienoic acid	$C_{19}H_{34}O_2$	899	294
5	16.722	63.000	13-methyltetradecanoate	$C_{16}H_{32}O_2$	955	256
6	15.552	63.000	Tridecanal	$C_{13}H_{26}O$	974	198
7	23.696	63.000	Propylhexedrin propionyl	$C_{13}H_{25}ON$	704	211

RT: Retention time, REV: Reverse fit factor, MW: Molecular weight, *A. fulica*: *Achatina fulica*, GC-MS: Gas chromatography-mass spectrometry

Table 2: Effect of *A. fulica* extract on body weight of alloxan-induced mice

Group	Body weight (g)
Group I (normal healthy control)	25.6±1.58
Group II (untreated diabetic control)	24.3±1.71
Group III (negative control)	21.5±0.89
Group IV (treated group)	29.7±1.46*

Values are expressed as mean±SD, *p<0.05. SD: Standard deviation, *A. fulica: Achatina fulica*

Table 3: Effect of *A. fulica* extract on organ weight of alloxan-induced mice

Group	Weight of liver (tissue weight/10 g b.wt)	Weight of kidney (tissue weight/10 g b.wt)
Group I (normal healthy control)	2.108±0.27	0.326±0.014
Group II (untreated diabetic control)	1.58±0.11*	0.55±0.031**
Group III (negative control)	1.6±0.04	0.51±0.07
Group IV (treated group)	1.88±0.15*	0.41±0.05*

Values are expressed as mean±SD, *p<0.05, **p<0.01. SD: Standard deviation, *A. fulica: Achatina fulica*

(25.6±1.58, p<0.001) but the alloxan-induced diabetic mice showed a significant reduction (24.3±1.71, p<0.001) during the period of study. The diabetic animals treated with *A. fulica* extract showed increments in their percentage mean body weights (29.7±1.46, p<0.001). Similarly, alloxan caused body weight loss was also regained to its normal control values by *Prosopis cineraria* bark extract treatment [25]. The decrease in body weight could be due to the excess breakdown of tissue proteins or may be due to derangement of metabolic pathways [26]. Mice treated with the extract showed a progressive increase in their percentage mean body weights. This could be explained by protein sparing action, i.e. gluconeogenesis from muscle protein (ketogenic amino acid) would result in a decrease in total protein [27].

Organ indices for liver and kidney were measured as tissue weight per 10 g body weight. The liver and kidney indices were significantly different in the diabetic control compared to the normal healthy control animals, and the liver index was significantly higher in the treated group (1.88±0.15 g) compared to the diabetic group (1.58±0.11 g). However, no such significant differences in kidney index were seen between the treated groups and the untreated diabetic group. Increased breakdown of glycogen, pronounced gluconeogenesis, enhanced catabolic processes such as glycogenolysis, lipolysis, and proteolysis triggered by a lack of insulin or cellular glucose in diabetes might be responsible for the reduction in liver weight of diabetic animals. Glomerular cell proliferation accompanied with glomerular enlargement caused an increase in kidney weight of alloxan-induced diabetic rats [28].

Effect of *A. fulica* extract on fasting blood glucose level of alloxan-induced mice

Mohan and Das (2001) reported that animals which received a single alloxan injection developed Type I diabetes demonstrated by high blood glucose level (350-400 mg/dl) and this is in agreement with our results that revealed a highly significant elevation in the blood glucose in diabetic mice as compared with the normal [29]. So, effective control of the blood glucose level is a key step in preventing diabetes. The mean blood glucose concentrations of all the experimental animals were estimated on the 2, 6, 16, and 24 hrs after the IP injection of alloxan and on 2nd, 4th, 6th, 8th, and 10th day after the induction of diabetes, respectively.

As shown in Table 4, a marked rise in fasting blood glucose level (263±16.2 mg/dl, p<0.001) was observed in diabetic control when compared to the normal control animals (92.55±2.6mg/dl, p<0.001). Diabetic mice treated with *A. fulica* extract for 10 days showed a significant reduction (222.0±0.9 mg/dl, p<0.001) in their blood glucose levels. The increase in blood glucose levels may be due to the result of the destruction of the beta cells of the pancreas by alloxan which was brought back to normal in the diabetic mice treated with *A. fulica* extract. The possible hypoglycemic mechanism of the extract may be through potentiation of pancreatic secretion of insulin from β-cell of islets or due to enhanced transport of blood glucose to the peripheral tissues [30].

Determination of the effect of *A. fulica* extract on lipid profile

Effect of A. fulica extract on TC, HDL, LDL, and VLDL of alloxan-induced mice

Hypercholesterolemia and hypertriglyceridemia have been reported to occur in diabetic mice [31]. The present results showed that the level of serum TC (147±56.6 mg/dl) was significantly elevated in the diabetic control group as compared to the normal healthy control (37.8±22.5 mg/dl, p<0.001). After supplementation with *A. fulica* extract, the alteration in lipid metabolism was partially attenuated as evidenced by decreased serum TC level (105±32.3, p<0.001) as shown in Table 5. The higher concentration of TC observed in diabetic rats is probably due to mobilization of free fatty acids from the peripheral fat depots. Alterations in the erythrocyte membranes lipid composition may be a reflection of alterations in the serum lipid profile [32]. Similarly, Sophia and Manoharan showed that oral administration of *Ficus racemosa* bark extract to diabetic rats significantly (p<0.05) normalized the levels of erythrocyte membrane cholesterol and phospholipids and restored the status of TC level [33].

Low HDL-C concentration is one of the distinctive features observed in diabetic dyslipidemia. The level of HDL-C was significantly reduced in untreated diabetic mice (38.5±4.6 mg/dl) and these lowered levels of HDL-C were enhanced significantly to 64.3±8.5 mg/dl (p<0.001) because of treatment with *A. fulica* extract (Table 6).

Compared to normal control group, the levels of LDL-C (94.14±41.1mg/dl) and VLDL-C (28.9±0.16 mg/dl) levels were significantly (p<0.005) elevated in the diabetic control group. Both LDL-C and VLDL-C levels were decreased to near normal level

Table 4: Effect of *A. fulica* extract on fasting blood glucose of alloxan-induced mice

Group	Blood glucose (mg/dl)					
	0th day	2nd day	4th day	6th day	8th day	10th day
Group I (normal healthy control)	85.93±2.3	89.28±1.1	90.23±1.7	92.21±2.1	96.50±2.0	92.55±2.6
Group II (untreated diabetic control)	284.0±22.1**	339.0±20.4**	328.0±25.2**	306.0±22.3**	291.0±19.3**	263.0±16.2**
Group III (negative control)	87.17±2.1	89.45±1.3	95.36±1.7	98.71±1.2	100.0±3.3	101.0±2.3
Group IV (treated group)	327.0±11.9**	302.0±22.3	334.0±12.6	270.0±9.3**	240.0±3.1**	222.0±0.9*

Values are expressed as mean±SD, *p<0.05, **p<0.01. SD: Standard deviation, *A. fulica: Achatina fulica*

(31.6±25.8 mg/dl and 22.76±0.27 mg/dl) in the extract treated group (Tables 7 and 8). The increased levels of LDL in the diabetic animals might be due to overproduction of LDL by the liver due to the stimulation of hepatic TG synthesis as a result of free fatty acid influx [34]. The reduction in the levels of LDL-C and VLDL-C were restored significantly following treatment with the extract. This may be attributed to increased clearance and decreased the production of the major transporters of endogenously synthesized cholesterol and TGs [35].

Serum TG levels were increased significantly in the diabetic group (144±2.07 mg/dl). Administration of *A. fulica* extract to diabetic mice brought down the level of TGs and thus reduced remarkably (113±3.3 mg/dl) in the treated group (Table 9). The deficiency of lipoprotein lipase activity may contribute significantly to the elevation of TGs in diabetes. Because Insulin is a potent inhibitor of lipolysis since it inhibits the activity of the hormone-sensitive lipases in adipose tissue and suppresses the release of TGs [36]. The diabetes-induced hyperlipidemia might be due to excess mobilization of fat from the adipose tissue because of underutilization of glucose. The extract normalized all the lipid profile parameters and thus this extract could attribute to antihyperlipidemic activity.

Effect of A. fulica extract on SGPT, SGOT, urea, and creatinine levels of alloxan-induced mice

SGPT and SGOT levels act as indicators of liver function and restoration of normal levels of these parameters indicate normal function of the liver. The increase in the levels of transaminase reflects a clear indication of cellular leakage and loss of functional integrity of the cell membrane. In diabetic mice, the level of SGPT elevated significantly from 0.81±0.001 to 0.92±0.001 IU/L and the enzyme levels were resettled to normal level (0.86±0.004 IU/L) after the IP administration of *A. fulica* extract as shown in Table 10. Likewise, the level of SGOT was also raised significantly from 0.066±0.001 to 0.07±0.001 IU/L in diabetic mice (Table 11). Following the administration of *A. fulica* extract, the level of SGOT (0.04±0.004 IU/L) was reduced significantly. The increased levels of transaminases, which are active in the absence of insulin, because of the availability of amino acids in the blood of diabetes, are responsible for the increased gluconeogenesis and ketogenesis observed in diabetes. Thus, the extract significantly reversed the elevated marker enzymes in alloxan-induced diabetic rats, i.e. SGOT and SGPT restored to normal values indicates a revival of insulin secretion into circulations and also its hepatoprotective effect.

The diabetic hyperglycemia induces the elevation of urea and creatinine levels in diabetic rats, which are considered a significant marker of renal dysfunction [37]. An increase in the level of serum urea in diabetic mice (6.31±1.38 mg/dl) were found to be slightly decreased in the treated mice (6.1±0.94 mg/dl, p<0.001) (Table 12). In the diabetic mice, the increased serum creatinine was noticed (11.4±2.9 mg/dl, p<0.001) and it was found to be reduced significantly (9.08±2.9, mg/dl, p<0.001) in the treated mice (Table 13). The significant elevations in serum urea and creatinine levels indicate impaired renal function in diabetic animals. Administration of *A. fulica* extract increased the serum urea and creatinine levels by enhancing the renal function that is generally impaired in the diabetic group.

Table 5: Effect of *A. fulica* extract on total cholesterol of alloxan-induced mice

Group	TC (mg/dl)
Group I (normal healthy control)	37.8±22.5
Group II (untreated diabetic control)	147±56.6*
Group III (negative control)	78.3±18.4
Group IV (treated group)	105±32.3*

Values are expressed as mean±SD, *p<0.05. TC: Total cholesterol, SD: Standard deviation, *A. fulica: Achatina fulica*

Table 6: Effect of *A. fulica* extract on serum HDL-C of alloxan-induced mice

Group	HDL-C (mg/dl)
Group I (normal healthy control)	46.7±2.98
Group II (untreated diabetic control)	38.5±4.6*
Group III (negative control)	44.8±4.97
Group IV (treated group)	64.3±8.5*

Values are expressed as mean±SD, *p<0.05. HDL-C: High-density lipoprotein-cholesterol, SD: Standard deviation, *A. fulica: Achatina fulica*

Table 7: Effect of *A. fulica* extract on serum LDL-C of alloxan-induced mice

Group	LDL-C (mg/dl)
Group I (normal healthy control)	32.9±14.4
Group II (untreated diabetic control)	94.14±41.1*
Group III (negative control)	18.2±7.19
Group IV (treated group)	31.6±25.8*

Values are expressed as mean±SD, *p<0.05. LDL-C: Low-density lipoprotein-cholesterol, SD: Standard deviation, *A. fulica: Achatina fulica*

Table 8: Effect of *A. fulica* extract on serum VLDL-C of alloxan-induced mice

Group	VLDL-C (mg/dl)
Group I (normal healthy control)	20.56±0.29
Group II (untreated diabetic control)	28.9±0.16**
Group III (negative control)	27.6±0.12
Group IV (treated group)	22.76±0.27**

Values are expressed as mean±SD, **p<0.01. SD: Standard deviation, *A. fulica: Achatina fulica*, VLDL-C: Very low-density lipoprotein-cholesterol

Table 9: Effect of *A. fulica* extract on serum TG of alloxan-induced mice

Group	Serum TG (mg/dl)
Group I (normal healthy control)	102±1.45
Group II (untreated diabetic control)	144±2.07**
Group III (negative control)	138±1.6
Group IV (treated group)	113±3.3*

Values are expressed as mean±SD, *p<0.05, **p<0.01. TG: Triglycerides, SD: Standard deviation, *A. fulica: Achatina fulica*

Table 10: Effect of *A. fulica* extract on SGPT of alloxan-induced mice

Group	SGPT (IU/L)
Group I (normal healthy control)	0.81±0.001
Group II (untreated diabetic control)	0.92±0.001**
Group III (negative control)	0.82±0.004
Group IV (treated group)	0.86±0.004**

Values are expressed as mean±SD, **p<0.01. SD: Standard deviation, *A. fulica: Achatina fulica*, SGPT: Serum glutamic pyruvic transaminase

Table 11: Effect of *A. fulica* extract on SGOT of alloxan-induced mice

Group	SGOT (IU/L)
Group I (normal healthy control)	0.066±0.001
Group II (untreated diabetic control)	0.07±0.001
Group III (negative control)	0.04±0.004
Group IV (treated group)	0.04±0.004*

Values are expressed as mean±SD, *p<0.05. SD: Standard deviation, *A. fulica: Achatina fulica*, SGOT: Serum glutamic oxaloacetic transaminase

Table 12: Effect of *A. fulica* extract on serum urea of alloxan-induced mice

Group	Serum urea (mg/dl)
Group I (normal healthy control)	3.39±0.36
Group II (untreated diabetic control)	6.31±1.38*
Group III (negative control)	5.1±0.23
Group IV (treated group)	6.1±0.94*

Values are expressed as mean±SD, *p<0.05. SD: Standard deviation, *A. fulica: Achatina fulica*

Table 13: Effect of *A. fulica* extract on serum creatinine of alloxan-induced mice

Group	Serum creatinine (mg/dl)
Group I (normal healthy control)	4.36±0.22
Group II (untreated diabetic control)	11.4±2.9*
Group III (negative control)	4.6±0.60
Group IV (treated group)	9.08±2.9*

Values are expressed as mean±SD, *p<0.05. SD: Standard deviation, *A. fulica: Achatina fulica*

CONCLUSION

The results of the present study clearly indicate that the methanolic extract of *A. fulica* was quite effective in lowering blood sugar levels, and the extract feeding showed definite improvement in the histopathology of liver. The gastropod extract also showed improvement in parameters such as body weight, lipid profile, liver marker enzymes such as SGPT, SGOT, serum urea, and creatinine and so might be of value in diabetes treatment. Thus, the present study suggests that the edible *A. fulica* extract can be successfully utilized for the management of DM due to their hypoglycemic, antihyperlipidemic, and hepatoprotective action. Further studies on the nature of active principles involved would enlighten the exact mechanism involved and thus help to rationalize their use in the treatment of DM more effectively.

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REFERENCES

1. American Diabetes Association: Screening for diabetes. Diabetes Care 1998;21:20-2.
2. Trivedi NA, Majumder B, Bhatt JD, Hemavathi KG. Effect of Shilajit on blood glucose and lipid profile in alloxan–Induced diabetic rats. Indian J Pharmacol 2004;36:373-6.
3. Pillai M. Reader's digest. In: Could you be a Diabetic. New Delhi: Living Media India Limited Press; 2006. p. 138-40.
4. Lukens FD. Alloxan diabetes. Physiol Rev 1948;28:304-30.
5. Lenzen S. The mechanisms of alloxan- and streptozotocin-induced diabetes. Diabetologia 2008;51(2):216-26.
6. Katsumata K, Katsumata Y, Ozawa T, Katsumata K Jr. Potentiating effects of combined usage of three sulfonylurea drugs on the occurrence of alloxan diabetes in rats. Horm Metab Res 1993;25(2):125-6.
7. Silverman HK. Travel Healthy, the Pill Book. New York: Pill Book Company; 1986. p. 166-74.
8. Li W, Zheng HC, Bukuru J, Kimpe DN. Natural medicines used in the traditional Chinese medical system for therapy of diabetes mellitus. J Ethnopharmacol 2004;92(1):1-21.
9. Seo C, Sohn JH, Oh H, Kim BY, Ahn JS. Isolation of the protein tyrosine phosphatase 1B inhibitory metabolite from the marine-derived fungus *Cosmospora* sp. SF-5060. Bioorg Med Chem Lett 2009;19:6095-7.
10. Kagoo EL, Ayyakannu K. Bioactive compounds from *Chicoreus ramosus* antibacterial activity-*in vivo*. Phuket. Mar. Biol Cent Spl Publ 1992;11:147-50.
11. Chellaram C, Anand PT, Kumaran S, Kesava D, Priya G. Central nervous system depressant properties of reef associated gastropods, *Drupa margariticola* and *Trochus tentorium* from Gulf of Mannar, Southeastern India. J Chem Pharm Res 2011;3:154-9.
12. Sasaki T, Matsui S. Effect of acetic acid concentration on the color reaction in the o-toluidine-boric acid method for blood glucose determination. Rinsbho Kagaku 1972;1:346-53.
13. Pierre J, Dagenais RG, Bergeron J. Total cholesterol/HDL cholesterol ratio vs LDL cholesterol/HDL cholesterol ratio as indices of ischemic heart disease risk in men. Arch Intern Med 1997;161:2685-92.
14. Friedewald WT, Levy RI, Fredrickson DS. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. Clin Chem 1972;18(6):499-502.
15. McGowan MW, Artiss JD, Strandbergh DR, Zak B. A peroxidase-coupled method for the colorimetric determination of serum triglycerides. Clin Chem 1983;29(3):538-42.
16. Reitman M, Frankel S. A colorimetric method for the determination of serum glutamic oxaloacetic and glutamic pyruvic transaminases. Am J Clin Pathol 1957;28(1):56-63.
17. Marsh WH, Fingerhut B, Miller H. Automated and manual direct methods for the determination of blood urea. Clin Chem 1965;11:624-7.
18. Brod J, Sirota JH. The renal clearance of endogenous "Creatinine" in man. J Clin Invest 1948;27(5):645-54.
19. Bancroft JD, Stevens A. Theory and Practice of Histology Techniques. New York: Churchill Livingstone; 1982. p. 680.
20. Moloney F, Yeow TP, Mullen A, Nolan JJ, Roche HM. Conjugated linoleic acid supplementation, insulin sensitivity, and lipoprotein metabolism in patients with type 2 diabetes mellitus. Am J Clin Nutr 2004;80(4):887-95.
21. Park Y, Pariza MW. Mechanisms of body fat modulation by conjugated linoleic acid (CLA). Food Res Int 2007;40(3):311-23.
22. Suresh Y, Das UN. Protective action of arachidonic acid against alloxan-induced cytotoxicity and diabetes mellitus. Prostaglandins Leukot Essent Fatty Acids 2001;64:37-52.
23. Van Pelt CK, Huang MC, Tschanz CL, Brenna JT. An octaene fatty acid, 4,7,10,13,16,19,22,25-octacosaoctaenoic acid (28:8n-3), found in marine oils. J Lipid Res 1999;40:1501-5.
24. Dunn JS, McLetchie NG. Experimental alloxan diabetes. Lancet 1943;2:384-6.
25. Sharma VK, Kumar S, Patel HJ, Hugar S. Hypoglycemic activity of *Ficus glomerata* in alloxan induced diabetic rats. Int J Pharm Sci Rev Res 2010;1(2):18-22.
26. Al-Shamaony L, Al-Khazraji SM, Twaij HA. Hypoglycaemic effect of *Artemisia herba alba*. II. Effect of a valuable extract on some blood parameters in diabetic animals. J Ethnopharmacol 1994;43:167-71.
27. Delvin TM. Protein Metabolism, Textbook of Biochemistry with Clinical Correction. New York: John Wiley and Sons Inc.; 1992. p. 585-7.
28. Sun L, Halaihel N, Zhang W, Rogers T, Levi M. Role of sterol regulatory element-binding protein 1 in regulation of renal lipid metabolism and glomerulosclerosis in diabetes mellitus. J Biol Chem 2002;227(21):18919-27.
29. Krishna Mohan I, Das UN. Prevention of chemically induced diabetes mellitus in experimental animals by polyunsaturated fatty acids. Nutrition 2001;17(2):126-51.

30. Kameswararao B, Kesavulu MM, Apparao C. Evaluation of antidiabetic effect of *Momordica cymbalaria* fruit in alloxan-diabetic rats. Fitoterapia 2003;74(1-2):7-13.
31. Sharma SR, Dwivewdi SK, Swarup D. Hypoglycaemic and hypolipidaemic effects of *Cinnamomum tamla* leaves. Indian J Exp Biol 1996;34:372-8.
32. Das S, Baliarshinha AK. Lipid and lipoprotein cholesterol in diabetic mellitus Indian Scene. Lipid India 1997;11:7-11.
33. Sophia D, Manoharan S. Hypolipidemic activities of *Ficus racemosa* linn. bark in alloxan induced diabetic rats. Afr J Tradit Comp Altern Med 2007;4(3):279-88.
34. Coppack SW, Jensen MD, Miles JM. *In vivo* regulation of lipolysis in humans. J Lipid Res 1994;35(2):177-93.
35. Rajasekaran S, Ravi K, Sivagnanam K, Subramanian S. Beneficial effects of *Aloe vera* leaf gel extract on lipid profile status in rats with streptozotocin diabetes. Clin Exp Pharmacol Physiol 2006;33(3):232-7.
36. Chahil TJ, Ginsberg HN. Diabetic dyslipidemia. Endocrinol Metab Clin North Am 2006;35(3):491-510.
37. Almdal TP, Vilstrup H. Strict insulin therapy normalises organ nitrogen contents and the capacity of urea nitrogen synthesis in experimental diabetes in rats. Diabetologia 1988;31(2):114-8.