RESEARCH ARTICLE

Evaluation of *in vivo* antidiabetic and antioxidant activity of *Achyranthes aspera* Linn. seeds by streptozotocin induced diabetic rats

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Abstract

Background: Diabetes mellitus is one of the most common endocrine metabolic disorders which have a significant impact on the health, quality of life, and life expectancy of patients as well as on the health-care system. Herbal remedies are convenient for the management of type 2 diabetes due to their traditional acceptability and availability, low costs, and lesser side effects. **Aim:** The aim of this study is to evaluate the antidiabetic and antioxidant effects of aqueous ethanolic extract of *Achyranthes aspera* Linn. seeds in normal and streptozotocin (STZ) 65 mg/kg body weight-induced diabetic rats. **Materials and Methods:** In this experiments, various dose ethanolic seed extracts of *A. aspera* were administrated to diabetic rats and observed a significant reduction in blood glucose by induced STZ at 28 days' treatment period. After the experimental period, the blood and tissue samples were collected and subjected to various biochemical parameters and histopathological studies. **Results:** The treatment group with the extract showed a significant increase in the blood glucose, hemoglobin levels, and insulin levels. The total cholesterol and serum triglycerides levels, low-density lipoprotein (LDL), and very LDL were also significantly reduced, and the high-density lipoprotein level was significantly increased on treatment with the *A. aspera* extract. A significant decrease in the levels of superoxide dismutase and glutathione (GSH) peroxidase and increase in the levels of Vitamin E, catalase, and reduced GSH were observed in *A. aspera*-treated diabetic rats. **Conclusion:** Thus, from this study, we conclude that ethanolic extract of *A. aspera* exhibited significant antihyperglycemic, hypolipidemic, and antioxidant activities in STZ-induced diabetic rats.

Key words: *Achyranthes aspera*, diabetes mellitus, glibenclamide, streptozotocin

INTRODUCTION

Diabetes mellitus is a serious complex chronic condition that is a major source of ill health worldwide. This metabolic disorder is characterized by hyperglycemia and disturbances of carbohydrate, protein, and fat metabolisms, secondary to an absolute or relative lack of the hormone insulin. DM has several other factor including duslipidemia or hyperlipidemia are involved in the development of micro vascular and macro vascular complications of diabetes, Which are the major causes of morbidity and death.[1] According to the World Health Organization projections, the prevalence of diabetes is likely to increase by 35%. Currently, there are over 150 million diabetics worldwide and this is likely to increase to 300 million or more by the year 2025. Statistical projection about India suggests that the number of diabetics will rise from 15 million in 1995 to 57 million in the year 2025, the highest number of diabetics in the world.[2] In modern medicine, no satisfactory effective therapy is still available to cure diabetes mellitus. There is increasing demand by patients to use natural products with antidiabetic activity due to side effects associated with the use of insulin and oral hypoglycemic agents. Recently, some medicinal plants have been reported to be useful in diabetes worldwide and have been used empirically as antidiabetic and antihyperlipidemic remedies.

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Antihyperglycemic effect of these attributed to their ability to restore the function of pancreatic tissues by causing an increase in insulin output or inhibit the intestinal absorption of glucose or to the facilitation of metabolites in insulin-dependent processes. More than 400 plant species having hypoglycemic activity is available in literature. In Tamil Nadu region (state of India), there is a great biodiversity of medicinal plants and there is a long tradition of using herbal products for skin and other problems by healers and old peoples. Besides, the plants have been used throughout the world as good reservoir of bioactive compounds, drugs, and remedies for various diseases since time immemorial. The most important of these bioactive compounds of plants are alkaloids, flavonoids, tannins, and phenolic compounds which can prove to be an important source of lead compounds in the development of new drugs.

In the present era of drug development and discovery of newer drug molecules derived from many plant products evaluated their traditional uses. One of the many plants which are being evaluated for their therapeutic efficacies is *Achyranthes aspera* L. belonging to the family Amaranthaceae. The plant shows many pharmacological activities such as spermicidal, anti-allergic, cardiovascular, nephroprotective, antiparasitic, antitumor, analgesic, antifertility, antipyretic, hypoglycemic activity, and thyroid-stimulating properties.[2] Therefore, the present study was carried out to investigate hypoglycemic and antioxidant activity of *A. aspera* seed extract and its effect on streptozotocin (STZ)-induced diabetic rats.

METERIALS AND METHODS

Collection and Authentication of Experimental Plants

Fresh, mature, *A. aspera* were collected from AMET Herbal Garden, Kanathur, Chennai, India. The plant was further identified and authenticated by a taxonomist, and an exemplar specimen was deposited at the Department of Botany, St. Joseph's College, Tiruchirappalli, India [Figure 1].

Preparation of Extraction

The coarse powder plant material was extracted with ethanol using Soxhlet apparatus. The solvent was removed under reduced pressure to get the crude extract. Preliminary phytochemical screenings were followed by the standard methods of Harborne.[3]

Gas Chromatography-Mass Spectrometry (GC-MS) Analysis

GC-MS analysis of the *A. aspera* was performed using a Shimadzu GC-MS-QP2010.

Figure 1: Collection and authentication of experimental plants

Animals

In this present study, healthy Wistar rats weighing approximately 110–150 g were used throughout the study. All the animals were maintained the animal house under the standard condition of temperature (24°C ± 2°C) and relative humidity (30–70) with a 12:12 light:dark cycle. The animals were fed with stranded pellet diet and water. The animal handling was performed according to good laboratory practice. Ethical clearance was obtained from the Institutional Animal Ethical Committee (CPCSEA/265/2015) and conducted according to the Indian National Science Academy guidelines for the use and care of experiments.

Acute Toxicity Study

The animals were grouped and each group comprising of 6 rats was administered orally with *A. aspera* logarithmic concentration (150, 300, 450, 600, and 750 mg/kg). The control group received only the normal saline (10 mL/kg body weight [b.w]). The toxicological effects were observed in terms of mortality and expressed as lethal concentration, 50% (LD_{50}). Toxic symptoms and behavioral changes of rats were also observed for each group at 24, 48, and 72 h after dose administration. The LD_{50} of the *A. aspera* extract was calculated by the method of Miller and Tainter.[4]

Animal Studies

Animal were divided into five groups (6 animals in each):
Group 1: Normal Control
Group 2: Negative Control (STZ 1% w/v)
Group 3: Positive control (glibenclamide, 5 mg/kg)
Group 4: Ethanolic extract of *A. aspera* (300 mg/kg)
Group 5: Ethanolic extract of *A. aspera* (600 mg/kg).

Induction of Diabetes Mellitus

STZ was used to induce diabetes mellitus in normoglycemic male albino Wistar rats. A freshly prepared solution of STZ

(65 mg/kg b.w) in 0.1 M citrate buffer, pH 4.5 was injected i.p., in a volume of 1 mL/kg b.w to overnight fasted rats. After 48 h of STZ administration, rats with moderate diabetes having glycosuria and hyperglycemia were selected for the experiment.

Examination of b.w

The b.w of rats from each group was measured on initial stage to 28 days. Weight was measured using standard digital weight balance to get accuracy.

Sample Collection

At the end of the treatment period, all rats were fasted for 12 h and sacrificed by cervical decapitation. The blood was collected into heparinized tubes, and plasma and serum were separated by centrifugation and used for biochemical analysis.

Biochemical Parameter

Blood glucose levels were analyzed using glucometer. Plasma insulin was assayed using the Ultrasensitive ELISA kit for rat insulin (Linco Research, St. Charles, MO, USA). Analysis of glycosylated hemoglobin was performed using HPLC (HBA1C) analyzer.

Estimation of Lipid Profile

Total cholesterol and triglyceride of serum were estimated using the standard method of Varley.[5] High-density lipoprotein (HDL) cholesterol was determined by phosphotungstate/magnesium method of Lyons.[6] VLDL cholesterol was calculated as triglycerides (mg/dL)/5. LDL cholesterol was calculated by the equation: LDL cholesterol = total serum cholesterol - (HDL+VLDL).

Estimation of Antioxidants

Enzymatic antioxidants

The estimation of enzymatic antioxidants superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), and glutathione (GSH) was measured. The activity of SOD was assayed by the method of Kakkar *et al*.,[7] CAT was estimated by the method of Sinha,[8] and GPx was measured by the method described by Rotruck *et al.*[9]

Non-enzymatic antioxidants

The estimation of non-enzymatic antioxidants such as Vitamins E, Vitamin C, and GSH was estimated. Vitamins C estimated by the method of Omaye *et al.,*[10] Vitamin E was estimated by the method of Baker *et al.,*[11] and GSH was measured by the method of Ellman.[12]

Histological Analysis

On the 28th day, pancreatic tissues were taken from animals which were fasted overnight under ether anesthesia. The whole pancreas from each animal was removed after sacrificing the animal and washed on ice cold saline immediately. A portion of pancreatic tissue was fixed in 10% neutral formalin fixative solution for histological studies. After fixation tissues were embedded in paraffin, solid sections were cut at 5 µm and the sections were stained with haematoxylin and eosin. The photomicrographs of histological studies are taken.

Statistical Analysis

All the results are presented as mean analyzed by the standard deviation method with the help of SPSS software. Results were considered statistically at $P < 0.001$.

RESULTS

Preliminary Photochemical Analysis

In the present study, the preliminary phytochemical content such as alkaloids (0.42%), flavonoids (0.62%), saponins (4.5%), tannins (1.0%), terpenoids (0.20%), carbohydrates (0.16%), and protein (2.5%) was recorded in *A. aspera* extract.

GC-MS Analysis of *A. aspera*

The presence of bioactive compounds in the *A. aspera* extract was identified using GC-MS analysis. The GC-MS chromatogram [Figure 2] expresses 13 major peaks and has been identified after comparison of the mass spectra with NIST library [Table 1], indicating the presence of bioactive compounds such as 1-monolinoleoylglycerol trimethylsilyl ether, stigmasterol, 9,12,15-octadecatrienoic acid, hexadecanoic acid, hexadecanoic acid–ethyl ester, phytol, squalene, digitoxin, 1-hexadecanol, octasilox ane,1,1,3,3,5,5,7,7,9,9.1,11,13,13,15,15,-hexadecamethyl, dl-alpha tocopherol, phytol acetate, oleic acid–eicosyl ester, Vitamin E, 13-docosenamide, ethyl iso-allocholate, 1-heptatriacotanol, and 6-octadecenoic acid.

Acute Toxicity Study

No significant changes were observed in the behavioral or autonomic responses in mice after treatment with different doses of ethanolic seed extracts of *A. aspera*.

Examination of b.w

The b.w changes in control and experimental groups were illustrated in Table 2. The b.w of diabetic rats

Table 1: GC-MS analysis of ethanolic extraction of *A. aspera* seeds

Name of compound	Nature of compound	Molecular weight	Activity
1-Monolinoleoylglycerol trimethylsilyl ether	Steroid	498	Antimicrobial
Stigmasterol		412	Antihepatotoxic,antioxidant,hypocholesterolemic,anti-inflammatory,estrogenic, antiviral
9,12,15-octadecatrienoic acid	Linolenic acid	278	Anti-inflammatory, anti-arthritic, anti-coronary, cancer preventive, nematicide, insectifuge
Hexadecanoic acid	Palmitic acid	258	Antioxidant, nematicide
Hexadecanoic acid, ethyl ester	Ester compound	284	Antioxidant, nematicide, flavor, pesticide
Phytol	Diterpene	296	Antimicrobial, Anticancer, Anti-inflammatory,
Squalene	Triterpene	410	Antibacterial, Antitumor, Antioxidant
Digitoxin	Steroid	764	Cardiotonic
1-Hexadecanol	Alcohol	242	Antioxidant
Octasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15,-hexadecamethyl	Volatile organic compounds	578	Antimicrobial
DL-alpha tocopherol	Vitamin E	430	Antiseptic, preservative flavor stomach, and internal hemorrhoids
Phytol acetate	Phytol compound	338	Antimicrobial, anti-inflammatory
Oleic acid, eicosyl ester	Oleic acid ester	562	Anti-inflammatory, cancer preventive, dermatitigenic
Vitamin E	Vitamin compound	430	Antidiabetic, analgesic, anti-inflammatory, antileukemic, anticancer
13-docosenamide, (z)-	Amide Compound	337	337 Antimicrobial
Ethyl iso-allocholate	Steroid	436	Antimicrobial
1-heptatriacotanol	Alcoholic compound	536	Antimicrobial
6-octadecenoic acid (z)	Stearic acid	282	Cancer preventive insectifuge

GC-MS: Gas chromatography-mass spectrometry

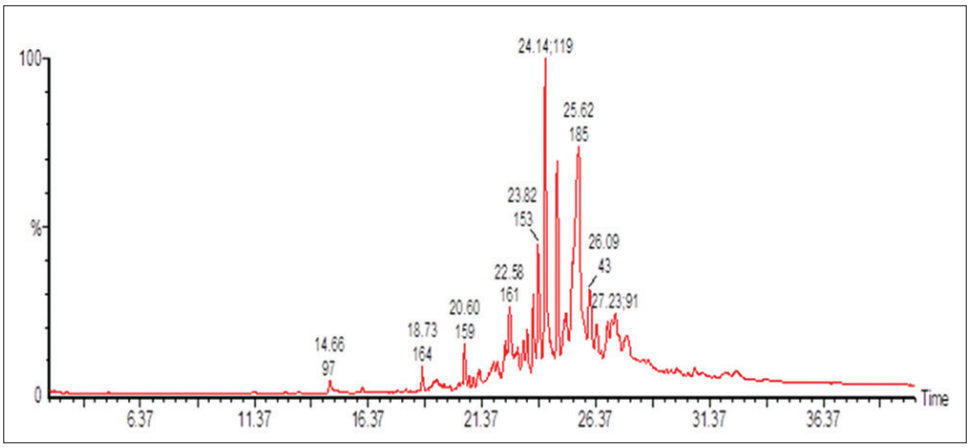

Figure 2: Gas chromatography-mass spectrometry analysis of ethanolic extraction of *Achyranthes aspera* seeds

significantly decreased when compared with control group. Supplementation of ethanolic seed extracts of *A. aspera* showed a significant improvement in the b.w of diabetic rats.

Estimation of Blood Glucose, Plasma insulin, and Hemoglobin

The blood glucose, plasma insulin, and hemoglobin levels of normal and experimental rats were express in Table 3. There was a significant increase in the level of blood glucose and plasma insulin, and decreased hemoglobin was observed in diabetic animals compared to the control group.

Estimation of Serum Lipid Profile

In the present study, Table 4 expresses the changes in serum lipids levels by administration of ethanolic seed extracts of *A. aspera* in diabetic rats. The triglycerides, VLDL, LDL, and the total cholesterol levels were significantly decreased, and HDL level improved in oral administration of ethanolic seed extracts of *A. aspera* 300 mg/kg of b.w, which was compared to that of glibenclamide group.

Estimation of enzymatic, non-enzymatic antioxidant, and lipid peroxidation

In the present study, Table 5 shows that inhibition of antioxidant activity during STZ induced diabetic animals may be improved generation of reactive free radicals, Which can create an oxidative stress in the cells. The administration ethanolic seed extracts of *A. aspera* 300 and 600 mg/kg b.w doses the SOD, CAT, GPx, GSH, Vitamin C, and Vitamin E activity as well as TBARS level in the blood plasma, which protected from the free radical-induced oxidative stress. This result supports that the antioxidant properties of the ethanolic seed extracts of *A. aspera* was excellent as compared with the standard drug glibenclamide.

Histological Assay

Multiple sections of pancreas were taken and studied for histological changes in the *A. aspera* extract-administered groups and control group [Figure 3a]. Histological findings of the pancreas in the extract-administered group and control group were tentatively similar. STZ-induced diabetic rats showed extensive damage on the islets of Langerhans cells [Figure 3b]. The orally administered extracts of *A. aspera* (200 and 300 mg/kg) and commercial drug glibenclamide (100 mg/kg) [Figure 3c and d] were showed restoration of normal cellular population and enlarged size of beta cells with hyperplasia found in islets of Langerhans cells in the pancreas. The pancreas present in the group of animals treated with the extracts of *A. aspera* extracts (300 mg/kg) clearly showed that Partial restoration of normal cellular population and enlarged size of beta cells. The islets were normal in size, shape, and number comparatively similar to that of standard-treated drug [Figure 3e].

Table 2: Effect of *A. aspera* ethanolic seed extract on the changes of b.w

Groups	b.w (g)	
	Initial (0 day)	Final (28 days)
Normal control (normal saline alone)	205.15±19.12	230.21 ± 19.14
Negative control (STZ 1% w/v)	182.04±1.2	149.11 ± 11.4*
Ethanolic extract of *A. aspera* (300 mg/kg)	185.05±3.02	188.19± 2.45
Ethanolic extract of *A. aspera* (600 mg/kg)	190.13±18.11*	195.14± 12.21*
Positive control (glibenclamide, 5 mg/kg)	202±11.25 **	225 ±12.35 **

Values are given as mean ± S.D (*n*=6 rats). **P*<0.01 versus control. ***P*<0.001 versus control by students *t*-test. Values are given as mean ± S.D (*n*=6 rats). S.D: Standard deviation, *A. aspera*: *Achyranthes aspera*, b.w: Body weight, STZ: Streptozotocin

Table 3: Effect of *A. aspera* ethanolic seed extract on the levels of blood glucose, plasma insulin and hemoglobin

Groups	Blood glucose (mg/dL)	Plasma insulin (µg/mL)	Hemoglobin (g/dL)
Normal control (normal saline alone)	86.44 ± 8.81	16.27 ±0.13	12.47 ± 1.35
Negative control (STZ 1% w/v)	285.11± 19.33	7.28 ± 1.54	8.97 ± 0.49
Ethanolic extract of *A. aspera* (300 mg/kg)	108.14 ± 4.57*	11.06 ± 1.21*	11.88 ± 0.45*
Ethanolic extract of *A. aspera* (600 mg/kg)	103.11 ± 4.57*	14.05 ± 1.21*	13.07 ± 1.58*
Positive control (glibenclamide, 5 mg/kg)	84.27 ± 6.23**	15.16 ± 2.43**	14.88 ± 3.12**

Values are given as mean ± S.D (*n*=6 rats). **P*<0.01 versus control. ***P*<0.001 versus control by students *t*-test. Values are given as mean ± S.D (*n*=6 rats). S.D: Standard deviation, *A. aspera: Achyranthes aspera*, STZ: Streptozotocin

Table 4: Effect of *A. aspera* ethanolic seed extract on lipid profile of experimental rats

Treatment	TGL mg/dl	HDL mg/dl	VLDL mg/dl	LDL mg/dl	Total C cholesterol	Serum phospholipid (mg/dL)
Normal control (Normal Saline alone)	77.15±6.68	39.6±2.71	17.03±1.51	43.9±4.25	98.16±8.76	106.61 ± 5.09
Negative control (STZ 1% w/v)	133.32±10.05	25.31±1.64	32.14±2.86	96.47±8.12	215.2±7.23	67.18 ± 4.27
Ethanolic extract of *A. aspera* (300 mg/kg)	104.45±11.14	27.56±2.62	31.24±1.64	94.19±6.77	182.86±10.05	71.12 ± 5.17
Ethanolic extract of *A. aspera* (600 mg/kg)	94.18±8.76*	37.23 ± 3.06*	30.19±2.8*	88.41±2.34	172.3±5.12*	87.51 ± 4.19*
Positive control (glibenclamide, 5 mg/kg)	82.04±6.71**	38.91±5.14**	16.92±1.34**	33.9±2.66**	96.2±4.8**	99.73 ± 5.44**

Values are given as mean ± S.D (*n*=6 rats). **P*<0.01 versus control. ***P*<0.001 versus control by students *t*-test. Values are given as mean ± S.D (*n*=6 rats). S.D: Standard deviation, *A. aspera: Achyranthes aspera*, STZ: Streptozotocin, HDL: High-density lipoprotein, LDL: Low-density lipoprotein

Table 5: Antioxidant activity of in *A. aspera* ethanolic seed extract

Groups	SOD (µmol/mg protein)	CAT (µmol/mg protein)	GPx (µmol/ mg protein)	Vitamin E (mg/dl)	Vitamin C (mg/dl)	GSH (nmol/mg protein)
Normal control (normal saline alone)	65.03±1.92	25.03±1.92	20.90±1.24	1.65±0.19	1.65±0.19	24.74±2.29
Negative control (STZ 1% w/v)	48.09±1.22	14.20±1.09	13.25±1.09	3.12±0.34	3.12±0.34	15.20±1.20
Ethanolic extract of *A. aspera* (300 mg/kg)	62.30±2.41*	22.35±1.92*	17.33±1.58*	2.14±0.21*	2.14±0.21*	22.35±1.03*
Ethanolic extract of *A. aspera* (600 mg/kg)	63.40±2.05**	24.22±1.90**	18.46±1.74**	1.63±0.18**	1.63±0.18**	23.44±1.98**
Positive control (glibenclamide, 5 mg/kg)	60.40±2.05**	23.22±1.90**	17.46±1.74**	1.53±0.18**	1.33±0.18**	22.44±1.98**

Values are given as mean ± S.D (*n*=6 rats). **P*<0.01 versus control. ***P*<0.001 versus control by students *t*-test. Values are given as mean ± S.D (*n*=6 rats). S.D: Standard deviation, *A. aspera: Achyranthes aspera,* STZ: Streptozotocin, SOD: Superoxide dismutase, CAT: Catalase, GPx: Glutathione peroxidase, GSH: Glutathione

DISCUSSION

In light of the results, our study indicates that ethanolic seed extracts of *A. aspera* have good antidiabetic activity. In GC-MS analysis, most of the bioactive compounds are responsible in diabetes mellitus. Ethanolic extracts of *A. aspera* exhibited significant anti-hyperglycemic activities in STZ-induced hyperglycemic rats without significant change in b.w; they can also improve the condition of diabetes mellitus as indicated by parameters such as b.w, blood glucose, plasma insulin, hemoglobulin, and lipid profile along with enzyme and non-enzymatic activity and histopathology analysis. In the present study, STZ-induced hyperglycemia is a widely used experimental model for screening the activity of hypoglycemic agents.

In this model, hyperglycemia arises because of irreversible destruction of the β-islet cells of the pancreas by STZ, causing a reduction of insulin secretion. The generation of reactive oxygen species (ROS) and the subsequent increase of local oxidative stress, DNA methylation, and protein modification are suggested as the pathophysiological mechanisms of STZ-induced diabetes.[13]

The fundamental mechanism underlying hyperglycemia in diabetes mellitus involves overproduction and decreased utilization of glucose by the tissues. In our study, the difference observed between the initial and final blood glucose levels of different groups under investigation revealed a significant elevation in blood glucose in the diabetic control group as compared to normal animals, at the end of the 28 days' experimental period, the blood glucose level was significantly increased in diabetic as compared with STZ as compared to normal control. Administration of *A. aspera* (300 mg/kg and 600 mg/kg) significantly reduced the raised blood glucose

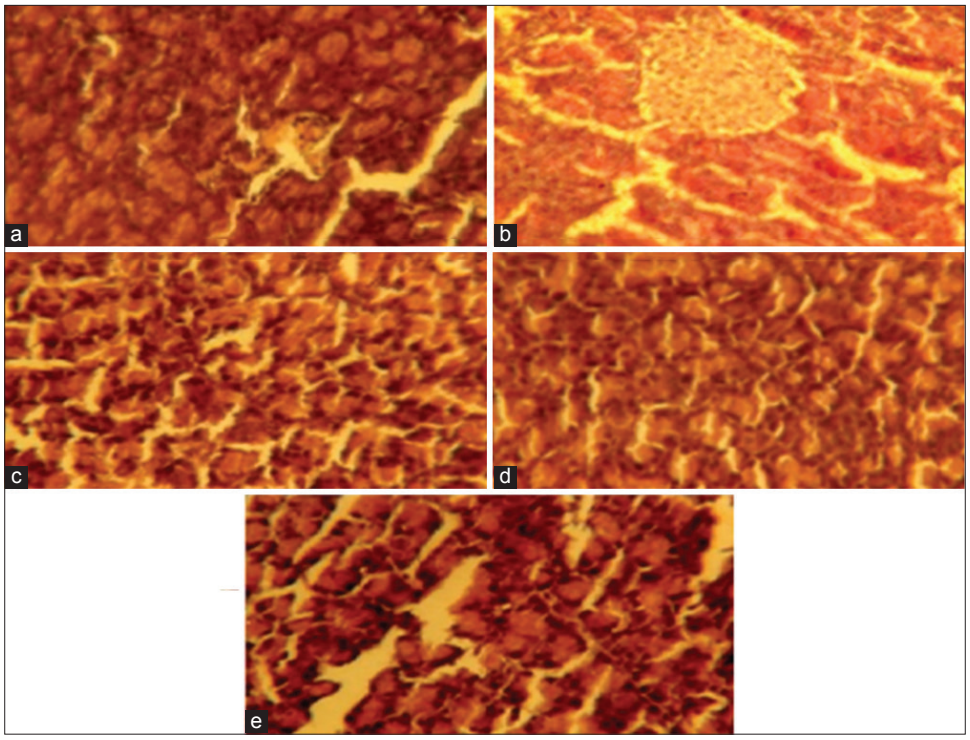

Figure 3: Histopathology analysis of *Achyranthes aspera* extraction of streptozotocin (STZ) induced of control and experimental rats on islets of Langerhans. (a) Control, (b) STZ induced, (c) ethanolic extract of *A. aspera* (300 mg/kg), (d) ethanolic extract of *A. aspera* (600 mg/kg), (e) glibenclamide

level ($P < 0.01$) in STZ-induced diabetic rats, and the lowering was almost comparable to glibenclamide. Further, this antidiabetic activity of *A. aspera* associated with an increase in the insulin level revealed that *A. aspera* may stimulate insulin secretion from regenerated β cells and remaining β-cells. Hemoglobin is routinely used as a marker for long-term glycemic control. Persistent hyperglycemia in diabetes manifests as increased HbA1C level as a result of glycation of hemoglobin. Diabetic patient was reported with an increased level of HbA1C up to 16%.[14] The raised hemoglobin level well correlates with the complication such as diabetic retinopathy, nephropathy, and neuropathy. In addition, there is a relative deficiency of insulin leading to decreased protein synthesis in all tissues consequently reduced the synthesis of hemoglobin in diabetes.[15] Thus, the findings of the present study showed that administration of *A. aspera* (300, 600 mg/kg) significantly decreased the raised hemoglobin level in STZ-induced diabetic rats when compared with glibenclamide and our findings were in agreement with the previous study reported the *A. aspera,*[16] *Caralluma edulis,*[17] *Calocybe indica,*[18] *Helianthus annuus,*[19] *Swertia chirayita*, *Andrographis paniculata*,[20] and *Xanthosoma sagittifolium*.[21]

Lipids play a vital role in the pathogenesis of diabetes mellitus. Diabetic is associated with profound alterations in the plasma lipid, triglycerides and lipoprotein profile and with an increased risk of coronary heart disease.[22] The most common lipid abnormalities in diabetes are hypertriglyceridemia and hypercholesterolemia. The increase in the levels of serum lipids such as cholesterol and triglycerides in the diabetic rats may be due to the fact that under normal circumstances, insulin activates lipoprotein lipase and hydrolyzes triglycerides. Insulin increases the uptake of fatty acids into adipose tissue and increases triglyceride synthesis. Moreover, insulin inhibits lipolysis. In case of insulin deficiency, lipolysis is not inhibited but increased lipolysis which finally leads to hyperlipidemia. In diabetic condition, the concentration of serum-free acids is elevated as a result of free fatty acid outflow from fat deposited, where the balance of the free fatty acid esterification-triglyceride lipolysis cycle is displaced in favor of lipolysis.[23] The lipoprotein pathway facilitates the movement of triglycerides synthesized in the liver to muscle and adipose tissue. Additionally, it also provides a pathway for the transport of cholesterol from the liver to peripheral tissues and thereby acts as a protective factor against coronary heart disease. The level of HDL cholesterol slightly increased after administration of ethanolic extract of *A. aspera* seeds at 300 mg/kg and 600 mg/kg b.w. This might be due to increase in the activity of lecithin cholesterol acyltransferase, which may contribute to the regulation of blood lipids.[24] Administration of ethanolic extract of *A. aspera* seeds lowered cholesterol level at all doses, while 300 mg/kg and 600 mg/kg b.w were able to reduce triglycerides and LDL cholesterol levels. Significant lowering of total cholesterol, triglycerides, LDL-cholesterol, and rise in HDL-cholesterol is a very desirable biochemical state for prevention of atherosclerosis and ischemic conditions.[25]

The free radicals have long been implicated as mediators of tissue, which are released in large amounts into the surrounding tissue. To neutralize this charge, free radicals

try to withdraw an electron from or donate an electron to a neighboring molecule. Other antioxidants work against the molecules that form free radicals, destroying them before they can begin the domino effect that leads to oxidative damage. For example, certain enzymes in the body, such as SOD, CAT, GPx, and GSH, work with other chemical to transfer free radical into harmless molecules.[26]

The antioxidant enzymes SOD and CAT play an important role in reducing cellular stress. SOD scavenges the superoxide radical by converting it to hydrogen peroxide and molecular oxygen, while CAT brings about the reduction of hydrogen peroxides and protects higher tissues from the highly reactive hydroxyl radicals.[27] In this experiment, antioxidant results are show in Table 5 in that the activities of SOD, CAT, and GPx were significantly decreased in tissue of diabetic control rats due to the inadequacy of the antioxidant defenses in combating ROS-mediated damage. The decreased levels of enzymatic antioxidants status were seen in STZ alone treated rats when compared with control group. Ethanolic extracts of *A. aspera* at a dose of 200 mg/kg b.w significantly normalized the enzymatic antioxidant such SOD, CAT, and GPx in diabetic-treated animals. Non-enzymatic antioxidant such as Vitamin C and Vitamin E plays a central role in the antioxidant protective system, protecting all lipids undergoing oxidation and diminishing the number of apoptotic cells. Table 5 shows that the activities of Vitamin C and Vitamin E decreased the level of STZ-induced diabetic rats when compared to control group. The levels of these antioxidants were significantly increased in diabetic rats by treating with seed extract of *A. aspera*. GSH has a multifaceted role in antioxidant defense. It is a direct scavenger of free radicals as well as a cosubstrate for peroxide detoxification by GPx.

The study suggested that diabetic animals are exposed to oxidative stress and *A. aspera* can partially reduce the imbalances between the generation of ROS and the scavenging enzyme activity. According to these results, *A. aspera* could be a supplement, as an antioxidant therapy, and may be beneficial for correcting the hyperglycemia and preventing diabetic complications due to lipid peroxidation and free radicals. The *A. aspera* plant is having a hypoglycemic effect, and it also controls the antioxidant level.

CONCLUSION

The ethanolic extract of *A. aspera* significantly controlled the diabetic condition including oxidative stress in the pancreas. Further investigations regarding the synergistic activity of the extract and bioactive compounds isolation are in the process.

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