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Original Article

ANTIDIABETIC ACTIVITY OF *ASYSTASIA GANGETICA* (L.) T. ANDERSON FLOWER EXTRACT IN STREPTOZOTOCIN INDUCED DIABETIC RATS

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ABSTRACT

Objective: *Asystasia gangetica* has long been used in the antiasthmatic medicinal preparations of traditional healers Nigeria of South Africa, in India the plant is being used for treating diabetes and microbial infections. The present study was evaluated for antidiabetic activity of *A. gangetica* flower ethanol extract (AGFEE) in streptozotocin induced diabetic rats.

M**ethods:** Diabetes was induced by 45 mg/kg STZ and after STZ induction; the hyperglycemic rats were treated with the extract orally at the dose 250 mg/kg body weight daily for 45 d. Antidibetic activity measured by blood glucose, insulin, hemoglobin, glycosylated hemoglobin levels. The oxidative stress was measured in liver and kidney by level of antioxidant markers i.e. lipid peroxidation (LPO), superoxide dismutase (SOD), glutathioneperoxidase (GPx), catalase (CAT) vitamin C and vitamin E and the lipid profile were also studied.

Results: The extract produced significant reduction in blood glucose level and increase insulin level as compared with the diabetic rats and also reduced total cholesterol (TC), triglycerides (TG), low-density lipoprotein (LDL) and increased high-density lipoprotein (HDL) levels in STZ-diabetic rats. It also significantly obliterated the increase in the levels of GPx, SOD and CAT activities in both liver and kidney. The levels of vitamin C and vitamin E were significantly augmented in extract treated diabetic rats in comparison with control group.

Conclusion: This study concludes that the antidiabetic effect of AGFEE proved the folkloric use for various treatments. Further investigations on identification and characterization of the active compounds to carried out.

Keywords: *Asystasia gangetica*, Streptozotocin, Insulin, β-cells, Diabetes

INTRODUCTION

Diabetes mellitus is a metabolic disorder characterized by hyperglycemia with impaired metabolism of carbohydrates, fat and proteins due to defects in insulin secretion, insulin action or both which may lead to the complications like atherosclerosis, cardiac dysfunction, retinopathy, neuropathy, and nephropathy [1, 2]. *In vivo* models provide valuable evidences in understanding the pathological mechanisms of diabetes and are useful for the screening of drugs for the prevention and treatment of diabetes. STZ is a commonly used chemical to generate diabetic animals in the laboratory for insulin-dependent diabetes mellitus characterized by high fasting blood glucose levels and a drastic reduction in plasma insulin concentration [3]. Oxidative stress is a major problem observed during diabetes induced by the generation of free radicals. The increase in the levels of reactive oxygen species and free radicals cause damage in the biological structures and also cause microvascular and macrovascular complications, cardiovascular diseases, kidney and nerve damage [4]. Several antioxidants of plant origin have been evaluated for their antioxidant property to protect the biological system against oxidative stress in diabetes. In addition plant secondary metabolites such as phenols, flavonoids and alkaloids are reported in preventing the formation of reactive oxygen induced diseases [5].

Medicinal plants have always been an exemplary source of drugs and many of the currently available drugs have been derived directly or indirectly from them. *Asystasia gangetica* (L) T. Anderson (Acanthaceae) is a fast growing, spreading, perennial herb, plant is used in ethnomedicine for the treatment of heart pains, stomach pains, rheumatism, while in Nigeria, the leaves are popularly used in the treatment of asthma [6]. In India, the plant is being used for rheumatism and the sap is applied to swellings [7]. In Tamil Nadu, the plant root paste is used for skin allergies [8]. It reported to is use as a folk remedy for the treatment of diabetes mellitus in parts of south India [9]. Till today no studies reported on the diabetic activity of flowers of *A. gangetica,* therefore in the present study, we evaluated antidiabetic activity of AGFEE in streptozotocin induced diabetic rats.

MATERIALS AND METHODS

Extraction of plant sample

The plant sample was collected from surrounding area of Karpagam University campus, Coimbatore, Tamil Nadu, India and was identified as *A. gangetica* (L.) T. Anderson (Acanthaceae) by Botanical Survey of India, Coimbatore (Voucher specimen number BSI/SRC/5/23/2011-12/Tech-667). Flowers were dried at room temperature for 2 w and ground into powder. Dry powder (200 g) was dissolved in 1000 ml of ethanol and kept in a rotary shaker for 72 h. The extract was filtered; the filtrate was concentrated in a room at 40 °C for 24 h and stored at 4 °C for further use.

Animals

Rats weighing (150-200 g) were maintained under standard conditions of humidity temperature (28±2ºC) and light (12 h light/dark). The animals were housed in polypropylene cages (45×24×15 cm) and were handled according to the university and institutional legislation, regulated by the ethical committee (Regd. No. 739/03/abc/CPCSEA).

Experimental induction of diabetes

The male albino Wistar rats weighing (150-200 g) were made diabetic by intraperitoneal injections of STZ. The animals were allowed to fast for 24 h and were given STZ injection (45 mg/kg bw), with freshly prepared aqueous solution of citrate buffer as a vehicle, pH 4.5. The control animals received buffer alone. STZ treated animals were allowed to drink 5% glucose solution overnight to overcome drug induced hypoglycemia. After 72 h of STZ administration, the blood glucose range above 250 mg/dl were considered as diabetic rats and used for the experiment.

Experimental design

In our previous studies, three different doses (125, 250 and 500 mg/kg bw)were tested for blood glucose lowering potentiality, among the three doses at the dose of 250 mg/kg bw the AGFEE showed maximum activity. Hence in this study, we selected 250 mg/kg bw dose for antidiabetic study. Animal were randomly divided into five groups of six animals each: Group I served as control rats, group II consisted of diabetic control rats, group III served as diabetic rats treated with AGFEE (250 mg/kg bw), group IV consisted of diabetic rats treated glibenclamide (5 mg/kg bw) and group V were normal rats treated with AGFEE alone (250 mg/kg bw).

Biochemical studies

After the treatment of 45 d, the animals were deprived of food overnight and sacrificed by cervical decapitation for biochemical parameters i.e. hemoglobin [10], urea [11] uric acid [12] total protein [13] creatinine [14] HDL [15] triglycerides [16] total cholesterol [17]. The liver and kidney were carefully removed, weighed and washed in ice-cold saline to remove the blood. Then both were sliced separately into pieces and homogenized with buffer containing 0.25 M sucrose and 0.1M Tris HCl buffer (pH7.4). The homogenate was centrifuged at 3000 ×g for 10 min at 0 °C in a cold centrifuge. The supernatant was separated and used for enzymatic antioxidant like superoxide dismutase [18], catalase [19], glutathione peroxidase [20], and non-enzymatic antioxidants like total reduced glutathione [21], vitamin C [22] and vitamin E [23], and also used for the determination of lipid peroxidation [24].

Histopathological study

One portion of pancreas of all the experimental groups was fixed in 10% formalin for histological observation by using the method of Dunn 1974 [25]

Statistical analysis

All quantitative measurements were expressed as mean±SD for control and experimental animals. The data were analyzed using one way analysis of variance (ANOVA) followed by Duncan's Multiple Range Test (DMRT) by using statistical package of social science (SPSS) version 10.0 for Windows. A difference in the mean values of P<0.05 was considered to be statistically significant.

RESULTS AND DISCUSSION

Diabetes mellitus is a common and very prevalent disease affecting the citizens of both developed and developing countries. It is estimated that 25% of the world population is affected by this disease. Diabetes mellitus is caused by the abnormality of carbohydrate metabolism which is linked to low blood insulin level or insensitivity of target organs to insulin. Currently, the use of alternative medicines especially consumption of phytochemicals have been rapidly increasing worldwide. As herbal medicines are less damaging than synthetic drugs they have better compatibility thus improving patient tolerance even on long-term use [26, 27].

The current study was carried out to determine the antidiabetic activity of *A. gangetica* flowers in STZ induced diabetic rats. STZ has been extensively used to induce diabetes mellitus in experimental rat models. In the present study at the dose of 45 mg/kg bw induced diabetes and produced a significant increase in plasma glucose level above 250 mg/dL by selectively destroying the pancreatic insulin secreting β cells. STZ produced significant (p<0.05) elevation in blood glucose levels of diabetic rats as compared to normal rats during the study (fig. 1). In our previous studies, we evaluated blood glucose lowering potentiality using three different doses (125, 250 and 500 mg/kg bw) of AGFEE, At the dose of 250 mg/kg bw the extract showed significant activity [28]. Hence, we selected this particular dose for this present study. After administration of extract at a dose of 250 mg/kg to diabetic rats significant (p<0.05) decreased level of blood glucose was observed. The significant reduction was evident from the second week onwards, the maximum decrease of blood glucose was observed on 5th week after administration of extract. Fig. 2 shows the body weight of control and experimental rats before and after administration of extract. Streptozotocin induced diabetic animals those are treated with extract at the dose of 250 mg/kg significantly (p<0.05) increased the body weight whereas diabetic control continued to lose weight till the end of the study.

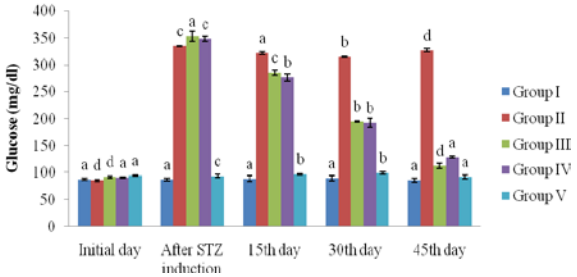

Fig. 1: Effect of AGFEE on glucose levels of control and experimental rate
Values are expressed as mean±SD (n=6). Values that have a different superscript letter (a-e) differ significantly with each other at P<0.05 (Duncan's multiple range test)

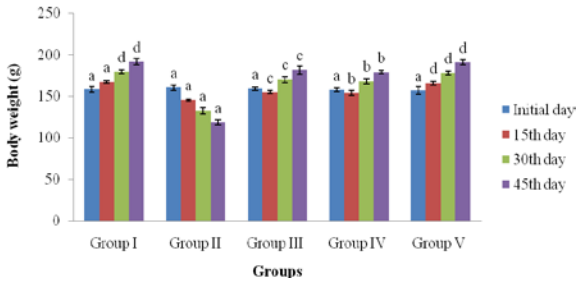

Fig. 2: Effect of AGFEE on body weight of control and experimental rate
Values are expressed as mean±SD (n=6). Values that have a different superscript letter (a-e) differ significantly with each other at P<0.05 (Duncan's multiple range test)

STZ-induced diabetes is characterized by a severe loss in body weight due to increased muscle wasting [29]. The decrease in body weight is due to the increased muscle destruction or degradation of structural proteins [30] When diabetic rats were treated with extract hey showed an improvement in body weight in comparison to the diabetic control group, which signifies its protective effect in controlling muscle wasting and the ability to protect body weight loss seems to be the result of its ability to reduce hyperglycemia.

Table 1 represents the effect of the extract on insulin, hemoglobin and glycosylated hemoglobin in normal and STZ induced diabetic rats. Administration of extract at the dose of 250 mg/kg bw caused significant (p<0.05) increase in insulin and hemoglobin level and decrease in glycosylated hemoglobin level. There were no significant differences observed in extract alone treated rats compared to normal control rats. A significant increase in serum urea, uric acid and creatinine levels and a significant decrease in plasma total protein levels were observed in diabetic rats whereas treatment with extract for 45 d produced reduced serum urea and creatinine level and increased total protein when compared to those of diabetic groups (table 1).

Serum TC, TG and LDL were significantly (p<0.05) higher and levels of HDL were lower in STZ-induced diabetic rats than those in normal Control rats (table 1). Treatment with extract and glibenclamide resulted in a significant decrease (p<0.05) in TC, TG and LDL and increase HDL levels compared to those in STZ-induced diabetic rats (table 1).

Table 1: Effect of AGFEE on biochemical parameters in normal and experimental rats

Parameters	Group I	Group II	Group III	Group IV	Group V
Insulin (µU/ml)	16.58±0.75^{d}	4.26±0.65^{a}	15.42±0.53^{c}	13.21±0.72^{b}	16.64±0.42^{d}
Hb (g/dl)	13.52±0.82^{d}	8.47±0.16^{a}	12.81±0.70^{c}	12.60±0.25^{b}	13.39±1.21^{d}
HbA1c (%)	5.36±0.32^{d}	12.89±0.13^{a}	5.64±0.56^{c}	5.57±0.54^{b}	5.28±0.33^{d}
Urea mg/dl	28.24±0.82^{a}	64.29±0.29^{c}	32.37±0.14^{b}	31.78±0.57^{b}	28.50±0.42^{a}
Uric acid	5.47±0.52^{a}	12.80±0.28^{c}	6.75±0.72^{b}	7.63±0.22^{b}	5.75±0.58^{a}
Total protein/dl	6.88±0.84^{a}	4.25±0.52^{c}	6.35±0.16^{b}	6.22±0.88^{b}	6.75±0.40^{a}
Crearinine/dl	0.855±0.34^{a}	1.327±0.21^{c}	0.904±0.44^{b}	0.962±0.28^{b}	0.882±0.26^{a}
HDL	32.48±6.2^{c}	17.36±1.22^{a}	26.71±3.44^{b}	25.63±2.89^{b}	4.48±2.46^{c}
Triglycerides	76.29±2.3^{a}	164.84±3.8^{e}	89.37±5.29^{c}	95.61±4.20^{d}	78.45±5.82^{b}
Cholesterol	68.48±1.84^{b}	182.26±2.84c	74.32±3.75^{a}	72.48±1.32^{a}	65.26±4.48b
LDL	45.58±1.37^{a}	188.24±0.06^{c}	54.86±2.62^{b}	62.32±1.08^{b}	43.18±2.16^{a}

Values are expressed as mean±SD (n=6). Values that have a different superscript letter (a-d) differ significantly with each other at P<0.05 (Duncan's multiple range test).

In the present study, the decreased level of total hemoglobin in diabetic rats is mainly due to the increased formation of HbA1c. During diabetes mellitus, the excess glucose present in the blood reacts with hemoglobin to form HbA1c. The amount of HbA1cincrease is directly proportional to the fasting blood glucose level [31]. Administration of extract to diabetic rats reduced the glycosylation of hemoglobin by virtue of its normoglycaemic activity and thus increases the levels of hemoglobin in diabetic rats.

Urea, uric acid and creatinine in the serum, which are considered significant markers of renal function [32], were significantly reverted to normal levels after treatment with extract and glibenclamide for 45 d. The decreased levels which indicate that the extract prevented the progression of renal damage in diabetic rats. The concentration of lipids, such as Triglycerides, Total cholesterol was significantly increased, whereas HDL cholesterol was decreased in the diabetic rats than normal rats. Apart from hyperglycemia, diabetes mellitus is accompanied by hypercholesterolemia, hyperlipidemia and hepatic steatosis [33] The hypercholesterolemia is a consequence of accelerated fatty acid oxidation to acetyl CoA which is the primary substrate for cholesterol synthesis [34]. Treatment with extract produced a significant reduction in elevated triglycerides, total cholesterol and significant (p<0.05) increase in (high density lipoprotein) HDL levels within 45 d in diabetic rats. The impairment of insulin secretion results in enhanced metabolism of lipids from the adipose tissue to the plasma. A variety of derangements in metabolic and regulatory mechanisms, due to insulin deficiency, are responsible for the observed accumulation of lipids [35]. Further, it has been reported previously that diabetic rats treated with insulin show normalized lipid levels [36]. Diabetic rats treated with extract and glibenclamide also normalized lipid levels which indicate that extract also may possess insulin like action by virtue of the ability to lower the lipid levels.

The effects of extract on the levels of serum lipid profile in experimental diabetic rats are given in table 1. In diabetic control rats, a significant increase in levels of cholesterol, triglycerides, LDL and decreased level of HDL were observed when compared to normal rats.

The oxidative stress and resultant tissue damage are important component in the pathogenesis of diabetic complications. Diabetes is associated with increased formation of free radicals and decreases in antioxidant potential. The increase in oxygen free radicals in diabetes could be due to increase in blood glucose levels, which upon autoxidation generate free radicals [37]. Superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx) are enzymatic antioxidants that play a vital role in preventing cells from being exposed to oxidative damage. SOD is capable of reducing the superoxide radical into hydrogen peroxide (H_2O_2) and CAT catalyzes the reduction of H_2O_2 and protects the tissues against reactive hydroxyl radicals [38]. In diabetes mellitus, high glucose can inactivate antioxidant enzymes SOD, CAT and GPx by glycating these proteins thus producing induced oxidative stress, which in turn, causes lipid peroxidation [39] table 2 reports the activities of enzymatic antioxidants i.e. superoxide dismutase (SOD), catalase (CAT) and Glutathione peroxidase (GPx) in kidney and liver of normal control, diabetic control and diabetic treated rats. Administration of extract for 45 d showed significantly (P<0.05) improvement in the activities of these enzymes in diabetic rats.

Table 2: Effect of AGFEE on enzymatic antioxidants in liver and kidney of control and experimental animals

Groups	Liver			Kidney		
	SOD	CAT	GPx	SOD	CAT	GPx
Group I	4.27±0.23^{a}	1.23±.76^{a}	4.12±.43^{a}	3.48±.92^{a}	1.58±.16^{a}	3.86±.10^{a}
Group II	2.02±.43^{e}	0.530±38^{d}	2.27±.25^{d}	1.65±.28^{e}	0.82±.59^{d}	1.87±.92^{d}
Group III	3.89±.08^{d}	1.18±.49^{c}	3.80±.13^{b}	3.26±.65^{b}	1.52±.42^{c}	3.72±.76^{c}
Group IV	3.65±.48^{c}	1.14±.14^{b}	3.52±.18^{b}	2.88±.58^{c}	1.36±.40^{b}	3.56±.63^{b}
Group V	4.15±.17^{b}	1.24±.72^{a}	4.02±.88^{c}	3.64±.35^{c}	1.57±.83^{a}	3.87±0.82^{a}

SOD: 50% inhibition of NBT reduction/min/mg protein for tissues, CAT: µ mole of H_2O_2 decomposed/min/mg protein, GPx: µg of glutathione consumed/min/mg protein, Values are expressed as mean±SD (n=6). Values that have a different superscript letter (a-e) differ significantly with each other at P<0.05 (Duncan's multiple range test).

In the present study, reduced activity of SOD and CAT were observed in the liver and the kidney; this may be due to the deleterious effect of the accumulation of superoxide anion radicals and hydrogen peroxide [40]. The result clearly shows that extract contains free radical scavenging activity which could exert a beneficial action against pathological alteration caused by the presence of O2•-and OH•. Glutathione peroxidase is a selenium containing enzyme present in significant concentrations that detoxifies H_2O_2 to H_2O through the oxidation of reduced glutathione [41].

Reduced glutathione is one of the most abundant non-enzymatic antioxidant bio-molecules present in tissues. Its functions are the removal of reactive oxygen species and provision of a substrate for GPx and glutathione S-transferase (GST) [42]. Vitamin C at high doses has been shown to reduce the accumulation of sorbitol in the erythrocytes of diabetes and to inhibit the glycosylation of proteins [43]. In the present study reduced vitamin C content level has improved after treatment with extract. The decreased level of vitamin C in diabetic rats may be either due to increased utilization

as an antioxidant defense against increased reactive oxygen species or due to decreased glutathione level since glutathione is required for recycling of ascorbic acid. Vitamin C at high doses has been shown to reduce the accumulation of sorbitol in the erythrocytes of diabetes and to inhibit the glycosylation of proteins [43].

Non-enzymatic antioxidants (GSH, vitamin C and E) of liver and kidney tissue of normal and treated rats are depicted in table 3. Activities of non-enzymatic antioxidants of liver and kidney were markedly reduced in diabetic group compared with control and treated groups. Treatment with extract at the dose of 250 mg/kg bw showed a significant (P<0.05) increase in the activities of non-enzymatic antioxidants. Vitamin E reduces lipid hydroperoxides generated during the process of peroxidation and protects cell structures against damages. The decreased level of vitamin E found in the liver and kidney of diabetic rats as compared with control rats could be due to increased oxidative stress, which accompanies the decrease in the level of antioxidant and may be related to the cause of diabetes mellitus [44, 45].

Table 3: Effect of AGFEE on non-enzymatic antioxidants in liver and kidney of control and experimental animals

	Liver (µg/g)			Kidney (µg/g)		
Groups	**GSH**	**Vitamin C**	**Vitamin E**	**GSH**	**Vitamin C**	**Vitamin E**
Group I	9.44±1.08^{a}	1.132±0.080^{a}	1.237±0.03^{a}	8.24±0.37^{a}	1.236±.116^{a}	1.108±0.064^{a}
Group II	6.28±0.86^{d}	0.812±0.021^{c}	0.853±0.10^{d}	4.22±0.42^{d}	0.836±0.04^{d}	0.755±0.085^{e}
Group III	9.14±0.44^{c}	1.114±.043^{b}	1.187±0.08^{c}	7.92±0.86^{c}	1.122±0.03^{c}	1.117±0.06^{d}
Group IV	8.68±0.27^{b}	1.117±0.068^{b}	1.165±0.03^{b}	7.58±0.61^{b}	1.104±0.05^{b}	1.065±0.04^{c}
Group V	9.47±0.35^{a}	1.137±0.052^{a}	1.235±0.04^{a}	8.27±0.45^{a}	1.222±0.07^{a}	1.114±0.09^{b}

Values are expressed as mean±SD (n=6). Values that have a different superscript letter (a-e) differ significantly with each other at P<0.05 (Duncan's multiple range test).

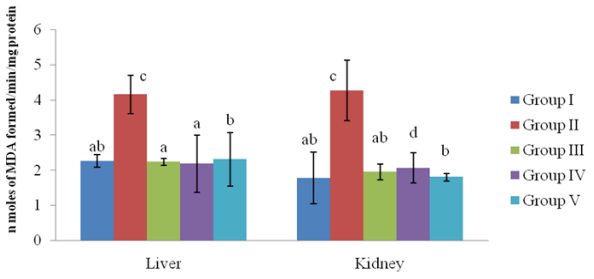

Fig. 3: Effect of AGFEE on the levels of lipid peroxide in liver and kidney of experimental animals
Values are expressed as mean±SD (n=6). Values that have a different superscript letter (a-e) differ significantly with each other at P<0.05 (Duncan's multiple range test)

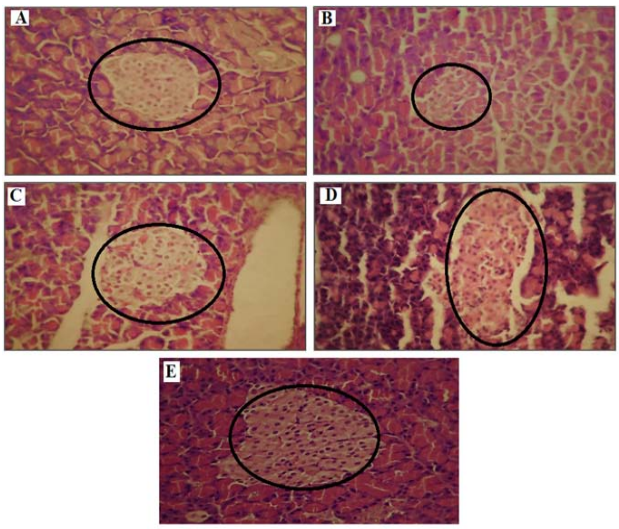

Fig. 4: Histological examination of pancreas of normal and AGFEE treated rats, A: Group I (Normal), B: Group II (Diabetic control),C: Group III (Diabetic+glibenclamide 5 mg/kg), D: Group IV (Diabetic+AGFEE 250 mg/kg), E: Group V (AGFEE 250 mg/kg)

Lipid peroxidation is one of the characteristic features of chronic diabetes and lipid peroxidation mediated tissue damage has been observed in diabetic conditions [46]. In the present study, the increased lipid peroxidation during diabetes may be due to the inefficient antioxidant system prevalent in diabetes. The increase in lipid peroxidation indicates an increased oxidative stress as a result of the excessive generation of free radicals. Administration of extract at the dose of 250 mg/kg bw per day for 45 d decreased the lipid peroxidation index significantly (fig. 3).

Histopathological results

Photomicrographs of pancreas of normal and experimental rats are shown in fig. 4. In control animals, a histopathological examination shows normal acini with normal cellular population in the islets (fig. 4 A). In diabetic control rats extensive damage to the islet of Langerhans and reduced dimensions of islets were visible (fig. 4 B), administration of extract in diabetic rats showed increased number of β cells and size of islets (fig. 4 C). Treatment with glibenclamide showed increased number of β cells and size of islets in diabetic rats (fig. 4 D). Extract treated in normal rats showed normal acini and normal cellular population in the islets (fig. 4 E).

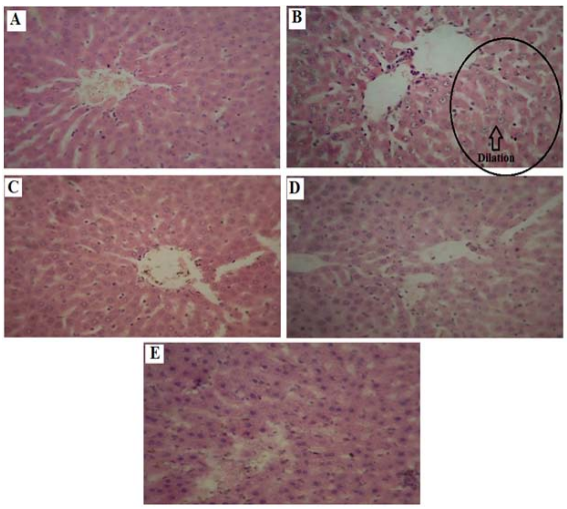

Fig. 5: Histological examination of liver of normal and AGFEE treated rats, A: Group I (Normal), B: Group II (Diabetic control), C: Group III (Diabetic+glibenclamide 5 mg/kg), D: Group IV (Diabetic+AGFEE 250 mg/kg), E: Group V (AGFEE 250 mg/kg)

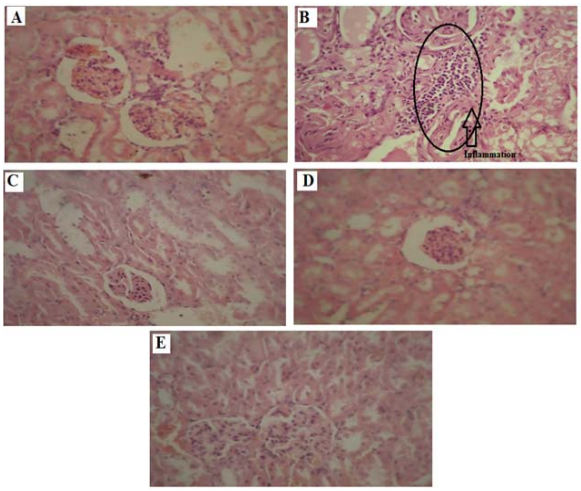

Fig. 6: Histological examination of kidney of normal and AGFEE treated rats, A: Group I (Normal), B: Group II (Diabetic control), C: Group III (Diabetic+glibenclamide 5 mg/kg), D: Group IV (Diabetic+AGFEE 250 mg/kg), E: Group V (AGFEE 250 mg/kg)

Fig. 5 shows the photomicrographs of the liver of normal and experimental rats. The liver of normal group rat shows (fig. 5 A) normal hepatic cells with well-preserved cytoplasm, nucleus, nucleolus and central vein. In diabetic control, the rat's liver section shows lobular architecture, but there was also sinusoidal dilation and reduced hepatocytes observed (fig. 5 B). Liver section of rats treated with extract and glibenclamide maintained lobular architecture normal hepatic cells with well-preserved cytoplasm, nucleus, nucleolus and central vein (fig. 5 C and D). Liver section of extract alone treated rat shows normal hepatic cells with well-preserved cytoplasm, nucleus, nucleolus and central vein (fig. 5 E).

Photomicrographs of kidney sections of normal and experimental rats were showed in fig. 6. The kidney section of normal rats showed well-arranged cells with compact glomerular basement membrane (fig. 6 A). The diabetic control rat's kidney section showed interstitial inflammation, sclerosed glomeruli and dilated tubules (fig. 6 B). Diabetic treated rats with glibenclamide and extract showed restoration of damage in glomeruli and tubules (fig. 6 C and D). The kidney section of extract alone treated rats showed well-arranged cells with compact glomerular basement membrane (fig. 6 E).

CONCLUSION

The present study showed that the supplementation of extract there was a significant decrease in plasma glucose level and simultaneously an increase in body weight and the insulin levels which clarify the antidiabetogenic action of extract. It also showed that the decreased blood glucose, increased antioxidant activity, hypolipidemic and decreased lipid peroxidation. The histopathological study revealed that the treatment with extract showed increase in the number of islets and also prevents the organs by suppressing the oxidative stress in associated with diabetes and increased insulin secretion are due to the insulin released from the regenerated β-cells of the pancreas.

CONFLICT OF INTERESTS

Declared none

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