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Research Article

ANTIHYPERGLYCEMIC ACTIVITY OF *SWERTIA CHIRAYITA* AND *ANDROGRAPHIS PANICULATA* PLANT EXTRACTS IN STREPTOZOTOCIN-INDUCED DIABETIC RATS

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ABSTRACT

Objective: The objective of the present study was undertaken to investigate and compare the antihyperglycemic effects of ethyl acetate and ethanol extract of two plants *Swertia chirayita* and *Andrographis paniculata* in normal and STZ-induced diabetic rats.

Methods: The diabetes was induced by single dose of STZ (60mg/kg bw, i.p.) in fresh citrate buffer, while normal control group was given vehicle only. Normal as well as diabetic rats were divided into groups (n=6) receiving different plant extracts treatment, the diabetic rats treated with 200mg/kg body weight of ethyl acetate and ethanol extract of two plants *S. chirayita* and *A. paniculata* and standard drug glibenclamide (5 mg/kg).

Results: The body weight of each group animals and blood glucose level estimation was performed every week up to the study period. At the end of study animals were sacrificed for biochemical studies. STZ-induced diabetic rats showed marked hyperglycemia, hypertriglycemia and hyper cholesterolemia at the end of study period. Body weight of animal, serum insulin, creatinine value and blood urea nitrogen were increase. Most pronounced the ethanol extract of *A. paniculata* plant showed significant ameliorated the alteration in body weight, blood glucose, serum triglyceride (TG), HDL-cholesterol, LDL-cholesterol, total cholesterol (TC), blood urea nitrogen (BUN), insulin and creatinine levels in diabetic rats. Histological examination of the pancreas of the animal treated with ethanol extract of both plant exhibit almost normalization of the damaged pancreas architecture in rats compare with diabetic rats.

Conclusion: From the results it may be concluded that supplements of the plant extracts mostly the ethanol extract of *A. paniculata* plant has better effects than *S. chirayita*.

Keyword: Streptozotacin, *Swertia chirayita*, *Andrographis paniculata*, Antihyperglycemic.

INTRODUCTION

The Type II diabetes mellitus (DM) is a chronic disease caused by inherited and/or acquired deficiency in production of insulin by the pancreas, or by the ineffectiveness of the insulin produced. Such a deficiency results in increased concentrations of glucose in the blood, which in turn damage many of the body's systems predominantly eye, kidneys and in particular the blood vessels and nerves. Present number of diabetics worldwide is 150 million and this is likely to increase to 300 million or more by the year 2025. Reasons for this rise include increase in sedentary lifestyle, consumption of energy rich diet, obesity, higher life span, etc. **[1,2]** Diabetes mellitus affects people of all ages and ethnic groups. It was estimated that 2.8% of the world's population was diabetic in 2000 and this figure would climb to be as high as 4.4% of the world's population by 2030 **[3,4]** and management of diabetes without any side effects is still a challenge to the medical system. **[5]**

Type II diabetes, which accounts for more than 90–95% of all diabetes, is characterized by metabolic defects, causing insulin resistance. **[6,7]** Sulfonylureas, biguanide, thiazolidinedione, and α-glycosidase inhibitors are widely used to control the hyperglycemia, hyperlipemia and insulin resistance of type II diabetes, but these drugs fail to significantly alter the course of diabetic complications and have limited use because of undesirable side effects hypoglycemia at higher doses, liver problems, lactic acidosis and diarrhea **[8,9]** and high rates of secondary failure. **[10]** Though different types of oral hypoglycemic agents are available along with insulin for the treatment of diabetes, there is an increased demand by patients to use natural products with less side effects antidiabetic activity. **[11]**

Apart from currently available therapeutic options, many herbal medicines have been recommended for the treatment of diabetes. Traditional plant medicines are used throughout the world for a range of diabetic complications. **[12]** Herbal drugs are prescribed widely because of their effectiveness, less side effects and relatively low cost. **[11]** Therefore, investigation on such agents from traditional medicinal plants has become more important. **[13,14]** India has a rich history of using various potent herbs and herbal components for treating diabetes. Many Indian plants have been investigated for their beneficial use in different types of diabetes and reported in numerous scientific journals. **[15,16]**

The genus *Swertia* (Gentianaceae) is an annual or perennial herb comprising more than 170 species. *Swertia* is a popular medicinal herb in Southeast Asia. The plant *Swertia chirayita* Buch-Ham is perhaps one of the oldest herbal medicines used against bronchial asthma and liver disorders in Western India. **[17]** It has been widely used in Ayurvedic and Unani medicine as an anthelmintic, febrifuge and stomach and liver tonic. Several studies have been carried out in animals concerning the hepatoprotective activity of the genus and have shown that the xanthone content of *Swertia* is the most responsible for its antihepatotoxic activity. **[18,19]** In the genus, many components such as xanthone derivatives, iridoids and secoiridoids, flavonoids, triterpenoids and alkaloids have been isolated, and their biological activities investigated. Among the different species of *Swertia* reported in India, *Swertia chirayita* is considered the most important for its medicinal properties. The bitterness, antihelmintic, hypoglycemic and antipyretic properties are attributed to amarogentin (most bitter compound isolated till date). It has also been reported that swerchirin, a compound with xanthone structure, has hypoglycaemic properties [**20,21**] and a protective effect on haematopoiesis in animal models. **[22]**

Andrographis paniculata (Burm.f.) Nees, also known commonly as "King of Bitters", is a member of the plant family Acanthaceae and is an ancient medicinal herb with an extensive ethnobotanical history in Asia. *A. paniculata* commonly known as Kalmegh is an important medicinal plant, occurring wild in India, and is used both in Ayurevda and Unani system of medicine. **[23]** The dried herb is a remedy for a number of ailments related to digestion, hepatoprotection, vermicidal, antiacne, analgesic, anti-inflammatory, antibacterial, antityphoid, hypoglycemic, besides immune enhancement. **[24,28]** *A. paniculata* is used for treating animal diseases, e.g. respiratory infection and diarrhoea, as an alternative to antibiotics. **[29]** A study conducted in Indonesia has also revealed anti HIV activity of the crude extract from the whole plant. **[30]** The therapeutic activities of this plant are attributed to andrographolide and related diterpens, i.e. dexoyandrographolide, 14-deoxy-11,12-didehydroandrographolide and neo-andrographolide. Modern

pharmacological studies indicate that andrographolide protects the liver and gallbladder, and has been found to be slightly more active than silymarin, a known hepatoprotective drug. **[31]** Neo-andrographolide show greater activity against malaria and is hepatoprotective against carbon tetrachloride. **[32,33]**

The present research deals with two selective Indian medicinal plants having pharmacologically established hypoglycemic potential was undertaken with the objective of evaluation and comparison of antihyperglycemic activities of these two plants.

MATERIALS AND METHODS

Plant material

The whole plants *Swertia chirata* Buch-Ham and *Andrographis paniculata* (Burm.f.) Nees was collected from north-east region of India in month of Sep.-Oct. The botanical identification of the plant material was confirmed by the Taxonomist Dr. B. K. Sinha (Scientist E-HOD) Botanical Survey of India, Shillong. A voucher specimen (DPSD-04) was deposited in the herbarium of Department of Pharmaceutical Sciences Dibrugarh University, Dibrugarh, Assam.

Preparation of the extracts

The plant materials were cut into pieces, shade dried for 5 days and then dried in an oven below 55ºc. The dried plant materials were then pulverized into coarse powder in a grinding machine. The powder plant materials were successive solvent extracted separately in petroleum ether, ethyl acetate and ethanol. Solvent from ethyl acetate and ethanol sample was filtered, squeezed off and evaporated off under reduced pressure in a rotary evaporator to obtain crude extract. The ethyl acetate and ethanol extract of both plants used for present study.

Animals

Male *Albino Wistar* rats weighing 200 ± 50 gm were used in this evaluation. These rats aged between 2.5 and 3 months procured from PBRI Bhopal. They were kept in polypropylene cages, under controlled temperature (24±2ºC), humidity and 12/12 h light/dark cycles. The animals were fed standard diet (golden feed, New Delhi) and water given *ad libitum*. These animal experiments were approved by Institutional Animal Ethics Committee (IAEC) of Pinnacle Biomedical Research Institute (PBRI) Bhopal (Reg No.-1283/c/09/CPCSEA). Protocol Approval Reference No. PBRI/IAEC/11/PN-120

Acute Oral Toxicity

The oral toxicity was performed as Organization for Economic Corporation and Development (OECD) guidelines 425. A total five female *Albino Wistar* rat were used, which received a single oral dose (2000 mg/kg body weight) of both plant extracts after overnight fasting. After administration of plant extracts, food was withheld for further 3-4 h. Animal were observed individually at least once during 30 min after dosing, periodically during the first 24h (with special attention during first 4h) and daily thereafter 14 days. At the end of the study the animal were observed for general toxic signs, morphological behavior and mortality. One tenth of LD_{50} (>2000 mg/kg body weight) was taken for the study. **[34]**

Oral hypoglycemic effect of extracts in normal rats

The oral glucose tolerance test was performed in overnight fasted normal animals. Rats divided into six groups were administered 2% gum acacia solution, glibenclamide 5mg/kg, *S. chirayita* and *A. paniculata* ethyl acetate extract (200mg/kg) dissolved in vehicle and *S. chirayita* and *A. paniculata* ethanol extract (200mg/kg) dissolved in vehicle respectively. Glucose (2g/kg) was fed 30min. after the administration of samples. Blood was withdrawn from retro-orbital sinus at 0, 30, 60, 90 and 120min of sample administration. **[35]** Blood glucose levels were estimated by glucose oxidase-peroxidase reactive strips.

Induction of diabetes and Experimental Procedure

A freshly prepared solution of Streptozotocin (60mg/kg bw, i.p.) in 0.1M freshly prepared citrate buffer, pH 4.5 was intraperitoneally in a volume of 1 mL/kg. After 48 hour Streptozotocin administration blood samples were drawn by retro-orbital sinus puncture and glucose levels was determined. The diabetic rats exhibiting blood glucose level in the range 300-340mg/100mL were selected for the studies, **[36]** glibenclamide (5mg) was used as reference standard drug. The dose of glibenclamide was selected based on previous reports. **[37]** A preliminary toxicity study was designed to demonstrate the appropriate safe dose range that could be used for subsequent experiment. Dose of plants extracts was selected by carrying out the acute toxicity study.

Following seven groups of rats (n=6) based on the plasma glucose levels and bodyweight the animals were assigned randomly.

Group I -Normal rats treated with vehicle 0.5mL of 5% Tween 80

Group II -Diabetic control rats received vehicle

Group III-Standard drug group rats received glibenclamide (5 mg/kg),

Group IV-Treated group rats received EAE-SC (200mg/kg),

Group V-Treated group rats received EAE-AP ethyl acetate extract (200mg/kg),

Group VI -Treated group rats received ETE-SC ethanol extract (200 mg/kg) and

Group VII -Treated group rats received ETE-AP ethanol extract (200mg/kg), with vehicle 0.5mL of 5% Tween 80 respectively.

Assessment of Antihyperglycemic activity

Bodyweight changes

Individual animal body weight was measured gravimetrically on 0, 7, 14 and 21 days respectively.

Blood glucose estimation

Fasting blood glucose levels were determine in all experimental rats initially to determine the diabetic status and thereafter every week during 21 days study period. Blood was obtained by snipping tail of rat with the help of sharp razor and blood glucose levels were determined using glucometer. Each time the tail of the rat was sterilized with spirit.

Biochemical estimation

At the end of 21th day, after the estimation of blood glucose level, the animals were sacrificed by decapitation. Serum separated for determination of parameters the level of serum triglyceride (TG), high density lipid cholesterol (HDL), low density lipid cholesterol (LDL), total cholesterol (TC), Blood urea nitrogen (BUN), insulin and Creatinine levels using commercial available kits. (SPAN Diagnostics)

Histopathological study

Sample from the splenic lobes of the pancreas were perfused via the left ventricle 30mL phosphate buffer saline (PBS) for 2 min at 37°C and then with PLP (2% paraformaldehyde, 75mM L-lysin, 10mM sodium periodate) fixative. The excised issues were, placed in PLP overnight at 4°C wash and stored in PBS containing 0.02% sodium azide at 4°C. Half-hemisected fixed tissue samples were washed with PBS three times for five min each, placed in PBS overnight, embedded in paraffin and cut into 4µm sections using a microtome. The sections were then stained with hematoxyline and eosine (H and E) staining and ten fields (0.4 mm^2/field) per tissue were used for histopathological study. **[38]**

Statistical analysis

All result were expressed as the mean± standard error of mean (SEM). The results were analyzed for statistical significance by One-way Analysis of Variance (ANOVA) followed by dunnett's multiple comparison tests. The significance was expressed by *P* value, as mention in the tables. P<0.01 was considered as statistically significant.

RESULTS

Acute Toxicity Test

No mortality was recorded among the rats at the dose of 2000mg/kg. Hence, one tenth of the dose tested, ie, 200mg/kg body weight was selected for the study.

Changes in Bodyweight

The STZ-induced hyperglycemic rats significantly decrease (P<0.01) in body weight as compared to control group from day 0, 7, 14 and 21 days show in Table 1. Daily treatment with both plants EAE-SC and EAE-AP and ETE-SC and ETE-AP supplement at 200mg/kg body weight to diabetic group animal resulted significantly increase body weight at end of the day compared to diabetic group. The most pronounced increase in body weight detected with ethanol extract of both plant. In standard drug at 5mg/kg body weight dosing group, there are no significance changes were observed as compared to diabetic group.

Oral Glucose Tolerance Test

The Blood glucose levels of control group reach a peak at 30 min. and continuously decrease to attain basal glucose level. Both plants EAE-SC, EAE-AP, ETE-SC and ETE-AP with 200mg/kg body weight doses showed significant (P<0.01) decrease in blood glucose level at 60 and 120 min. after glucose administration as compared to the control group showed in Table 2.

Changes in Blood Glucose Level

STZ-induced hyperglycemic rats showed significant (P<0.01) increasing in fasting glucose on the successive days of experiment starting from 0, 7, 14 and 21 days as compared to the base values. Daily oral treatment with both plants ethanol extract 200mg/kg dose produced significant (P<0.01) reduction in blood glucose on successive days of the experiment as compared to diabetic control group as well as standard drug group showed in Table 3.

Changes in Biomarkers

Serum content LDL-cholesterol, total cholesterol (TC) and triglyceride (TG) showed elevation where as HLD-cholesterol were significantly (P<0.01) decreases in STZ-induced hyperglycemic rats as compared to the control group. On daily oral treatment with alcoholic extracts of both plants *A. paniculata* and *S. chirayita* at 200mg/kg dose showed significant (P<0.01) reduction of LDL, TC and TG and simultaneously increase the HLD levels as compared to the diabetic control group (Table 4). The renal function marker such as blood urea nitrogen (BUN) and Creatinine level elevated whereas in insulin level in STZ-induced hyperglycemic rats significantly decreases (P<0.01) as compared to control group showed in Table 5.

Histological changes

In STZ-induced hyperglycemic rats decrease in pancreatic islet number and size, atrophy and vacuolation, and connective tissue invasion in the parenchyma of pancreas islet was detected. On treatment of both plants EAE-SC, EAE-AP, ETE-SC and ETE-AP with 200mg/kg body weight doses mainly alcoholic extract of plants showed dramatic decrease in all abnormal histological changes as compared to the diabetic control group (Figure 1). Significantly lesser histological changes occurred in the standard drug glibenclamide treated group.

Table 1: Effects of Plant extracts on body weight changes in normal and streptozotocin-induced diabetic rats

Animal Groups	0 days	7 days	14 days	21 days
G-I Vehicle	233.83±2.21	241.33±0.98[b]	250.16±1.81 [b]	256.66±1.25 [b]
G-II STZ Diabetic Control	227.50±1.31	196.16±3.32[a]	186.66±2.40 [a]	177.50±2.72 [a]
G-III Standard Drug (Glebenclamide)	230.50±1.83	232.66±1.66 [a b]	235.83±1.47 [a b]	242.66±2.02 [a b]
G-IV EAE-SC (200mg/kg)	236.16±1.07	235.16±2.18 [a b]	240.33±2.12 [a b]	248.50±3.87 [b]
G-V EAE-AP (200mg/kg)	235.00±1.59	237.50±1.08 [a b]	241.00±1.29 [a b]	248.16±3.19 [b]
G-VI ETE-SC (200mg/kg)	236.00±1.91	235.50±2.91 [a b]	241.83±1.072 [a b]	249.66±3.15 [b]
G-VII ETE-AP (200mg/kg)	234.50±1.87	232.66±1.82 [a b]	237.16±1.70 [a b]	243.33±3.08 [b]

Values are mean± SEM (n=6), statistical significance: [a]P<0.01, compared with vehicle group I; [b]P<0.01, compared with STZ diabetic control group II;

Table 2: Blood glucose concentration (mg/dl) in Oral Glucose Tolerance Test (OGTT)

Animal Group	0 min	30 min	60 min	90 min	120 min
G-I Vehicle	77.66±1.83	157.83±5.26 [b]	140.33±3.25 [b]	137.00±2.92 [b]	124.5±2.23 [b]
G-II Standard Drug	75.16±1.49	132.66±2.76[a]	124.66±1.16 [a]	114.66±1.68 [a]	80.16±1.72 [a]
G-III EAE-SC(200mg/kg)	75.66±3.13	150.16±2.42 [b]	143.33±3.08 [b]	136.66±1.92 [b]	110.16±2.38 [a b]
G-IV EAE-AP(200mg/kg)	77.70±2.21	147.83±2.31 [b]	141.16±1.32 [b]	133.83±2.30 [b]	105.16±1.62 [a b]
G-V ETE-SC (200mg/kg)	77.00±2.94	145.16±1.55	136.16±0.79 [b]	130.00±1.46 [a b]	90.83±1.19 [a b]
G-VI ETE-AP(200mg/kg)	77.16±2.68	143.66±1.28 [a]	128.83±0.94 [a]	123.16±2.08 [a b]	87.00±1.31 [a b]

Values are mean± SEM (n=6), statistical significance: [a]P<0.01, compared with vehicle group I; [b]P<0.01, compared with standard drug group II;

Table 3: Blood glucose level (mg/dl) in streptozotocin-induced diabetic rats in experimental groups

Animal Groups	0 days	7 days	14 days	21 days
G-I Vehicle	79.00±1.39	77.66±1.22[b]	76.33±0.84 [b]	76.83±1.55 [b]
G-II STZ Diabetic Control	340.66±0.66	337.16±1.37 [a]	340.00±1.23 [a]	345.00±1.40 [a]
G-III Standard Drug (Glebenclamide)	320.80±1.55	266.00±1.50 [ab]	193.83±1.64 [ab]	117.83±0.94 [ab]
G-IV EAE-SC (200mg/kg)	338.80±2.27	285.33±2.34 [ab]	210.66±1.85 [ab]	147.33±2.57 [ab]
G-V EAE-AP (200mg/kg)	340.33±0.88	289.50±1.25 [ab]	215.16±2.22 [ab]	146.00±1.46 [ab]
G-VI ETE-SC (200mg/kg)	329.16±0.74	282.16±1.90 [ab]	207.83±0.94 [ab]	126.50±1.25 [ab]
G-VII ETE-AP (200mg/kg)	327.33±1.14	273.66±0.76 [ab]	205.50±1.08 [ab]	123.83±0.90 [ab]

Values are mean± SEM (n=6), statistical significance: [a]P<0.01, compared with vehicle group I; [b]P<0.01, compared with STZ diabetic control group II;

Table 4: Serum biochemical parameters in STZ-induced diabetic rats in experimental groups

Animal Groups	HDL (mg/dL)	LDL (mg/dL)	Total Cholesterol (mg/dL)	Triglyceride (mg/dL)
G-I Vehicle	42.00±1.67 [b]	39.33±1.30[b]	91.50±2.81[b]	84.33±5.00 [b]
G-II STZ Diabetic Control	21.66±1.05 [a]	90.66±1.80 [a]	174.80±3.49 [a]	169.70±2.02 [a]
G-III Standard Drug (Glebenclamide)	38.5±0.99 [b]	37.50±0.95 [b]	102.80±1.86 [b]	108.30±2.76 [a b]
G-IV EAE-SC (200mg/kg)	28.66±0.88 [a b]	81.33±1.72 [b]	130.80±2.90 [a b]	132.50±1.58 [a b]
G-V EAE-AP (200mg/kg)	30.16±1.99 [a b]	82.66±0.95 [a b]	135.50±3.48 [a b]	133.00±3.61 [a b]
G-VI ETE-SC (200mg/kg)	31.16±1.30 [a b]	42.16±1.75 [b]	126.50±1.40 [a b]	123.00±3.20 [a b]
G-VII ETE-AP (200mg/kg)	35.83±1.13 [a b]	39.33±0.80 [b]	115.50±3.58 [a b]	121.20±1.49 [a b]

Values are mean± SEM (n=6), statistical significance: [a]P<0.01, compared with vehicle group I; [b]P<0.01, compared with STZ diabetic control group II;

Table 5: Serum biochemical parameters in STZ-induced diabetic rats in experimental groups

Animal Groups	Blood Urea Nitrogen (BUN)	Insulin (ng/mL)	Creatinine (mg/dL)
G-I Vehicle	32.33±0.84 [b]	0.444±0.008 [b]	0.55±0.09 [b]
G-II STZ Diabetic Control	229.50±1.23 [a]	0.225±0.001 [a]	3.39±0.04 [a]
G-III Standard Drug (Glebenclamide)	160.66±1.02 [a b]	0.444±0.034 [b]	2.42±0.03 [a b]
G-IV EAE-SC (200mg/kg)	218.66±1.62 [a b]	0.223±0.003 [a]	3.38±0.03 [a]
G-V EAE-AP (200mg/kg)	216.50±1.85 [a b]	0.223±0.003 [a]	3.38±0.02 [a]
G-VI ETE-SC (200mg/kg)	204.33±1.52 [a b]	0.280±0.003 [a]	2.80±0.02 [a b]
G-VII ETE-AP (200mg/kg)	183.83±2.37 [a b]	0.364±0.014 [a b]	2.31±0.02 [a b]

Values are mean± SEM (n=6), statistical significance: [a]P<0.01, compared with vehicle group I; [b]P<0.01, compared with STZ diabetic control group II;

DISCUSSION

Diabetes mellitus causes probably due to the changes in life style, changes in diet pattern commonly rich sugar fast food diet in place of traditional fibers food and also because of genetic basis. The disease characterized by high glucose levels due to the absolute or relative deficiency of insulin circulation. The presently available pharmacotherapy for the treatment of diabetes in modern health care system include the oral hypoglycemic agents and insulin is not possible to the people of majority of economic constraints of developing countries to used these drugs on regular basis, moreover these synthetic drugs are associated with various large number of side effects. Hence there is growing interest to use traditional indigenous plants for the treatments of diabetes mellitus were increased. Over 150 plant extracts and plant active ingredients including flavonoids, terpenoids, tannins, alkaloids and polyphenols etc. are used for the treatment however few of them has been screened pharmacologically. **[39-42]**

STZ-induced diabetes may be due to the selective destroying pancreatic β-cells which is responsible for the insulin production from endocrine cells. However the animal survived without insulin treatment and shows improvement by glibenclaimde drug which acts stimulating β-cells of pancreas indicates incomplete destruction of pancreatic β-cells of diabetic animals. **[43-46]**

In present study increased blood glucose level and rats body weight loss confirmed the induction of hyperglycemia by streptozotocin (STZ). Oral supplements of ethanolic extracts of both the plants under study caused a rapid decrease in hyperglycemic peak after glucose loading in rats. A significantly decrease blood glucose 60 min after administration of a dose of 200mg/kg ethanolic extracts of both plant *A. paniculata* and *S. chirayita*. The above suggested that the plant extracts may have post-prandial hyperglycemia therapeutic potential. The body weights usually decrease as diabetes progresses, but plant extract inhibited the decrease in body weight during the period of study from the start of supplement. The results led us to suppose that the effects of ethanolic extracts could take place through a sulfonylurea like mechanism. Since it cause a decrease in blood glucose levels during glucose tolerance test. **[47]**

The ethanol extract of both plants decrease blood concentration to normal glycemic concentration. Significantly increase the plasma insulin levels with treatment of 200mg/kg ethanol extract of plants as compared to the diabetic control. The β-cells are highly susceptible to cytotoxic agent like STZ. The insulin secretion impairments result abnormal glucose homeostasis, leading to type I diabetes which are due to selective and progressive destruction of pancreatic β-cells. In present study increasing in insulin levels in ethanolic extracts of plants with 200mg/kg may have been due to the increase in secretion from β-cells and insulin synthesis of cells in the treatment group as compared to diabetic control group. The possible mechanism of the extracts may be an induction of insulin secretion through interaction with sulfonylurea receptors in plasma membrane of pancreatic β–cells or may be due to the property to promote insulin secretion by stimulation of Ca^{2+} influx, membrane depolarization, and closure of K^{+}-ATP channels an initial key step in insulin secretion. **[48-51]**

The long term damage, dysfunction and failure of various organs especially associated with eyes, kidney, nerve, heart and blood vessels in hyperglycemia of diabetes. The disturbance in lipid metabolism in diabetes is often important determination of course and status of disease. The lipid profile of STZ-induced has been shown to the important indicator for metabolic disturbance including diabetes. The higher concentration of serum TC may be attributed to inhibition of cholesterol catabolism or may be insulin deficiency and mobilizations of fatty acids from adipose tissues by lipolysis **[53-54]** and increase in serum TG level due to increase biosynthesis or dismissed clearance from the blood, elevation of serum TG are also associated with an increased risk of pancreatitis. Lipids particularly oxidized lipids, cause glomerular injury according to numerous experimental and clinical observations **[55,56]** and serum triglyceride play a role in the development and progression of renal disease in type II diabetes mellitus. **[57,58]** On treatment with ethanol extract of *A. paniculata* and *S. chirayita* the TG and TC decrease while with HDL-cholesterol increases significantly as compare to diabetic control group.

The levels of blood urea nitrogen (BUN) and serum creatinine are significant marker of renal insufficiency. Urea is the major nitrogen containing metabolic product of protein metabolism; creatinine is endogenously produced and released in to body fluid and its clearance as measures for the indicator of renal function. **[59]** In the present study the STZ-induced diabetic rats had increase level of serum BUN and serum creatinine, and on treatment of ethanol extracts of *A. paniculata* and *S. chirayita* showed significant reduction in parameter which could be due to the decrease disturbance in nucleic acid and protein metabolism for better glycemic control. However, involved protection component is still unclear.

Insulin deficiency of β–cells was increased by immunocytochemical intensity. In STZ-induced diabetic group reduced the number of

immunoreactive insulin producing β–cells and they were distributed in restricted pancreatic islets. On treatment of plant extract mostly alcoholic extracts of *A. paniculata* and *S. chirayita* inhibited these abnormal changes. However, slightly more insulin producing β–cells in the standard drug gilbenclamide treated group was observed than in the diabetic group. Improved pancreatic exocrine activities can be ascribed to insulin secretion from existing residual β–cells of islets or due to enhanced transport of blood glucose to peripheral.

Histopathological report showed that the insulin producing β–cells are located generally in central region of pancreatic islets. However the cells are destroyed in STZ-inducing diabetes. Inhibition of these histomorphological changes and maintenance of normal architecture of insulin producing cells by *A. paniculata* and *S. chirayita* ethanol extract found to protect from the destruction of these cells by STZ.

CONCLUSION

In conclusion, the result showed that supplements of the plant extracts mostly the ethanol extract of *A. paniculata* plant has better effects than *S. chirayita*. The supplement of the ethanol extract having favorable effects in changes of body weight, blood glucose level, lipid profile and renal marker, it also increase the number of insulin producing β–cells. Thus it is attributed the antihyperglycemic effects of plant extract were partly due to the restoration of pancreatic tissue and insulinotropic effects is very similar to sulphonylureas.

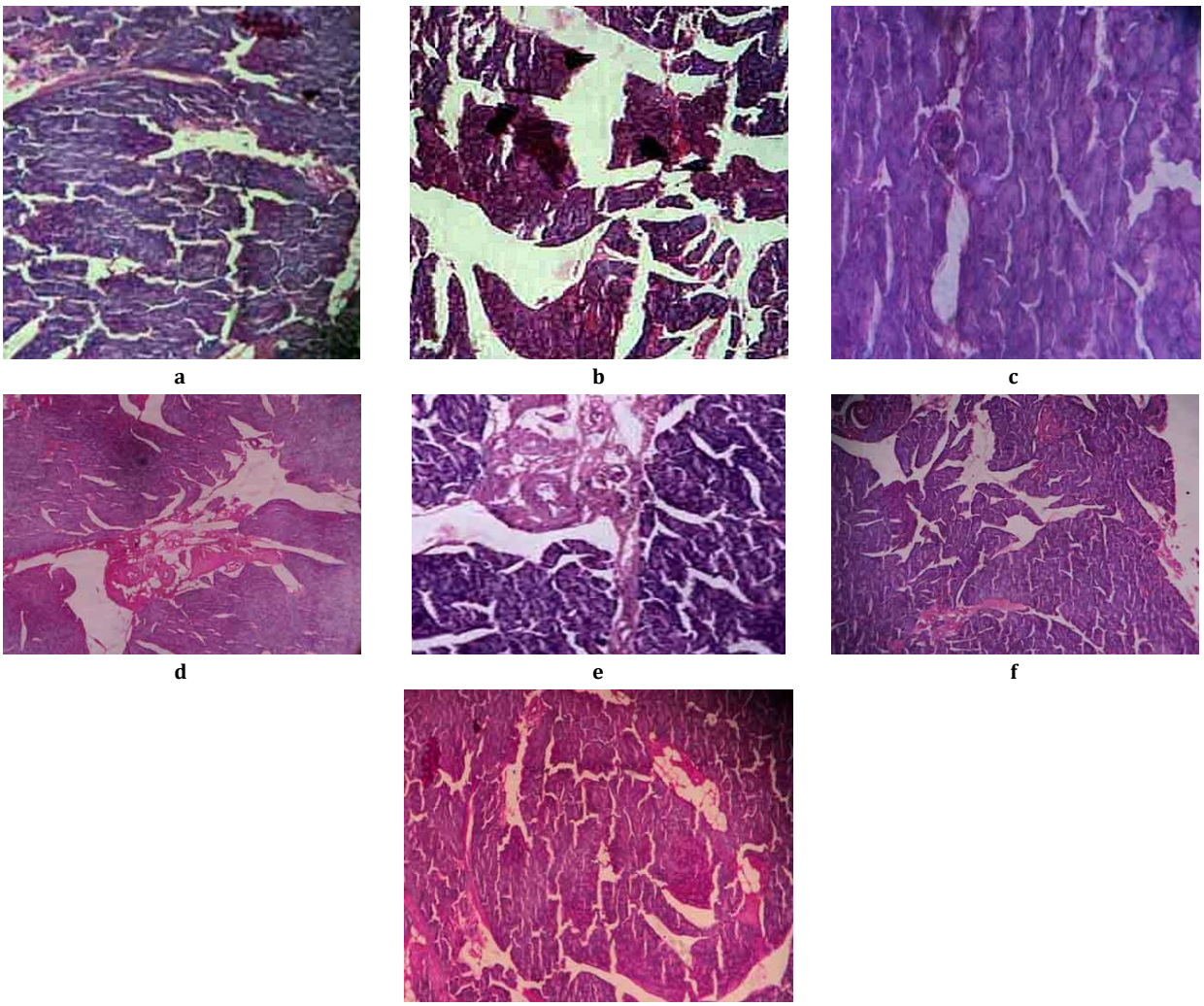

Fig. 1: Shows histopathology of pancreas a. Vehicle control rat showed acini and normal cellular population in islet of legerhans b. STZ - diabetic control showed damaged islets and reduced islet size c. Standard Drug -GLB (5mg/kg) showed restoration of normal cellular population size of islets of langerhans and absence of islet damage and presence of hyperplasia. d. & e. EAE-AP and EAE-SC showed less restoration of normal cellular population size of islets of langerhans and damaged islets. f. & g. ETE-SC and ETE-AP showed less restoration of normal cellular population size of islets of langerhans and absence of islet damage and presence of hyperplasia.

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