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Research Article

ANTIDIABETIC ACTIVITY OF *Sebastiania chamaelea* Muell. ARG. LEAF EXTRACTS IN ALLOXAN INDUCED DIABETIC ALBINO RATS.

N. YASODAMMA*, K. S.SHANTHI SREE, C. ALEKHYA

Department of Botany, S.V. University, Tirupati 517502, A.P, India. Email: yasodanpalli@gmail.com

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ABSTRACT

Background: Several drugs such as biguanides and sulfonylureas are presently available against hyperglycaemia. But these drugs have side effects and thus there is a need for searching new biologically active compounds which are essential to overcome diabetic problem. One such herbal drug is *Sebastiania chamaelea* possessing active secondary metabolites. It has been reported as a tonic in overcoming the vertigo, plant juice acts as astringent and remedy against syphilis and diarrhoea. It is also reported with high amounts of significant amino acids as argenine along with commendable phenolic constituents and other secondary metabolites. Hence the leaf extracts of *S. Chamaelea* was subjected for antidiabetic activity.

Materials and Methods: Acute Toxicity studies were carried out as per the guidelines of OECD (Organisation for Economic Co- operation and development). Further antidiabetic activity was carried out with aqueous and methanolic leaf extracts of *S. chamaelea* in Alloxan induced diabetic Wistar albino rats by Resmi and Fathima, Satyanarayan, Mangathayary and Chude methods.

Results: Due to non toxicity of the aqueous and methanolic leaf extracts 300 mg /kg b.w daily dose up to 21 days against diabetic rats resulted reduction of blood glucose levels to that of the standard drug Glibenclamide 10mg/kg b.w, increased body weights to that of diabetic control rats and reduction of TC, VLDL-C, LDL-C, TG respectively. An increase in HDL-C also observed to that of the control rats. Histopathological studies of showed regeneration of tissues to their normal status in the islets of pancreas, central veins and fibrous tissues of liver, glomeruli tubules of kidneys.

Conclusion: Leaf aqueous and methanolic extracts of *S. chamaelea* at the dose of 300 mg/kg body weight brings about significant beneficial effects in various physiological and histological parameters altered during diabetic manifestations and these effects are quite comparable with the standard drug, glibenclamide.

Keywords: Acute toxicity, Blood glucose, Cholesterol, Glibenclamide, Histopathological, Secondary metabolites.

INTRODUCTION

Several drugs such as biguanides and sulfonylureas are presently available to reduce hyperglycemia in diabetes mellitus. These drugs have side effects and thus searching for new biologically active compounds are essential to overcome diabetes mellitus **[1]**. The ethnobotanical reports state that about 800 plants may possess antidiabetic potential **[2]**. Leaf decoction of *S.chamaelea* in ghee is given as tonic and applied to the head in vertigo. The juice of the plant is astringent and is used as a remedy for syphilis and diarrhoea **[3, 4]**. *S.chamaelea* accounted for 77.5% of human necessary amino acids of which argenine stands highest with 60% of the free amino acids, may promoted the role for its medicament activity **[5]**. Preliminary Phytochemical screening of aqueous and methanolic leaf extracts revealed the presence of flavonoids. Phenols, steroids, tannins in higher quantities and glycosides, terpenoids in low quantities. Saponins present only in methanol extract. Methanol extract showed effective antimicrobial activity. Qualitative analysis of leaf has been reported 15 Phenolic acids such as caffeic acid, melilotic acid, aesculetin, p-hydroxy benzoic acid, coumarin, cinnamic acid, salicylic acid and scopoletin along with five flavonoids like myrecetin, quercetin, kaempferol, luteolin, and apigenin **[6]**. These natural compounds could act individually or synergistically cause the hypoglycaemic effect **[7]**. Hence the present study with leaf aqueous and methanol extracts of were tested for their effect on the alloxan induced diabetes rats, on blood glucose, body weight and physiological effects on pancreatic, liver and kidney tissues.

Acalypha indica methanol and acetone (70:30) extracts on Alloxan induced diabetic rats showed effective hypoglycemic activity at 300 and 500 mg/kg after 5 hrs of treatment and also slightly reduced cholesterol, urea and triglycerides levels **[8].** Oral administration of petroleum ether, chloroform, acetone and methanol extracts of *A.indica* with 100 mg/kg b.w for 21 days caused a decrease in fasting blood sugar with methanol extract significantly ($p<0.001$) in diabetic rats **[9].**

Bridelia retusa bark n-butanol extracts showed effective anti hypoglycemic effects upto 12 days in alloxan induced diabetic rats at 200 - 400mg/kg compared to glibenclamide 5 mg/kg than methanol and petroleum ether extracts **[10].** Similar results were observed with stem bark ethanolic extracts of *B. ndellensis* and *B. ferrugenea* **[11].**

Croton zambesicus methanolic leaf extract at 100-200mg/kg evaluated against alloxan induced (150mg/kg) diabetic rats was compared to chlorpropamide resulted effective hypoglycemic activity about 60% reduction in BGL after 12hrs with single dose and also prolonged treatment about 7days. LD 50 values of the drug was 1400±148 mg/kg, and produced physical signs of toxicity at 100-200 mg/kg **[12]**. *C. klozchianus* aerial part extract of an Ayurvedic diabetic drug at 100 and 300 mg/kg bw, for three weeks against diabetic rats showed significant reduction in blood glucose levels about 46% and also increase in insulin secretion by 8 fold at 2 mg/ml, and also increase in high density Lipoprotein and reduction in cholesterol and Triglycerides **[13] .**

Euphorbia hirta a traditional antidiabetic medicine ethanolic extracts 250 and 500mg/kg of leaf, flower and stem against STZ induced diabetic mice about 21days administration resulted reduction of 80% of blood glucose levels, body weights remains constant, decreased triglycerides, cholesterol and triglycerides increased HDL levels may be due to the presence of tannins and flavonoids. And also no toxicity was observed up to 2000 mg/kg up to 14 days **[14].**

Phyllanthus emblica and *P.acidus* fruit aqueous extracts significantly reduced serum glucose cholesterol, triglycerides and increased HDL-cholesterol in alloxan induced diabetic rats **[15]**. *P.emblica* and *P.acidus* aqueous fruit extracts of at 350 mg/kg bw after 84 days of treatment on Alloxan induced diabetic rats resulted an effective inhibition on blood glucose levels and insulin secretions to that of control rats and also reduced cholesterol, triglycerides serum, urea and creasine and increased HDL - cholesterol levels **[16].** *P.emblica* ethanol extracts fruits at the dose of 200 mg/kg for 45 days resulted in a significant reduction in blood glucose levels and increase in plasma insulin in diabetic rats; reduction in TC, VLDL-C, LDL-C, FFA, PL, TG and an increase in HDL-C **[17]**. *P. simplex* methanol and

aqueous extracts on alloxan induced diabetic rats showed effective anti hyperglycemic and anti oxidant potential effect at 125 to 250 mg/kg **[18].** Srilankan Ayurvedic medicine *P. debilis* whole plant aqueous extract a with 497.5, 995 and 1990 mg/kg was showed effective hypoglycemic activity against tolbufanide induced diabetic rats. **[19].** *Putranjiva roxburghii* ethanolic leaf extract with 250 and 500 mg/kg exhibited a dose dependant significant anti-hyperglycemic activity on 4th, 7th and 10th day post treatment **[20]**. Leaf methanolic extract of *Securinega virosa* on Streptozocin induced diabetic rats at 100-600mg/kg b. wt dose dependent hypoglycemic activity was observed **[21].**

MATERIALS AND METHODS

Collection and identification of Plant material

Sebastiania chamaelea was collected from the fields of S.V.Veterinary College, Tirupati, A.P, India during the month of June.2010. The taxonomic identity was confirmed by Prof. N. Yasodamma, Department of Botany, Sri Venkateswara University, Tirupati. Voucher specimen KS711 was preserved in the Herbarium Department of Botany as per the standard method **[22].** Leaves were thoroughly washed and then dried under shade for one week. Dried leaves were ground in a mixer grinder and sieved. Powder was stored in air sealed polythene bags at room temperature until further use.

Preparation of extracts

Dried leaf powder (70 g) was soak in distilled water. The crude drug was extracted after 72 hr. and filtered extract was dried on water bath. The dried powder (40 g) was extracted in a soxhlet apparatus using 200 ml of methanol, extracts were concentrated on rotavapour. These extracts were further mixed with 1% Tween 80 and used as test solutions.

Maintenance of Experimental Animals

Adult male albino rats of wistar strain of 180±20g were collected. The animals were acclimatized to standard laboratory conditions. The experiment was conducted according to the ethical norms approved by CPCSEA, Ministry of social justice and empowerment, Government of India and ethical clearance was granted by Institutional Ethical Committee resolution no. 11/2011-2012/(i)/a/CPCSEA/IAEC/SVU/NY-KSS/dt:14/11/2011.

Acute toxicity study

The acute toxicity studies of aqueous and methanol extracts at the dose level of 10, 100, 1000, 1500 and 3000mg/kg body weight by gavage method and observed for 14 days. As per the 423 guidelines set by OECD (Organisation for Economic Co- operation and Development) **[23]**. Albino rats (n=6) of either sex selected by random sampling technique were used for the study.

Antidiabetic activity [24, 25, 26]

Fasting blood glucose was determined after depriving food for 16 hrs with free access of drinking water. Hyperglycemia was induced by a single i.p. injection of 120 mg/kg of alloxan in sterile saline. After 5 days of alloxan injection, the hyperglycaemic rats (glucose level > 250 mg/dl) were separated and divided into different groups comprising of 6 rats each for the anti-diabetic study. The treatment with plant extracts (p.o) was started from the same day (except normal control and diabetic control groups) for a period of 21 days. During this period, animals in all groups had free access to standard diet and water. Body weights and blood glucose levels were estimated on 1st, 7th, 14th and 21st day of the treatment. On the 21st day all the overnight fasted rats were killed by cervical dislocation and blood samples were collected for biochemical studies by cardiac puncture. Liver, pancreas and Kidneys were dissected out, washed in ice cold saline, and immediately stored in deep freeze at -80^{0}C and processed for histopathological studies.

Various groups used in experiment

Forty two rats were divided into seven groups each containing six rats. Control group-I received vehicle (1% Tween 80) at a dose of 10 ml/kg orally. Group II and III non diabetic received aqueous and methanol extracts at 300 mg/kg of b.w for 21 days. The positive control Group IV received alloxan 100 mg/kg body weight (i. p) for a single dose. Test groups V and VI diabetes induced rats received the aqueous and methanol extracts at the doses of 300mg/kg of b.w orally for 21 days. The positive control group-VII diabetic rats received glibenclamide at the dose of 10 mg/kg orally.

Biochemical investigation

Estimation of Blood glucose levels was carried out by using Accu Chek glucometer. Cholesterol; Serum triglycerides; HDL- Cholesterol was carried out by the standard methods **[27, 28]**. By using Friedwald formula the concentrations of VLDL and LDL cholesterol in serum was calculated.

LDL = (Total cholesterol- VLDL- Cholesterol) + (HDL – Cholesterol)

Histopathological studies

A small portion of Pancreas, liver and kidney was fixed in 10% formalin for histopathological studies. Pancreas, liver and kidney cross sections were taken with microtome consisting 5µm thick, and stained with hematoxylin and eosin. Sections were observed under microscope for histopathological changes.

Statistical Analysis

The results were subjected for statistical significance using one way Anova followed by Dunnett's test. The p< 0.01 was considered significant.

RESULTS

Acute Toxicity Studies

Acute toxicity studies revealed that there is no toxicity and death of the rats with aqueous and methanolic leaf extracts of *S. chamaelea* up to 3000 mg/kg body weight even after 21 days.

Antidiabetic activity: (Figure-I)

Changes in Body weights

Diabetes is characterized by weight loss. Alloxan administration brought about marked reduction in body weights of rats. These reduced body weights were found to be increased significantly (p< 0.01) in aqueous, methanol extracts and glibenclamide treated rats when compared to the respective diabetic control group showing decrease in body weights. Whereas the relative efficacy in increasing or maintaining body weights in the rats treated with aqueous extract seems to be the most promising than methanol extract, when compared with the glibenclamide treated rats showing almost equal. No significant changes were observed in the non-diabetic control and non diabetic rats treated with plant extracts when compared with diabetic control rats.

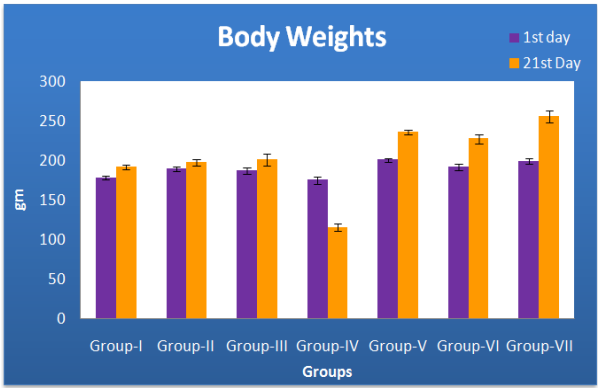


Fig. I: Changes in Body Weights (g)

Blood glucose levels (Figure-II)

Alloxan administration at the dose of 120 mg/kg b.w causes significant diabetogenic response in albino rats. These raised levels of blood glucose in diabetic rats were declined sharply after oral

feeding of aqueous and methanol extracts and glibenclamide. When comparisons made between diabetic and drug treated animals, blood glucose levels were found to be declined sharply from 279.83-110.00 mg/ dL and from 289.66-113.00 mg/ dL on 21st day after oral feeding of leaf aqueous and methanol extracts respectively and also compare with control drug Glibenclamide treatment blood glucose levels decreased from 295.66-115.01 mg/ dL. No significant changes were observed in the non-diabetic control and non diabetic rats treated with plant extracts when compared with diabetic control rats.

Biochemical estimation (Table-I) (Figure III- VII)

Oral administration of aqueous, methanol extracts and glibenclamide for 21 days had shown a significant ($p< 0.01$) reduction in Cholesterol (TC), Very Low Density Lipoprotein-Cholesterol (VLDL-C), LDL-Cholesterol, Triglycerides (TG) and increase in HDL-cholesterol levels. Diabetic control rats showed increased levels of Cholesterol, VLDL-C, LDL-C, TG and decreased HDL-cholesterol. No significant changes were observed in the non-diabetic control and non diabetic rats treated with plant extracts.

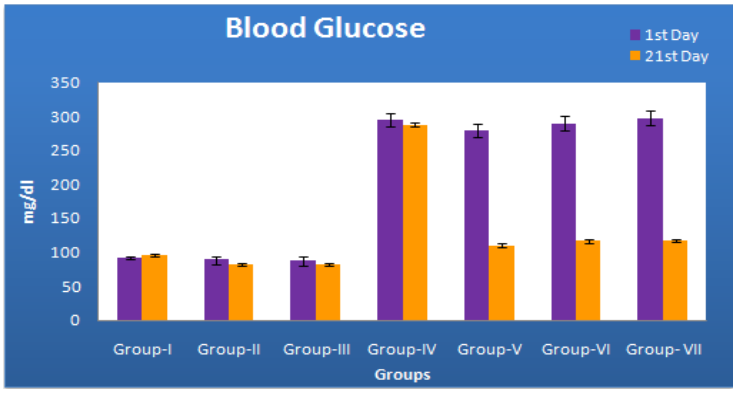


Fig. II: Blood glucose levels (mg/dil)

Table I: Biochemical Estimation (mg/ dil)

Groups	Cholesterol	VLDL	HDL	LDL	TGL
Group I	70.00±0.74**	15.46±0.35**	85.41±2.37**	-30.87±2.37	77.37±1.75**
Group II	76.17±2.91**	15.25±0.31**	87±2.35**	-26.08±2.35	76.31±1.57**
Group III	79.72±1.51**	15.28±0.58**	84.49±1.67**	-19.02±2.38	76.43±2.89**
Group IV	140.09±4.43	32.62±1.37	60.94±2.38	48.61±4.90	163.12±6.87
Group V	88.68±1.89**	17.37±0.38**	66.68±1.29	4.615±2.06	86.9±4.72
Group VI	89.05±1.52**	17.5±0.44**	91.00±1.11	-19.45±2.12**	87.50±2.23**
Group VII	88.24±1.45**	17.50±0.45**	107.01±1.52	-36.27±2.80**	87.53±2.27**

All the data are expressed as Mean ±SEM: **indicate $p<0.01$ as compared to control group, n=6: One -way ANOVA followed by Dunnett's test.

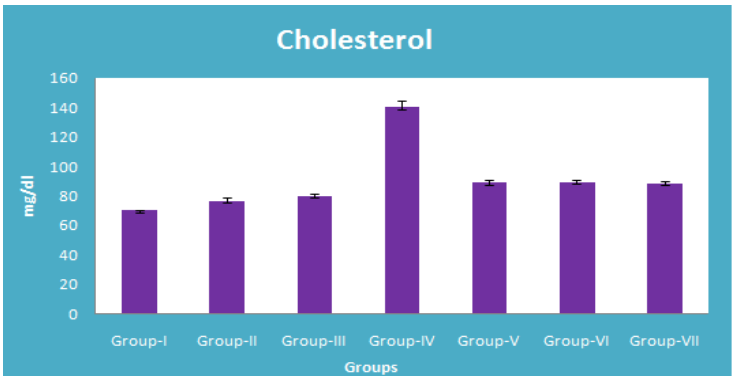


Fig. III: Cholesterol levels (mg/dil)

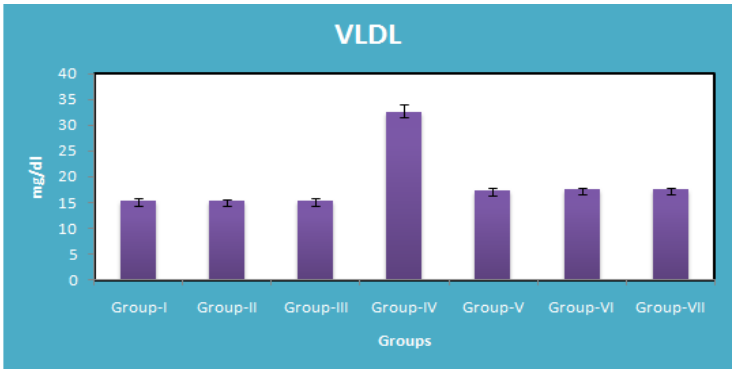


Fig. IV: VLDL-C levels (mg/dil)

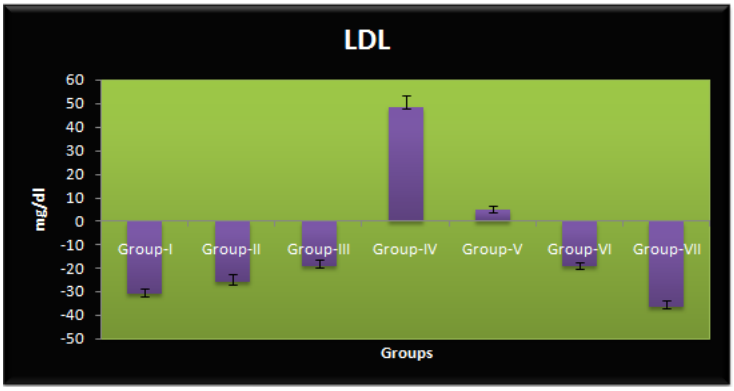


Fig. V: LDL-Cholesterol levels (mg/dil)

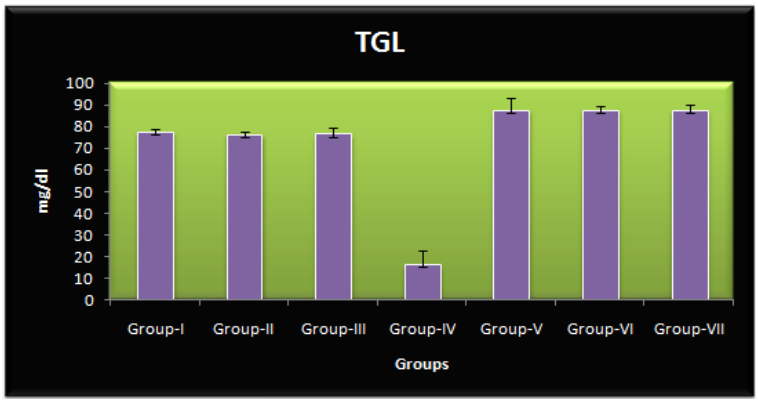


Fig. VI: Triglycerides (TGL) levels (mg/dil)

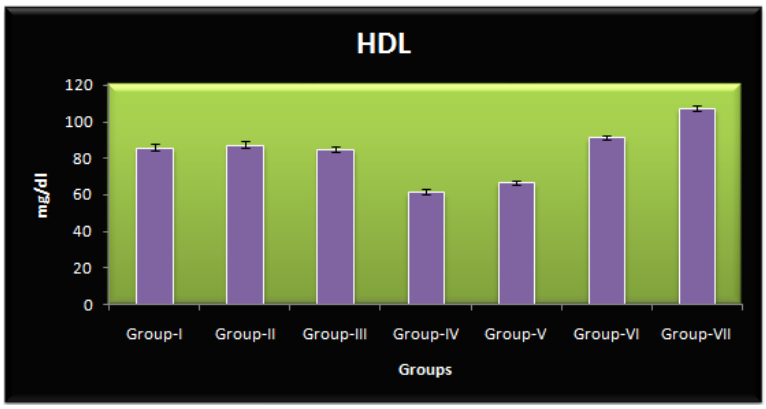


Fig. VII: HDL – Cholesterol levels (mg/dil)

Histopathological study (Plate: I)

Pancreas

Histologically, the islets of langerhans of normal control group I were unevenly scattered in the pancreatic tissue and they were often quite abundantly distributed. The islet cells were compactly arranged, with negligible intercellular space and no inflammatory cells were observed. Group II and group III treated with only plant aqueous and methanol extracts showed no difference to that of the control rats. Pancreatic islets of diabetic control group IV revealed significant architectural disarray. Pancreatic islets of group V (treated with aqueous extract), group VI (treated with methanol extract) and group VII (treated with glibenclamide) also showed architectural disarray but to a lesser extent as compared to alloxan induced diabetic rats (group IV).

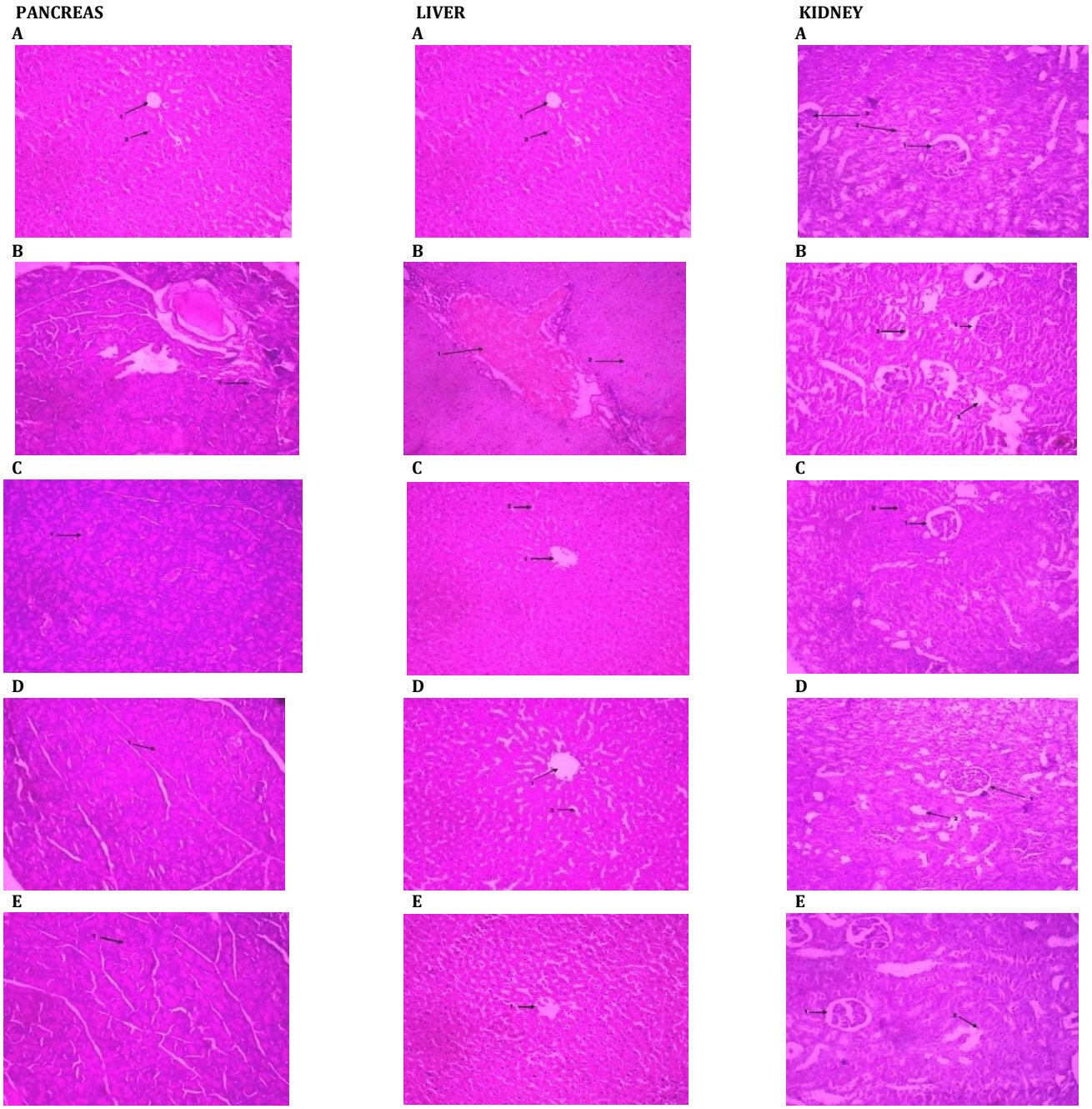
Liver

Normal control rats liver contain hexagonal lobules with number of hepatocytes with central vein. Whereas in diabetic rats lumen of central vein extensively filled with fibrous tissue. However in diabetic rats treated with aqueous and methanol extracts the liver looked almost normal with central vein and lesser vacuolization. The non diabetic control rats treated with plant extracts were not showing any demarcating changes from normal rats.

Kidney

The normal control rat kidney contains abundant glomeruli, tubular epithelial cells and Bowman's capsule. The diabetic control rats kidney showed degenerated glomeruli, enlarged tubules and intouchbular vacuolization. Whereas the diabetic rats treated with aqueous and methanol extracts showed atropic glomeruli and regeneration of tubular epithelial cells. The non-diabetic control rats treated with plant extract were not showing any demarcating changes from normal rats.

Plate: I Histopathological Observations of Pancreas, Liver and Kidney



A: Group - I; B: Group- IV; C: Group - V; D: Group -VI; E: Group – VII

Legends

PANCREAS	LIVER	KIDNEY
A. Normal islets of langerhans. **B.** Destruction of islets **C &D.** Langerhans with mild regeneration. **E.** Mild recovery of β-cells	**A.** Normal central vein of hepatic cells. **B.** Central vein with high hemarage (1), dense kupper cells (2). **C.** Mild regenerative changes (1), vacuolization (2). **D.** Normal central vein with mild regenerative changes (1), vacuolization (2). **E.** Central vein with regenerative cells	**A.** Normal glomeruli (1), tubular epithelial cells (2), Bowman's capsule (3). **B.** Complete degeneration of glomeruli (1), enlarged tubules (2), intouchbular vacuolization (3). **C.** Atropic glomeruli (1), regeneration of tubular epithelial cells (2). **D.** Atropic glomeruli (1), regeneration of tubular epithelial cells (2) **E.** Regenerating architecture of glomeruli (1) picnotic nuclei (2).

DISCUSSION

Alloxan causes a massive reduction in insulin release by the destruction of β-cells of the islets of langerhans and thereby induces hyperglycaemia **[29]**. It is evident from the present investigation that Alloxan administration at the dose of 120 mg/kg body causes significant diabetogenic response in albino rats.

Flavonoids are reported to regenerate the damaged pancreatic β-cells in diabetic animals **[30]**. Polyphenolics such as tannins and Saponins from several plant extracts also shown to reduce blood glucose levels through inhibition of α- amylase and sucrose from the intestine **[31, 32]**. Quercetin supplementation promotes regeneration of the pancreatic islets and increases insulin release in alloxan induced diabetic rats **[33]**. The inhibitory effect of some flavonoids on C- AMP- Phosphodiesterase activity that eventually stimulates the insulin secretion reduces blood glucose concentration **[34]**. Myricetin is a naturally occurring flavonoid was found to lower blood glucose through improved glucose utilization in diabetic animals. Flavonoids have reported to activate peroxisome proliferators activated receptors (PPARs) **[35]**. Qualitative analysis of leaf Flavonoids of *S.chamaelea* has been reported to have myrecetin, quercetin which also may be responsible for the antihyperglycemic activity **[8].**

The hypoglycaemic action of the herbal extracts in diabetic rats may be possible through the insulinomimictic action or by other mechanism such as stimulation of glucose uptake by peripheral tissue, inhibition of endogenous glucose production or activation of gluconeogenesis in liver and muscles [**36]**. This is an interesting finding and suggests that *S. chamaelea* leaf aqueous and methanol extracts at 300 mg/kg b.wt may have antioxidant and free radical scavenging properties, which also supports the antidiabetic activity **(37).**

CONCLUSION

Treatment with leaf aqueous and methanol extracts of *S.chamaelea* against anti diabetic rats when compare the results with standard drug glibenclamide, aqueous extract has been proved the highest activity than the methanol extracts. It is also proved to that of the antidiabetic activity of *Acalypha, Bridelia, Croton, Euphorbia, Phyllanthus and Securinega* species of the family.

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