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Biochemical and histological study of traditional plant: *Dodonaea viscosa* Linn extracts in diabetic rats

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Abstract

Aim of this study was to evaluate the individual biochemical changes of leaf extracts of *Dodonaea viscosa*-A traditional plant in alloxan induced diabetic rats. Methanol and chloroform extract of *Dodonaea viscosa* were obtained by cold maceration and were administered to alloxan induced diabetic albino rats. Parameters including blood glucose, triglycerides, cholesterol, protein, urea, creatinine, SGPT, SGOT were checked using standard test kits and methods after administration of extracts. Histological changes in pancreas and liver of the animal were also examined. Extract treated groups shown reduction in blood glucose level to normal. Increased levels of all other biochemical parameters like SGPT, SGOT, Triglycerides, Cholesterol, Protein, Creatinine and Urea with alloxan treatment have been significantly reduced in extract treated groups. Histological changes supported this claim. Finally, the implications of results after administration of the extracts show their potential use in management of diabetes.

Keywords: Medicinal plants, Hypoglycemia, Histopathology, Diabetes, *Dodonaea viscosa*.

Introduction

Traditional knowledge is integral to identity of most local communities. The Preservation, protection and promotion of traditional knowledge based innovations and practices of local communities are particularly important for developing countries. Interest in medicinal plants as a re-emerging health aid has been fuelled by rising costs of prescription drugs in the maintenance of personal health and well-being, and bio-prospecting of new plant derived drugs. Based on current research and financial investments, medicinal plants will, seemingly, continue to play an important role as a health aid.¹ The essential values of some plants have long been published, but a large number of them have remained unexplored till date. Therefore there is a need and necessity to explore their uses and to conduct pharmacological studies to ascertain their therapeutic properties. Although herbal medicine are effective in the treatment of various ailments, very often these drugs are improperly used, and only few of them have been validated by scientific criteria. So these plant drugs do need a detailed study in the light of modern medicine. Since the modern world is full of stress, the incidence of diabetes is on increasing trend. By definition, diabetes mellitus is categorized as a metabolic disease characterized by hyperglycemia resulting from defects in insulin secretion and insulin action, or both.² Diabetes mellitus is a potential morbid condition with high prevalence worldwide thus the disease constitutes a major health concern.³

Currently the global prevalence of the disease is around 200 million and would increase to 300million by 2025 as per WHO estimation.⁴

In view of the increasing prevalence, there is a growing need to develop integrated approaches towards the management and prevention of Diabetes mellitus by exploring the potentials offered by the traditional phytotherapies. Moreover uncontrolled diabetes appears to involve oxidative stress known to exhibit direct tissue damage properties, which may lead to many complications. It has been already been established that chronic hyperglycemia of diabetes is associated with long term damage, dysfunction and eventually the failure of organs, especially the eyes, kidney, liver nerves, heart and blood vessels.⁵

A scientific investigation of traditional herbal remedies for diabetes may provide valuable lead for the development of an alternative drug and therapeutic strategies.⁶ Experimental evidences suggest the involvement of free radicals in the pathogenesis of diabetes. So plants capable of neutralizing free radicals are effective in preventing diabetes and reduce the severity of diabetic complications. Phenolics and Flavonoids can exert their antioxidant activity by various mechanisms like quenching the free radicals, by chelating of metal ions by inhibiting enzymatic systems responsible for free radical generation⁷ so polyphenolics and flavonoids incorporation into diet could contribute to potential management of hyperglycemia and disturbed lipid pattern and also helps to alleviate complications of organ functions.

But selection of inappropriate animal models has been identified as one of the common problem associated with ethno botanical researches.⁸ Alloxan and its reduction product di-aluric acid establish a redox cycle with the formation of superoxide radicals. These radicals undergo dismutation to hydrogen peroxide. Thereafter, highly reactive hydroxyl radicals are formed by fenton reaction. The action of reactive oxygen species with a simultaneous massive increase in cytosolic calcium concentration causes rapid destruction of beta cells.⁹ Thus alloxan induced diabetes mellitus served as a pathological biomodel for testing a substance with supposed antioxidant activities in vivo.¹⁰ One of the targets of reactive oxygen species is DNA of pancreatic islets. Its fragmentation takes place in beta cells exposed to alloxan. The increase in oxygen free radicals in diabetic conditions is mainly because of the effect of the diabetogenic agent alloxan. The simplistic argument often made against the use of alloxan to induce type II diabetes mellitus is that, alloxan administration produces beta cells damage and thus leading to type I

rather than type II Diabetes mellitus. Alloxan administration in experimental animals has been reported to produce pancreatic lesion which is proportional to the dose of the drug administered. And the size of the lesion also correlates with the pancreatic insulin content.¹¹ This perhaps explains why the drug at a low or medium dose does not produce absolute but insufficient insulin deficiency in experimental animals. Experimental dose of the drug must be carefully selected in order to avoid excessive pancreatic tissue damage.¹²

Dodonaea viscosa (L). Jacq., (Family-Sapindaceae) is an evergreen shrub, which can grow well on poor soils and rocky sites. It is a traditional medicine worldwide, administered orally to treat a great variety of ailments. Leaves, seeds are used to treat fever, sore throat. Leaves are used to treat aches and can be used as antispasmodic agents.¹³ Experimental studies have demonstrated the activity of anti-inflammatory¹, antimicrobial¹⁵, antiulcer¹⁶ and wound healing.¹⁷

Previous chemical studies on this species resulted in the isolation and characterization of several flavonoids¹⁸, diterpenoid acids¹⁹, biologically active saponins²⁰, p-coumarin acid ester²¹, plant acids²² and tannins from aerial parts of *D. viscosa*.²³

This study was carried to clarify the effect of *D. viscosa* extracts (Methanol and chloroform) as beneficial in the treatment of diabetes on blood glucose, biochemical parameters and their possible effects on pancreatic tissue and liver. The most fascinating phyto nutrients flavonoids and tannins in plant leaves that give strong antioxidant activity made this plant to be more concern about the study. Active solvent extracts of the plants are commonly used because they may contain more than one active ingredient and less expensive than a purified single compound. Keeping these facts in view the present study has been undertaken to identify the active anti-diabetic extract of *Dodonaea viscosa*.

Materials and methods

Plant material

The leaves of *D. viscosa* (Family:Sapindaceae) were collected in the month of February from Moinabad, Ranga Reddy District, Hyderabad, A.P, India. The plant was identified and authenticated by Prof. Badraiah, Department

of Botany, Osmania University, Hyderabad, India and a voucher specimen number 0164 was deposited at herbarium of the University, Hyderabad for future reference.

Preparation of extracts

The leaves were dried in shade at room temperature and powdered to coarse powder. Extraction was done using Methanol, Chloroform by simple maceration technique for seven days. The excess solvent was removed using Rotary flash evaporator. The obtained crude extract was stored in air tight container in refrigerator below 10°C for further studies. Chloroform and methanol extracts were selected for the study based on the preliminary phytochemical evaluation.

Toxicity studies were conducted as per OECD Guidelines. No mortality observed up to 4000mg/kg, for methanol and 2000mg/kg for chloroform extract, so these doses were considered as the maximum tolerated doses. From this 1/10 of the dose was selected for further Pharmacological studies.

Alloxan Monohydrate

Alloxan monohydrate was used in physiological saline. The solution was injected as a single dose of 120mg/kg/B.W intraperitoneally within 50-75 seconds.

Experimental animals

Albino male rats (wistar strain) weighing between 150-200g were used in this investigation. Animals were purchased from the Teena Biolabs Pvt. Ltd, Hyderabad. Experimental procedure was approved by the Institutional Animal Ethical Committee (IAEC) of Gokaraju and Rangarju College of Pharmacy Osmania University, Hyderabad with registration number 177/99/CPCSEA. All the animals were kept for acclimatization for 2 weeks under laboratory conditions and fed with pellet diet and tap water ad libitum.

Induction of Experimental diabetes

The diabetes was induced in rats as described by Trivedi and his collaborators, animals were allowed to fast for 18 hrs prior to injection with freshly prepared solution of alloxan monohydrate 120 mg/kg, I.P.²⁴ The rats were kept on 5% glucose solution in the cages to prevent hypoglycemia. After 5 days, the rats with fasting serum

glucose levels more than 250mg/dl were considered as diabetic and were used in subsequent experimental procedures.

Experimental Design

The rats were divided in as normal animals and hyperglycemia induced animals. The hyperglycemic rats were divided into 4 groups consisting of six animals each.

Group 1: Normal control

Group 2: Diabetic control

Group 3: Diabetic rats treated with 10mg/kg of glibenclamide orally

Group 4: Diabetic rats treated with 400mg/kg (body weight) methanol extract orally (DVM)

Group 5: Diabetic rats treated with 200mg/kg (body weight) chloroform extract orally (DVC)

Group 1 and 2 animals were fed with 2% carboxy methyl cellulose. Group 3 animals were treated with standard drug glibenclamide dissolved in 2% carboxy methyl cellulose.

Group 4 and 5 animals were treated with DVM and DVC dissolved in 2% carboxy methyl cellulose daily from the day 1 to 14.

Blood glucose level and body weight were monitored at regular intervals for 2 weeks. Animals were sacrificed after 14 days of treatment four hours after dosing. Blood was collected and serum was separated by centrifugation at 5000 rpm for 20 min. collected serum was used for biochemical analysis.

Biochemical Estimation

Serum glucose level was determined according to Trinder et al., total protein was determined according to Lowry and his coworkers, triglycerides, cholesterol, urea, creatinine was determined according to the procedures given in the kits.^{25, 26} SGPT and SGOT were evaluated using Reitman and Frankel.²⁷

Histopathological Examination

Pancreas and liver were isolated and subjected to histopathological examination after fixing in 10%

Formalin. Sections were cut at 5µm thickness and stained with Haematoxylin and Eosin.²⁸ Microscope examination of sections was then carried out for histological changes.²⁹

Statistical Analysis

The values were expressed as mean± SEM. The obtained results were statistically analyzed using One-Way analysis of variance (ANOVA) followed by student-Newman Keuls Multiple comparison test. Values with p<0.05 were considered as significant.

Results

Body weight

Significant reduction in body weight of the animals was observed in diabetic group. After treatment with DVM and DVC for 14 days, the body weight was recovered significantly (p<0.05, p<0.01 respectively) compared to diabetic control (Table 1).

Blood Glucose Levels

High percentage of reduction in blood glucose levels were noted after giving DVM and DVC extracts, which are comparable to glibenclamide effect. Out of both the extracts DVC has shown maximum reduction in blood glucose (Table 2).

Lipid Profile

The effects of DVM and DVC on lipid profile of alloxan induced diabetic rats were identified and its significance can be tested (Table 3).

SGPT and SGOT

The effects of DVM and DVC on SGPT and SGOT levels of alloxan induced diabetic rats were recorded (Table 4).

The effects of DVM and DVC on Urea and Creatinine levels of alloxan induced diabetic rats were measured and recorded in table (Table 5).

Table 1: Effect of DVM and DVC on body weight profile of alloxan induced diabetic rats

Group	Initial body weight(gms)	Final body weight(gms)
Diabetic Control	229.16±10.03	178.33±8.72
Diabetic+ Standard	229.16±10.03	206±8.21*
Diabetic+ DVM	241.66±5.27	211.66±5.27*
Diabetic+ DVC	235.833±8.20	230±7.95**

***p<0.001**p<0.01*p<0.05 NS-Non significant

Table 2: Effect of DVM and DVC on glucose levels of alloxan induced diabetic rats

Group	Initial glucose level of diabetic rats	Final glucose level of diabetic rats	Percentage reduction
Diabetic Control	292.489±3.95	258.296±3.415	11.6%
Diabetic+ Standard	329.313±8.226	187.106±6.00***	43.18%
Diabetic+ DVM	328.115±7.63	184.451 ±4.20***	43.78%
Diabetic+ DVC	343.868±11.72	99.931±3.52***	70.93%

***p<0.001**p<0.01*p<0.05 NS-Non significant

Table 3: Effect of DVM and DVC on lipid profile of alloxan induced diabetic rats

Animal groups	Total cholesterol(mg/dl)	Total protein(g/dl)	Triglycerides(mg/dl)
Diabetic Control	117.3±3.29	5.15±0.18	185.6±8.22
Diabetic+ Standard	72.55±2.09***	6.62±0.14***	110.78±2.18***
Diabetic+ DVM	126.116±3.34**	8.20±0.08***	126.11±3.34***
Diabetic+ DVC	85.67±1.147***	7.36±0.13***	92.16±2.29***

***p<0.001**p<0.01*p<0.05 NS-Non significant

Table 4: Effect of DVM and DVC on SGPT and SGOT levels of alloxan induced diabetic rats.

Animal groups	SGPT(U/L)	SGOT(U/L)
Diabetic control	75.33±2.02	186.08±5.57
Diabetic+ Standard	27.4±1.47***	60.14±4.69***
Diabetic+ DVM	41.15±0.38***	88.55±4.01***
Diabetic+ DVC	39.21±1.28***	174.08±4.84 NS

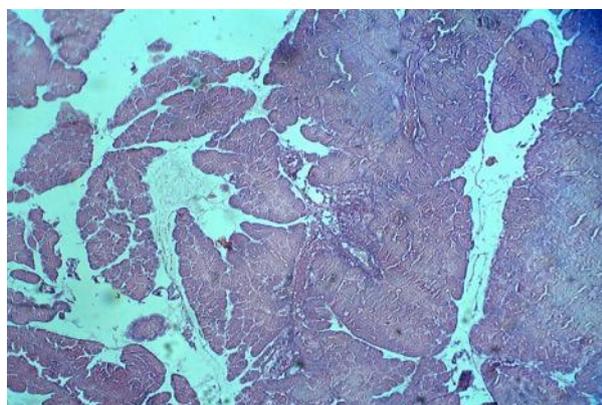
***p<0.001**p<0.01*p<0.05 NS-Non significant

Table 5: Effect of DVM and DVC on Urea and Creatinine levels of alloxan induced diabetic rats.

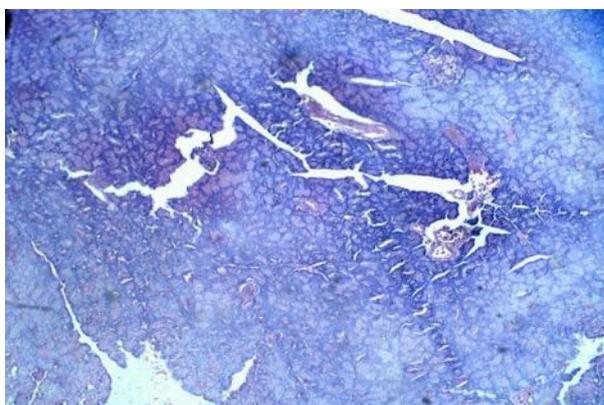
Animal groups	Urea(mg/dl)	Creatinine(mg/dl)
Diabetic Control	37.15±2.88	0.96±0.05
Diabetic+ Standard	18.38±0.89***	0.62±0.01***
Diabetic+ DVM	32.85±0.93*	0.08±0.008**
Diabetic+ DVC	16.06±0.68***	0.81±0.01**

***p<0.001**p<0.01*p<0.05 NS-Non significant

Histopathology of Pancreas



a



b

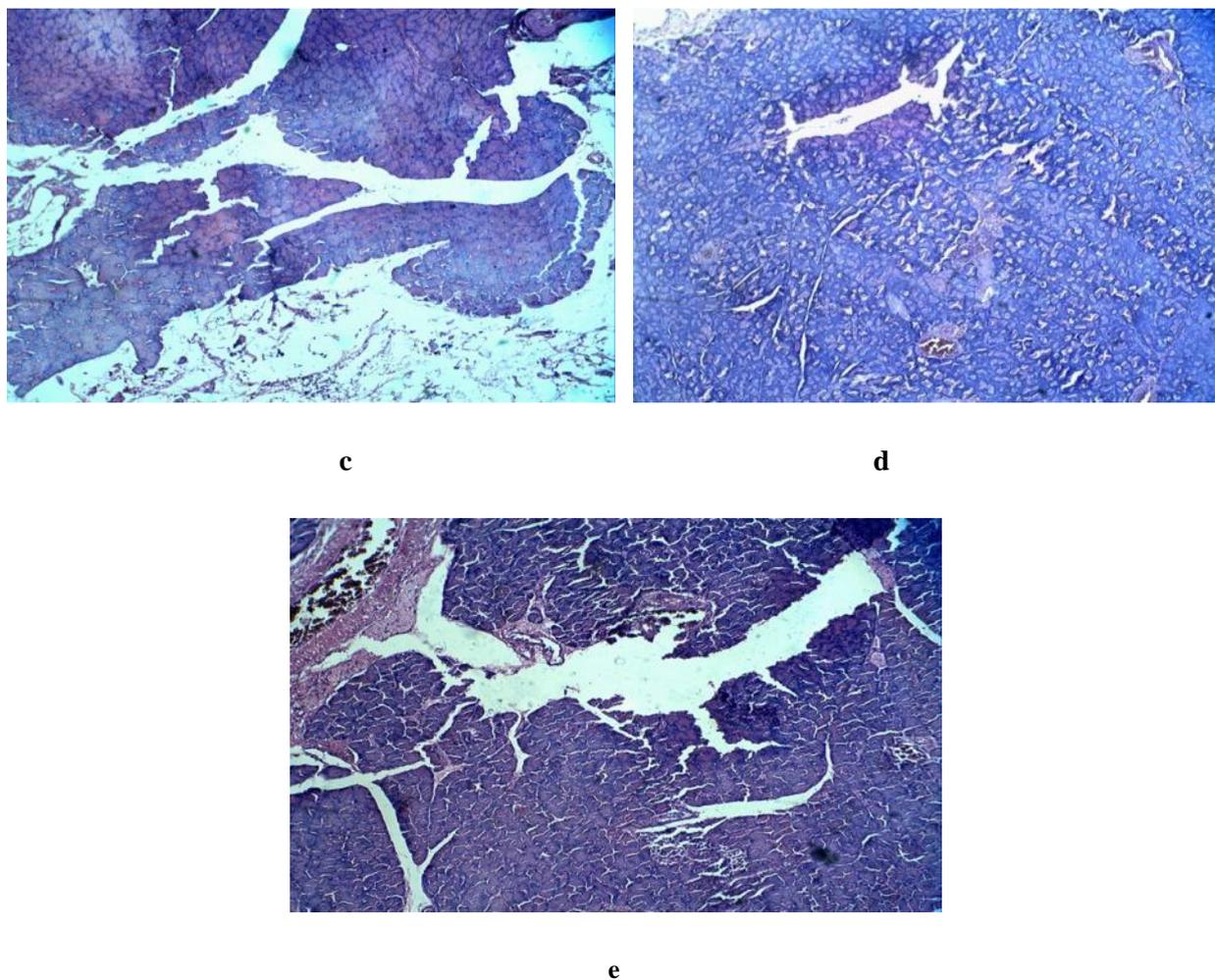
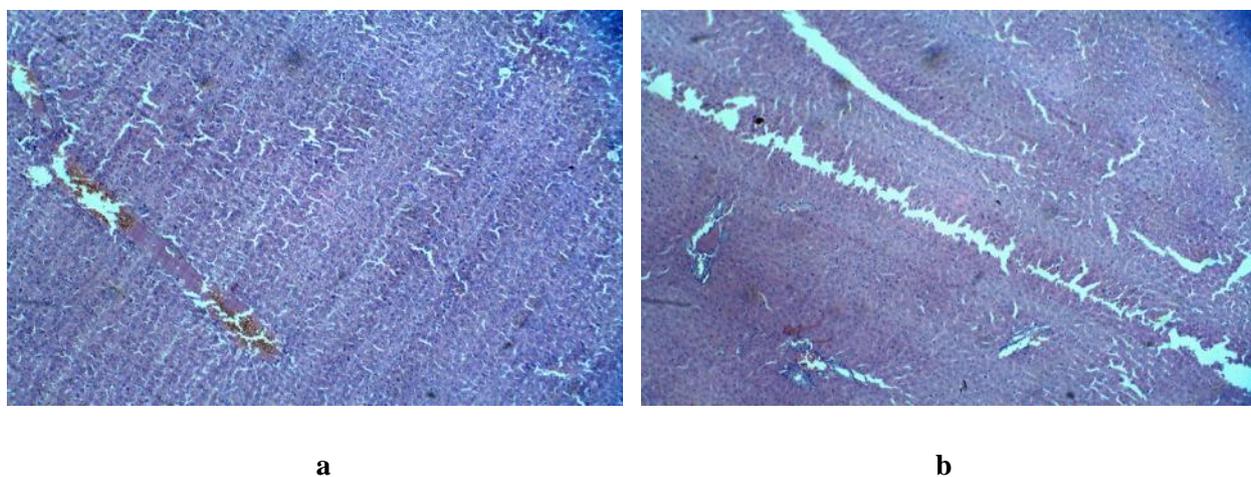


Figure 1: Sections of the pancreatic tissue of animals treated with extracts of *Dodonaea viscosa*. (a) Section of the pancreatic tissue of control animal showing normal islets; (b) Section of the pancreatic tissue of alloxan treated animals showing necrosis of islets (120mg/kg I.P); (c) Section of pancreatic tissue of animals treated with Glibenclamide (10mg/kg); (d) Section of pancreatic tissue of animals treated with DVM (400mg/kg); (e) Section of the pancreatic tissue of animals treated with DVC (200mg/kg).

Histopathology of Liver



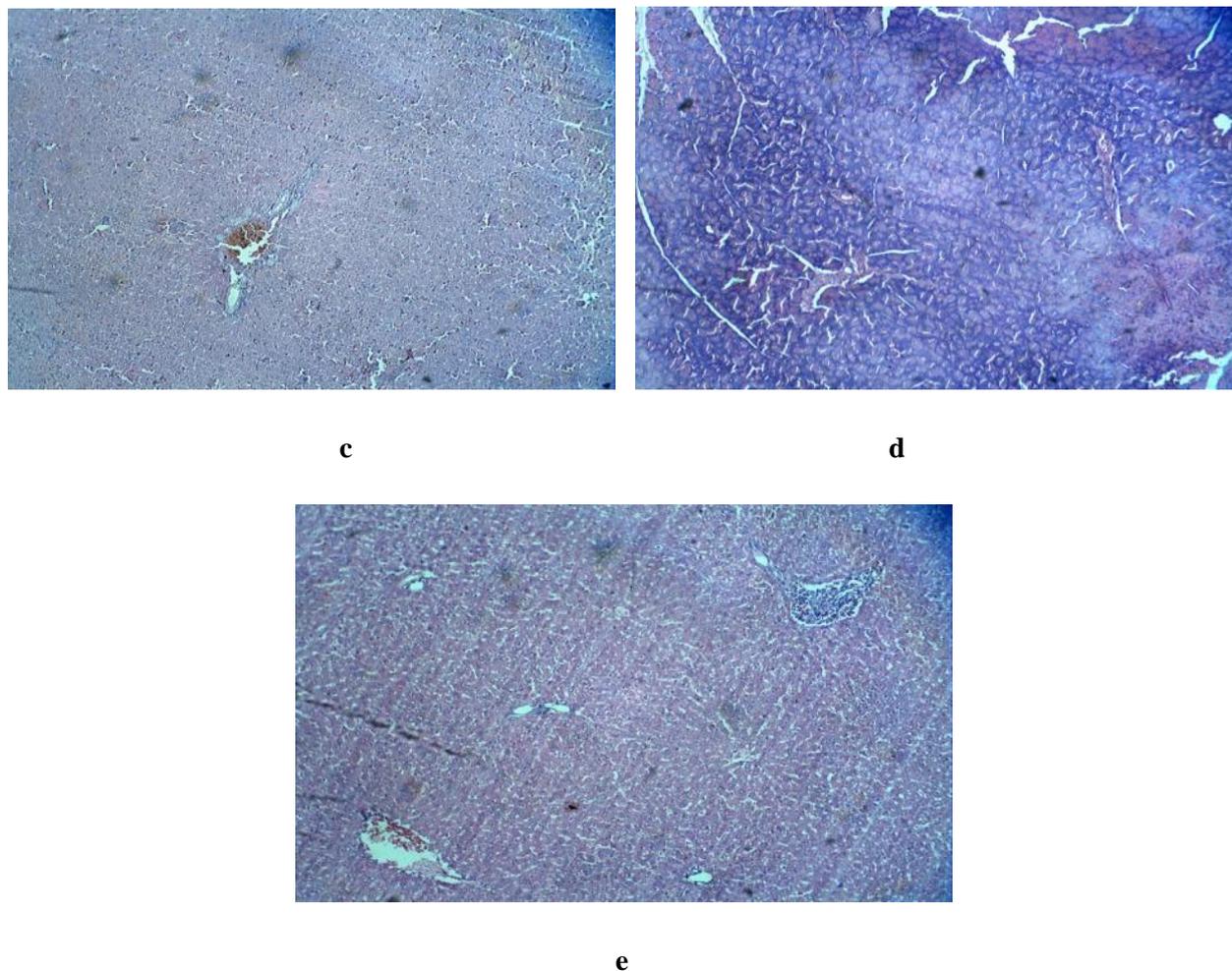


Figure 1: Sections of the liver tissue of animals treated with DVM and DVC. (a) Section of the liver tissue of control animal showing normal islets; (b) Section of the liver tissue of alloxan treated animals showing necrosis of islets (120mg/kg i.p); (c) Section of liver tissue of animals treated with Glibenclamide (10mg/kg); (d) Section of liver tissue of animals treated with DVM (400mg/kg); (e) Section of the liver tissue of animals treated with DVC (200mg/kg).

Discussion

Body weight

In diabetic rats, decrease in body weight was observed. This indicates the polyphagic condition and loss of weight due to excessive breakdown of tissue proteins. The decrease in body weight in diabetic rats could be due to dehydration and catabolism of fats and proteins, which further leads to muscle wasting, might also be the reason for the reduced body weight in diabetic rats.

Oral administration of extracts for consecutive 14 days to diabetic rats improves body weight. This could be due to better control of hyperglycemic state in the diabetic rats. Reversal of weight loss in extract treated diabetic group

indicates the restorative effect of the extract which may be due to reversal of gluconeogenesis and glycogenolysis.

Bio chemical parameters

In diabetic animals, insulin deficiency leads to various metabolic alterations in animals i.e. increased blood glucose, increased cholesterol, Triglyceride, SGPT, SGOT levels. Treatment with extracts reduced glucose levels and also reduced Triglycerides, Cholesterol, SGPT, SGOT levels thus reducing the complications caused due to diabetes.

It confirms the possibility that major function of the extract is the protection of vital tissues i.e. liver and pancreas and thereby reducing the causation of diabetes in the experimental animals. The present study also indicates that

D.viscosa can partially inhibit alloxan renal toxicity as observed from serum urea and creatinine levels.

Histological changes

Pancreas is the primary organ involved in sensing the dietary and energetic states. In histological study alloxan induced animals showed necrosis and reduction in the number of islets cells due to decrease in the antioxidant defense. The extract treated animals showed decreased cellular necrosis and regeneration.

Histopathological Evaluation of pancreas

Diabetic rats revealed degeneration cells in islets of langerhans of pancreas. It was also observed the islets were shrunken, inflammatory cellular infiltration with fibrosis. Treatment of these diabetic rats with glibenclamide inhibited alloxan induced shrinking of islets of langerhans of the pancreas, inflammatory cellular infiltration and enlarged pancreatic cells. Treatment with *Dodonaea viscosa* extracts (Both methanolic and chloroform extracts) reversed all the effects of alloxan dose dependently and supports biochemical tests.

Histopathological Evaluation of Liver

Liver sections showed necrosis and reduction in the number of cells due to decrease in the antioxidant defense after inducing diabetes with alloxan. Treatment with *Dodonaea viscosa* extracts (Both methanolic and chloroform extracts) reversed all the effects of alloxan dose dependently and supports biochemical tests.

The literature reports reveal that flavonoids and tannins present in plant extract known to possess antioxidant activity. In present investigation also the observed antidiabetic potential of test extracts may be due to the presence of similar phytoconstituents.

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