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Evaluation of hypoglycemic effect of ethanolic seed extracts of *Citrullus lanatus*

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Abstract

Background: Diabetes is one of the most serious, chronic diseases characterized by hyperglycemia resulting from defects in insulin secretion, insulin action, or both. It is becoming the third "killer" of the health of mankind after cancer and cardiovascular diseases. Diabetes is a metabolic disorder affecting about 220 million people worldwide. A number of plants have been described in Ayurveda for the management of diabetes. The present study was aimed to find out the hypoglycemic and non-toxic effect of *Citrullus lanatus* seed extracts. **Materials and Methods:** In acute toxicity study, there was no mortality observed up to the maximum dose level of 2000 mg/kg body weight of the extract after administered orally. After toxicity studies the various extracts of plant were used for hypoglycaemic activity in which the ethanolic extract showed very good reduction in blood glucose level. From that three doses were selected for oral glucose tolerance test and 400 mg/kg of ethanolic seed extract showed better glycaemic control. Hence antidiabetic studies were conducted with 400 mg/kg dose level for 30 days and the tissues antioxidant levels and histopathological studies were carried out by using standard protocols. **Results:** The ethanolic extract of *Citrullus lanatus* helps to maintain the antioxidant level in various organs and also helps to protect the organs from oxidative damage. **Conclusion:** From our study, the ethanolic seed extract of *Citrullus lanatus* controls the blood glucose level and also helps to prevent the organ from oxidative damage. Hence it can be used in the management of diabetes mellitus.

Keywords: *Citrullus lanatus*, Antioxidants, Streptozotocin (STZ), Glucose level, Tissues, Histopathology.

Introduction

Diabetes mellitus has been described as a metabolic disorder characterized by hyperglycemia and alteration in carbohydrate, fat and protein metabolism associated with absolute or relative deficiency of insulin secretion and/or insulin action.[1] The disease has affected millions of people all over the world.[2] Drugs of natural origin are considered to be less toxic and free from adverse effects than synthetic ones. Even though active compounds of many herbal drugs were unknown, they have been widely prescribed by the practitioners of traditional medicines due to their minimal adverse effects and low cost.[3]

Patients have been classified clinically as having either type 1 diabetes mellitus (insulin-dependent diabetes mellitus, IDDM), or type 2 diabetes mellitus (noninsulin dependent diabetes mellitus, NIDDM).[4] Type 2 diabetes mellitus, more commonly referred to as diabetes, is more prevalent and considered to be a world-wide epidemic,

which is projected to affect 366 million people by 2030.[5, 6] Oral agents are clearly a popular method of treatment in the battle for glycemic control. These drugs are, however, associated with a number of side effects. They cause severe hypoglycemia, renal impairments, flatulence, diarrhea and abdominal bloating.[7] These associated problems necessitate the search for better drugs with fewer side effects. Plant materials that have been used as traditional medicine for the treatment of diabetes are considered one of the good sources for a new drug or a lead to make a new drug.[8-10] Many plants are seen to possess hypoglycemic and antihyperglycemic properties.[11]

Citrullus lanatus (Cucurbitaceae) can be used for smoothes, sorbets or granite depending on the texture whether smooth or coarse. The fruit is also diuretic, being effective in the treatment of dropsy and renal stones. The rind of the fruit is prescribed in cases of alcoholic poisoning and diabetes. The root is purgative and in large dose is said to be emetic. The seed is demulcent, diuretic, pectoral and tonic. The main objective of the present research work was to evaluate the hypoglycaemic activity of the *Citrullus lanatus* seed extract by using animal model.

Materials and Methods

Collection of plant material

The seeds of *Citrullus lanatus* (Thunb.) were collected from Kerala, authenticated by Dr. C.V. Moorthy, Botanical Survey of India, Tamilnadu Agricultural University Campus, Coimbatore (Voucher No.BSI/SRC/5/23/2010-Tech 1728). The seeds were washed with water. They were air dried at room temperature for 10 days in the absence of sunlight and powdered well using a mixer. Then they were weighed and kept in an airtight container.

Sample extraction

The powdered plant material was subjected to successive solvent extraction using different solvents (petroleum ether, chloroform, ethyl acetate, ethanol and water) in the increasing order of polarity. 50g of dried plant powder was extracted in 250 ml of various solvent in an orbitory shaker for 72hrs. Obtained extract was evaporated to dryness by using a rotary vacuum evaporator at 40-50°C and stored at 0-4ºC in an air tight container for further use.

Experimental animals

Adult albino rats weighing about 150-200 g were obtained from the animal house of Karpagam University, Coimbatore and were used for the study. Rats were housed in polycarbonate cages in a room with a 12-hour day-night cycle, at constant temperature of 22ºC and humidity of 45-64%. During the experimental the study rats were fed on pellets (Gulmohur rat feed, Lipton India, Bangalore) with free access to tap water. The rats received humane care according to the criteria outlined in Principles of Laboratory Animal Care, 1985.

Induction of experimental diabetes

Rats were rendered diabetic by a single intraperitoneal injection of freshly prepared streptozotocin (45 mg/kg body weight) in 0.1M citrate buffer (pH 4.5) in a volume of 1ml/kg body weight. Normal rats received 1 ml citrate buffer as vehicle. Diabetes was identified in rats by moderate polydypsia and marked polyuria. After 48 hours of streptozotocin administration, blood glucose levels were estimated in rats following overnight fasting. Rats with a blood glucose ranging between 200–300 mg/dl were considered as diabetic and used for the experiments.

Glycemic index

After induction of diabetes, five different extracts namely petroleum ether, chloroform, ethyl acetate, ethanol and water extract at the concentration of 200 mg /kg were given orally to different group of rats containing 3 rats each, by a single intraperitoneal administration of streptozotocin (45 mg/kg body weight) for the period of 7 days. The body weight, blood glucose level and glycemic index of these rats were calculated at the end of 7th day. The glycemic index was found to be maximum in the water extract with 81.3 %, so it is used for the further study.

Glucose tolerance test (GTT)

The glucose tolerance tests on glucose loaded rats were performed on overnight fasted normal rats. The animals were divided into 4 groups as control (n=4); rats treated with 200, 400 and 600 mg/kg body weight of seed ethanolic extract of *Citrullus lanatus* for 7 days along with normal diet. At the end of the seventh day the rats were put on overnight fasting for 14hrs.On the next day, blood was drawn from tail vein and blood sugar was determined and reported as fasting blood glucose. The animals were then fed with glucose (4 g/kg) and blood was withdrawn from the tail vein every 30 min for the next 3hours and the

glucose level was estimated using one touch electronic glucometer using heamoglucostrips (Lifescan, Johnson and Johnson Ltd) and it was confirmed by the results of O-Toluidine method.

Experimental design for antidiabetic study

The Wister strain of albino rats weighing between 150-180g was used for the study. The animals were divided into five groups of six animals each. Group 1 serves as control rats, group 2 consisted of streptozotocin-induced rats (diabetic control), group 3 consisted of diabetic rats treated with 5mg/kg of reference drug Glibenclamide, group 4 rats consisted of diabetic rats treated with 400 mg/kg of *C. lanatus* and group 5 consisted of normal rats treated with 400 mg/kg of *C. lanatus*. The animals were weighed and the respective dosage was administered orally, daily using oral intragastric tube for 30 days. They were kept under controlled room temperature and photoperiod. Standard food in the form of pellets and water were provided.

Biochemical studies

After 30 days of treatment the animals were sacrificed under chloroform anaesthesia. Liver and kidney was quickly excised off, a portion of organs were washed with saline and their homogenates were prepared using 0.1 M phosphate buffer, pH 7.4. The liver and kidney homogenate was centrifuged and the supernatant was used for the determination of basal lipid peroxidation[12], ascorbate induced lipid peroxidation (13), peroxide induced lipid peroxidation[13], enzymatic antioxidant like superoxide dismutase[14], catalase[15], glutathione peroxidase[16], glutathione S transferase[17] and non-enzymatic antioxidants like total reduced glutathione[17] and Vitamin C.[18]

Histopathological studies

At the end of the study all the rats were sacrificed and the organs like liver, kidney and pancreas were collected. The tissue was fixed in 10% formalin immediately after removal from the animal to avoid decomposition for one hour. It was dehydrated by three changes of 500 ml of acetone and cleaned off from acetone by three changes of 500 ml of xylene for 3 hrs. Embedding in paraffin wax was carried out by removal of water using alcohol from 10-30% and then stained with hemotoxylin, which has an aqueous base. The sections were dehydrated using increasing concentrations of alcohol and then stained with eosin. They were then treated with diphenylxylene (DPX) and examined under the microscope to find the morphology.[19]

Statistical analysis

The values were expressed as Mean ± SD (n=6). The statistical analysis was carried out by one way analysis of variance using SPSS (version 10) statistical analysis program. Statistical significance was considered at $p<0.05$.

Result and Discussion

Diabetes is a major disease characterized by derangement of carbohydrates, fats and protein metabolism, affecting about 10% of the population.[20] Streptozotocin (STZ) selectively destroys β- cells of pancreas by generating excess ROS and carbonium ion leading to DNA breaks by alkylating DNA bases. The N-nitroso- N methyl urea portion of the molecule exhibits diabetogenic activity. Glucose may act as a carrier for this cytotoxic group.[21] The treatment of DM in clinical agents and insulin, the former being reported to be endowed with characteristic profiles of serious side effects.[22] This leads to increasing demand for herbal products with antidiabetic factor with little side effects.[23] A large number of plants have been recognized to be effective in the treatment of diabetes mellitus.[24] The present study was carried out to assess the protective effects of the ethanoic extract of *C. lanatus* seed on impaired glucose level induced by streptozotocin.

Acute toxicity study was conducted to evaluate the potential toxicity of high exposure of *C. lanatus* to experimental animals. Three groups of six animals each were orally administered with a single dose of the ethanolic extract of *C. lanatus* at different concentrations (500, 1000, 2000 mg/kg body weight). Rats were then monitored for mortality, signs of gross toxicity such as frequent urination, diarrhea and behavioral changes for first 24 hours and also for the next 72 hours to evaluate toxicity. No toxic effect was seen even with 4- 15 times the effective dose of ethanolic extract and there were no death in any groups. Result drawn from this study shows the relative oral safety of the extract at the dose of 2000 mg/kg. According to Clarke and Clarke[25] 1977, any compound or drug with the oral LD_{50} estimate greater than 1000 mg/kg could be considered of low toxicity and safe. Hence it can be concluded that ethanolic extract of *Citrullus lanatus* seeds is safe to use upto 2000 mg/kg body weight.

The five different extracts namely petroleum ether, chloroform, ethyl acetate, ethanol and water extract at the concentration of 200 mg/kg were orally administered to different group of rats containing four rats each in which diabetes was induced by a single intraperitoneal injection of streptozotocin (45 mg/kg body weight). The blood glucose level and glycemic index of these rats were calculated and compared to other extracts. The glycemic index was found to be maximum in the ethanolic extract (78.6%) which was followed by aqueous extract (74.6%). So, ethanolic extract was selected for further studies. The results were given in Table 1.

Table 1: Effect of various extracts of *Citrullus lanatus* seeds on blood glucose levels of Streptozotocin induced rats

Extacts (mg/kg body weight)	**Blood glucose level (mg/dl)**		
	Intial	**Final**	**Glycemic index (%)**
Petroleum ether	562.0±0.43	300.0±0.45	46.61
Chloroform	406.0±0.31	200.0±0.25	50.73
Ethyl acetate	569.0±0.54	330.0±0.35	42.00
Ethanol	500.0±0.21	107.0±0.46	78.60
Water	552.0±0.57	140.0±0.55	74.6

Values are expressed as Mean±S.D. Values are taken as a mean of three individual experiments

From the above result, the ethanolic extract of *Citrullus lanatus* seeds was chosen as the best among the other extracts with a glycemic index of 78.60% and a significant increase in body weight. The phytochemical screening of the ethanolic extract of *Citrullus lanatus* showed the presence of many chemical components like flavanoids, terpenoids, alkaloids, carbohydrates, glycosides and tannin which may be responsible for its antidiabetic property. Though the aqueous extract also showed competitive glycemic index, they had been ruled out because it did not help in gaining the deteriorated body weight. Further studies were carried out with the ethanolic seed extract of *Citrullus lanatus*.

The glucose tolerance test determines the hypoglycemic effect of various doses of *C. lanatus* extract. Figure 1 depicts the hypoglycemic effect of single administration of 200, 400, 600 mg/kg of ethanolic extract of *C. lanatus* seeds on blood glucose level of normal rats during GTT studies. The oral glucose tolerance test showed that the dose of 400 mg/kg of *C. lanatus* continuously reduced the blood glucose level. The dose 400 mg/kg showed a maximum fall of 24.29% in normal rats after the administration of glucose whereas a fall of 11.85% and 14.89% were observed in 200 and 600 mg/kg respectively. This phenomenon of less hypoglycemic response at a higher dose is not uncommon with indigenous plants and has already been observed in *Annona squamosa*, *Trichosanthes diocia* and *Ficus bengalensis*.[26] Since the dose 400 mg/kg showed a maximum fall in blood glucose level and it was identified as the most effective dose. Hence this dosage was used for the further studies in future to identify the actual mechanism for it hypoglycemic activity in Wistar albino rats.

Oxidative stress is produced during normal metabolic process in the body as well as a variety of environmental factors and chemical substances. Oxidative stress has been shown to have a significant effect in the causation of diabetes mellitus as well as diabetes related completions in human beings. Oxidative stress in diabetes mellitus has been shown to coexist with a reduction in the antioxidant status and glycation of proteins, inactivation of enzymes, and alteration in structural functions of collagen basement membrane. Scavengers of oxidative stress may have an effect in reducing the increased serum glucose level in diabetes mellitus and may alleviate the diabetes mellitus as well as reduce its secondary complications.[27]

SOD and GPx constitute a mutually supportive team of defense against Reactive Oxygen Species (ROS). SOD is a metalloprotein and is the first enzyme involved in the antioxidant defense by lowering the steady state level of O_2. In hyperglycaemia, glucose undergoes auto oxidation and produces superoxide and it produce free radicals that in turn leads to lipid peroxidation in lipoproteins. GPx catalyses the reaction of hydroperoxides with reduced glutathione to form glutathione disulphide (GSSG) and the reduction product of the hydroperoxide.[28] In our study, decline in the activities of these enzymes in STZ induced

animals and attainment of near normalcy in *C. lanatus* treated rats indicates oxidative stress elicited by STZ had been nullified due to the effect of the extract. This observation perfectly agrees with those of[29] who have demonstrated hypoglycaemic and antioxidant activity of *Salscia oblonga* wall extract in STZ induced diabetic rats. Similar to the finding in this study, a decrease has been observed in the activities of SOD and GPx in some of the tissues of diabetic rats.[30]

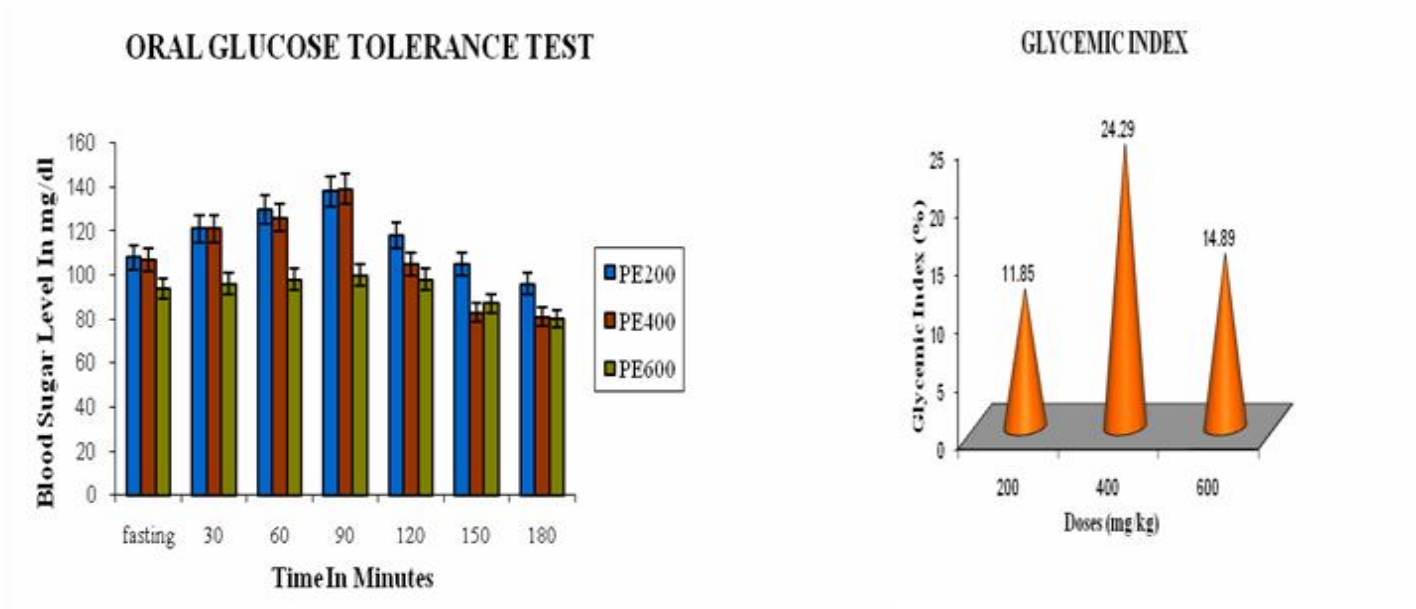

Figure 1: Oral glucose tolerance test (OGTT); Values are expressed as Mean S.D. Values are expressed as mean of four different experiments

From the Table 2 and 3 it was observed that GST level decreased in streptozotocin induced diabetices rats. The decreases in the activity of GST result in the involvement of deleterious oxidative changes and also insufficient availability of GSH. After the treatment with *C. lanatus* there was a marked elevation GST level in both the organs. Similar results were observed when the protective effect of *T. arjuna* and its potential to elevate the antioxidant status in liver and heart of diabetic rats were reported.[31]

Table 2: Effect of *C. lanatus* on the activities of antioxidant enzymes GPx, SOD, GST, polyphenol oxidase and catalase in the liver of control and experimental groups

Particulars	Control	Diabetic control	Diabetic + Glibenclamide	Diabetic + *C. lanatus*	*C. lanatus* alone
GPx	31.13±0.38[a]	13.12±0.37[b]	26.97±0.11[c]	25.50±0.41[d]	31.40±0.41[a]
Superoxide dismutase	6.96±0.04[a]	3.06±0.30[b]	6.19±0.08[c]	6.9±0.23[a]	7.09±0.35[a]
Catalase	68.0±0.07[a]	38.05±0.16[b]	64.17±0.12[c]	63.37±0.37[d]	68.01±0.13[a]
GST	8.3±0.11[a]	4.22±0.12[b]	7.5±0.61[c]	7.14±0.22[c]	8.26±0.24[a]

Values are expressed as Mean±S.D. Values are expressed as mean of four different experiments. Values not sharing a common superscript letter differ significantly (DMRT)

Units: GPx - µg of GSH/ mg of protein, Superoxide dismutase - Units/ g tissue, GST - µ moles of CDNB Conjugate formed/ mg of protein

Table 3: Effect of *C. lanatus* on the activities of antioxidant enzymes GPx, SOD, GST, polyphenol oxidase and catalase in the kidney of control and experimental groups

Particulars	Control	Diabetic control	Diabetic + Glibenclamide	Diabetic + *C. lanatus*	*C. lanatus* alone
GPx	31.15±0.16[a]	17.03±0.13[b]	27.80±0.43[c]	26.96±0.22[d]	31.18±0.11[a]
Superoxide dismutase	2.98±0.14[a]	0.84±0.02[b]	2.24±0.09[c]	2.12±0.69[c]	2.85±0.40[ac]
Catalase	26.23±0.10[a]	16.98±0.10[b]	24.22±0.10[c]	21.44±5.04[d]	26.32±0.12[a]
GST	7.16±0.15[a]	3.19±0.05[b]	6.61±0.03[c]	5.68±0.05[d]	7.42±0.13[e]

Values are expressed as Mean±S.D. Values are expressed as mean of four different experiments. Values not sharing a common superscript letter differ significantly (DMRT)

Units: GPx - µg of GSH/ mg of protein, Superoxide dismutase - Units/ g tissue, GST - µ moles of CDNB Conjugate formed/ mg of protein

Diabetes can increase oxidative stress and change the redox potential of glutathione. The decreased level of GSH in liver and kidney of diabetic rats may increase the susceptibility to oxidative stress. Reduction of oxidised form of glutathione requires NADPH, as a cofactor and enzyme glutathione reductase.[32] The reduced availability of NADPH could be either due to reduced synthesis or increased mobilization during diabetes. Some workers also reported that this could be the reason for the low levels of reduced glutathione in diabetic rats.[33] Administration of *C. lanatus* extract (400 mg/kg) and glibenclamide increases the GSH in liver and kidney of diabetic rats which may be due to the less production of ROS (Table 3).

Non enzymatic antioxidants are involved in scavenging free radicals in vivo. They are significantly impaired in STZ induced diabetic rats. Reduced level of non enzymatic antioxidants observed in plasma and tissue in STZ induced diabetic rats as compared to diabetic rats could be due to increased oxidative stress. From the Table 3 and 4 the administration of *C. lanatus* seed extract improve the level of vitamin C in liver and kidney of diabetic rats, may be expected to enhance the GSH level or stimulation of the system to recycle dehydroascorbic acid back to ascorbic acid.[33]

Table 4: Effect of *C. lanatus* on concentration of vitamin C and glutathione in the liver and kidney of control and experimental groups

Particulars		Control	Diabetic control	Diabetic + Glibenclamide	Diabetic + *C. lanatus*	*C. lanatus* alone
Vitamin C	Liver	1.85±0.03[a]	0.70±0.03[b]	1.68±0.03[c]	1.60±0.02[d]	1.85±0.011[a]
	Kidney	1.66±0.03[a]	0.62±0.01[b]	1.5±0.022[c]	1.52±0.01[d]	1.92±0.022[e]
Glutathione	Liver	52.7±0.22[a]	30.3±0.18[b]	46.7±0.09[c]	43.9±0.23[d]	52.04±0.17[a]
	Kidney	47.1±0.09[a]	25.3±0.15[b]	44.9±0.45[c]	41.9±0.24[d]	45.14±0.10[c]

Values are expressed as Mean±S.D. Values are expressed as mean of four different experiments. Values not sharing a common superscript letter differ significantly (DMRT)

Units: Vitamin C – mg/g of protein, Glutathione - µg/mg protein

Table 5: Effect of *C. lanatus* on the concentration of lipid peroxidation, ascorbate induced lipid peroxidation and peroxide induced lipid peroxidation in the liver and kidney of control and experimental groups

Particulars		Control	Diabetic control	Diabetic + Glibenclamide	Diabetic + *C. lanatus*	*C. lanatus* alone
Basal LPO	Liver	14.13±0.14[a]	29.17±0.11[b]	19.18±0.17[c]	21.0±0.19[d]	15.24±0.22[e]
	Kidney	10.14±0.30[a]	26.82±0.51[b]	14.11±0.55[c]	15.18±0.18[d]	10.14±0.57[a]
Ascorbate induced LPO	Liver	15.1±0.17[a]	35.31±0.64[b]	19.48±0.27[c]	20.21±0.19[d]	15.21±0.14[a]
	Kidney	12.1±0.17[a]	30.31±0.64[b]	15.48±0.27[c]	16.21±0.19[d]	12.21±0.14[a]
Peroxide induced LPO	Liver	9.9±0.11[a]	25.24±0.24[b]	13.24±0.14[c]	15.43±0.28[d]	9.45±0.04[a]
	Kidney	8.9±0.11[a]	22.24±0.24[b]	11.24±0.14[c]	12.43±0.28[d]	8.45±0.04[a]

Values are expressed as Mean±S.D. Values are expressed as mean of four different experiments. Values not sharing a common superscript letter differ significantly (DMRT)

Units: Basal Lipid peroxidation - nm of MDA/ gm tissue, Ascorbate induced lipid peroxidation - nm of MDA/ gm tissue, Peroxide induced lipid peroxidation - nm of MDA/ gm tissue

Lipid peroxidation is one of the characteristic features of diabetes mellitus. Increased lipid peroxidation in diabetic condition may be due to the oxidative stress in the cells as a result of depletion of antioxidant scavenging enzymes like SOD, CAT, GPx and GSH. In diabetes, hypoinsulineamia increases the activity of fatty acyl co enzyme A oxidase, which intiates β oxidation of fatty acids, resulting in lipid peroxidation. Lipid peroxidation alters the membrane permeability and also produces substances which are harmful to the cells in the body which are associated with atherosclerosis, brain and kidney damage.[31] The extract showed a significant reduction in lipid peroxidationin liver and kidney when compared to diabetic control (Table 5). Thus it can be concluded that the ethanolic extract of C.lanatus has the ability of providing good antioxidant potential.

The histopathology of Liver and kidney of various groups were shown in figure 2 and 3.

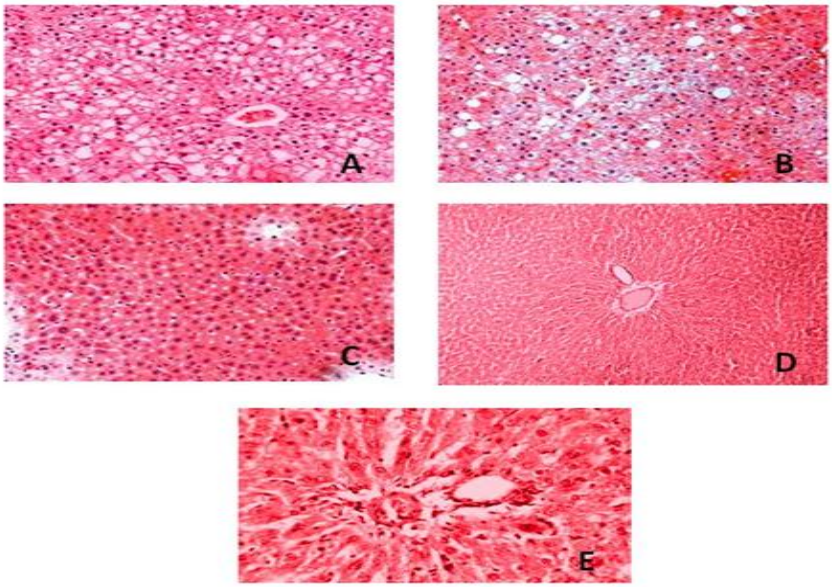

Figure 2: Histopathology of rat liver. **A** represents the liver of control animals showing normal histology, **B** served as diabetic control which showed mild fatty changes and necrosis, **C** represent liver of streptozotocin rats treated with standard drug glibenclamide showing normal histology, **D** depicts the liver of streptozotocin rats treated with the ethanolic extract of *C. lanatus*

(400 mg/kg) showing normal histology as that of control and **E** denotes the liver of normal rats treated with the ethanolic extract of *C. lanatus* (400 mg/kg) showing normal histological structure.

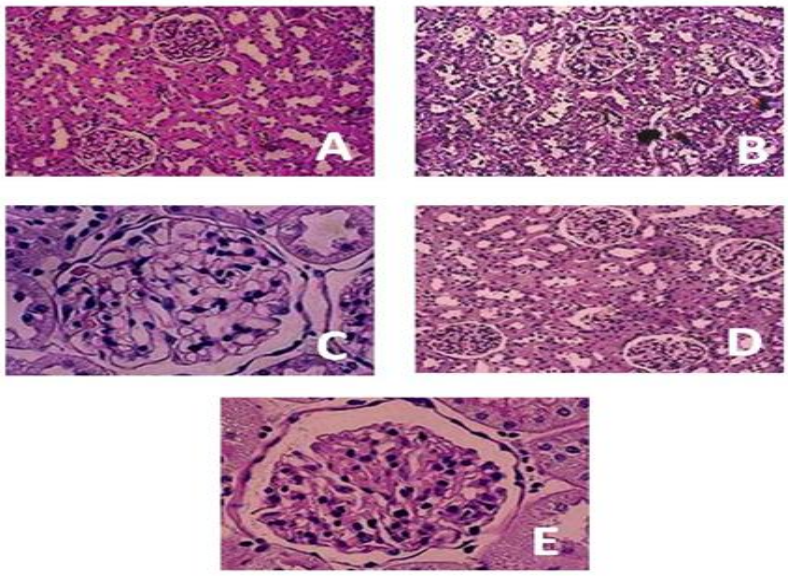

Figure 3: Histopathology of kidney of various groups. **A** denotes the kidney of control animals showing normal histology, **B** showing focal renal tubular atrophy (diabetic control), **C** served as kidney of streptozotocin rats treated with standard drug, Glibenclamide showing normal histology, **D** represents kidney of streptozotocin rats treated with the ethanolic extract of *C. lanatus* (400 mg/kg) showing normal histology and **E** was kidney of normal rats treated with the ethanolic extract of *C. lanatus* (400 mg/kg) showing normal histological structure.

Conclusion

The present investigation indicates that ethanol seed extract of *C. lanatus* exerts significant protection against streptozotocin induced oxidative stress in diabetes by its potential to ameliorate the lipid peroxidation through the free radicals scavenging activity, which enhanced the levels of antioxidant defense system. This effect may be attributed to the antioxidant principles due to the present of secondary metabolites in plant material. Further studies are needed for the identification exact mechanism for this activity.

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Conflict of Interest

We declare that we have no conflict of interest.

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