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Research Article

HYPOGLYCEMIC EFFECT OF METHANOLIC EXTRACT OF*CITRULLUS LANATUS* SEEDS

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ABSTRACT

The aim of present work is to study the hypoglycemic effect of methanolic extract of *Citrulluslanatus* seeds in streptozotocin (STZ) induced diabetic rats. The diabetes was induced by a single dose of STZ (65 mg/kg) in citrate buffer, while the normal control group was given the vehicle (citrate buffer) only. After three days of induction of diabetes, the diabetic animals were treated further four weeks with methanolic extract of *Citrulluslanatus* seeds (MECL) (200,400 and 600 mg/kg) and glibenclamide (4 mg/kg). Blood glucose estimation was performed every week of the study. Food and water consumption, urine output and urine frequency were observed every week in 24 hours. At the end of study period, animals were sacrificed to perform the histopathological study of liver, kidney and pancreatic tissue. STZ-induced diabetic rats showed marked hyperglycemia, along with polyphagia, polydipsia andpolyureathroughout the study period. Microscopically, liver tissue showed Kupffer cells activation, dilation and congestion of central vein and hepatic sinusoids.Kidney tissue showedglomerular lesions, irregular and widened capillaries and necrosis of tissues.Whereas pancreatic tissue showeddisrupted islets of Langerhans exhibiting hydrophobic cells, necrotic cells, vacuolizations andirregular hyperchromic nuclei.Severeand diffused inflammatory cell infiltration was observed in all tissues. The four-week treatment with methanolicextract of *Citrulluslanatus*(MECL) seeds (200, 400 and 600 mg/kg) significantly reduced the elevated fasting blood glucose level and recovery of polyphagia, polyurea and polydipsia in diabetic rats. Microscopically, there was attenuated morphological disturbance, reduction in necrosis and inflammatory cell infiltration and thus further tissue damage in liver, kidney and pancreas. Thus, present study suggested the antidiabeticpotential of *Citrulluslanatus*seeds and thereby preventing otherrelated diabetic complications.

Keywords: *Streptozotocin, Citrulluslanatus, Kupffer cells, Vacuolization.*

INTRODUCTION

The incidence of diabetes is growing rapidly both in the United States and worldwide. For example, it is estimated that more than 180 million people worldwide are afflicted with diabetes, and the prevalence is expected to more than double by the year 2030. Diabetes is not a single disease. Rather, it is a heterogeneous group of syndromes characterized by an elevation of blood glucose caused by a relative or absolute deficiency of insulin. Diabetes mellitus is a metabolic disorder characterized by hyperglycemia, glycosuria, hyperlipidemia, negative nitrogen balance and sometimes ketonemia.

The American Diabetes Association (ADA) recognizes four clinical classifications of diabetes: Type 1 diabetes (formerly insulin-dependent diabetes mellitus),Type 2 diabetes (formerly non-insulin dependent

diabetes mellitus), Gestational diabetes, and Diabetes due to other causes (e.g. genetic defects or medication induced)(Manisha*et al*., 2011).The presence of DM shows increased risk of many complications such as cardiovascular diseases, peripheral vascular diseases, stroke, neuropathy, renal failure, retinopathy, blindness, amputations etc (Arora*et al*., 2009).Drugs such as insulin and oral hypoglycemic drugs are used primarily to save life and alleviate symptoms. Secondary aims are to prevent long-term diabetic complications and, by eliminating various risk factors, to increase longevity(Ismail MY, 2009). However none of these medications is ideal due to their toxic side effects and diminution of responses is observed sometimes in their prolonged use(Jothivel*et al*., 2007). The main disadvantage of currently available drugs is that they have to be given throughout the life and they do not restore normal glucose homeostasis(Bastaki S, 2005).Moreover, due to the high cost of allopathic drugs it is difficult to provide modern medical healthcare, especially in developing countries. It is therefore become necessary to make use of vast reserves of plant origins for medical purposes which will help to search effective as well as safer drug remedy for diabetes mellitus.

Cucurbitaceae plants are known to contain bioactive compounds such as cucurbitacin, triterpenes, sterols and alkaloids. Scientific studies mainly refer to the Middle East and Asia where cucurbit plants were used actively as herbal remedies which shown tremendous results regarding the use of this plant. *Citrulluslanatus* (Water melon), is used widely in traditional herbal medicine. 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 a large dose is said to be emetic. The seed is demulcent, diuretic, pectoral and tonic. It is sometimes used in the treatment of the urinary passages and bed wetting. The seed is also a good vermifuge and has hypotensive action.The fatty oils in the seed, as well aqueous or alcoholic extracts, paralyze tapeworms and roundworms. In Northern Sudan, it is often used for the treatment of burns, swelling, arthritis and as alaxative(Dixit V and Joshi S, 1985). Studies have shown that fruits and vegetables contain vital nutrients, vitamins, phytochemicals, fibers, antioxidants and consumption of at least 5-10 serving daily significantly reduce the risk of chronic diseases and meet nutrient requirement for optimum good health(Bandavane*et al.,* 2011). It also contains steroids, terpenoids, carotenoids, flavonoids, alkaloids, tannins and glycosides which act as antibiotics. These photochemical have been reported to exhibit antifungal, anti-inflammatory, fungistaticactivities(Hassan *et al.,* 2011). Although leaves root, stem, flowers and whole plant were examined for useful photochemical in many studies, few reports refer to seeds as a source of phytoconstituents and their useful effects. The seeds are often considered waste part of plant, being a good source of phytoconstituents. Consumptions of varieties of plant food including watermelon seeds in diabetes may provide additional health benefits. This paper,therefore,represents the hypoglycemic potential of phytoconstituents present in watermelon seeds.

MATERIAL AND METHODS

Collection and authentication of *Citrulluslanatus*

Freshseeds of watermelon were purchased from local markets of Pune and authenticated by J. Jayanthi at Botanical Survey of India, Koregaon road, Pune-01. They were washed, dried and crushed to make the coarse powder. (Voucher No. BSI/WRC/Tech/2012).

Preparation of extract

The crushed material was weighed exactly about 650 gm and minced using mixer grinder for the fine powder. It was then extracted with 80% methanol by Soxhlet apparatus for 7 days in dark under room temperature with intermittent shaking. After 7 days, the extracted material was collected and filtered using muslin cloth at first and then through filter paper. It was evaporated to dryness under low pressure and temperature to get the concentrated extract. It was stored first in the desiccator for 3 days then in the refrigerator for pharmacological studies(Jain *et al*., 2013).

Chemicals

Streptozotocin (STZ) and Glibenclamide was purchased from New Neeta Chemicals, Pune(Bill No.1486). Glucose, Triglyceride, Total cholesterol and HDL cholesterol kits were purchased from Universal Diagnostics, Pune (Bill No.256). All other chemicals purchased from SD Fine Chemicals, Mumbai, were of analytical reagent grade. Accu-check glucometer with 100 strips was purchased online from Bantom Laboratories Pvt Ltd, Paharganj, New Delhi (Invoice no.C29131/14-15/8224).

Animals

Male Wistar rats of seven weeks old (150-200 g) were obtained from National Institute of Bioscience, Sinhgad Road, Pune (Invoice no.B-

30). Before and during experiment rats were fed with standard pellet diet (Nutrivit life sciences, Pune) and free access to water. After randomization into various groups and before initiation of experiment rats were acclimatized for a period of 7 days under the standard environmental condition of temperature, relative humidity, and light/dark cycle. Animals stated as fasting were deprived of food and water for 16 hours ad libitum.All the experimental procedures were carried out in accordance with the committee for the purpose of control and supervision of experiments on animal (CPCSEA) guidelines. The study was reviewed and approved by the Institutional Animal Ethical Committee, Maharashtra Institute of Pharmacy, Kothrud, Pune-38.(Approval no. MIP/IAEC/2013-2014/M1/Apr/001).

Acute and short-term Toxicity study

The methanolic extract of *Citrulluslanatus* seeds was tested for its acute and short-term toxicity in mice. To determine acute toxicity of the drug, overnight fasted Wistar albino mice were orally fed with extract in increasing dose of 100, 300, 500, 1000, 1300, 1500 to 5000 mg/kg body weight. The mortality and general behavior of animals were observed periodically for 72 hours and observed continuously for initial period of 4 hours, intermittently for next 6 h, then again at 24 h, 48 h and 72 hours following drug administration. The parameters observed with the higher dose more than 1300 mg/kg were grooming, sedation, loss of reflex, etc(Bandavane*et al.,* 2011;Arambewela*et al.,* 2005).

Determination of test dose

During preliminary toxicity study, no any mortality was observed in experimental animals with oral administration of seed extract up-to high dose of 5 gm/kg body weight observed for 48 hours. Hence, sub-maximal doses of 200, 400 and 600 mg/kg were selected as a test dose.

Experimental induction of diabetes

Diabetes was induced by using Streptozotocin (STZ) as diabetes inducing agent. The solution was injected once intra-peritonealy in a dose of 65 mg/kg in rats. 5% glucose solution was administered orally for 24 hours to prevent mortality due to initial hypoglycemia induced by streptozotocin. After 72 hrs of STZ injection, fasting blood glucose levels were tested using glucose oxidase-peroxidase reactive strips (Accu-Chek glucometer) Rats showing fasting blood glucose level more than 200 mg/dl were considered diabetic and used for further study(Bandavane*et al.,* 2011;Adaramoye*et al*., 2006).

Experimental animal groups

Rats were divided into different groups as follows

Group I- as normal control where rats received citrate buffer daily

Group II- as diabetic control where rats received citrate buffer daily and STZ-65 mg/kg once on the first day (diabetic rats)

Group III- diabetic rats received 200 mg/kg methanolic extract of seeds of*Citrulluslanatus*

Group IV- diabetic rats received 400 mg/kg methanolic extract of seeds of*Citrulluslanatus*

Group V- diabetic rats received 600 mg/kg methanolic extract of seeds of*Citrulluslanatus*

Group VI-diabetic rats received 4 mg/kg Glibenclamide as an oral hypoglycemic agent.

EXPERIMENTAL PROCEDURE

Blood glucose estimation

Fasting blood glucose levels were determined in all experimental rats initially to determine the diabetic status of animals and thereafter every week during the 28 day study period. Blood was obtained fromthe retro-orbital sinus ofanesthetized rats with the help of capillary tube and blood glucose levels were determined using Accu-ChekGlucometer (Bandavane*et al.,* 2011).

Determination of food consumption

The amount of food consumption of rats was estimated in 24 hours in day 0, 7, 14, 21 and 28. Individual animal were kept in a small single cage and predetermined quantity of food pellets was kept in cages 24 hours before day 0, 7, 14, 21 and 28. The remaining food pellets after 24 hours were weighed and subtracted from initial food weight to know the amount of food consumed (Ulman et al., 2008).

Determination of water consumption

The amount of water consumption of rats was estimated in 24 hours in day 0, 7, 14, 21 and 28. Individual animal were kept in a small single cage and predetermined quantity of water was kept in cages 24 hours before day 0, 7, 14, 21 and 28. The remaining water after 24 hours were measured and subtracted from initial water quantity to know the amount of water consumed(Ulman et al., 2008).

Determination of urine output

Urine quantity of rats was estimated in 24 hours in day 0, 7, 14, 21 and 28. Individual animal was kept in ametabolic cage and allowed free access to water 24 hours before day 0, 7, 14, 21 and 28.

The urine was collected in a beakerduring 24 hours and measured(Marijke et al., 1997).

Determination of urine frequency

Urine frequency was determined in 24 hours in day 0, 7, 14, 21 and 28 by counting colored spots on filter paper. The individual rat was kept in a metabolic cage with free access to water. Filter paper marked with 12 X 12 cm square was kept and spread securely on the base of cage. Numbers of spots were determined by counting it under UV light after 24 hours to know urine frequency(Bruce 2013).

Histopathological study

At the end of the study, all the rats were sacrificed and the organs like liver, kidney and pancreas were collected. The tissues were 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 (Sumane*t al.,* 2013).

Statistical analysis

All results are expressed as the mean ± SEM. The results were analyzed for statistical significance by one-way ANOVA followed by Dunnets's Multiple Test of Comparison.

RESULTS

The effect of STZ and methanolic extract *Citrulluslanatus* seed (MECL) on fasting blood glucose level is shown in Table 1. On repeated administration of MECL, for 28 days, a sustained and significant ($p<0.01$) decrease in blood glucose level of diabetic rats was observed in dose-dependent manner as compared with diabetic control group. In diabetic rats, blood glucose level was reduced by 57.9 mg%, 66.4mg% and 93 mg% in 200, 400 and 600 mg/kg doses of MECL respectively (on 28th day). The standard oral hypoglycemic drug glibenclamide (4 mg/kg) showed more potent antidiabetic activity by reducing blood glucose level by 108.2 mg% as compared with diabetic control group (on 28th day).

As shown in Table 2, STZ diabetic rat's treated with MECL showed significant ($p<0.01$) reduction in the food consumption as compared to diabetic rats. Chronic treatment of extract (600 mg/kg) and glibenclamide (4 mg/kg) reduced total food consumption (in 24 hours of day 28) by 3.37 gm and 4.27 gm respectively. Similarly chronic treatment of MECL in doses 200 mg/kg and 400 mg/kg also found to lower food consumption significantly by 2.17 gm and 2.8 gm respectively(in 24 hours of day 28). These effects indicate an anti-polyphagic property of the drug.

As shown in Table 3, STZ diabetic rat's treated with MECL showed significant ($p<0.01$) reduction in the water consumption as compared to diabetic rats. Chronic treatment of extract (600 mg/kg) and glibenclamide (4 mg/kg) reduced water consumption (in 24 hours of day 28) by 4.77 ml and 4.3 ml respectively. Similarly chronic treatment of MECL in doses 200 mg/kg and 400 mg/kg also found to reduce water consumption significantly by 1.9 ml and 2.77 ml respectively(in 24 hours of day 28).These effects indicate the reduction of thirst in diabetes.

As shown in Table 4, urine output is significantly elevated in the diabetic control group as compared with a normal group throughout the study period. MECL in a dose of 200 mg/kg and 400 mg/kg significantly reduced urine output (in 24 hours of day 28) by 2.93 ml and 4.2 ml respectively indicating hypoglycemic effect. The more pronounced effect is observed when MECL is given in a dose of 600 mg/kg i.e. 5.1 ml reduction as compared with diabetes induced group. The same effect is observed in glibenclamide (4 mg/kg) treated groupi.e.5.07 ml reduction as compared with diabetes induced group. These effects were observed in 24 hours of day 28. This shows that watermelon seeds are very effective in the treatment of polyurea.

As shown in Table 5, urine frequency is significantly elevated in the diabetic control group as compared with the normal group throughout the study period. MECL in a dose of 600 mg/kg and glibenclamide (4 mg/kg) significantly lowered the urine frequency by 5.01 and 6.01 respectively in 24 hours of day 28. Similarly, MECL in a dose of 200 and 400 mg/kg also reduced urine frequency by 3.67 and 4.34. This effect is also an evidence of hypoglycemic activity of the drug.

Histopathological Results

As per shown in Figure 1a, liver sections of STZ induced group showsvacuolated hepatocytes and necrosis, infiltration of inflammatory cells, and disruption of normal architecture. It also shows Kupffer cell activation and dilation and congestion of central vein and hepatic sinusoids.

A liver section of drug treated group isshowing reduced vacuolations in hepatocytes, focal necrosisand infiltration of inflammatory cells in a dose dependant manner as compared with diabetic control group. A liver section of Glibenclamide treated group restores almost completely degeneration of hepatocytes and normal morphology of tissue.

As per the shown in Figure 1b, severe tubular degeneration, irregular and widened capillaries with necrosis of tissuesand vacuolation in glomeruli were observed in kidney sections of diabetes-induced group. The severity of all these changes is reduced in drug treated group in dose dependant manner. The tubular degeneration, inflammation and necrosis of glomerulus were recovered in glibenclamide treated group.

As per shown in Figure 1c, disrupted islets of Langerhans exhibiting hydrophobic cells, necrotic cells, vacuolizations and irregular hyperchromic nuclei are observed in pancreatic tissue in the diabetic induced group. The number and size of islets cell are also reduced in this group. Lower, middle and higher concentration of drug treated groups showing restoration islets of langerhans cells, reduction in necrosis and vacuolizations in dose dependant manner as compared to the diabetic induced group. Glibenclamide treated group showing almost normal restoration and morphology of islets cell.

DISCUSSION

The development of type 2 diabetes is associated with pancreatic β-cell dysfunction occurring together with insulin resistance. Normal β-cell can compensate for insulin resistance by increasing insulin secretion, but insufficient compensation leads to the onset of glucose intolerance. Once hyperglycemia becomes apparent, β-cell function progressively deteriorates, glucose-induced insulin secretion becomes further impaired and degranulation of β-cell becomes evident, often accompanied by the decrease in the number of β-cell.Chronic hyperglycemia may impair β-cell function at the level of insulin synthesis as well as insulin secretion (Kaneto*et al*., 1999). This diabetes is associated with factors which directly contribute to cardiovascular disorder including resistance, dyslipidemia, atherosclerosis, hypertension(Lio*et al*., 1998; Ivorra*et al*., 1989)endothelial dysfunction and vascular inflammation(Marles RJ andFamsmith NR, 1995; Kesari*et al*., 2006).Obesity is another risk factor in the development of diabetes and CHD(Gupta *et al.,* 2005). Pharmacotherapy is available for treatment of diabetes in the modern healthcare system and that include insulin and oral hypoglycemic drugs(Tripathi KD, 2003). However due to economic problems, it is not possible to take these medications on daily basis. Plants are the more potent healer because they promote the repair mechanism in a natural way(Chitra*et al.,* 2009). Various herbs, plants, leaves, spices have been described for the treatment of diabetes (Gurib, 2006; Pareez*et al*., 1998; Son 2003). Over 150 plants extract and some of their active principles including flavonoids, tannins, alkaloids etc are used and very few of these plants have been screen pharmacologically(Erememisoglu*et al.,* 1995; Grover *et al*., 2002).

Streptozotocin-induced diabetes model is an important and valuable model for induction of diabetes mellitus in experimental animals. Streptozotocin is toxic to beta cells in the pancreas; it probably induces diabetes by making pancreas swell and causing degeneration of beta cells in islets of langerhans in pancreas in 2-4 days after induction. Streptozotocin induces one type of diabetes which is similar to diabetes mellitus with non-ketosis hyperglycemia in some animal species(Akbarzadeh*et al.,* 2007). However, animals survived without insulin treatment and showed improvement by glibenclamide which act by stimulating beta cells and preventing their further damage and destruction in the present study. This model shows symptoms like hyperglycemia, polyphagia, polyuria, polydipsia which can be easily tested in animals.

In the present study, STZ exhibited a significant increase in blood glucose level in rats. Chronic treatment with the methanolic extract of *Citrulluslanatus* (MECL) reduced blood glucose level throughout the experimental period in dose-dependent manner indicating its hypoglycemic activity. With a decrease in glucose, consumption of food and water also decreases along with the reduction of urine output and frequency. Microscopically, all morphological alterations such as necrosis, inflammation, vacuolations, in liver, kidney and pancreas are restored with chronic administration of MECL.

CONCLUSION

The present study showed that methanolic extract of *Citrulluslanatus* seeds significantly lowered elevated blood glucose level in STZ induced diabetic rats without showing hypoglycemic effects in normal rats. The exact mechanism of *Citrulluslanatus* in the treatment of diabetes is unclear, probably methanolic extract may reduced blood glucose by either stimulation of insulin release from beta cells

that are less damaged or may be survived in the presence of streptozotocin. This antidiabetic effect may further be due to increased utilization of glucose by tissues or by improved sensitivity of target tissues for insulin or it may be due to improved metabolism of glucose. This also reduced symptoms of diabetes. Thus, the present study concludes that the *Citrulluslanatus* possesses remarkable hypoglycemic effects in STZ induced diabetic rats and thereby this drug may be successfully used for the treatment of diabetes and all associated diabetic complications. However, comprehensive research is required to identify the active constituents responsible for this effect.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest in the publication of this paper.

Table 1: Effect of chronic administration (28 days) of MECL on fasting blood glucose in STZ induced diabetic rats

Experimental Groups	Fasting Blood glucose (mg %)				
	Day 0	Day 7	Day 14	Day 21	Day 28
Normal control	112.5±7.50	103.3±5.34	130.7±5.78	121.7±7.23	105.8±9.18
Diabetic control	434.5±9.47	450±11.61	405.7±9.42	277.3±21.24	199.7±15.01
Diabetic + 200 mg/kg MECL	429.5±3.04	396.5±8.0*	358.0±17.02**	185.2±37.84**	141.8±16.68**
Diabetic + 400 mg/kg MECL	423.7±4.85	387.8±4.36**	331.8±13.75**	176.8±15.66**	133.3±12.60**
Diabetic + 600 mg/kg MECL	402.0±20.81	379.5±17.81**	314.8±16.81**	175.5±12.52**	106.7±3.75**
Diabetic + 4 mg/kg GL	417.7±14.47	281.0±8.05	259.7±12.55	153.2±12.17	91.50±4.829

n=6, *p<0.05, **p<0.01. Values are mean ± SEM., MECL: Methanolic extract of *Citrulluslanatus* seeds; GL:Glibenclamide, Data analyzed by one way Analysis of Variance (ANOVA) followed by Dunnet's multiple test of comparison.

Table 2: Effect of chronic administration (28 days) of MECL on food consumption (24 hours) in STZ induced diabetic rats

Experimental Groups	Food consumption in 24 hours (gm)				
	Day 0	Day 7	Day 14	Day 21	Day 28
Normal control	10±0.57	11±0.36	11.23±0.63	12.07±078	11.27±0.72
Diabetic control	8.93±0.46	16.37±0.69	15.93±0.47	16.33±0.81	16.37±0.34
Diabetic + 200 mg/kg MECL	9.56±0.57	15.30±0.75	14.37±0.40	14.80±0.45	14.20±0.37
Diabetic + 400 mg/kg MECL	9.96±0.57**	14.47±0.71	13.83±0.60*	14.03±0.23**	13.57±0.24**
Diabetic + 600 mg/kg MECL	9.63±0.34**	13.13±0.84	12.80±0.11	13.33±0.33**	13±0.45
Diabetic + 4 mg/kg GL	9±0.57	12.50±0.23	11.93±0.52	12.93±0.46	12.10±0.47*

n=6, *p<0.05, **p<0.01. Values are mean ± SEM., MECL: Methanolic extract of *Citrulluslanatus* seeds; GL:Glibenclamide, Data analyzed by one way Analysis of Variance (ANOVA) followed by Dunnet's multiple test of comparison

Table 3: Effect of chronic administration (28 days) of MECL on water consumption (24 hours) in STZ induced diabetic rats

Experimental Groups	Water consumption in 24 hours (ml)				
	Day 0	Day 7	Day 14	Day 21	Day 28
Normal control	6.43±0.55	7.16±0.66	8.06±0.29	8.16±0.44	9.16±0.44
Diabetic control	7.96±0.63	14.30±0.43	14.03±0.31	13.43±0.34	13.50±0.36
Diabetic + 200 mg/kg MECL	6.53±0.36	12.83±0.16	12.70±0.28	12.50±0.50	11.60±0.83
Diabetic + 400 mg/kg MECL	6.73±0.21*	12.57±0.28**	11.97±0.27	11.90±0.70**	10.73±0.37**
Diabetic + 600 mg/kg MECL	7.90±0.83*	12.23±1.22	11.07±0.29	10.80±0.65	8.73±0.37
Diabetic + 4 mg/kg GL	6.96±1.00*	11.90±0.45	10.33±0.24	9.16±0.44	9.20±0.41*

n=6, *p<0.05, **p<0.01. Values are mean ± SEM., MECL: Methanolic extract of *Citrulluslanatus* seeds; GL: Glibenclamide, Data analyzed by one way Analysis of Variance (ANOVA) followed by Dunnet's multiple test of comparison.

Table 4: Effect of chronic administration (28 days) of MECL on urine output (24 hours) in STZ induced diabetic rats

Experimental Groups	Urine output in 24 hours (ml)				
	Day 0	Day 7	Day 14	Day 21	Day 28
Normal control	10.0±1.52	10.45±0.99	11.27±0.37	9.76±1.01	10.57±0.66
Diabetic control	11.50±0.76	17.27±0.43*	16.90±0.23	16.33±0.55	16.00±0.05
Diabetic + 200 mg/kg MECL	12.63±0.13	16.10±0.49	14.40±0.49	14.20±0.51	13.07±0.29
Diabetic + 400 mg/kg MECL	12.53±0.57	16.47±0.51*	12.83±0.44**	12.17±0.58	11.80±0.17**
Diabetic + 600 mg/kg MECL	11.13±0.63**	15.43±0.8*4	13.40±0.32	11.33±0.44*	10.90±0.11
Diabetic + 4 mg/kg GL	10.80±1.12*	12.17±0.52	11.37±0.54	11.47±0.55**	10.93±0.54**

n=6, *p<0.05, **p<0.01. Values are mean ± SEM., MECL: Methanolic extract of *Citrulluslanatus* seeds; GL:Glibenclamide, Data analyzed by one way Analysis of Variance (ANOVA) followed by Dunnet's multiple test of comparison

Table 5: Effect of chronic administration (28 days) of MECL on urine spots counted on filter paper in (24 hours) STZ induced diabetic rats

Experimental Groups	Urine frequency (spots counted in 24 hour)				
	Day 0	Day 7	Day 14	Day 21	Day 28
Normal control	3.66±0.33	5.00±0.57	3.33±0.88	5.33±0.88	5.33±0.33
Diabetic control	4.00±0.57	13.67±1.20	15.00±0.57	15.00±0.57**	14.67±0.33*
Diabetic + 200 mg/kg MECL	3.66±0.88*	11.33±0.88*	12.00±0.57	12.00±0.57	11.00±0.57
Diabetic + 400 mg/kg MECL	3.66±0.66	12.67±1.20**	11.33±0.66	11.00±0.57	10.33±0.33
Diabetic + 600 mg/kg MECL	4.33±0.33	11.67±0.88**	10.67±0.33*	9.66±1.33*	9.66±0.33
Diabetic + 4 mg/kg GL	4.33±0.33**	8.33±0.88	8.0±0.57	8.00±0.57	8.66±0.33*

n=6, *p<0.05, **p<0.01. Values are mean ± SEM., MECL: Methanolic extract of *Citrulluslanatus* seeds; GL:Glibenclamide, Data analyzed by one way Analysis of Variance (ANOVA) followed by Dunnet's multiple test of comparison.

HISTOPATHOLOGICAL SLIDES OF TISSUES

Liver

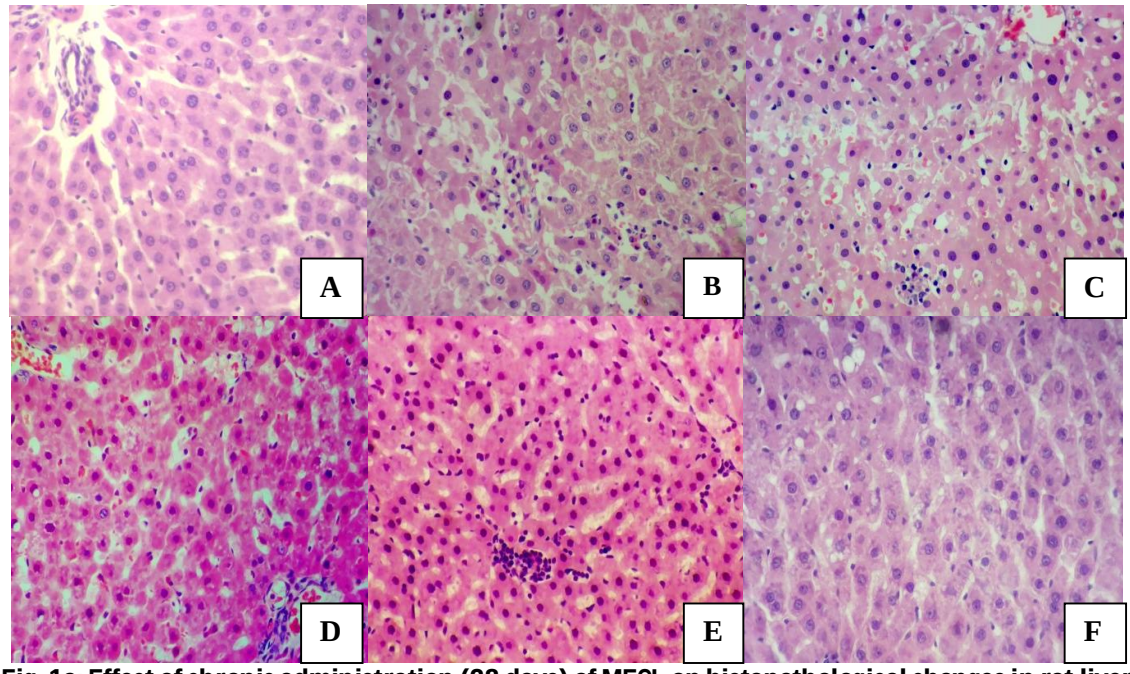

Fig. 1a: Effect of chronic administration (28 days) of MECL on histopathological changes in rat liver tissue in STZ induced diabetic rats. [A] Normal control group [B] Diabetes control group (65 mg/kg STZ) [C] MECL (200 mg/kg) treated group [D] MECL (400 mg/kg) treated group [E] MECL (600 mg/kg) treated group [F] Glibenclamide (4 mg/kg) treated group (H&E 40X)

Kidney

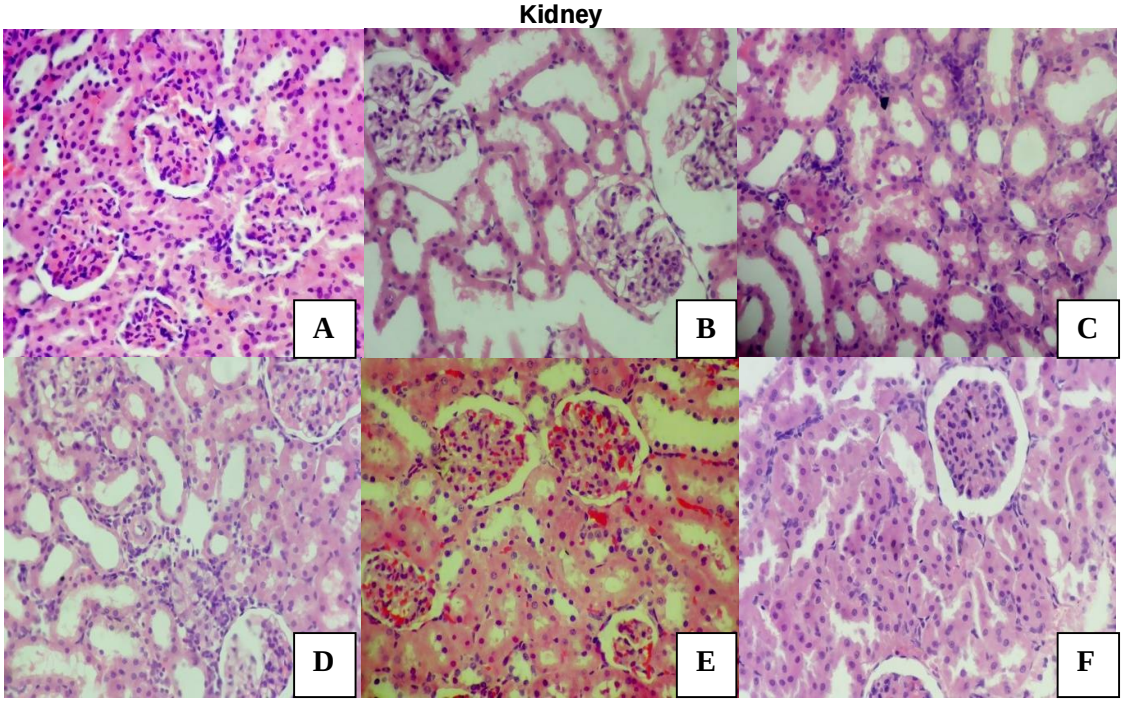

Fig. 1b: Effect of chronic administration (28 days) of MECL on histopathological changes in rat kidney tissue in STZ induced diabetic rats. [A] Normal control group [B] Diabetes control group (65 mg/kg STZ) [C] MECL (200 mg/kg) treated group [D] MECL (400 mg/kg) treated group [E] MECL (600 mg/kg) treated group [F] Glibenclamide (4 mg/kg) treated group (H&E 40X)

Pancreas

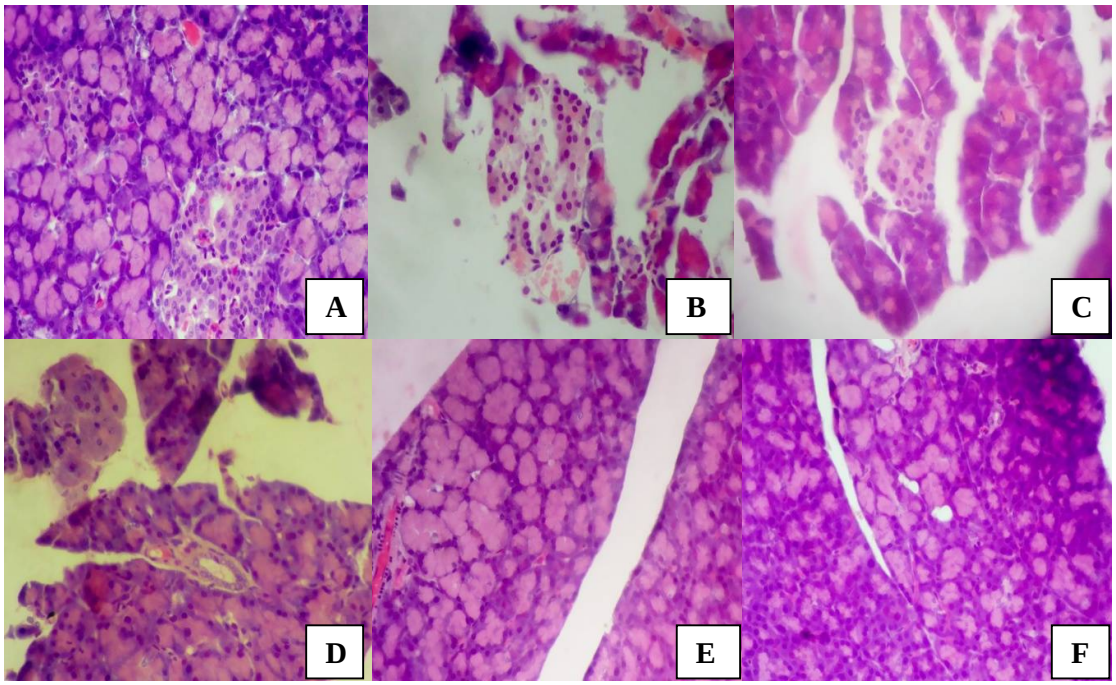

Fig. 1c: Effect of chronic administration (28 days) of MECL on histopathological changes in rat pancreatic tissue in STZ induced diabetic rats. [A] Normal control group [B] Diabetes control group (65 mg/kg STZ) [C] MECL (200 mg/kg) treated group [D] MECL (400 mg/kg) treated group [E] MECL (600 mg/kg) treated group [F] Glibenclamide (4 mg/kg) treated group (H&E 40X)

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