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Nephroprotective effect of *Mollugo cerviana* in streptozotocin induced diabetic rats

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Abstract
The underlying causes of diabetes complications are protein glycosylation, oxidation, and changes in enzyme activity, all of which are brought on by hyperglycemia. Diabetic Nephropathy remains a major cause of morbidity and mortality in patients suffering from renal dysfunction. In this study, the ethanolic extract of *Mollugo cerviana* was tested for its ability to protect rats' kidneys against streptozotocin (STZ)-induced diabetic nephropathy. The oldest method of treating various diseases used by humans is the use of ethno-medicinal plants. The evaluation of medicinal plants' benefits and safety has expanded quickly along with the interest in their use. A significant decrease in blood glucose, urea, and creatinine was observed after treatment with *Mollugo cerviana* ethanolic extract at doses of 250 mg/body weight and 500 mg/body weight, along with an improvement in the electrolyte balance.

Keywords: *Mollugo cerviana*, antidiabetic, nephroproetective

Introduction
Diabetes mellitus (DM) is well-known endocrine disease induced via acquired or inherited lack of insulin production in pancreas tissue. According to the report, 300 million people will get affected by DM until 2025 [1]. Increased free radical generation and oxidative stress may play a pivotal role in pathogenesis of diabetes mellitus (DM) and its late complications [2]. The most common pathological features of DM complications are diabetic nephropathy, neuropathy, cardiovascular disorder and retinopathy. Especially, diabetic nephropathy (DN) is a common complication of DM since 30–40% patients with DM develop diabetes-linked renal disease. Renal disease is the main complication of both types of diabetes (type I and type II) even if diabetes under control. DN remains a major cause of morbidity and mortality in patients suffering from renal dysfunction. DN induces end-stage renal disease, which is categorized via a series of renal structure dysfunction including glomerulosclerosis, membrane thickening, mesangial expansion, basement membrane thickening, inflammatory reaction and oxidative stress. Therefore, urgent need to find a more potential drug for treating diabetic nephropathy and diabetes mellitus [3].
The chosen medicinal plant namely as Mollugo cerviana L belongs to the Molluginaceae family. Mollugo cerviana. (Molluginaceae) is widely distributed in India, Nepal and Bhutan. The literature survey revealed activity was screened against the microorganisms causing skin allergies, diarrhea and dysentery. A recent study with methanol extract of mature leaves reported anti- inflammatory and antinociceptive activity. The objective of this study to assess the potential effects of ethanolic extract of mollugo cerviana on streptozotocin induced daibetic rat for metabolic and renal alterations [4]

Materals and Methods
Plant collection and extraction
The entire mollugo cerviana plant, also called as thread storm carpet weed locally, was collected from various locations in southern India identified and authentified. The plant material was carefully cleaned, dried in the shade, powdered, and the bioactive components were extracted [5].

Animals
Adult male Wistar rats weighing 250-300 g were housed in standard environmental conditions maintained at 23 ± 2 ° C with 12 hour light dark cycle. Animals were fed standard rodent diet and water. Experimental protocol was approved by Institutional Animal Ethical Committee ((IAEC Approval number KAHE/IAEC/2020/16-10/002) and the experiments were performed according to the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) guidelines.

Chemicals
Nicotinamide (NAD) was purchased from Finar India Ltd. and STZ from Sigma Aldrich in Milwaukee, Wisconsin, the United States. Reckon Diagnostics Pvt. Ltd. provided the diagnostic tools for the biochemical estimations. Analytical-grade reagents were used throughout the experiments.

Acute Toxicity Test
Using Lorke's up and down approach, the acute toxicity test of mollugo cerviana ethanol leaf extract was conducted. The rats were fasted overnight with free access to water before the dose after colonization for 7 days The rats were weighed and dosed with a limit dose of 5000 mg/kg body weight of the freshly prepared ethanol extracts of mollugo cerviana. For 72 hours, the rats' behaviour was observed for any changes. The fact that the rats tolerated the maximum dose led researchers to believe that the plant extract's lethal dose (LD50) is more than 5000 mg/kg [6].

Induction of Diabetes Mellitus
Prior to the induction of diabetes, the animals were fasted for 16–18 hours while having free access to water. Streptozotocin was administered intraperitoneally once at a dose of 65 mg/kg body weight, dissolved in 0.1 M citrate buffer (pH 4.5), to induce diabetes. To avoid hypoglycemia, the rats were subsequently kept in their cages for the following 24 hours on bottles of 5% glucose solution. Rats with a fasting blood glucose level of more than 180 mg/dL were labeled diabetic and enrolled in the study. Blood glucose was monitored using a glucometer 72 hours after Streptozotocin administration [7].
Laboratory Design for this investigation, 25 Wistar albino female rats weighing on average 180 g were employed. The animals were divided into five groups (A-E) of five rats each once the diabetes was induced.
Group A: Normal control group was given 1 ml of normal saline orally.
Group B: Diabetic rats treated with 25 mg/kg body weight of Glibenclamide orally
Group C: Diabetic rats treated with 250 mg/kg/b.wt of Mollugo Cerviana orally.
Group D: Diabetic rats treated with 500 mg/kg/b.wt of Mollugo Cerviana orally.
All treatments were given once daily for a period of 14 days.

Biochemical Assays
Electrolyte (Na+, K+, Cl- and HCO3 -), creatinine, urea, and total proteins were estimated according to the manufacturer's instruction on the assay kits [8]

Statistical Analysis
Data were expressed as Means ± S.D (n=5). The results were analyzed using one-way ANOVA followed by Dunnet's test to compare the diseased and the treated groups. At the same time, the statistical difference between the normal and diseased was analyzed by an unpaired t-test. The results were considered statistically significant, if $p<0.05$.

Results
Experimental rats treated with an acute dose of 5000 mg/kg/body weight of the ethanol leaf extract of demonstrated no change in physical activity and no apparent toxicity symptoms or mortality up to 72 hours post treatment. Therefore, it was concluded that the LD_{50} of the ethanol crude extract of the plant in rats is greater than 5000 mg/kg/body weight.

Table 1

Group	Initial fasting blood sugar	Fasting blood sugar on 72 hr	Fasting blood sugar on 10th day	Fasting blood sugar on 15th day	Fasting blood sugar on 28th day
Control	86.5±3.95	88.5±4.05	88±3.63	90.5±4.11	94.5±2.63
ONLY STZ	97±3.14^{ns}	480±45.5***	493±49.9***	343±119^{ns}	293±101
STZ+STD	89.3±1.89^{ns}	473±82.6***	445±44.3***	375±39.3*	113±8.54
STZ +EXT 250mg/kg	93.5±3.62^{ns}	470±41.4***	420±33.4***	243±84.6^{ns}	108±36.8
STZ +EXT 500mg/kg	93.8±2.39^{ns}	463±51.1***	433±33.3***	405±56.8*	97.5±37.1

Effects of treatment with aqueous extract of Mollugo cerviana on blood glucose level (mg/dL).

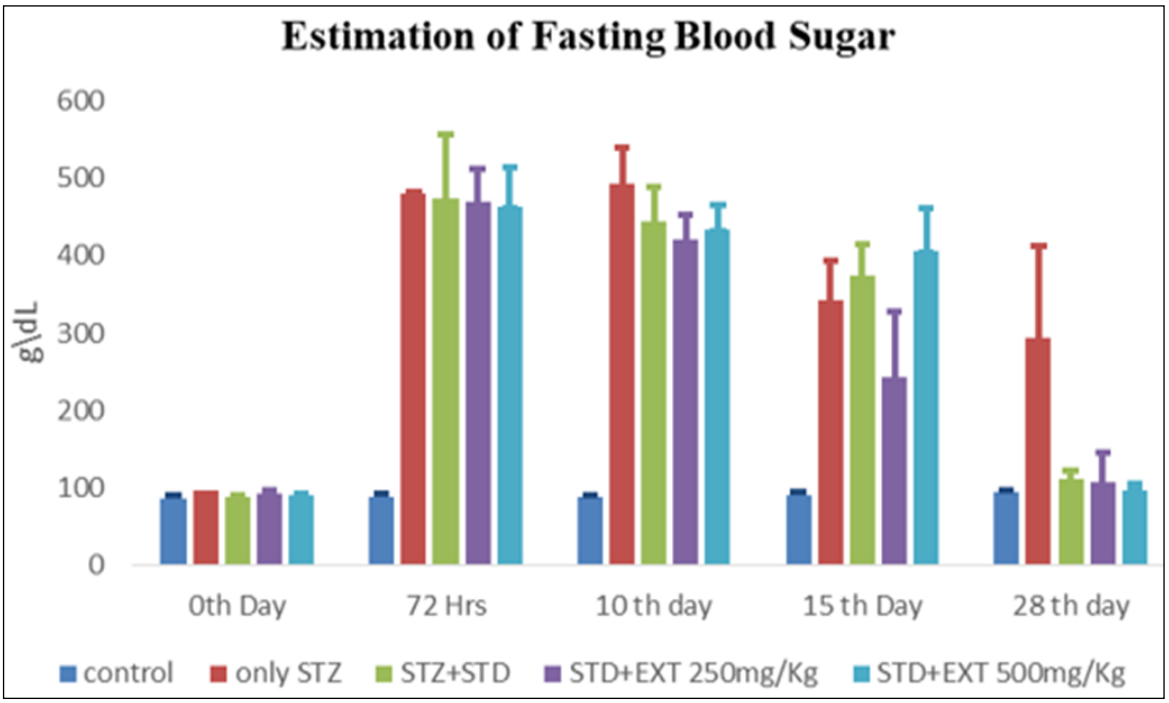
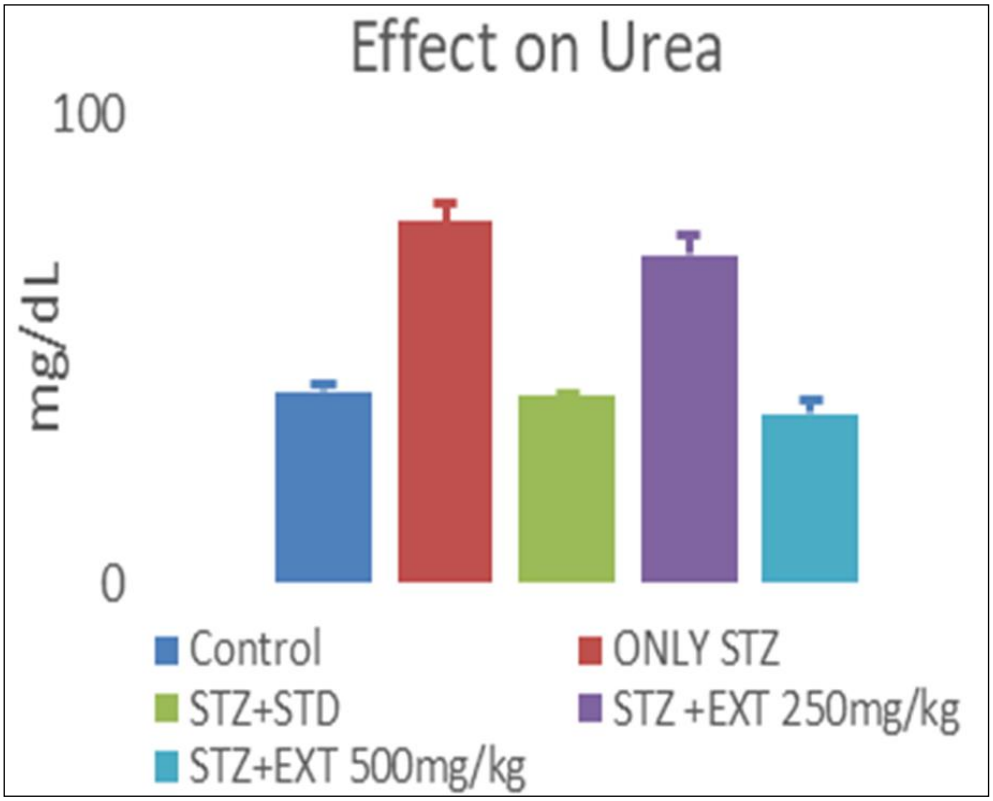


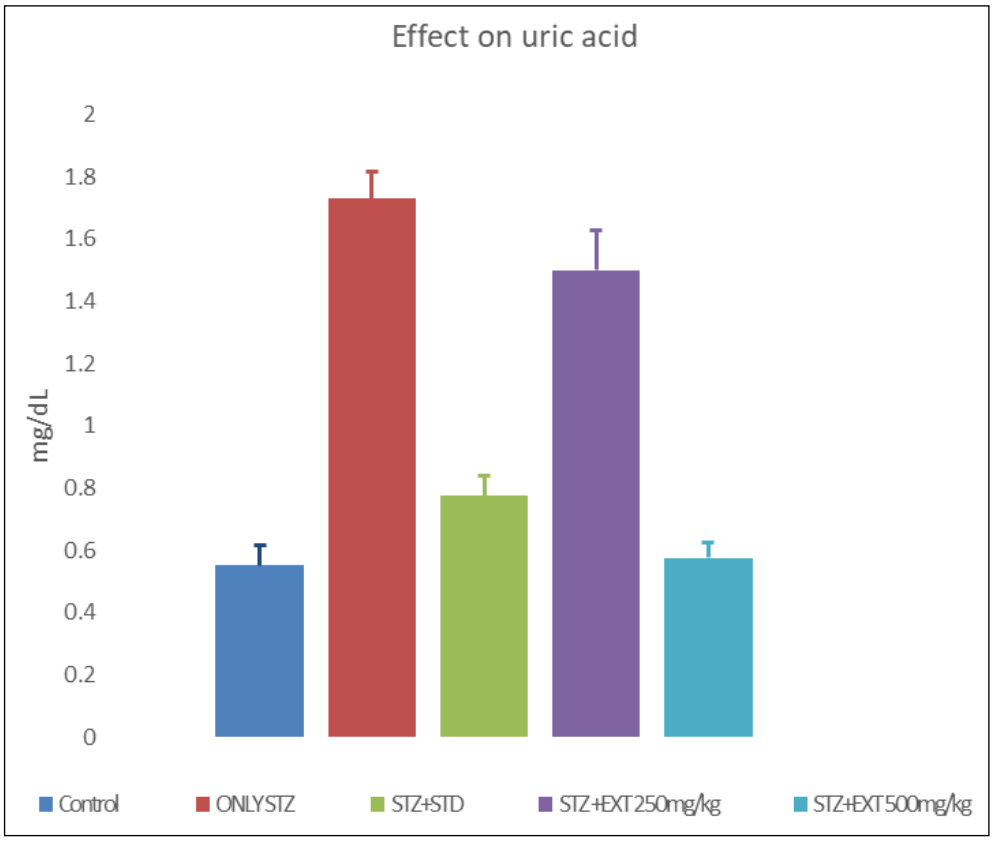
Fig 1: Effect of ethanolic extracts of Mollugo cerviana on blood sugar level.

Table 2: Effect Ethanolic extracts of Mollugo cerviana on renal function estimation in diabetic-nephropathy Wistar rats.

Group	Control	ONLY STZ	STZ+STD	STZ +EXT 250mg/kg	STZ+EXT 500mg/kg
Urea (mg/dl	41±1.08	77±4.8***	40±0.408^{ns}	70±4.16***	35.3±2.56^{ns}
Uric acid (mg/dl)	0.55±0.064	1.73±0.085	0.775±0.063	1.5±0.129	0.575±0.048
Creatinine (mg/dl)	0.625±0.103	1.825±0.025***	0.8±0.0408^{ns}	1.55±0.1708***	0.575±0.075^{ns}

Values are Mean ± SD, n=5. *Values are significantly different from the normal control group at ($p<0.05$) #insignificant difference from the normal control





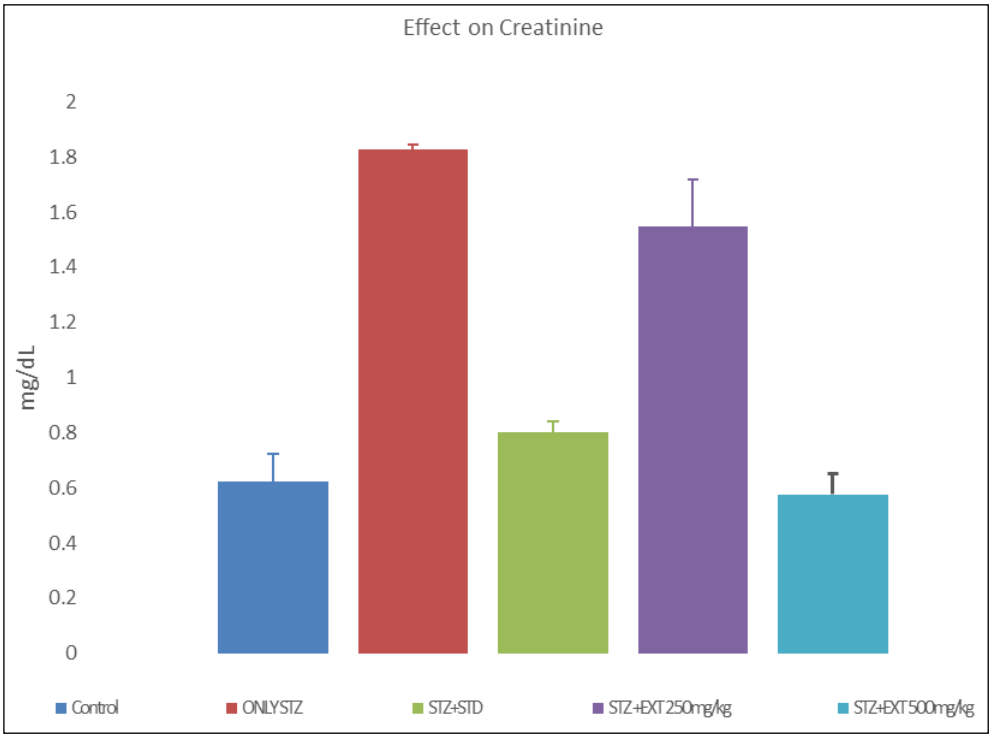


Fig 2: Effect Ethanolic extracts of Mollugo cerviana on renal parameters.

Table 3: Effect Ethanolic extracts of Mollugo cerviana on intake Parameters.

Parameter	**Control**	**Diabetic** (STZ)	**Diabetic** (STZ)+STD	**STD+EXT** 250mg/bod wt	**STD+EXT** 500mg/bod wt
Water consumption (mL)	21.8±0.23	41.09±0.42	24.508±0.403	27.334±0.92	25.28±0.73
Food intake (g)	19.33±0.87	29.94±0.56	21.223±0.987	21.987±0.345	20.676±0.898
Urine Volume	21.65±0.88	30.76±0.67	20.343+0.567	22.786+0.964	20.343+0.156

p+Values are Mean ± SD, n=5. *Values are significantly different from the normal control group at ($p<0.05$) #insignificant difference from the normal control

Table 4: Effect Ethanolic extracts of Mollugo cerviana on Electrolyte balance

Group	Potassium	Sodium	Chlorine	HCO_3^-
Control	5.25±0.82	147.00±.602	106.00±2.33	30.25 ± 0.78
Diabetic (STZ)	5.95±0.47	110.29±4.74*	86.44±1.84*	22.67± 1.12
Diabetic(STZ)+ STD	5.27±0.678	143.92±2.43	105.12±0.165	30.01 ± 1.07
STD+EXT 250mg/bod wt	5.67±0.907	138.81±3.46	101.59±4.604	25.11 ± 1.20
STD+EXT 500mg/bod wt	5.41±0.65	140.67±0.943	103.630±1.64	29.56 ±0.91

Values are Mean ± SD, n=5. *Values are significantly different from the normal control group at ($p<0.05$) #insignificant difference from the normal control

Discussion

Diabetes-related chronic hyperglycemia is linked to long-term harm, dysfunction, and failure of many organs, particularly the kidneys, nerves, heart, and blood vessels. Long-term diabetic problems include retinopathy, which could cause blindness, nephropathy, which can result in renal failure, and cardiovascular issues, which raise the risk of coronary heart disease and cardiomyopathy [9].

When compared to the control group, there was a significant rise in the levels of urea and creatinine in the diabetic control group, which may indicate sufficiently impaired renal function that is most likely asymptomatic [10]. We therefore postulate that the minor but insignificant rise in blood urea and creatinine may be caused by a lack of insulin, an anabolic hormone, and the difficulty of glucose to reach the extra hepatic tissues Furthermore, it was mentioned that enhanced proteolysis may have generated glucogenic amino acids, which could be the cause of hyperuricemia [11].

Uncontrolled hyperglycemia has been reported to alter electrolyte balance. It has been postulated that hyperglycemia induced osmotic diuresis leads to severe loss of electrolytes such as sodium, potassium, calcium, chloride, and phosphates. reason for the alteration in serum levels of the electrolytes in Streptozotocin-induced diabetic rats was numerous that includes renal function impairment or may be due to hypoglycemia induced osmotic diuresis, accompanied with the marked urinary loss of water and electrolytes [12]. It may also be aggravated by urinary excretion of ketones which involved additional electrolyte loss. Furthermore, decreased serum Na+ ion level may also be due to the translocation of Na+ K+-ATPase pumps from the basolateral membrane of proximal convoluted tubules to the cytosol which resulted in a decrease in sodium pumping from renal tubules to the blood Additionally, the expression of sodium channel proteins in the collecting ducts and distal convoluted tubules was altered, leading to an increased fractional excretion of sodium in urine [13]. The mechanism follows in tandem for chloride ion, which is known to have similar cellular tran-smembrane transport might increase the uptake of glucose by peripheral tissue and thereby presumably decreased the serum glucose level.

The insignificant increase in Urea and creatinine levels in the diabetic control when compared to the control group may suggest moderate impaired renal function which is most likely to be asymptomatic We therefore hypothesize that the deficiency of insulin, an anabolic hormone, and inability of glucose to reach the extra hepatic tissues (which then induces gluconeogenesis as an alternate route of glucose supply)[14] Additionally, the slight, insignificant increase in creatinine level in the untreated diabetic group could be attributed to the decreased body weight and reduced muscle mass Oral administration of the extracts insignificantly reversed this condition. This is in line with our earlier suggestion that the anti-hyperglycaemic effect of the plant may be responsible for reduction in serum glucose concentration and consequently the reintroduction of insulin effect, thereby leading to proteolysis reclines via hormonal stimulation [15]. This study suggests that the extract of Mollugo cerviana apart from reducing high blood glucose levels in blood, may also improve the basal metabolic rate and Kidney functions electrolyte profile and suggested that the plant might contain biochemical agents, which could protect the kidney against impairment due to diabetes

Conclusion

This study validates the traditional use of Mollugo Cerviana and suggested that the plant might contain biochemical agents, which could protect the kidney against impairment due to diabetes.

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