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Evaluation of nephroprotective and cytotoxic effect of ethanolic extract of *Mikania scandens* leaves by using alloxan-induced diabetic nephropathy mice

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Abstract

Background: Diabetic is one of the fundamental persuasive of diabetic nephropathy and significantly sparks off kidney diseases and end stage renal failure globally.

Method: The current research was carried out to evaluate hypoglycemic potential and nephroprotective effect of ethanolic extract of *Mikania scandens* leaves by using alloxan-induced diabetic nephropathy (DN) mice. The mice were intraperitoneally treated with (200 mg/kg) *Mikania scandens* leaves extract and standard (120 mg/kg) metformin HCL solution up to 22 days. During this treatment period, we collected blood for evaluation of different types of parameters such as blood glucose level body weight at 0, 15, 22th days, serum creatinine, uric acid, total protein were estimated at the end of the experiment (day 22).

Result: *Mikania scandens* leaves extract significantly ($p < 0.05$) lessen blood glucose level, serum creatinine, total protein and uric acid. Apart this, cytotoxicity studies were assessed by Brine Shrimp Lethality Bioassay. By this method, we measure the dose of LC_{50}. The plant has high LC_{50} dose thus cytotoxicity has ensued at massive amount and safe to administer.

Conclusion: Lowering of serum creatinine, uric acid and total protein recommend that the ethanolic extract of *Mikania scandens* possess potent nephroprotective activity and assure the traditional avail of this plant in the management of diabetes nephropathy.

Keywords: *Mikania scandens*, Diabetic, Nephroprotective, Cytotoxic

Background

Diabetic kidney disease is a leading phenomenon of chronic and end-stage-renal disease worldwide and the prime predictor of mortality in patient with diabetes [1]. Diabetic nephropathy is a chronic complication of both type 1 DM and type 2 DM [2]. Approximately, 30% of all diabetic patients metamorphose into diabetic nephropathy after 10–20 years of diabetes [3]. DN characterized by high blood pressure, proteinuria, a progressive decline in renal function and hike the risk of cardiovascular disease, is becoming more and more prevalent to the extent that it has touched epidemic proportion [4]. Abnormalities in DN with long-standing poorly control blood glucose level. This is followed by multiple alterations in filtration units in the kidneys, the nephrons [5]. Initially, there is constriction of the efferent arteriole and dilation of the afferent arteriole, with resulting glomerular capillary hypertension

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Table 1 Effects of EMSL on body weight in alloxan induced diabetic nephropathy mice

Groups	Body weight comparison level		
	At 0th day	At 15th day	At 22th day
Control	21.26 ± 0.90973	28.32 ± 1.34625	30.84 ± 1.85408
MS-200 mg/kg	22.86 ± 0.96208	20.54 ± 0.65468	20.02 ± 0.55082
Diabetic control	20.00 ± 2.49319	24.78 ± 2.04436	27.90 ± 1.96061
Standard	19.48 ± 1.01755	19.18 ± 0.57219	18.70 ± 1.45327

Values are expressed as mean ± SEM (Standard Error of Mean) of five experiments

and hyperfiltration; this gradually changes to hypofiltration over time [6].

Mikania scandens (L) is popularly used as a herbal remedy for various ailments of Bangladesh. The genus Mikania is a member of family Asteraceae (Compositae). In Bangladesh, *M. scandens* is known as "Jarmany lota" [7]. The main phytochemical groups of the plant are coumarins and derivatives, sesquiterpenes, sesquiterpenes lactones, diterpenes, phytosterol or terpenoids and flavonoids [8]. *M. scandens* is used in folk medicine for the treatment of stomach ulcers, diarrhea, blood coagulant and scabies [9–11]. In-vitro experiments asserted that the flowers exposed anti-inflammatory properties [12]. It has analgesic, in vitro antioxidant and antidiabetic activities of leaf material [13].

Brine shrimp lethality bioassay is a simple, high throughput cytotoxicity test of bioactive chemicals. It is based on the killing ability of test compounds on sample zoological organism brine shrimp (*Artemia salina)* [14]. It's a preliminary toxicity screen for further experiment on mammalian animal models.

The purpose of the study is to investigate the ethanolic extract of *Mikania scandens* leaves experimentally induce diabetic nephropathy mice and assess the toxic level of this plant. In future, we want to isolate the compound of plant leaves extract and run further experiment.

Materials and methods

Plant collection

Fresh leaves of *Mikania scandens* were collected from medicinal plant garden at Jashore University of Science and Technology, Jashore, Bangladesh. Leaves were shed dried and grind with electric grinder into coarse powder.

Preparation of crude extract

Coarse powder of *Mikania scandens* leaves soaked in 95% ethanol for 7 days at room temperature with occasional shaking and stirring. The solvent were filtered through cotton and then through a filter paper. The ethanolic solution was allowed to evaporate using a rotary evaporator. Then the collected extract was preserved in a refrigerator for the analysis of cytotoxic and pharmacologic properties.

Experimental animals

Healthy male *Swiss albino* mice were procured from Jahangir Nagar University. They were housed in polypropylene cages and maintained under standard conditions. The study protocol was approved by institutional ethical committee (Ref: ERC/FBS/JUST/2018–12).

Chemical and drug collection

Standard antidiabetic agent metformin hydrochloride was the generous gift sample from square pharmaceuticals Ltd. Bangladesh. Alloxan was purchased from seico research laboratories Ltd. Mumbai, India. Tween-80 was obtained from BDH chemical, UK and saline solution was collected from Beximco infusion Ltd., Bangladesh. URCA

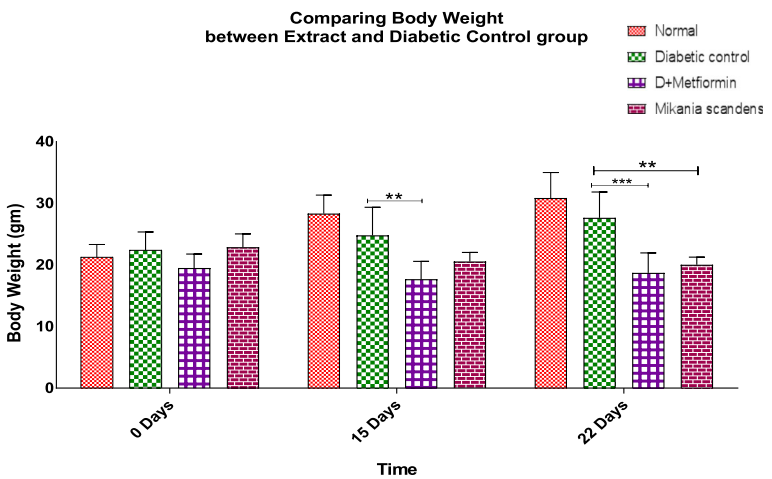

Fig. 1 Comparing body weight among diabetic control, Metformin HCL and *Mikania scandens* animal group. Significantly different ($p < 0.05$) from the diabetic control. Data was analyzed by two way ANOVA followed by Scheffe's post-hoc test

Table 2 Effects of EMSL on blood glucose level in alloxan induced diabetic nephropathy mice

Groups	Blood glucose comparison level		
	At 0th day	At 15th day	At 22th day
Control	5.22 ± 0.75987	5.68 ± 0.35693	7.80 ± 0.97929
MS-200 mg/kg	24.30 ± 2.8066	6.68 ± 1.31848	5.68 ± 1.35956
Diabetic control	14.86 ± 1.00230	12.56 ± 3.78188	21.08 ± 2.85577
Standard	24.18 ± 2.52456	8.20 ± 1.61555	7.72 ± 1.43611

Values are expressed as mean ± SEM (Standard Error of Mean) of five experiments

Flex reagent cartilage, CRE2 Flex reagent cartilage, TP Flex reagent cartilage, is registered trade mark of Fresenius, Kabi AG, Bad Humburg, Germany.

Hypoglycemic effect of leave extract

Induction of diabetes

After overnight fasting, a freshly prepared solution of alloxan monohydrate (200 mg/kg body weight in normal saline) was administered intraperitoneally. After 48 h blood glucose content was measured by a Glucometer (SAFE TOUCH Glucometer, HMD Biomedical Inc., Taiwan technology of USA). Mice with blood glucose level above 11.1 mmol/L were selected for the study [15]. Their base line blood glucose level was also measured just prior to the administration of alloxan.

Experimental design

Mice were divided into four groups consisting of five animals in each group. Group 1 (Normal control): Normal mice treated with saline 1 ml/kg. Group 2 (Diabetic control): Diabetic mice give no treatment. Group 3 (EMSL 200 mg/kg): Diabetic mice treated with 200 mg/kg body weight p.o. of *Mikania scandens* leave extract once a day. Group 4 (Positive control): Diabetic mice treated with 120 mg/kg p.o. body weight of metformin hydrochloride once a day.

Determination of blood glucose level

All blood samples were collected by cutting the tail tip of the mice. Blood samples were collected from the tail at intervals of 0, 15, 22th days.

Diabetic nephroprotective effect of leave extract

After completing the 22th day's blood glucose testing, the mice were sacrificed and 3–5 ml of blood was collected direct from the heart by syringes, centrifuged at 4000 rpm for 10 min and the serum was obtained.

Uric acid test

Uric acid was analyzed by URCA Flex reagent cartilage. Uric acid, which absorbs light at 293 nm, is converted by uricase allantoin which is non-absorbing at 293 nm. The change in absorbance at 293 nm due to disappearance of uric acid is directly proportional to the concentration of uric acid in the sample and is measured by using a bichromatic (293, 700) end point technique.

Total protein test

Total protein analyzed by TP Flex reagent cartilage. Cupric ion reacts with the peptide linkage of the protein in a basic solution. The blue copper (II) protein complex thus formed is proportional to the total protein concentration in the sample and is measured by using a bichromate (540, 700) end point technique.

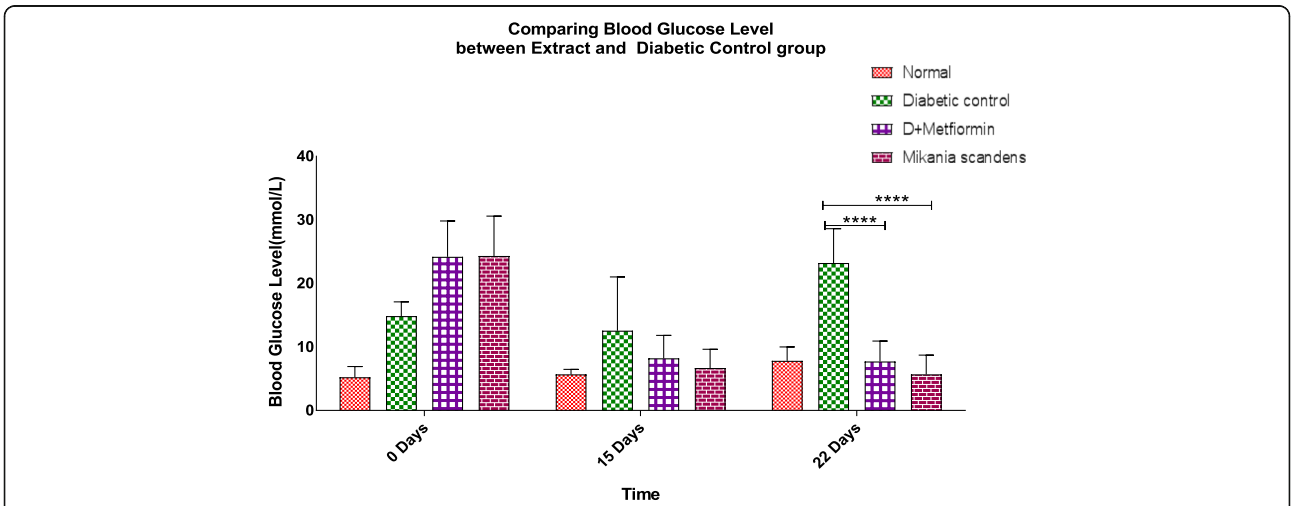

Fig. 2 Comparing blood glucose level among diabetic control, metformin HCL and *Mikania scandens* animal group. Significantly different ($p < 0.05$) from the diabetic control. Data were analyzed by two way ANOVA followed by scheffe's post-hoc test

Table 3 Effect of EMSL on serum creatinine level on alloxan induced diabetic nephropathy mice

Group	Serum creatinine comparison level (22th day)
Control	0.5800 ± 0.01528
MS-200 mg/kg	0.5800 ± 0.02082
Diabetic control	0.5667 ± 0.04410
Standard	0.8300 ± 0.04041

Values are expressed as mean ± SEM (Standard Error of Mean) of five experiments

Serum creatinine test

Creatinine is analyzed by CRE2 Flex reagent cartilage. The CRE2 method uses a modified kinetic Jaffe Technique. In presence of a strong bases such a NaOH, picrate reacts with creatinine to form a red chromophore. The rate of increasing absorbance at 520 nm due to formation of this chromophore directly proportional to the total protein concentration in the sample and is measured by a bi-chromatic (510, 600) rate technique. Bilirubin is oxidized by potassium fericyanide to prevent interference.

Cytotoxic effect of leaf extract

Brine shrimp lethality bioassay

Cytotoxicity of plant extract can be determined through brine shrimp lethality bioassay followed by the method of Meyer et al. [16]. Sea water was used for performing the hatching of *Artemia salina* Leach (Brine shrimp eggs) into mature nauplii (Larvae) within 48 h at 25 °C. The sea water contained 10 nauplii, where the test solutions were added that were diluted serially then the number of alive larvae was counted after 24 h incubation period that was carried out at 25 °C.

Statistical analysis

Data are expressed as mean ± SEM (standard error of mean). For statistical evaluation of all test results, one-way ANOVA following Dunnett's test ($P < 0.05$, vs. diabetic control) was utilized. For the analysis of all data and graph generation, SPSS software (version 20; IBM Corporation, New York, USA) and Graph Pad Prism software (version 5; San Diego, California, USA) were used, respectively. The obtained results are compared with the diabetic control group. The significance is determined at the level of $P < 0.05$.

Results

Effects of EMSL on body weight in alloxan induced diabetic nephropathy mice

The effect of intra-peritoneal administration of alcoholic leaves extract of *Mikania scandens* in diabetic nephropathy mice is presented in Table 1 and Fig. 1. A gradual raise in body weight gain was observed in the control group of animals. A slight improvement in body weight gain was seen in diabetic control group whether standard and *Mikania scandens* groups were showed a little loss of body weight.

Effects of EMSL on blood glucose level in alloxan induced diabetic nephropathy mice

The effect of intra-peritoneal administration of alcoholic leaves extract of *Mikania scandens* in diabetic nephropathy mice is shown in Table 2 and Fig. 2. Alloxan-induced diabetic mice showed approximately two fold uplift of blood glucose level at 22th day. Administration of alcoholic extract at dose of 200 mg/kg to alloxan-induced diabetic mice cause diminution of blood glucose level which was significant ($p < 0.05$). Metformin HCL at 120 mg/kg

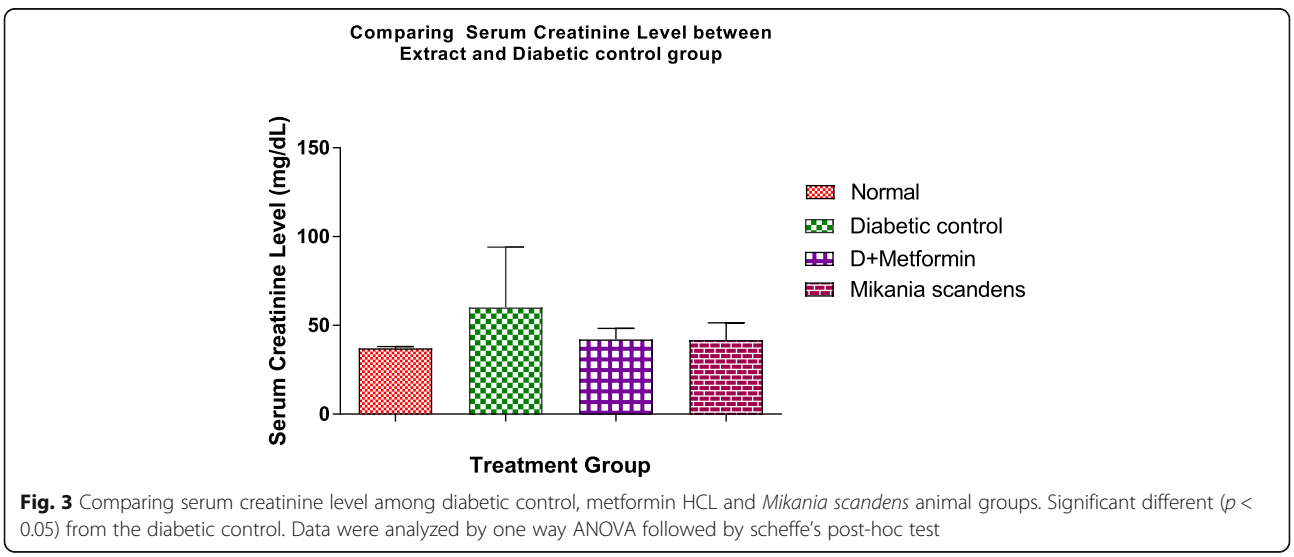

Fig. 3 Comparing serum creatinine level among diabetic control, metformin HCL and *Mikania scandens* animal groups. Significant different ($p < 0.05$) from the diabetic control. Data were analyzed by one way ANOVA followed by scheffe's post-hoc test

Table 4 Effect of EMSL on serum uric acid level in alloxan induced diabetic nephropathy mice

Group	Serum uric acid comparison level (22th day)
Control	6.3167 ± 0.10990
MS-200 mg/kg	4.2333 ± 0.22400
Diabetic control	8.8433 ± 0.14881
Standard	3.1000 ± 0.23692

Values are expressed as mean ± SEM (Standard Error of Mean) of five experiments

Table 5 Effects of EMSL on total protein level in alloxan induced diabetic nephropathy mice

Group	Protein comparison level (22th day)
Control	5.3333 ± 0.13383
MS-200 mg/kg	4.6800 ± 0.19732
Diabetic control	7.7333 ± 0.32441
Standard	6.7333 ± 0.23786

Values are expressed as mean ± SEM (Standard Error of Mean) of five experiments

exhibited significant ($p < 0.05$) reduction in blood glucose level when compared to diabetic control.

Effect of EMSL on serum creatinine level on alloxan induced diabetic nephropathy mice

Comparing serum creatinine level among diabetic control, Metformin HCL and *Mikania scandens* groups by using alloxan induce diabetic nephropathy mice are displayed in the Table 3 and Fig. 3. No significant result was found among diabetic control, Metformin HCL and *Mikania scandens* groups.

Effect of EMSL on serum uric acid level in alloxan induced diabetic nephropathy mice

Comparing serum uric acid among diabetic control, metformin HCL and *Mikania scandens* groups by using alloxan induce diabetic nephropathy mice are presented in the Table 4 and Fig. 4. Alcoholic extract at dose 200 mg/kg and metformin HCL 120 mg/kg cause decrement of serum uric acid level which was significant ($p < 0.05$).

Effects of EMSL on total protein level in alloxan induced diabetic nephropathy mice

Comparing total protein level among diabetic control, metformin HCL and *Mikania scandens* groups are shown in the Table 5 and Fig. 5. *Mikania scandens* and standard group showed significant ($p < 0.05$) downfall of total protein level when compared to diabetic control group.

Cytotoxic effect of EMSL on brine shrimp lethality bioassay

As a dose dependent manner, the mortality rate of brine shrimp was found to be onward with accretive concentration of the sample. The median lethal concentrations at which 50% lethality (LC_{50}) of brine shrimp nauplii occurrence were found to be 1083 μg/ml for the crude extract of *Mikania scandens* which was displayed at Table 6 and Fig. 6.

Discussion

Medicinal plants being the potential sources of bioactive agents are gaining acceptability worldwide. Safe, effective and indigenous remedies are gaining popularity equally among the people of both the urban and rural areas [17]. The major reason of death among patients with diabetic nephropathy was uraemia (66%) [18]. Anti-hyperglycemic effect was determined by blood glucose measuring at different intervals, while nephroprotective activity analysis against alloxan induced toxicity was performed. Sustained decrease in hyperglycemia will diminish the danger of

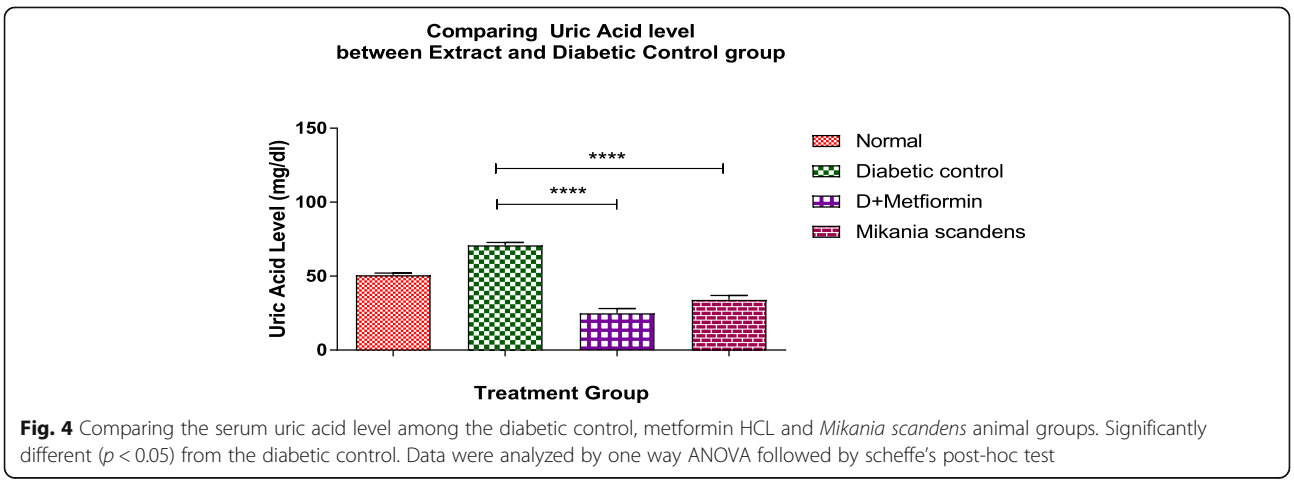

Fig. 4 Comparing the serum uric acid level among the diabetic control, metformin HCL and *Mikania scandens* animal groups. Significantly different ($p < 0.05$) from the diabetic control. Data were analyzed by one way ANOVA followed by scheffe's post-hoc test

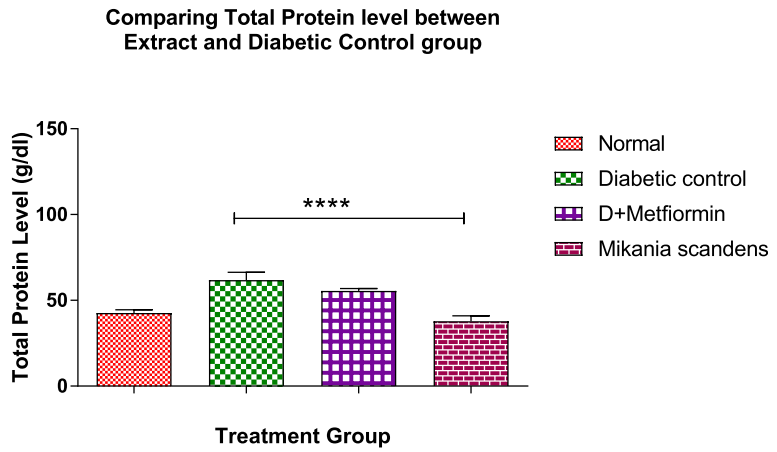

Fig. 5 Comparing total protein among diabetic control, metformin HCL and *Mikania scandens* animal group. Significantly different ($p < 0.05$) from the diabetic control. Data were analyzed by one way ANOVA followed by Scheffe's post-hoc test

micro vascular difficulties & doubtlessly decrease the danger of macro vascular deforms [19].

Notable chemical compounds such as, alkaloids, flavonoids, phytosterol, phenolic compounds, tannins and glycosides were revealed in the ethanolic extract of *M. scandens* leaves [20]. Due to containing of phytosterol in *Mikanina scandens* leaves extract, it shows potent nephroprotective effect [21].

In body weight experiment, it was observed that normal control 47.6% weight gaining, *Mikania scandens* leave extract 9% weight loss, standard treatment mice 6% weight loss, diabetic control 22.7% weight gaining at 22th day. Various plants extracts contain tannins, compounds that may exert an anti-nutritional impact by interfering with gut function [22] and minimize the glycaemic response to carbohydrate foods [23]. In standard group weight loss observed because the total adiposity or plasma leptin level, liver weight were significantly reduced after treatment with metformin [24]. In diabetic control group weight gaining occurred due to uric acid level that causes fluid accumulation [25].

In observation of blood glucose level *Mikania scandens* leaves extract decreased 77% blood glucose level, standard mice reduced 67% blood glucose level at 22th day. Both group lowered blood glucose at 15th and 22th day. In diabetic control mice, it was noticed that fluctuation of blood glucose level occurred. At 15th day 12.8% decreased and 22th day 60% increased blood glucose level. The fluctuation of blood glucose level of diabetic control mice is unknown. Further study is needed to explain this phenomenon.

Table 6 Cytotoxic effect of EMSL on Brine shrimp lethality bioassay

Concentration	% inhibition	LC_{50}
100 μg/ml	10	
200 μg/ml	20	
400 μg/ml	20	1083 μg/ml
800 μg/ml	40	

The role of serum creatinine may also differ in patients with chronic kidney disease compared with healthy persons [26]. In present study, there was no significant result found of serum creatinine level among experimental, diabetic control and standard group on serum creatinine level. The cause of this result is unknown. Further study is needed to evaluate this result.

Uric acid is known to cause endothelial dysfunction, vascular smooth muscle cell proliferation, increased IL-6 synthesis, and impairment of nitric oxide production, all of which may contribute to the progression of chronic kidney disease [27]. In current study, there was increased in the level of uric acid level in alloxan induce diabetic nephropathy mice at 22th day when compared with no diabetic animal. Treatment with ethanolic extract of *Mikania scandens* leaves and metformin HCL groups exhibited significant ($p < 0.05$) reduction in uric acid when compared to diabetic control group animals.

High level of serum total protein level gradually diminishes renal function and formation of kidney stone [28]. In this study, there was increased in the level of total protein in alloxan induce diabetic nephropathy mice at 22th day when compared with no diabetic animals. Treatment with ethanolic extract of *Mikania scandens* leaves and Metformin HCL groups exhibited significant ($p < 0.05$) reduction total protein level on 22th day when compared to diabetic control group animal, which are mostly statically significant and show effective treatment era with highly nephroprotective activity.

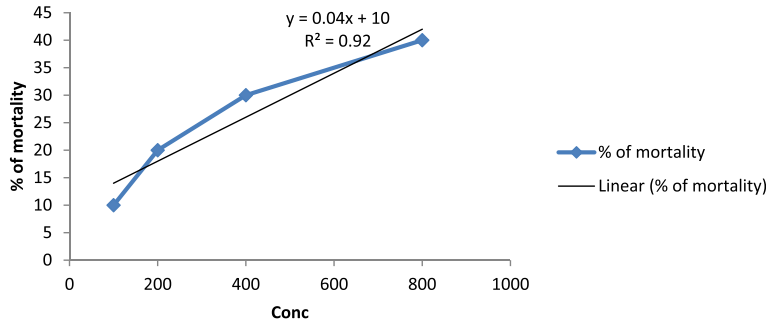

Fig. 6 Cytotoxic effect of *Mikania scandens* leaves extract

In cytotoxic test gradual increase of concentration of *Mikania scandens* leaves extract enhance the rate of mortality. LC_{50} value obtain from the test is 1083 μg/ml that indicates the 50 % death occurs in this concentration. High dose of LC_{50} value indicates that it is out of danger to administer.

Conclusion

In this investigation, evaluation of body weight, blood glucose level, serum creatinine, uric acid and total protein levels in diabetic control group were significantly reversed by an ethanolic extract of *Mikania scandens* leaves in alloxan induce diabetic nephropathy mice. Therefore, this study suggested that ethanolic extract of *Mikania scandens* leaves showed their ability to attenuate the renal damage in diabetes.

Abbreviations

DM: Diabetes Mellitus; DN: Diabetic Nephropathy; LC_{50}: Lethal Concentration

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Consent of publication

Not applicable.

Authors' contributions

KA carried out all part of the experiment & planning of this research, writing and editing of research paper. MHHJ helped in experimental analysis. MI helped in planning of the experiment and editing of this research paper. MBY helped in animal handling and take part in experiment. SMMR also helped in animal handling, take part in experiment and editing of this research paper. This research was supervised by RS. The authors read and approved the final manuscript.

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Availability of data and materials

Not applicable.

Ethics approval and consent to participate

The study protocol was approved by institutional ethical committee (Ref No: ERC/FBS/JUST/2018–12).

Competing interests

The authors declare that they have no competing interests.

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