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.
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

| 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.

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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 1 Effects of EMSL on body weight in alloxan induced diabetic nephropathy mice
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 bi-chromatic (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 bi-chromate (540, 700) end point technique.

### 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 ± 0.35963  | 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

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 as 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
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 $\mu$g/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 ureaemia (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...
micro vascular difficulties & doubtlessly decrease the
danger of macro vascular deforms [19].

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

In body weight experiment, it was observed that nor-
mal 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, com-
ponds that may exert an anti-nutritional impact by
interfering with gut function [22] and minimize the gly-
caemic response to carbohydrate foods [23]. In standard
group weight loss observed because the total adiposity
or plasma leptin level, liver weight were significantly re-
duced 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 scan-
dens* 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 fluctu-
ation 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 ex-
plain this phenomenon.

The role of serum creatinine may also differ in patients
with chronic kidney disease compared with healthy per-
sons [26]. In present study, there was no significant result
found of serum creatinine level among experimental, dia-
betic 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 ex-
hibited significant (*p* < 0.05) reduction in uric acid when
compared to diabetic control group animals.

High level of serum total protein level gradually dimin-
ishes 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.

### Table 6 Cytotoxic effect of EMSL on Brine shrimp lethality bioassay

| Concentration | % inhibition | \( \text{LC}_{50} \) |
|---------------|-------------|-----------------|
| 100 µg/ml     | 10          |                 |
| 200 µg/ml     | 20          |                 |
| 400 µg/ml     | 20          | 1083 µg/ml      |
| 800 µg/ml     | 40          |                 |

![Comparing Total Protein level between Extract and Diabetic Control group](image)

**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.
In cytotoxic test gradual increase of concentration of *Mikania scandens* leaves extract enhance the rate of mortality. LC<sub>50</sub> value obtain from the test is 1083 μg/ml that indicates the 50 % death occurs in this concentration. High dose of LC<sub>50</sub> 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<sub>50</sub>: 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. MHHU helped in experimental analysis. MBY helped in planning of the 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.

**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<sub>50</sub> value obtain from the test is 1083 μg/ml that indicates the 50 % death occurs in this concentration. High dose of LC<sub>50</sub> value indicates that it is out of danger to administer.

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DM: Diabetes Mellitus; DN: Diabetic Nephropathy; LC<sub>50</sub>: 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. MHHU helped in experimental analysis. MBY helped in animal handling and take part in experiment. SWMR 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.

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

**Cytoxicty**

| Conc | % of mortality |
|------|---------------|
| 0    | 0             |
| 200  | 5             |
| 400  | 10            |
| 600  | 15            |
| 800  | 20            |
| 1000 | 25            |

 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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