

## HYPOGLYCEMIC STUDY OF ETHANOLIC EXTRACT OF *MIKANIA CORDATA* LEAF

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### ABSTRACT

Besides synthetic drugs, plants derived drugs are also used to manage diabetes. In this study ethanolic leaf extracts (200 & 400 mg/kg body weight) of *Mikania cordata* were evaluated for hypoglycemic potency. Oral glucose tolerance test was performed on mice at 0, 30, 60, 90 and 120 minutes after glucose (2 mg/kg) administration to determine the rapidity of the extracts to reduce blood glucose level. The extracts exhibited rapid glucose reduction rates and achieved statistical significance ( $p < 0.05$  &  $P < 0.01$ ) compared to normal control. An intraperitoneal injection of alloxan (150 mg/kg body weight) was given per mouse to induce diabetes and the animals with

blood glucose levels more than 11 mmol/L selected for the experiment. During a study of 14 days both extracts reduced blood glucose level (38.04% for 200 mg/kg & 47.72% for 400 mg/kg body weight) effectively as well as achieved statistical significance ( $p < 0.01$ ) compared to diabetic control, metformin hydrochloride (100 mg/kg body weight) served as positive control. One way analysis of variance (ANOVA) followed by Dunnett's multiple comparison test was performed to analyze the statistical significance of the values. The extract was found safe up to 2000 mg/kg body weight consumption by following acute toxic class method and reduction of body weight in diabetic mice (3.25% & 5.24% respectively for 200 & 400 mg/kg body weight) was observed. Thus the study revealed and corroborated the hypoglycemic significance of the leaf of *Mikania cordata*.

**KEYWORDS:** *Mikania cordata*, Alloxan, Diabetes mellitus, Metformin hydrochloride, Oral glucose tolerance test.

## INTRODUCTION

Diabetes mellitus, a chronic metabolic disorder has become a common global health menace that affects more than 170 million people worldwide. It is estimated that by 2030, the number will rise to 366 million.<sup>[1]</sup> The worldwide survey on diabetes reveals that among the entire diabetes cases more than 90% are accounted to type 2. The death rate due to diabetes is twice than that of without diabetes.<sup>[2]</sup> Though there are numerous hypoglycemic drugs to manage diabetes, but patients are not satisfied for the adverse effects of the existing drugs.

*Mikania cordata* (Family: Asteraceae) is a creeping, perennial vine up to 10 m long, twining around young tree crops, smothering them and forming dense, tangled mass. Leaves are opposite, heart shaped. The leaves of the plant are abundant with various phytochemicals like alkaloids, steroids, tannins, glycosides, saponins, flavonoids.<sup>[3-5]</sup> Previous studies revealed antimicrobial,<sup>[3-4]</sup> cytotoxic,<sup>[3-5]</sup> antinociceptive,<sup>[3]</sup> antiulcer<sup>[6]</sup> characteristics of the leaves and the whole plant is reported to exert anthelmintic and antiemetic properties.<sup>[7]</sup>

The current global market for plant-derived drugs is worth more than 20 billion and it is continuously expanding.<sup>[8]</sup> It was estimated that natural products and related drugs are used to treat 87% of all human diseases.<sup>[9]</sup> Herbal medicines are free of side effects and it is found that herbal medicines with rich content of phytochemicals like flavonoids, phenols, glycosides, alkaloids, terpenoids etc exert potent hypoglycemic activity. In this study the hypoglycemic potency of the leaves of *Mikania cordata* was evaluated.

## MATERIALS AND METHODS

### Collection and Identification of Plant

The leaves of *Mikania cordata* were obtained from Sher-e-Bangla Agricultural University campus, Dhaka. The authentication of the plant was performed by the taxonomists of Bangladesh National Herbarium, Mirpur, Dhaka. The voucher specimen number of 40255 was preserved for further reference.

### Preparation of Extract

The leaves were washed in running tap water to remove adhering dirt, cut into small pieces and sun dried for 4 days. The dried leaves were ground into coarse powders by means of a grinding machine. The powders (200 g) were extracted with 1 L of ethanol through occasional shaking and stirring for 7 days. The extract was filtered through cotton and finally through Whatman No. 1 filter paper. The obtained filtrate then concentrated at 40°C by rotary evaporator, air dried to obtain solid residue and dissolved in 2% tween solution.

**Experimental animals**

The experiment was carried out on young swiss albino mice of either sex, aged 7 to 8 weeks, weighing about 30-35 g. Mice were purchased from the Animal Research Branch of International Centre for Diarrhoeal Disease Research, Bangladesh (ICDDR, B). Animals were kept in standard environmental conditions (Temperature: 25<sup>0</sup>C, Relative humidity: 55-65% and 12 h light and 12 h dark cycle). The mice were supplied with filter water and ICDDR, B formulated rodent food.

**Chemicals**

Alloxan was purchased from Fluka, Germany. Metformin hydrochloride from Square Pharmaceuticals Ltd, Bangladesh. Tween-80 was obtained from BDH Chemicals, UK and saline solution was collected from Beximco Infusion Ltd. All the chemicals used were of analytical grade.

**Ethical approval**

All experiments regarding animals were accomplished under the guidelines of the institutional animal ethical committee.<sup>[10]</sup> Experiments were done by following the instructions of ICDDR, B as well as Southeast University ethical committee monitored and approved the experiment.

**Acute toxicity study**

According to OECD-423 guidelines (acute toxic class method) the acute toxicity study of the extract was carried out on female mice at different doses of 5, 50, 300 and 2000 mg/kg body weight. The mice were observed continuously 24 hours for any behavioral, neurological and autonomic changes and next 14 days for lethality.<sup>[11]</sup>

**Oral glucose tolerance test**

To perform Oral glucose tolerance test non-diabetic mice were divided into 4 groups, each containing 6 animals (n = 6).

Group 1 (Normal Control): Treated with distilled water at 1 ml/kg p.o.

Group 2 (Positive Control): Treated with Metformin hydrochloride at 100 mg/kg body weight p.o.

Group 3 (Test group, MCL 200): Treated with 200 mg/kg body weight p.o. of the leaf extract of *Mikania cordata*.

Group 4 (Test group, MCL 400): Treated with 400 mg/kg body weight p.o. of the leaf extract of *Mikania cordata*.

Mice were fasted overnight (12-16 h) before the administration of the above treatments. After 30 min each group is orally treated with 2 mg/kg glucose solution. The blood is withdrawn from the tail vein of each mouse at 0 (just before glucose administration), 30, 60 and 120 min of glucose administration and measured by means of glucometer.

### Induction of diabetes

Diabetes was induced in mice by intra peritoneal injection of alloxan monohydrate at 150 mg/kg body weight in saline solution. To avoid hypoglycemia, mice were supplied with 10% glucose solution for the next 24 h. After one week, animals with fasting blood glucose level more than 11 mmol/L were considered as diabetic mice and included for further experiment.

### Experimental design

Mice were divided into 5 groups consisting of 6 animals each (n = 6).

Group 1 (Normal Control): Normal mice treated with saline 1 ml/kg p.o.

Group 2 (Glucose Control): Diabetic mice treated with saline 1 ml/kg p.o.

Group 3 (MCL 200): Diabetic mice treated with 200 mg/kg body weight p.o. of *Mikania cordata* leaf extract once a day.

Group 4 (MCL 400): Diabetic mice treated with 400 mg/kg body weight p.o. of *Mikania cordata* leaf extract once a day.

Group 5 (Positive Control): Diabetic mice treated with 100 mg/kg p.o. body weight of Metformin hydrochloride once a day.

The experiment was performed for 14 days. On the starting (0 day), 7<sup>th</sup> and 14<sup>th</sup> day of experiment fasting blood glucose levels of different groups were determined from tail veins of mice by using a glucometer. The percentage glycemic and weight changes of different groups were also calculated.

### Statistical significance

All values regarding experiments are expressed as mean  $\pm$  SEM, One way analysis of variance (ANOVA) followed by post hoc Dunnett's multiple comparison test using Graphpad prism version 6.05 was performed to determine the statistical significance.

## RESULTS

### Acute toxicity study

No mortality nor any behavioral change was observed in mice up to the dose of 2000 mg/kg body weight consumption of the experimental leaf extract of *Mikania cordata* by following acute toxic class method.

### Oral glucose tolerance test

**Table 1: Oral glucose tolerance test of *Mikania cordata* leaf extracts (200 & 400 mg/kg) compared with other groups.**

Groups	Blood glucose levels in mmol/L (Mean $\pm$ SEM)				
	0 min	30 min	60 min	90 min	120 min
Normal control	4.22 $\pm$ 0.18	11.60 $\pm$ 0.48	9.38 $\pm$ 0.44	7.37 $\pm$ 0.38	6.49 $\pm$ 0.39
Positive control	4.33 $\pm$ 0.12	8.83 $\pm$ 0.44*	7.13 $\pm$ 0.33**	5.58 $\pm$ 0.17**	4.18 $\pm$ 0.05**
MCL 200	4.52 $\pm$ 0.11	9.75 $\pm$ 0.27*	8.17 $\pm$ 0.39*	6.69 $\pm$ 0.25*	6.00 $\pm$ 0.43**
MCL 400	4.58 $\pm$ 0.33	9.53 $\pm$ 0.25*	7.52 $\pm$ 0.40**	6.28 $\pm$ 0.15*	5.31 $\pm$ 0.26**

Results are presented as Mean  $\pm$  SEM, \*P<0.05 and \*\*P<0.01 when compared to Normal control group, One way ANOVA followed by Dunnett's multiple comparison test was performed in Graphpad Prism version 6.05.

In the oral glucose tolerance test blood glucose levels of different groups were determined at 0, 30, 60, 90 and 120 minutes after glucose administration. All groups exerted their peak blood glucose level at 30 minutes. Then the glucose levels of all the groups started to decrease at a continuous pace. Both the extracts (200 & 400 mg/kg) along with standard metformin hydrochloride (100 mg/kg) reduced the blood glucose level effectively and achieved statistical significance (p<0.05 & P<0.01). The inhibition of blood glucose levels between 30 minutes and 120 minutes were recorded 38.46% and 44.28% for 200 and 400 mg/kg body weight of *Mikania cordata* leaf extracts respectively whereas for standard Metformin hydrochloride it was 52.66%.

**Long term hypoglycemic study****Table 2: Effect of *Mikania cordata* leaf extracts (200 & 400 mg/kg) on alloxan induced diabetic mice compared with other groups**

Groups	Fasting blood glucose levels in mmol/L (Mean $\pm$ SEM)			% reduction in blood glucose levels between day 0 & day 14
	Day 0	Day 7	Day 14	
Normal control	4.05 $\pm$ 0.31	4.10 $\pm$ 0.23	4.28 $\pm$ 0.43	---
Diabetic control	12.52 $\pm$ 0.53	13.67 $\pm$ 0.78	14.48 $\pm$ 0.80	---
Positive control	13.40 $\pm$ 0.45	7.20 $\pm$ 0.25**	5.25 $\pm$ 0.38***	60.82
MKL 200	12.30 $\pm$ 0.29	8.48 $\pm$ 0.37**	7.62 $\pm$ 0.39**	38.04
MKL 400	12.28 $\pm$ 0.46	7.92 $\pm$ 0.32**	6.42 $\pm$ 0.30**	47.72

Results are presented as Mean  $\pm$  SEM, \*\*P<0.01 and \*\*\*P<0.001 when compared to Diabetic control group, One way ANOVA followed by Dunnett's multiple comparison test was performed in Graphpad Prism version 6.05.

The experiment was carried out 14 days on diabetic mice where the blood glucose levels were determined on initial (0 day), 7<sup>th</sup> and 14<sup>th</sup> day. Both the experimental extracts (200 & 400 mg/kg) reduced blood glucose level, ameliorating the diabetic condition. The reduction of blood glucose levels between beginning (0 day) and final (14<sup>th</sup> day) of the treatment were 38.04% and 47.72% for 200 and 400 mg/kg extracts respectively whereas standard metformin hydrochloride achieved the highest reduction rate (60.82%). Both extracts as well as positive control (metformin treated) exhibited hypoglycemic effects and achieved statistical significance (p<0.01). Between the extracts better hypoglycemic activity was observed for 400 mg/kg than the 200 mg/kg body weight of the plant.

**Weight change****Table 3: Effect of *Mikania cordata* leaf extracts (200 & 400 mg/kg) on alloxan induced diabetic mice compared with other groups**

Groups	Body weight in grams (Mean $\pm$ SEM)			% change in body weights between day 0 & day 14
	Day 0	Day 7	Day 14	
Normal control	32.13 $\pm$ 0.76	33.73 $\pm$ 0.44	34.97 $\pm$ 0.78	+ 8.84
Diabetic control	31.81 $\pm$ 0.49	27.56 $\pm$ 1.00	24.90 $\pm$ 0.98	-21.72
Positive control	33.39 $\pm$ 0.47	32.00 $\pm$ 0.66	30.20 $\pm$ 0.59	- 9.55
MKL 200	33.85 $\pm$ 0.73	33.00 $\pm$ 0.64	32.75 $\pm$ 0.63	- 3.25
MKL 400	34.18 $\pm$ 0.36	33.42 $\pm$ 0.67	32.39 $\pm$ 0.71	- 5.24

Results are presented as Mean  $\pm$  SEM, (+) indicates addition and (–) indicates a reduction of body weight.

During the 14 days of study a decrease of weight in positive, diabetic and extracts treated groups was observed, whereas gaining of weight (8.84%) was exhibited only in the normal control group. The highest decrease of weight was recorded for the diabetic group (21.72%) whereas the positive group (metformin 100 mg/kg) stood for a reduction of 9.55%. Though both the extracts reduced the weight (3.25% in 200 mg/kg & 5.24% in 400 mg/kg) but these were not as high as the diabetic control group.

## DISCUSSION

To reveal the hypoglycemic potency of *Mikania cordata*, the ethanolic leaf extracts (200 & 400 mg/kg body weight) of the plant were experimented on mice. In glucose tolerance test both the extracts were able to reduce the blood glucose level rapidly at different time intervals (30, 60, 90, 120 minutes) compared to the normal control group and achieved statistical significance ( $p < 0.05$  &  $P < 0.01$ ). Thus, from the oral glucose tolerance test it is proved that the leaf extracts of *Mikania cordata* have the capacity to reduce blood glucose level within very short period of time.

Consumption of leaf extracts (200 & 400 mg/kg) of the plant on a daily basis for 14 consecutive days reduced blood glucose levels successfully. At initial (0 day), mice treated with 200 mg/kg body weight of leaf extract had a blood glucose level of 12.30 mmol/L and finally (14<sup>th</sup> day) it reduced to 7.62 mmol/L, resulting 38.04% decrease in blood glucose level. Between extracts higher dose (400 mg/kg) yielded better activity, lessened blood glucose level from 12.28 to 6.42 mmol/L, achieved 47.72% of blood glucose inhibition. The hypoglycemic activity of the plant might be due to increased peripheral glucose utilization or the amelioration of the destroyed  $\beta$ -cells of the pancreas to secrete insulin or the combination of both.<sup>[12]</sup> Moreover, the content of phytochemicals like flavonoids, phenols, glycosides, alkaloids, terpenoids that are believed to yield hypoglycemic activity might play an important role to exert hypoglycemic potency of the plant.

In diabetes, body used triglycerides as an alternative energy source accompanied by catabolism of tissue proteins, resulting in the loss of both fat and as well as lean masses, leading to a significant loss of total body weight.<sup>[13]</sup> In the experiment of 14 days body weight change was determined on 7<sup>th</sup> and 14<sup>th</sup> day. An increase of 8.84% weight was



observed in normal control, whereas all other groups including extracts reduced the weight. Though the dose of one experimental extract (400 mg/kg) was twice of other (200 mg/kg) there was not a high weight reduction difference between the two extracts (3.25% for 200 mg/kg & 5.24% for 400 mg/kg).

## CONCLUSION

The present study supports the hypoglycemic potency of ethanolic leaf extract of *Mikania cordata*. But the mechanism of action is not yet known. So further investigations should be performed to identify the exact mechanism and purification of the chemicals or molecules of the plant producing hypoglycemic effect.

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