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## HYPOGLYCEMIC AND HEPATOPROTECTIVE EFFECT OF *MERREMIA VITIFOLIA* IN MICE

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### Keywords:

*Merremia*, Hypoglycemic, Hepatoprotective,  $\alpha$ -glucosidase, Alanine aminotransferase

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**ABSTRACT:** Traditionally, *Merremia vitifolia* meets multipurpose medicinal uses in tribal areas. Therefore, the study worked on hypoglycemic and hepatoprotective effects on animal models to meet the research gap on this plant. Methanolic stem extract at 200 mg/kg and 400mg/kg were administrated orally to determine the effects on blood glucose and hepatic enzymes. The highest dose showed a significant ( $p < 0.01$ ) reduction in blood glucose. Maximum glucose level reduction (52.1%) was observed at 400 mg/kg extract with  $3.5 \pm 0.6$  mmol/L after 3 h, while reference glibenclamide (10mg/kg) reduced 25.2% with  $4.4 \pm 0.8$  mmol/L. The plasma levels of alkaline phosphatase, alanine aminotransferase, and aspartate aminotransferase of  $\text{CCl}_4$  intoxicated mice were 108.8, 43.4, and 268.8 IU/L at 400 mg/kg, where the values for reference silymarin (100 mg/kg) were 103.4, 54.3, and 237.7 IU/L, respectively. The reduction of alkaline phosphate and alanine aminotransferase levels were moderately ( $p < 0.01$ ) and minimally ( $p < 0.05$ ) significant at concentration 400 mg/kg, respectively. Therefore, the study explored the plant's potential hypoglycemic and hepatoprotective effects, where the hypoglycemic effect was strongly significant.

**INTRODUCTION:** Traditional herbal medicine has a long history to use for hypoglycemic effects and hepatoprotective effects all over the world <sup>1, 2</sup>. A study describes the uses of 800 and 150 plants in Mexico and India for hypoglycemic effects, respectively. It is considered that the plant metabolites contain active compounds that reduce blood glucose levels by the following mechanisms, such as stimulating endogenous insulin release or drop of sugar absorption or improving insulin sensitivity <sup>3</sup>.

On the other hand, traditional natural products convey any probable modes of hepatoprotection, including immunomodulation, stimulation of reductive enzymes, modulation of hepatic DNA synthesis, lipid accumulation Suppression, and reduction of hepatic fibrosis <sup>4, 5</sup>.

A study included 509 species belonging to 140 genera that exhibit significant anti-diabetic activity <sup>6</sup>. *Ficus benghalensis* <sup>7</sup>, *Momordica charantia* <sup>8</sup>, *Ficus hispida* <sup>9</sup>, *Ficus elastic* <sup>10</sup> and *Pterocarpus marsupium* <sup>11</sup> are some prominent traditional medicines used for the treatment of diabetes. Another study enlisted 100 medicinal plants clinically proven for hepatoprotective effects <sup>12</sup> and some of the significant plants are *Phoenix dactylifera* <sup>13</sup>, *Aquilaria agallocha* <sup>14</sup> and *Salix caprea* <sup>15</sup>.

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The genus *Merremia* of over 100 plants belongs to family Convolvulaceae and is an abundant source of structurally varied phytochemicals with medicinal potential<sup>16</sup>. Additionally, its species meet nutrition values up to a safety level and are prohibited from chronic and prolonged administration to humans as well as animals<sup>17</sup>. Some therapeutically valuable *Merremia* plants are *M. borneensis*, *M. emarginata*, *M. mammosa*, *M. peltate*, *M. tridentate*, and *M. vitifolia*<sup>18-20</sup>. The plants provide a wide spectrum of activities, including antimicrobial, analgesic, anticancer, antidiabetic, hepatoprotective activities. Specifically, *M. mammosa* and *M. tridentate* for antidiabetic activity<sup>21, 22</sup>, *M. emarginata* for hepatoprotective action<sup>23</sup>. *Merremia vitifolia* is a perennial and climbing plant widely distributed in South and East Asia. Its leaf and rhizome are mainly used in tribal areas to treat fever, inflammation, rheumatism, jaundice, dysentery, and urinary diseases<sup>24-26</sup>. Nevertheless, only a few studies on the plant are available. A recent study revealed the substantial antioxidant, thrombolytic, and anti-nociceptive potential of the methanolic leaf extract<sup>27</sup>. However, no work on hypoglycemic effects was determined on any animal model. Moreover, no evidence of hepatotoxicity or hepatoprotective was presented. As chemical constituents metabolized in the liver and are an essential mark for drug safety, our present aim was to investigate the level of hypoglycemic and hepatoprotective effect of value addition on traditional medicine.

## MATERIALS AND METHODS:

**Chemicals and Drugs:** Analytical grade Methanol (Merck, Germany); Standard Drugs: Glibenclamide and Silymarin (Square Pharmaceuticals Limited, Bangladesh); Dextrose (Glaxo SmithKline Bangladesh).

**Experimental Animals:** Six-seven weeks old Swiss albino mice of both sexes with a mean body weight of  $25 \pm 5.0$  g were procured from Animal House at the Department of Pharmacy, Jahangirnagar University, Savar, Bangladesh. The animals were fed with a commercial rat pellet diet *ad libitum* during the entire experimental period.

**Collection and Identification of the Plant:** *M. vitifolia* was collected from Noakhali Science and

Technology University, Sonapur, Noakhali and identified by Bangladesh National Herbarium, Mirpur-1, Dhaka. The identification code was DACB46503.

**Preparation of Plant Sample:** The collected plant stem was dried in the dark for ten days and ground into a coarse powder. Roughly 500g of powder was produced and stored in an airtight container in at cool, dark, and dry place until extraction commenced.

**Cold extraction for *M. vitifolia*:** The powder was soaked in 1500 ml of 80% methanol at room temperature for two weeks, accompanied by occasional shaking and stirring. Then the solution was filtered using a filter cloth and Whitman filter paper every seven days. The rotary evaporation (Yamato Scientific, Japan) at 45°C and evaporation at ambient conditions gave nearly 65 g of gummy, concentrate and greenish-black extract. Then, the extract was stored in a refrigerator in a beaker covering it with aluminum foil.

**Hypoglycemic Test:** A total of four groups of mice (n=10) and equally designed by sex were taken to perform hypoglycemic tests according to the described method with a little bit of modification<sup>28</sup>. For Group I (Control), mice were given only distilled water (10 mL/kg of mouse body weight), while Group II was used as the standard Group and treated with glibenclamide (10mg/kg). Group III and IV were treated with 200 mg/kg of stem extract and 400mg/kg body weight, respectively. Fasting blood glucose measurement followed by immediate administration (p.o.) of test samples. After 1 hour, dextrose (2 g/kg) solution was administered to all groups, and blood was collected from the mouse tail vein after 1 h, 2 h, and 3 h of administration. Glucose concentration was measured by Accu-Check electronic glucometer (Roche, Germany).

**Hepatoprotective Effect on CCl<sub>4</sub>-induced Mice:** The mice were divided into five groups (n = 10) and followed the method described by Li *et al.*<sup>29</sup>. Group I (negative control) animals were administered a single dose of water (25 ml/kg, p.o.) daily for 7 days and received olive oil (8 ml/kg, i.p.) on day 7. Group II (Toxicant control) received water (25 ml/kg, p.o.) once daily for 7 days and received 0.2% CCl<sub>4</sub> in olive oil (8 ml/kg, i.p.) on

day 7. Group III (positive control) received standard drug silymarin (100 mg/kg, p.o.) once daily for 7 days and received 0.2% CCl<sub>4</sub> in olive oil (8 ml/kg, i.p.) on day 7. Groups IV–V were administered orally 200 & 400 mg/kg of the extract once daily for 7 days, respectively. The Groups III–V animals were simultaneously administered 0.1% CCl<sub>4</sub> in olive oil (8 ml/kg, i.p.) on day 7 after 2 h of the silymarin and extract administration. Animals were sacrificed after 24 h of treatment, and blood was collected through cardiac puncture, and the serum was separated by centrifugation at 6000 × g for 15 min. Liver function biochemical markers alkalinephosphatase (ALP) levels, alanine aminotransferase (ALT), and aspartate aminotransferase (AST) were evaluated according to the supplier's specifications from the standard kits.

**Statistical Analysis:** Data are expressed as mean ± standard error of the mean (SEM) and analyzed using the Statistical Package for the Social Sciences (SPSS), version 20.0 software. The difference between group means was analyzed with

a one-way analysis of variance (ANOVA) followed by Dunnett 't' test. P <0.05 was considered as statistically significant.

## RESULTS:

**Hypoglycemic Effects:** The blood glucose levels of all groups were nearly 7 mmol/L before 1 hr of administration. In contrast, several changes were observed in all administrations except control **Table 1**. After 1 hr of administration, both standard and extracts showed a minute decrease of glucose level, and the most minor reduction was exhibited by the standard at 6.5±0.5 mmol/L. In 2<sup>nd</sup> hour, the standard also exhibited the lowest glucose level, although MV 400 had a substantial fall of that level with 5.6±0.8 mmol/L. Dramatically, in 3<sup>rd</sup> hour, MV 400 showed a significant decrease (p<0.01) in glucose level, where it surpassed the hypoglycemic effect of glibenclamide (10mg/kg) and reached the maximum reducing figure at 3.5±0.6 mmol/L. At that end point, MV 400 exhibited 52.1 hypoglycemic effect compared to the blood glucose level before administration.

**TABLE 1: EFFECT OF METHANOLIC STEM EXTRACT OF *M. VITIFOLIA* ON BLOOD GLUCOSE LEVEL (MEAN ± SEM)**

Treatment	Blood Glucose Concentration (mmol/L)			
	-1 Hour	+1 Hour	+2 Hour	+3 Hour
Control	7.0±0.5	7.1±0.5	7.4±0.2	7.2±0.3
Standard	7.0±0.4	6.5±0.5	5.1±0.6	4.4±0.8*
MV200	7.3±0.4	7.1±0.5	7.0±0.4	5.7±0.5
MV400	7.2±0.4	7.1±0.6	5.6±0.8*	3.5±0.6**

Here, MV stands for methanol extract of *M. vitifolia*; \*p<0.05; \*\*p<0.01

**Hepatoprotective Test:** The results of the hepatoprotective effect of extracts on CCl<sub>4</sub>-intoxicated rats are shown in **Table 2**. As indicated from the results, CCl<sub>4</sub>-intoxicated animals showed an increase in the alkalinephosphatase, alanine aminotransferase, and aspartate aminotransferase

levels compared to the negative control group. Treatment of animals with *M. vitifolia* at 200 and 400 mg/kg, p.o., or silymarin 100 mg/kg, p.o., significantly decreased the level of serum marker enzymes.

**TABLE 2: PROTECTIVE EFFECT OF *M. VITIFOLIA* AND SILYMARIN ON CARBON TETRACHLORIDE (CCL<sub>4</sub>) INDUCED ELEVATION IN ALKALINEPHOSPHATASE, ALANINE AMINOTRANSFERASE, AND ASPARTATE AMINOTRANSFERASE LEVELS (MEAN ± SEM)**

Group	Alkaline phosphatase (IU/L)	Alanine aminotransferase (IU/L)	Aspartate aminotransferase (IU/L)
Negative Control	188.2±15.6	55.9±10.0	274.0±66.8
Toxicant Control	204.2±22.9	63.7±14.2	327.0±36.1
Positive Control	103.4±21.7**	54.3±8.3	237.7±45.6*
MV200	188.6±27.0	54.3±12.4	282.7±30.7
MV400	108.8±15.3**	43.4±4.0*	268.8±59.9

Here, MV stands for methanol extract of *M. vitifolia*; \*p<0.05; \*\*p<0.01

*M. vitifolia* at 400 mg/kg exhibited  $108.81 \pm 15.3$  IU/L of ALP concentration, almost a 50% decrease from the toxicant control group. Alanine aminotransferase concentration showed surprising results; 400 mg/kg dose had a value of  $43.4 \pm 4.0$  U/L, whereas positive control provided a higher value of  $54.3 \pm 8.3$  IU/L. Regarding aspartate aminotransferase level, 400 mg dose also showed the least value between the two concentrations, and positive control exhibited the least among all groups. The reduction of alkaline phosphate and alanine aminotransferase levels were moderately ( $p < 0.01$ ) and minimally ( $p < 0.05$ ) significant at concentration 400 mg/kg, respectively.

**DISCUSSION:** The study determined the hypoglycemic and hepatoprotective effects of the methanolic extract of the stem of *M. vitifolia* on mice models. Upon administration of the extract with two concentrations, the results showed a significant lowering of blood glucose and liver enzyme levels that marked potential phytoconstituents at the plant metabolite.

Empirically, the people in Luwu, Southern Sulawesi, use *M. vitifolia* to cure diabetes mellitus (Hasanah et al., 2019). However, the 400 mg/kg dose surpasses the blood glucose level for 10 mg/kg glibenclamide in this study. Although no in vivo activity on hypoglycemic activity is available, one study on n-hexane extract of the plant leaves showed the inhibition of  $\alpha$ -glucosidase, which is associated with glucose absorption inhibition by the intestinal epithelium membrane. Phytol, neophytadiene,  $\beta$ -caryophyllene, stigmaterol,  $\gamma$ -sitosterol, and lup-20(29)-en-3-one probably contribute to  $\alpha$ -glucosidase inhibitory activity<sup>31</sup>. Some plants under the genus *Merremia* also exhibited hypoglycemic activity. A study confirmed the potent antidiabetic activity of *M. emarginata* in streptozotocin-induced diabetic rats. The enzymatic activities for carbohydrate metabolism were significantly increased; however, glucose-6-phosphatase, fructose-1, 6-bisphosphatase were significantly decreased, and interestingly it showed the pancreatic  $\beta$ -cells regeneration in this study<sup>32</sup>. Another study described that flavonoid-rich fraction of *M. tridentata* revealed strong hypoglycemic effects in alloxan-induced diabetic mice compared with the references, glibenclamide, and metformin. At the

same time, the plant exhibited good  $\alpha$ -amylase and  $\alpha$ -glucosidase inhibitory activity compared with acarbose<sup>33</sup>. The root extract of *M. tridentata* also possessed significant antidiabetic activity in streptozotocin (STZ)-induced diabetic rats<sup>34</sup>. Another *Merremia* species *M. mammosa* exhibited wound healing in a diabetic rat model<sup>35</sup>.

In the case of hepatoprotective effects against  $\text{CCl}_4$  intoxicated mice, alkaline phosphatase, alanine aminotransferase, and aspartate aminotransferase levels were decreased significantly. Additionally, the ingestion of the plant extracts reduced comparatively enlarged intoxicated liver size. However, a recent study on a genus of the plant explored that *M. tridentata* ameliorated the liver damage caused by the  $\text{CCl}_4$  and reduced serum aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, acyl carrier protein and total Bilirubin content to normal levels<sup>36</sup>. Another species showed a hepatoprotective effect, where four out of ten elevated enzymes level by  $\text{CCl}_4$  were significantly reduced upon administration of *M. borneensis*. Moreover, pretreatment with *M. borneensis* against rats treated with  $\text{CCl}_4$  showed hepatic enzymatic and non-enzymatic antioxidant molecules in increased level, and histopathological improvement, substantially<sup>37</sup>.

One recent study confirmed the isolation of 27 phytochemicals in this plant and some proposed compounds are azedarachin C, stigmastan-3,6-dione, pheophorbide A, and isoquinaldonitrile, 3-methyl-, 2-oxide, however, comprehensive bioactivity testing had not been done on these compounds<sup>25</sup>. The present finding must keep a query for extensive investigation on the plant's phytochemicals that exactly impart in these actions.

**Limitation of this Study:** The study evaluated the pharmacological insight on hypoglycemic and hepatoprotective effects only that is not enough for the plant's therapeutic profiling. It should take more than two doses to determine the dose-response relationship meaningful. Moreover, no hint can be conveyed about the molecules responsible for the two pharmacological aspects.

**CONCLUSION:** The study is the scientific evidence of hypoglycemic and hepatoprotective

activities of *M. vitifolia* that justify the ethnopharmacological diversity of the plant. Although plant extract exhibited less significant hepatoprotection, it may be highlighted for antidiabetic activity as an alternative medicine.

#### Declaration:

**Ethical Issue:** The ethical committee, Faculty of Science of Noakhali Science and Technology University, Bangladesh, had approved the research with the code: NSTU/SCI/EC/2020/132.

**Consent for Publication:** Not Applicable

**Availability of Data and Materials:** There has no restriction to sharing any data.

**CONFLICTS OF INTEREST:** There is no conflict of interest

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