Jagannath University Journal of Life and Earth Sciences, **5**(1):114-121, 2019 ISSN 2414-1402

- Short Communication

DICHLOROMETHANE EXTRACT OF *SWIETENIA MACROPHYLLA* KING SEED SHOWS HYPOGLYCEMIC ACTIVITY BOTH *IN VITRO* AND *IN VIVO*

Subrata Kumar Barman[1], Md. Mohibullah[1], Muaj Ibne Sahid[1,] Shahenul Islam[1], Md. Tanvir Ashraf[1], S.H. Youn[2] and Sukumar Bepary[1*]

Abstract

Dichloromethane extract and methanol extracts of *Swietenia macrophylla* King seed were extracted separately and then evaluated for *in vivo* hypoglycemic activity in Swiss albino mice. Here, by oral dose of 500 mg/kg of body weight, both the extracts were found to show consistent reduction in the blood glucose level when tested for 120 minutes. The dichloromethane extract was more potent to reduce the blood glucose level than the methanol extract (29% vs 16% reduction respectively). Subsequently, *in vitro* study was performed with the dichloromethane extract using 250 µg/ml and 1000 µg/ml dose levels against the α-amylase enzyme. Percentage (%) inhibition of the enzyme was found to be dose dependent and the IC_{50} value was 3500 µg/ml. Thus the extracts possessed potential hypoglycemic constituent(s) as observed from this study. But the fractions were found to be different in terms of chemical contents. Thus extraction by dichloromethane should be considered independently for phytochemistry and phytomedicinal researches. However *Swietenia macrophylla* King seed exhibits hypoglycemic activity both *in vivo* and *in vitro* thereby indicating that the phytoconstituents present here not only increase the glucose update by the cells but also inhibit enzyme dependent starch hydrolysis and subsequent glucose absorption from the gut.

Keywords: *Hypoglycemic, Swietenia macrophylla, α-amylase enzyme.*

Diabetes is a life-long disease condition resulting in 3.2 million deaths every year worldwide (WHO, 2004). Hyperglycemia is a common effect of uncontrolled diabetes and over time leads to serious damage to many of the body's systems, especially the nerves and blood vessels. Thus, diabetes is a major cause of blindness, kidney failure,

[1]Department of Pharmacy, Jagannath University, Dhaka-1100, Bangladesh
[2]Korea Ginseng Corporation, 30 Gajeong-ro, Yuseong-gu, Daejeon, South Korea
[*] Corresponding Author: Sukumar Bepary, Email- sukumarsb@yahoo.com

heart attacks, stroke and lower limb amputation (WHO, 2018). It was reported that in 2017 there were 451 million (age 18-99 years) people with diabetes worldwide and by 2045 this has been expected to increase to 693 million. The global healthcare expenditure on people with diabetes was estimated to be USD 850 billion in 2017. However, the major antidiabetic therapy include subcutaneous administration of various forms of insulin or oral administration of insulin secretagogues, where the major limitations are hypoglycemic episode, weight gain, premature atherosclerosis, etc. These limitations as well as the alarming increase in the disease prevalence is keeping a lot of researchers busy for developing better antidiabetic agents worldwide.

The herbal anti-diabetic agents are generally regarded as the safer option (Rao *et al.*, 2003). According to the World Health Organization, up to 80% of populations of the developing country rely on traditional medicine for their primary health care (WHO News, 2004).Plants have been being used as medicines for 60,000 years and about 25% of the drugs prescribed worldwide come from plants (Yuan *et al*., 2016 & Rates, 2001). In Malaysia, Indonesia and West Bengal *Swietenia macrophylla* King seeds are used as a raditional medicine for treating diabetes (Dewanjee *et al*., 2009; Goh *et al*., 2011 and Haldar *et al*., 2011).

Though several research studies have reported the anti-diabetic activity of the aqueous extract or alcoholic extract of *Swietenia macrophylla* King seed (Dutta *et al*., 2013; Kalpana *et al*., 2011; Maiti *et al*., 2008 and Maiti *et al*., 2009) none of these used dichloromethane as the solvent for extraction. Thus dichloromethane has been used as the solvent in this case for making the seed extract and subsequent evaluation of the hypoglycemic activity. The results have been reported here.

The fruits of *Swietenia macrophylla* King were collected from the area of Sundarganj, Gaibandha, Bangladesh (25.5644° N, 89.5191° E). The plant was identified and authenticated by Bangladesh National Herbarium (BNH). A voucher specimen was submitted to Bangladesh National Herbarium. The accession number provided by BNH is 46739.

All chemicals and solvents used were of analytical grade. Methanol and dichloromethane were collected from Duksan Pure Chemicals Co. Ltd, South Korea. Starch (soluble) and n-Hexane were collected from Daejung Chemical & Metal Co. Ltd., South Korea. Dimethyl sulphoxide (DMSO), α-Amylase enzyme, iodine and potassium chloride, hydrochloric acid, gum acacia, sucrose were collected from Loba Chemie, India. Anhydrous sodium sulfate was collected from Merck Specialties Private Ltd., India.

Phosphate buffer (pH 7.0) was collected from Chem-Lab NV, Belgium. Distilled water was collected from Al-Shifa Chemicals (ASC), Gazipur, Bangladesh. Glipizide as a reference was collected from Beximco Pharmaceuticals Ltd., Bangladesh.

For *in vivo* hypoglycemic test, healthy Swiss albino mice including male and female of 18-23 gram were procured from the Animal Resources Facility of icddr,b. The animals were kept in plastic cages in a laboratory room in the department of Pharmacy of Jagannath University. Room temperature was maintained of 22±1°C under cycle of 12 h light/dark with free access to water and standard pellet food. The animals were acclimatized for 7 days before the experiment. All procedures concerning animals were carried out in an ethically proper way by following guidelines as set by the WHO and conform to the European Community guiding principles in the care and use of animals (86/609/CEE,CE Off J No.L358, 18 December 1986).

The *Swietenia macrophylla* seed kernel was collected and crushed properly. Weighted amount of the crushed mass was soaked in desired solvent (Methanol or dichloromethane). After continuous stirring at 50^{o}c for 2.5 hours the solid was filtered off. The filtrate was collected and subsequently the solvent was evaporated under vacuum by rotary evaporator. The residue was then washed with n-Hexane to remove the oils to get the desired fraction for subsequent biological evaluations.

The mice were divided into 4 groups each having 5 mice ensuring mice of both sexes. The groups were treated as negative control, positive control having glipizide, methanol extract 500 mg/kg and dichloromethane extract 500 mg/kg. Blood sugar was tested by using Finetest single touch glucometer before administration of the samples orally. For collecting the blood, tail picking was done and the blood drops were used for the reading by the Finetest Premium Test Strips. Subsequently, the mice were orally given the test compounds as per their treatment groupings. Blood sugar levels were tested at 30 min, 60 min and 120 min after the administration. Finally the results were analyzed to have the comments.

Preparation of iodine reagent

635 mg iodine and 1 gm potassium iodide were dissolved in 250 ml distilled water with continuous stirring.

Experimental procedure

For observing the *in vitro* antidiabetic effects the published method (Sudha *et al*., 2011) was applied with minor modification. In this procedure the mixture of 6.0ml phosphate buffer (pH 6.9), 0.2 ml of enzyme solution containing 10 mg of α-amylase in 100 ml of phosphate buffer,0.2 ml of the desired test extract (250 μg/ml and 1000 μg/ml) dissolved in distilled water were incubated at 37°C for 10 minutes. After subsequent addition of 0.2 ml starch solution (1% w/w) the mixture was incubated for 15 minutes. The reaction was stopped by addition of 0.2 ml of 1M HCl. To the final mixture was added 0.3 ml iodine reagent and then the absorbance was measured at 620 nm. Enzyme solution was made by replacing test drug with vehicle. The control was made by replacing both the test drug and enzyme with vehicle. Enzyme activity was calculated by the following equation-

Enzyme activity (EA) = Absorbance of control – Absorbance of sample

The % inhibition of the enzyme activity was calculated by the following equation-

$$\% \text{ inhibition of the enzyme activity} = (EA_{[Enzyme]} - EA_{[Test]}) / EA_{[Enzyme]} \times 100$$

Finally the IC_{50} value of the test drug was observed from plots of percentage inhibition vs concentration.

While comparing the dichloromethane extract with the methanol extract, the former has produced more pronounced hypoglycemic effect at 120 minute after the administration of the drug (16% and 29% respectively) as shown in Figure 1. However reduction at 30 minute was more potent in case of methanol extract as shown in Figure 2. The dichloromethane extract showed consistent decrease in the reduction of blood glucose level throughout the observation time (Dotted line).

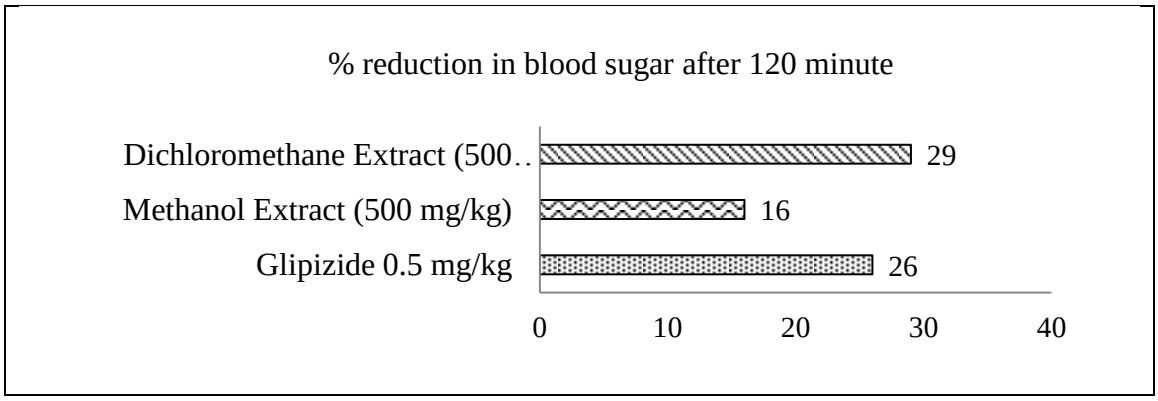

Figure 1. Average blood sugar reduction after 120 minutes

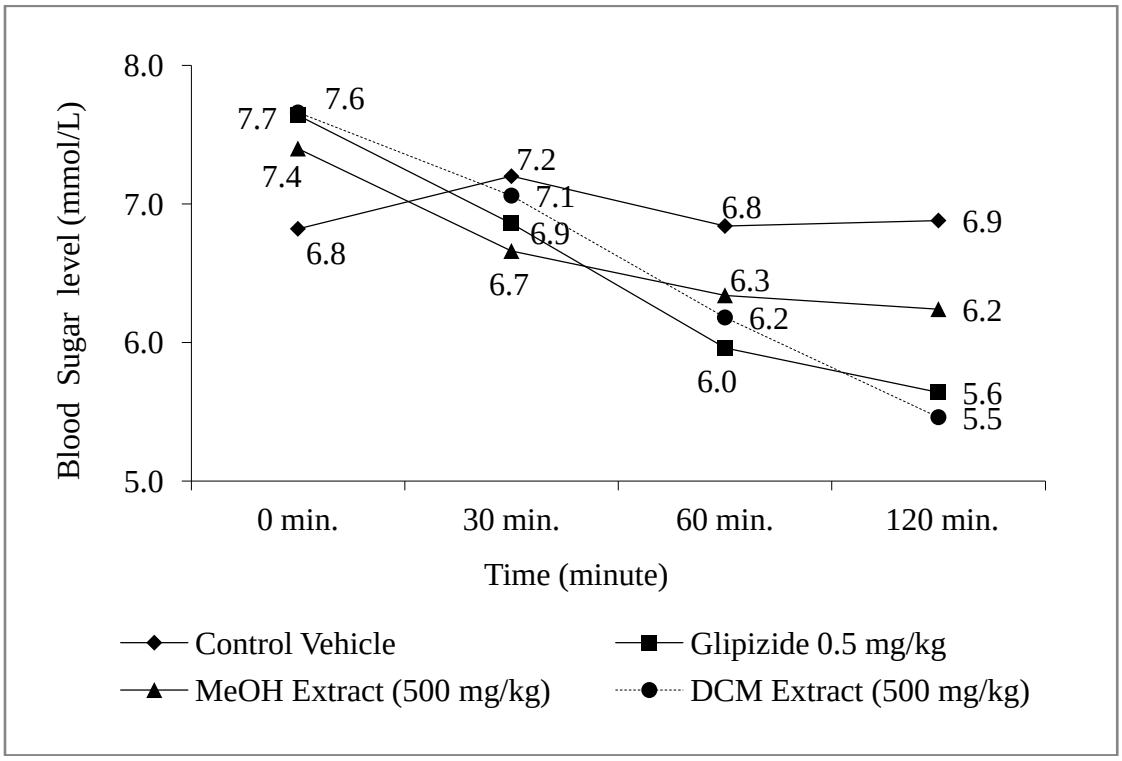

Figure 2. Average blood sugar levels at various time intervals

When observed the hypoglycemic potency *in vitro* through observation of the α-amylase inhibitory potency of the *Swietenia macrophylla* King seed, dichloromethane extract was found to inhibit the α-amylase enzyme. As shown in Figure 3 and Figure 4, though the inhibitory action was of moderate intensity, there was a dose dependent increase in the

inhibitory potential when the dose was increased to 1 mg/ml. Thus the dichloromethane extract appears to contain phytoconstituents(s) having α-amylase inhibitory potential.

Thus there seems to have another interesting observation from this study. The phytoconstituents from this plant appear to have important role in preventing the hydrolysis of starch. Thus there will be reduced availability of absorbable glucose in the gut. This will specially help the obese people because there will be enhances glucose uptake by the cell as well as there will be reduced absorption of glucose from the gut. This dual action may be exploited to discover better antidiabetic agents in future.

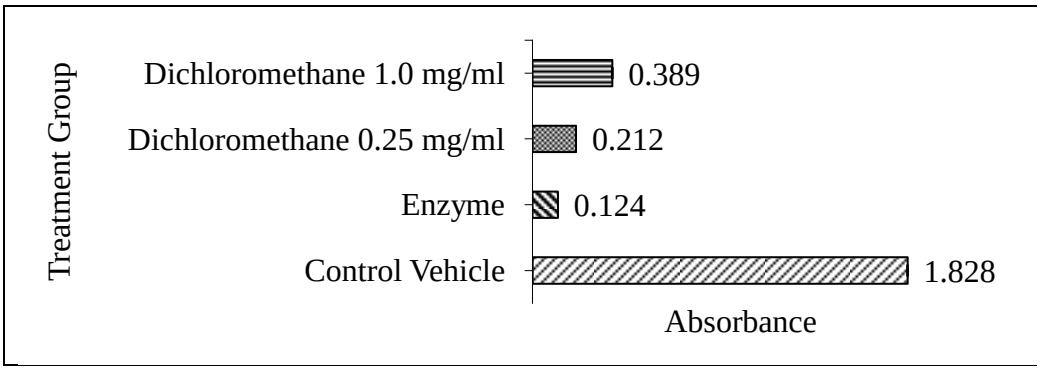

Figure 3. Inhibitory activity of dichloromethane extract of *Swietenia macrophylla* seed

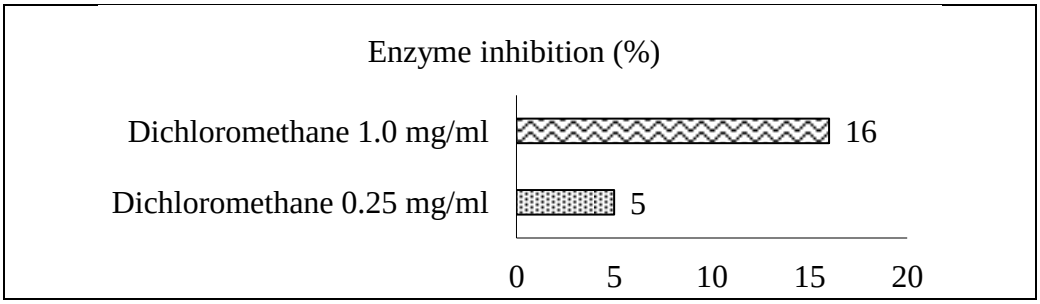

Figure 4. Dose dependent enzyme inhibition by dichloromethane extract of *Swietenia macrophylla* seed

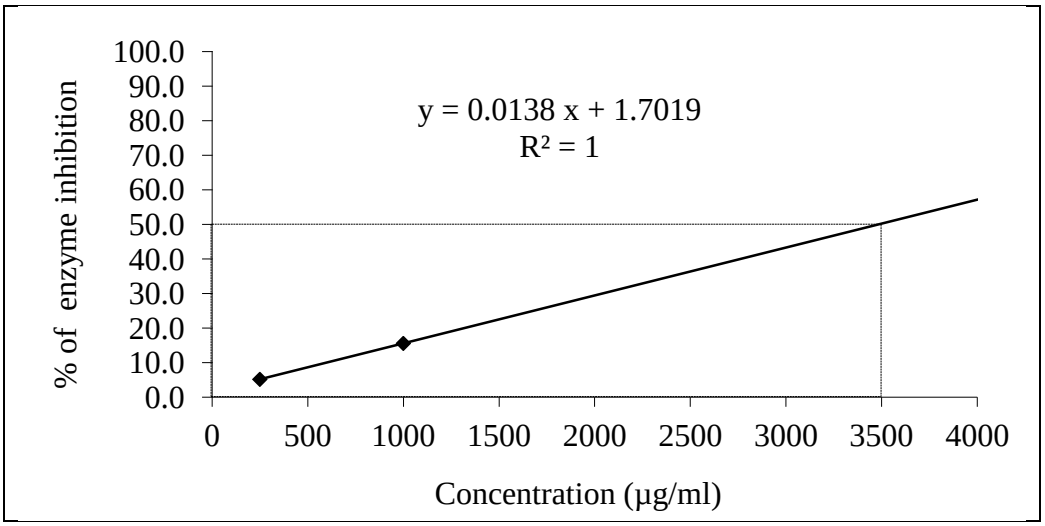

Figure 5. IC_{50} of dichloromethane extract of *Swietenia macrophylla* seed against α-amylase

Since there was dose dependent increase action, the two-point result was used to have an idea about the IC_{50} value of the extract. As shown in Figure 5, the dichloromethane extract shows the IC_{50} value of 3.5 mg/ml while applied against the α-amylase enzyme.

In this study, the dichloromethane extract and methanol extract of *Swietenia macrophylla* King seed were found to show consistent reduction in the blood glucose level. Interestingly, the dichloromethane extract was also found to be active *in vitro* study where the inhibitory was observed against the α-amylase enzyme. Thus *Swietenia macrophylla* King seed exhibits hypoglycemic activity both *in vivo* and *in vitro* thereby indicating that the phytoconstituents present here not only increase the glucose update by the cells but also inhibit enzyme dependent starch hydrolysis and subsequent glucose absorption from the gut. Thus further researches are being suggested for bioactivity-guided separation and isolation of hypoglycemic constituents with for developing new oral hypoglycemic agents in future.

Acknowledgements

Authors are cordially grateful to Jagannath University, Dhaka, Bangladesh, for providing the necessary financial support for completion of this work.

References

Dewanjee, S., Maiti, A., Das, A.K., Mandal, S.C. and Dey, S.P. (2009). Swietenine: A potential oral hypoglycemic from Swietenia macrophylla seed. *Fitoterapia*. 80: 249-251.

Goh, B.H. and Kadir, H.A. (2011). In vitro cytotoxic potential of Swietenia macrophylla King seeds against human carcinoma cell lines. *J Med Plants Res.* 5: 1395-1404.

Haldar, P.K., Adhikari, S., Bera, S., Bhattacharya, S., Panda, S.P. and Kandar, C.C. (2011). Hepatoprotective efficacy of Swietenia mahagoni L. Jacq. (Meliaceae) bark against paracetamol-induced hepatic damage in rats. *Indian J. Pharm. Educ. Res.* 45: 108-113.

Dutta, M., Biswas, U.K., Chakraborty, R., Banerjee, P., Maji, D., Mondal, M.C. and Raychaudhuri, U. (2013). Antidiabetic and antioxidant effect of Swietenia macrophylla seeds in experimental type 2 diabetic rats. *Int. J. Diabetes Dev. Ctries.* 33: 60-65

Kalpana, K. and Pugalendi, K.V. (2011). Antioxidative and hypolipidemic efficacy of alcoholic seed extract of Swietenia macrophylla in streptozotocin diabetic rats. *J. Basic Clin. Physiol. Pharmacol.* 22: 11-21.

Maiti, A., Dewanjee, S., Jana, G. and Mandal, S.C. (2008). Hypoglycemic effect of Swietenia macrophylla seeds against type II diabetes. *Int. J. Green Pharm.* 2:224.

Maiti, A., Dewanjee, S., Kundu, M. and Mandal, S.C. (2009). Evaluation of antidiabetic activity of the seeds of Swietenia macrophylla in diabetic rats. *Pharm. Biol.* 47: 132-136.

Rao, B.K., Sudarshan, P.R., Rajasekhar, M.D., Nagaraju, N. and Rao, C.A. (2003). Antidiabetic activity of Terminalia pallida fruit in alloxan induced diabetic rats. *J. Ethnopharmacol.*; 85: 169-172.

Rates, S.M.K. (2001). Plants as source of drugs. *Toxicon*. 39: 603-613.

Sudha, P., Smita, S.Z., Shobha, Y.B. and Ameeta, R.K. (2011). Potent α-amylase inhibitory activity of Indian Ayurvedic medicinal plants. *BMC Complement Altern Med.* 11: 5.

WHO, *International Diabetes Federation* (2004). Diabetes action now : an initiative of the World Health Organization and the International Diabetes Federation. World Health Organization, Geneva, Switzerland. https://doi.org/924159151 X

WHO, *Diabetes*. (2018). https://www.who.int/news-room/fact-sheets/detail/diabetes (March 5, 2019).

WHO News (2004). New WHO guidelines to promote proper use of alternative medicines. https://www.who.int/mediacentre/news/releases/2004/pr44/en/

Yuan, H., Ma, Q., Ye, L. and Piao, G. (2016). The traditional medicine and modern medicine from natural products. *Molecules,* 21.

Jagannath University Journal of Life and Earth Sciences

ISSN 2414-1402

Number 2, December 2019