

Research Journal of Pharmaceutical, Biological and Chemical Sciences

Potential Antidiabetic Activity of *Plantago Major* Leaves Extract in Streptozocin-Induced Diabetic Rats.

Mahfoudh A. Abdulghani^{1,2*}, Ismat Hamid³, Redhwan Ahmed Al-Naggar⁴, and Muhamed T Osman⁵.

¹Pharmacology Department, Unaizah College of pharmacy (UCP), Qassim University, 51911, Unaizah, Al Qassim, Saudi Arabia.

²Pharmacology Department, International Medical School, Management and Science University, 40100, Shah Alam, Malaysia.

³Faculty of Health and Life Sciences, Management and Science University, 40100, Shah Alam, Malaysia.

⁴Population Health and Preventive Medicine Department, Faculty of Medicine, Universiti Teknologi MARA (UiTM), 47000, Selangor, Malaysia.

⁵Department of Pathology, Faculty of Medicine, Universiti Teknologi MARA (UiTM), Sg., Selangor, Malaysia.

ABSTRACT

Plantago major (PM) is one of a perennial plant and it belonging to the family of Plantaginaceae. It has been used in Malaysia as a folk remedy for diabetes and other illness. PM grows widely in whole of Europe and temperate Asia. The objective of study was evaluating antidiabetic activity of alcoholic extract of PM using STZ-induced diabetic rats. Methodology: adult male Sprague dually (SD) rats were used in the experiment for hypoglycaemic activity in oral glucose tolerance test (OGTT) and antidiabetic activity in streptozotocin induced rats. Results: showed that the continuous post-treatment for 14 days with the 1000 mg/kg of PM showed potential hypoglycaemic activity in OGTT and antidiabetic activity in streptozotocin induced rat models. Furthermore, isolation and establishment of exact mechanism of action of specific compound from PM is to be carried out in the future.

Keywords: *Plantago major*, diabetes mellitus, hyperglycemia, oral glucose tolerance test.

*Corresponding author

INTRODUCTION

Diabetes mellitus (DM) is one of the biggest world health problems with high incidence and mortality [1]. Though oral hypoglycemic agents and/or insulin are currently available for the DM control, various medicinal plants are also used empirically and some of them have been pharmacologically investigated [1,2].

Plantago major (PM) is a perennial plant, belonging to the family *Plantaginaceae*, grows widely in Europe and temperate Asia. PM has been used in Malaysia as a folk remedy as antidiabetic and other illnesses [3]. The PM is worldwide known and it has several local names commonly called weed [3]. In Malaysia called 'Ekor Anjing [4], in India known as 'White man's footprint' and in Europe known 'grobad' meaning 'healing leaves' [5]. It was traditionally used in treatment of many of illnesses such as cough, asthma, diarrhoea, urinary tract infections, skin diseases, menstrual disorders and stone [5]. Phytochemical studies of PM extract showed that it contains phenol's, flavonoids and terpenoids [3]. Other studies showed that PM contains such as polysaccharides, lipids, caffeic acid derivatives, iridoid glycosides, alkaloids, organic acids and vitamines derivatives e.g; vit. A and vit. C [3-5]. PM shows many biological activities such as antiulcer [6], anticancer as prophylactic oncology agent and antitumor activity [7], antiinflammatory [8], antifungal [9], antibacterial activities [10], antimalaria [11], immunodulatory effect [12], Antioxidant [13], intermediate diuretic [14] and antidiabetic activity [15,16].

However, no extensive work has been performed for possible hypoglycaemic properties of this plant, hence, the present study was planned to evaluate the antidiabetic potential of PM in streptozotocin-induced diabetic rats. It was an attempt has been undertaken to establish a possible mechanism of action to be considered in management of diabetes.

MATERIALS AND METHODS

Chemicals and reagents

Streptozotocin was procured from Sigma chemical Co. (St. Louis, USA), buffer phosphate and Dimethyl sulfoxide (DMSO) used in the present study were of analytical grade (AR).

Plant material and extraction

Methanol standard extract of leaves of *Plantago major* was provided by Faculty of Medicine and Health Science, Universiti Sains Islam Malaysia [3].

Animals

Male Sprague Dawley (SD) rats weighing 200–250 g were used in this study (international medical school, Management and Science University, Malaysia), Animals were housed four-six per standard rat cage, in a room with a 12:12 h light/dark cycle (lights on 07:00 h) and controlled temperature (22 ± 1 °C). Commercial rodent pellets and tap water

were available *ad libitum*. They were allowed to adapt to the laboratory conditions for 1 week before the study. There were six rats per group in each experiment. The procedures were performed in accordance with institutional guidelines for animal care and use.

Oral toxicity studies

Acute toxicity in rats

The present study was conducted according to the Organisation for Economic Cooperation and Development (OECD) revised up-and-down procedure for acute toxicity testing (OECD guideline 425, 2001). A dose limit of 5000 mg/kg of the standardised methanolic extract was used in five healthy female adult SD rats. Rats were fasted overnight from food, but free to access water, prior to dosing and weighed before the extract was administered orally. Alcohol extract was prepared in 5000 mg/10ml of 0.1 of DMSO. A dose of 5000 mg/kg was given orally to the first rat, and this rat was observed for mortality and clinical signs for the first hour, then hourly for 3 h and, finally periodically until 48 h. The visual observation included changes in the skin and fur, eyes and mucous membrane and bizarre behavior such as unusual aggressiveness, unusual vocalisation, restlessness, sedation and somnolence; movements: twitch, tremor, ataxia, catatonia, paralysis, convulsion, fasciculation, prostration and unusual locomotion; convulsion. If the animal survived, then four additional animals were given the same 5000 mg/kg dose sequentially at 48-h intervals. All of the experimental animals were maintained under close observation for 14 days, and the number of rats that died within the study period was noted. The LD₅₀ was predicted to be above 5000 mg/kg if three or more rats survived.

EXPERIMENTAL DESIGN

Study on Oral Glucose Tolerance Test (OGTT)

OGTT of plant extracts were carried out in overnight fasted normal rats, which were equally divided into four groups of six rats each. Group of normal control received only vehicle (1 ml of 0.1% DMSO; p.o.) and positive group received 1 ml of reference drug glibenclamide (GL) suspended in the vehicle (0.25 mg/kg, p.o.), while group from third to fourth were administered with 1 ml of PM (500 and 1000 mg/kg, p.o.) respectively. Thereafter, following 30 min post extract administration all the animals were fed with glucose (2 g/kg). Blood samples were collected from tail vein prior to dosing and then at 30, 60, 90 and 120 min after glucose administration. The fasting blood glucose level was analyzed using glucose-oxidase-peroxide reactive strips (Accu-chek, Roche Diagnostics, GmbH, Germany).

Study on STZ-induced diabetic rats

Diabetes was induced by intraperitoneal injection (single dose) of streptomycin (45 mg /kg b.w) in buffer phosphate solution to overnight fasted normal rats. Blood glucose level was checked by using one-touch glucometer and diabetes was confirmed after 72 hr of all STZ-treated rats. Rats shown FBG > 15 mM/dl were considered to be diabetic and were selected for studies. Animals selected were fasted over night and then divided into four

groups (n=6) as follows: Group-I: Normal control rats (non-STZ-treated) that was administered with vehicle (1 ml of 0.1% v/v DMSO in distilled water; p.o.) only; Group-II: Diabetic control rats (Untreated, STZ -treated); Group-III: Diabetic rats administered once with glibenclamide (0.25 mg/kg b.w) as reference standard drug while; Group-III and IV: Diabetic rats administered with PM (500, 1000 mg/kg/day) respectively. Treatment was continued for a period of 14 days following oral

Statistical Analysis

The results are expressed as mean \pm S.E.M. The statistical significance was determined by One-Way *Pos-hoc* Dunnet's test. $P < 0.05$ was considered to be statistically significant.

RESULTS

LD₅₀ of *Planto major*

In acute toxicity study, alcoholic extracts of *Planto major* (PM) leaves at the tested dose level of 5000 mg/kg body weight. did not show significant toxicity signs when observed for the parameters during the first four hours and followed by daily observations for 14 days. No mortality was observed and the drug was found to be safe. The LD₅₀ of PM was predicted to be above 5000 mg/kg.

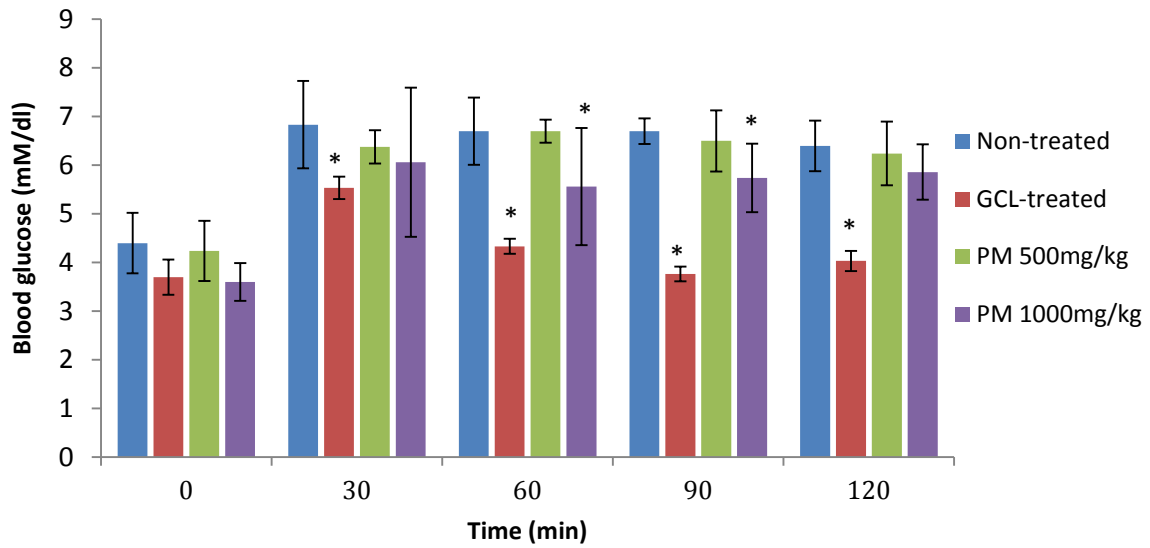
Oral Glucose Tolerance Test (OGTT)

The effects of different extracts on glucose tolerance test in normal rats were evaluated. At 30 min after glucose administration the peak of blood glucose level increased rapidly from the fasting value and then subsequently decreased. Glibenclamide treated group (0.25 mg/kg b.w.) prevented glucose induced hyperglycemia significantly at 60 min, 90min and 120 mins (4.33 ± 0.15 , 3.77 ± 0.15 and 4.03 ± 0.21) as compare to normal control (6.70 ± 0.69 , 6.70 ± 0.26 and 6.40 ± 0.52) respectively. Maximum glucose tolerance in *Planta major* extracts was observed in 1000 mg/kg (5.56 ± 1.20 , 5.74 ± 0.71 , 5.86 ± 0.57) and minimum glucose tolerance was observed in 500 mg/kg (6.7 ± 0.23 , 6.5 ± 0.65 and 6.24 ± 0.65) in 60, 90 and 120 minutes compared with the normal control (Fig.1).

Effect of *Planto major* on BG of STZ-induced diabetic rats

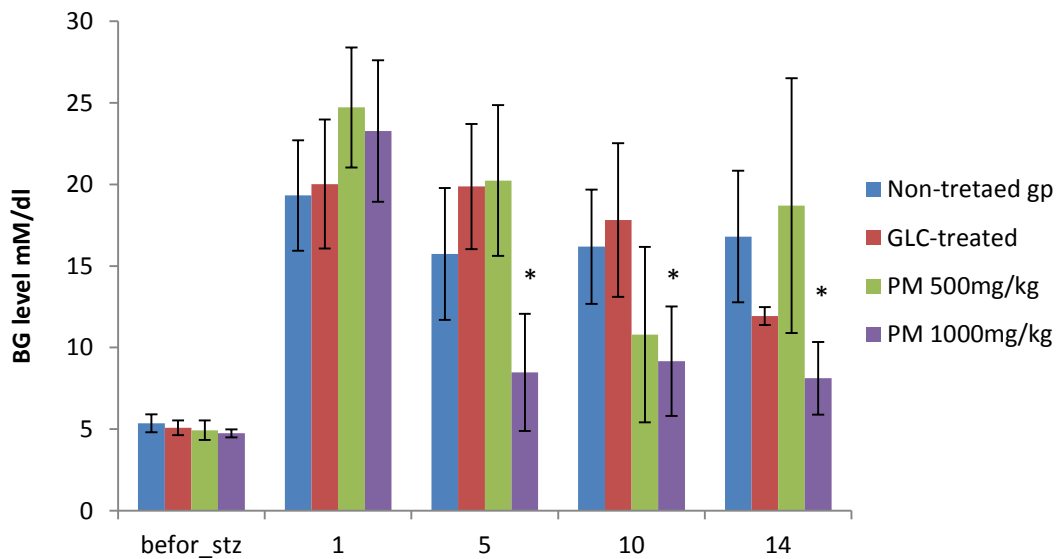
After 5, 10 and 14 days, 8.48 ± 3.59 ($p < 0.05$), 9.17 ± 3.36 ($p < 0.05$) and 8.12 ± 2.22 ($p < 0.05$) significant fall in blood glucose level with the 1000 mg/kg of PM methanolic extract; 24.72 ± 3.68 , 20.23 ± 4.62 , 10.80 ± 5.38 and 18.70 ± 7.81 diminution with the 500 mg/kg of methanolic extract; 20.02 ± 3.95 , 19.87 ± 3.84 , 17.82 ± 4.71 and 11.93 ± 0.55 diminution with glibenclamide and 19.32 ± 3.38 , 15.74 ± 4.04 , 16.18 ± 3.50 and 16.80 ± 4.03 rise in the control, respectively, were noticed (Fig.2).

Figure 1: Hypoglycaemic effect of PM in oral glucose tolerance test (OGTT)



Values are expressed as mean \pm SEM (n = 6). $P < 0.05$ as compared to respective control (one-way ANOVA followed by Dunnet's test).

Figure 2: Hypoglycaemic effect of PM in diabetic rats



Values are expressed as mean \pm SEM (n = 6). $P < 0.05$ as compared to respective control (one-way ANOVA followed by Dunnet's test).

DISCUSSION

Few previous experimental studies, performed with PM to validate its antidiabetic properties in rats, an ethanol extract was given orally at a dose of 500 mg/kg and the results did not show a significant effect [17]. However, in the present experiment, the continuous post-treatment for 14 days with the PM showed potential hypoglycaemic activity in OGTT and anti-diabetic activity in streptozotocin induced rat models. In order to establish a

scientific base for the usefulness of this plant in the treatment of diabetes, it was decided to evaluate experimental design of anti-diabetic activity by following glucose tolerance test and STZ-induced model. As expected, in the diabetic control, there was severe hyperglycaemia as compared to the normal animals.

Our results showed that in glucose tolerance test, the standard drug glibenclamide (GCL) 0.25 mg/kg b.w and the 1000 mg/kg b.w of PM significantly ($p < 0.05$) reduced blood glucose after one hour whereas 500 mg/kg b.w did not show significant reduction [Fig 1]. In antidiabetic experiment, 0.25 mg/kg daily of the GCL didn't lower the blood glucose level significantly of diabetic rats so have not brought it nearly back to normal. Because may be the duration of pharmacological action of GCL is not enough to control blood sugar for 24 hrs. The single dose of methanolic extract (1000 mg/kg b.w.) of PM significantly ($p < 0.01$) reduced the blood glucose level as compare to diabetic control at 5th day of the study (Fig.2). There is no report of *Planto major* possessing anti-diabetic activity.

Decreased blood glucose level in diabetic rats, this may be due to improving the glycemic control mechanisms from remnant pancreatic-cells in diabetic rats. The exact biologically active constituents responsible for the said effect have not been reported nor was the exact mode of action of the anti-diabetic activity reported earlier, with the lone observation that it is used in folklore diabetic treatments. However, some studies reported the preliminary hypoglycemic effect of PM due to the presence of tannins in the hexane extract, as well as flavonoids, sterols and sugars in the dichloromethane extract. Some of these phytochemicals are characterized because they typically contain at least one aromatic ring with hydroxyl groups (polyphenolic type), which have been associated with antioxidant activity and glucose-lowering effects [18]. Several flavonoids have been isolated from *P. major*, which have been associated with antioxidant and anti-inflammatory activities [5]. On the other hand, the seeds of *P. major* contain polysaccharides, which have also been associated with immunological and hypoglycemic activity, among other biological actions [5,19-20]. Moreover, the anti-diabetic activity may be due to antioxidant constituents like phenol's group, different variation of organic acid groups, flavonoids and terpenoids [3]. *Plantago* species showed antioxidant activity [13]. Further study is required to investigate the exact mechanism of anti-diabetic activity of PM.

CONCLUSIONS

We concluded that *Planto major* have potent antidiabetic effects in STZ-induced diabetic rats. The present investigation has also opened paths for further research especially with reference to the development of potent formulation for diabetes mellitus from *Planto major* leaves. Activity guided fractionation, formulation and its evaluation is in progress and will be available in a short period of time.

ACKNOWLEDGEMENTS

The financial assistance provided to the correspondent author in the form of seed grand by postgraduate institute, MSU, Malaysia is gratefully acknowledged. Authors are grateful to Dr. *Jamilah*, Dr. *Sharifa* and Dr. *Sharifah* from Faculty of Medicine and Health Science, Universiti Sains Islam Malaysia for providing us the standard extract.

REFERENCES

- [1] Alarcon AFJ. Traditional Medicines for Modern Times: Antidiabetic Plants. CRC Press, Taylor and Francis Group., FL. 2006.
- [2] Alarcón-Aguilar F, Vega-Ávila E, Almanza-Perez J, et al. Proceedings-Western Pharmacology Society 2006;199B:51.
- [3] Jamilah J, Sharifa A, Sharifah N. World App Sci J 2012;17:67-70.
- [4] Noor H, Juing M, Chee B, Kueh B, Othman Z. Pertanika J Trop Agr Sci 2000;23:29-35
- [5] Samuelsen AB. J Ethnopharmacol 2000;71:1-21 .
- [6] Atta A, Nasr SM, Mouneir SM. Nat Prod Rad 2005;4:258-263.
- [7] Ozaslan M, Didem Karagoz I, Kalender ME, et al. The American J Chinese Med 2007;35:841-851.
- [8] Ringbom T, Segura L, Noreen Y, Perera P, Bohlin L. J Nat Prod 1998;61:1212-1215.
- [9] Mathur A, Singh R, Yousuf S, et al. Adv Appl Sci Res 2011;2:260-264.
- [10] Kumar VP, Chauhan NS, Padh H, Rajani M. Search for antibacterial and antifungal agents from selected Indian medicinal plants. J Ethnopharmacol 2006;107:182-188.
- [11] Ali AA, Al-Rahwi K, Lindequist U. Afr J Trad CAM 2004;1:72 – 76.
- [12] Chiang L-C, Chiang W, Chang M-Y, Lin C-C. The American J Chinese Med 2003;31:225-234.
- [13] Oto G, Ekin S, Ozdemir H, et al. Asian Pacific J Cancer Prev 2011;12:531-535.
- [14] Wright C, Van-Buren L, Kroner C, Koning M. J Ethnopharmacol 2007;114:1-31.
- [15] Marles R, Farnsworth N. Phytomed 1995;2:137-189.
- [16] Hernandez-Galicia E, Aguilar-Contreras A, Aguilar-Santamaria L, et al. Proceedings of the Western Pharmacology Society 2002:118-124 .
- [17] Rodriguez J, Loyola J, Maulen G, Schmeda-Hirschmann G. Phytother Res 1994;8:372-374.
- [18] Timothy J, Letitia MM, Patrick O. Traditional Medicines for Modern Times: CRC Press; 2005:293-299.
- [19] Ding X, Zhang J, Jiang P, Xu X, Liu Z. Lett App Microbiol 2004;38:223-228.
- [20] Guo Y, Kong L, Wang Y, Huang Z. Phytother Res 2004;18:204-207.