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Effect of Ethanolic Extract of *Moringa oleifera* Lam. Leaves on Body Weight and Hyperglycemia of Diabetic Rats

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Abstract: *Moringa oleifera* is cognizant locally as mungai in Malaysia and commonly used traditionally. Therefore, the 14-day study was conducted on STZ-induced diabetic rats to evaluate the effect of 95% ethanolic extract of *Moringa oleifera* leaves on body weight, hyperglycemia and lipids. Group-I was negative diabetic control, received distilled water (10 ml/kg bw). Metformine (500 mg/kg bw) treated group-II was specified as positive control while group-III to VI received leaves extract with variable doses (1000, 500, 250, 125 mg/kg bw orally). In multiple dose experiment, the blood glucose and body weight monitoring was executed at day 0, 7 and 14 while in single dose at 0, 1, 3, 5 and 7 h. At the end of treatment, animals were sacrificed and blood was collected through cardiac puncture for lipid profile. The acute and sub-chronic treatment exhibited highly significant ($p < 0.01$) fall in blood glucose at 500 and 1000 mg/kg dose and 25.8% decline in body weight was observed. The treated group also offered reduction in total cholesterol ($p < 0.01$), triglycerides ($p < 0.05$) and low density lipoprotein ($p < 0.01$). It also appeared that by reducing the dose of extract, both the antihyperglycemic and loss of body weight decreases in treated groups. It can be recommended in obese diabetic patients to prevent macrovascular complication pertinent to body weight and lipids.

Key words: *Moringa oleifera*, ethanolic extract, antihyperglycemic, body weight, plasma lipids

INTRODUCTION

Diabetes mellitus has become an epidemic (Chaurasia *et al.*, 2010), affecting majorly the adults (69%) in developing countries (Shaw *et al.*, 2010). According to WHO, there is expectation of 592 million in the year 2035 particularly in low and middle-income developing countries (International diabetes federation atlas, 2013). The Malaysian National morbidity survey III claimed that the overall prevalence of diabetes mellitus in Malaysia was 11.6% in 2006 (Letchuman *et al.*, 2010) but now it went to 22.6% among Malaysians aged ≥ 30 years.

Moringa oleifera is familiar with its pharmacological actions particularly against diabetes mellitus (Babu and Chaudhuri, 2005; Bhishagratna, 1991). The leaves, pods, seeds, stem and barks have been tried for antidiabetic effects (Anwar *et al.*, 2007) and phenolic compounds assert about this (Fahmy *et al.*, 2015). Furthermore, it was also investigated that ethanolic extract represented more concentration of such compounds in contrast to aqueous extract (Khalid *et al.*, 2013). Therefore, the current study was designed to test out the effect of 95% ethanolic extract of *Moringa oleifera* Lam. leaves on glycemic control, body weight and plasma lipids.

MATERIALS AND METHODS

Plant materials: *Moringa oleifera* dried leaves were locally purchased from Harbagus Sdn Bhd. Butterworth of Pulau Penang, Malaysia, identified by Dr. Rahmad Zakaria, school of botany, University Sains Malaysia (voucher specimen, 11626). The leaves were grinded into powder with herbal grinder (Chyun Tsch Industrial Co; LTD) and extracted with 95% ethanol (Technical Avenue Bhd) via maceration, placing 24hr in water bath at 45°C. The filtrate obtained concentrated using rotary evaporator (Buchi Labortechnik, CH-9230 Flawil Switzerland) under reduced pressure with temperature 40°C and kept in oven to dry.

Experimental animals: The male Sprague Dawley (SD) rats 5-6 weeks old (170-200 g) were obtained from the Animal Research And Service Centre (ARASC), Universiti Sains Malaysia. Acclimatized for 7-days in the transit room of School of Pharmaceutical Sciences, provided with temperature (30±3°C) and 12 h dark and light cycles. The experiments on animals were done after approval from USM Animal ethical committee (Animal ethics approval no. AEA/2015/665).

Diabetic model: Streptozotocin (45 mg/kg body weight intraperitoneally, freshly prepared in 0.9% NaCl) diabetic model was used to induce diabetes in rats. 72 h post injection of streptozotocin (Lenzen, 2008), rats with fasting blood glucose (FBG) concentrations more than 15 mmol/L was used for study.

Study design: Thirty six male diabetic rats were divided into six groups (VI). Group I considered as negative control, given distilled water (10 ml/kg body weight orally), group II administered metformine with dose 500 mg/kg body weight orally as positive control, group III treated with 1000 mg/kg, group IV given 500 mg/kg, group V administered 250 mg/kg while group VI received the lowest dose 125 mg/kg body weight orally using 16G oral needle. All the measurements were made after 14 h overnight fasting. For acute antihyperglycemic effect, glucose concentration was noted at 0, 1, 3, 5 and 7 h with glucometer (ACCU-CHEK Performa No. 55404068967, a trade mark of Roche, USA). In case of 14-day study, the blood glucose and body weight was determined at day 0, 7 and 14. During the whole experiment, the bedding was changed regularly as diabetic rats produced a lot of urine. The percentage of reduction was calculated by the following formula:

$$\text{Percentage reduction} = \frac{\text{Initial value} - \text{final value}}{\text{Initial value}} \times 100$$

Biochemical analysis: At the completion of sub-chronic treatment, the rats were anaesthetized by inhalational anesthetic and blood samples were collected in tubes containing heparin through cardiac puncture. The blood samples immediately sent to pathology laboratory (Gribbles Pathology, Sdn Bhd, Malaysia) for total cholesterol (TC), triglycerides (TG), low density lipoprotein (LDL) and high density lipoprotein (HDL).

Phytochemical analysis: The presence of tannins and phenols was done by heating extract solution for two minutes in water bath and then few drops of 5% FeCl₃ were added to the test solution. To test saponins, extract solution was observed after vigorous shaking. For flavonoids, 1% aluminium chloride solution was added to the extract solution. Alkaloid was detected by adding few drops of conc. HCl, Meyer's and Wagner's reagents to extract dissolved in methanol. Terpenoids screening was carried out by mixing extract with 2 ml chloroform and conc. H₂SO₄ added gently. Glycosides were indicated by adding extract in chloroform and glacial acetic acid. After cooling, conc. H₂SO₄ was added slowly (Harborne, 2005; Sofowora, 1993; Trease and Evans, 1989).

Statistical analysis: The results were expressed as mean±SEM and statistically analyzed by using ONE-

WAY Analysis of Variance (ANOVA) (IBM SPSS Statistics 22) followed by Dunnett test as post hoc test. The difference in the means was considered statistically significant at p<0.05.

RESULTS

Effect of single dose treatment on blood glucose level:

The Table 1 describes the acute effect of ethanolic extract of *Moringa oleifera*. The extract at dose 250 and 125 mg/kg bw did not amend blood glucose concentration. The group treated with 1000 mg/kg dose showed 16.0% reduction while metformine stood at 54.95% after 7 h.

Evaluation of sub-chronic treatment on hyperglycemia:

Fig. 1 reveals that sub-chronic treatment with gradually reducing doses of ethanolic extract of *Moringa oleifera*, significantly (p<0.001) reduced fasting blood glucose concentration at day 7 and 14. The maximum reduction in glucose concentration was 53.44% with 1000 mg/kg dose. The fall in glucose was not observed in case of treatment with 125 mg/kg bw even at day-14.

Evaluation of two weeks treatment on body weight:

Table 2 describes that the diabetic rats treated with extract showed maximum loss in body weight a day 7 and furthermore, loss in body weight at the end of oral doses was gradually decreasing with dose. While the controlled diabetic rats showed almost equal reduction in body weight from day 0 to 7(19.13%), then from 7 to day 14 (19.10%).

Effect of 14-day treatment on plasma lipids: The result indicated that HDL level remained high (non significant) in rats with 500 and 250 mg/kg dose (0.84±0.12) and (0.60±0.16), respectively compared to diabetic control rats (0.47±0.032) except metformine treated diabetic rats (1.02±0.17). The maximum dose of extract had no significant effect on LDL (0.50±0.052) as shown in Fig. 2.

DISCUSSION

The natural products have contributed a lot to the knowledge about new therapeutically active agents (Cechinel *et al.*, 1998). These can be used directly as leads for the development of new drugs (Kaplan *et al.*, 1990) or to identify active one, with desired pharmacological activity (Secco, 1990). About 80% of patients in developing countries still depend on herbal medicines for their primary health (Kamboj, 2000) and many have been tested in chemically induced diabetic models (Frode and Medeiros, 2008). Usually, these extracts by altering the metabolic pathways (glycolysis, citric acid cycle, glycogen and lipid metabolism), are beneficial in diabetes (Prabhakar and Doble, 2008).

Table 1: Effect of variable doses of ethanolic extract on glucose concentration

Groups (n = 6)	Dose orally/kg b.w	BGL after treatment (mmol/l) at different time (h)				
		0	1	3	5	7
NDC	10 ml	18.57±0.39	20.07±0.86	20.15±0.77	20.17±0.39	19.88±0.41
PDC	500 mg	17.47±0.40	18.47±0.71	10.6±1.20 ^a	9.33±0.57 ^c	7.87±0.82 ^c
EE	1000 mg	18.43±0.48	21.83±0.38	19.52±0.49	17.60±0.50 ^a	15.48±0.78 ^b
	500 mg	18.62±0.24	22.75±0.44	20.7±1.08	18.75±0.90	17.17±0.77 ^a
	250 mg	19.08±0.47	21.08±0.70	19.83±0.72	19.25±0.83	19.40±0.53
	125 mg	17.25±0.86	18.83±0.75	18.45±0.65	18.48±0.45	18.28±0.74

Expressed as mean±SEM, BGL (blood glucose level), FBG (fasting blood glucose), NDC (negative diabetic control), PDC (positive diabetic control), EE (ethanolic extract). ^ap<0.05, ^bp<0.01, ^cp<0.001

Table 2: Effect of graded doses of ethanolic extract of *M. oleifera* on body weight

Groups (n = 6)	Dose orally/kg bw	Body weight (g)				Reduction (%)
		Day 0	Day 7	Day 14		
NDC	10 ml	172.5±8.59	139.5±8.01	113.0±8.33	34.49	
PDC	500 mg	181.17±4.76	155.0±4.60	144.33±4.81 ^b	20.33	
EE	1000 mg	200.83±6.12	169.17±5.54 ^b	149.17±5.31 ^b	25.72	
	500 mg	184.67±4.35	155.5±3.86	146.0±3.42 ^b	20.94	
	250 mg	163.67±4.93	142.5±3.74	135.83±3.54	17	
	125 mg	197.33±8.08	180.5±9.45	175.17±9.22	11.23	

Expressed as Mean±SEM, ^bp<0.01, bw: body weight.

Percentage reduction in body weight = body weight (day-0)-body weight (day-14)/body weight (day-0) x 100, n = 6 (no. of rats/group)

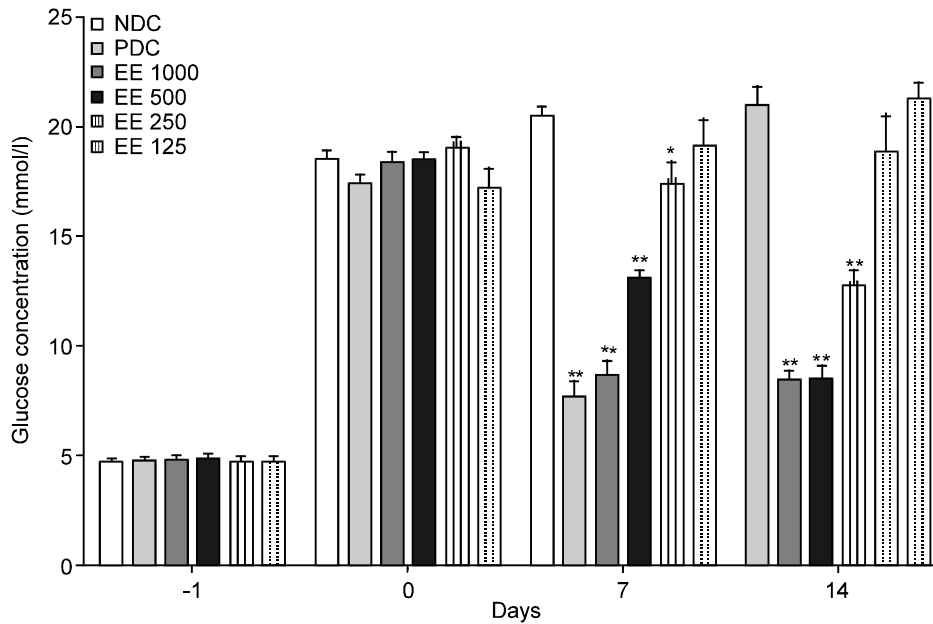


Fig. 1: Showing glucose concentration at day 0, 7 and 14 as administration of ethanolic extract of *Moringa oleifera*. In the figure (-1day) represent FBG concentration in normal rats, (day 0) diabetic rat groups showing FBG before treatment and day 7 and day 14 after treatment, the treated groups showing significant difference with NDC (*p<0.05, **p<0.001). Number of rats per group 6

Therefore, the 95% ethanolic extract with variable doses was evaluated on hyperglycemia, body weight and plasma lipids in acute and sub-chronic studies. The results of the study clearly describes that after single administration of ethanolic extract of *M. oleifera*, there was encouraging fall in blood glucose in diabetic rats with 500 and 1000 mg/kg dose. The most favorable

results were obtained with 1000 mg/kg bw dose of *M. oleifera* at 5 and 7 h. In contrast, decline was not observed at both 250 and 125 mg/kg dose. The multiple dose (14-day) treatment indicated that 95% ethanolic extract of *M. oleifera* proved to be successful up to 250 mg/kg dose. But the reduction was consistent only at 500 and 1000 mg/kg dose of extract even at the

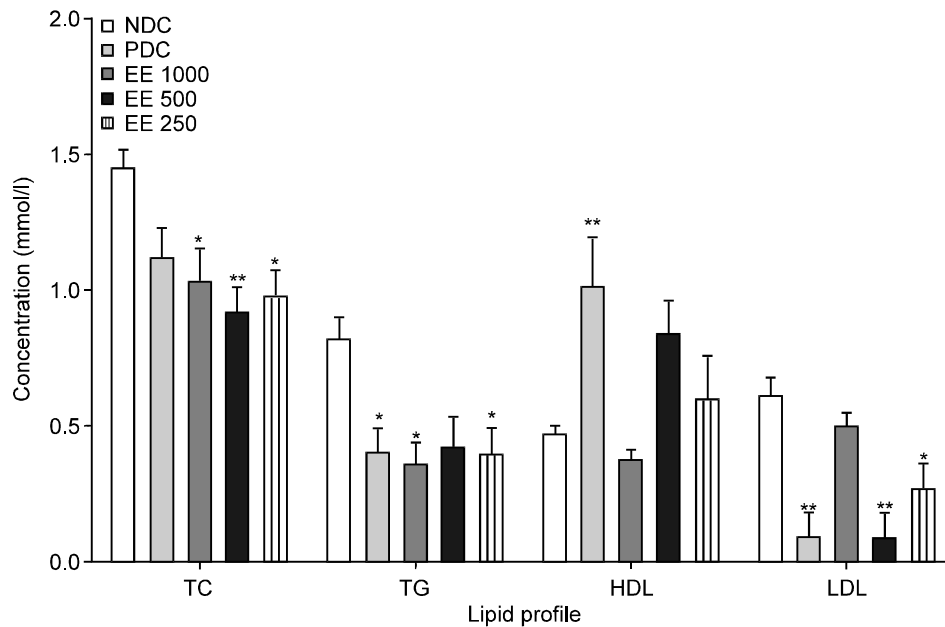


Fig. 2: Comparison of treated groups with diabetic control on lipids. Value presented as Mean±S.E.M, (1) TC: total cholesterol, (2) TG: triglycerides, (3) HDL: high density lipoprotein, (4) LDL: low density lipoprotein, *p<0.05, **p<0.01

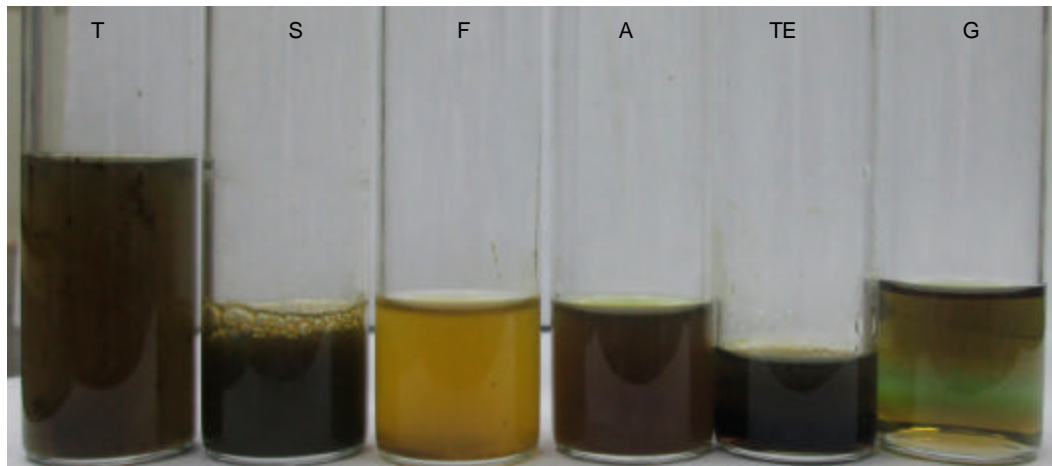


Fig. 3: Phytochemical screening of 95% ethanolic extract. T (Tannins) and phenols = brownish green coloration (+iv), S (Saponins) = Foaming (+iv), F (Flavonoids) = Yellow color (+iv), A (Alkaloids) = Precipitation (-iv), TE (Terpenoids) = Grayish color (-iv), G (Glycosides) = Blue to green coloration (+iv)

last day of experiment. Furthermore, the group receiving the dose 250 mg/kg produced significant reduction only at day-7 while nothing observed in terms of fall in diabetic rats acquiring 125 mg/kg dose of ethanolic extract of *M. oleifera*. In glycolysis, glucose-6-phosphatase is the key enzyme to release glucose in the blood (Berg *et al.*, 2001). The antidiabetic mechanism might be due to inhibition of hepatic gluconeogenesis. As the decreased activity of glucose-6-phosphatase enzyme after 14-day administration of

aqueous extract inhibit hepatic glucose synthesis in the liver (Maiti *et al.*, 2004). The extract also indicated the presence of phenols, tannins, flavonoids, saponins and glycosides. These phyto-compounds combat the symptoms of diabetes mellitus and further damage to β -cells of pancreas due to their antioxidant effects (National Nutrition Council, 1999; Murthy *et al.*, 1992) and moreover inhibit glucose absorption by inhibiting sodium glucose co-transporter-1 (S-GLUT-1) in the intestine (Hakkim *et al.*, 2007).

Additionally, the standard drug (metformine) also expressed highly significant reduction in blood glucose but did not lower glucose than normal glucose likewise ethanolic extract of *M. oleifera*. In alloxan induced diabetic rats, the extract did not bring glucose values lower than normal fasting glucose (NFG), exhibiting antihyperglycemic action (Pari and Umamaheswari, 2000). Metformine works by various mechanisms like by (i) enhancing insulin sensitivity, (ii) inducing peripheral uptake of glucose by tissues and (iii) inhibition of hepatic gluconeogenesis (Cusi and Consoli, 1996) and lower hyperglycemia without increase in body weight (Bailey, 1992).

Commonly, the metformine, exenatide and alpha-glucosidase inhibitors promote weight loss (Mavian *et al.*, 2010). The results of this study showed that by increasing the dose of 95% ethanolic extract, more reduction in body weight was observed. As the extract caused the suppression of daily food intake and increased level of cholecystokinin that is responsible in reduction of body weight (Wenya *et al.*, 2012). Furthermore, weight loss has been proved to lower the risk of coronary heart disease (Hermansen and Mortensen, 2007).

Lipids have strong connection with diabetes mellitus and increased the risk of coronary heart diseases (Tan *et al.*, 2005). These are potent inhibitors of insulin signaling and result in an acquired insulin resistance. The reduction in fatty acids promotes insulin signaling pathway and reduce insulin resistance (Shulman, 2000) might be due to β -sitosterol as reported in *Moringa oleifera* (Ghasi *et al.*, 2000). The 95% ethanolic extract as well lowered total cholesterol, triglycerides and low density lipoprotein, can involve in glycemic control. The literature claimed that flavonoids lower the levels of lipids by decreasing HMG-CoA reductase enzyme activity (Jung *et al.*, 2006).

Conclusion: The current research study concerning measurement of blood glucose describes that antidiabetic study with 95% ethanolic extract of *Moringa oleifera* leaves is the first study that reported highly significant acute antihyperglycemic effect. The study also discloses that ethanolic extract ($p < 0.05$) reduced body weight, cholesterol and triglycerides, thus reduces the risk of coronary heart diseases.

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Conflict of interest: The authors have no conflict of interest.

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