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Physalis minima Linn Methanolic Extract Reduces Blood Glucose Level without Compromising Sperm Quality in Normoglycaemic Mice

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ABSTRACT

Effects of *Physalis minima* Linn (PML) methanolic extract on blood glucose level and sperm quality of normoglycaemic mice have been investigated. Twenty four ICR male mice were randomly assigned to four groups and fed with maintenance diets (commercial rat chow, 5 g/head/day and water *ad-libitum*). Group A (n=6) served as a control and received additional 2 ml/kg bodyweight (bwt) of distilled water. Group B, C and D were supplemented with 50, 100 and 200 mg/kg bwt of PML, respectively. Bodyweight and blood glucose level were monitored on a weekly basis. After four weeks of treatment, all animals were sacrificed by cervical dislocation and their epididymis was collected and subjected to sperm analysis. Bodyweight was increased ($p<0.05$) over time but no differences ($p>0.05$) were observed between treatments. Blood glucose was decreased significantly ($p<0.05$) in PML treated groups (dose dependent manner) compared to control. Sperm quality was not affected with PML supplementation. In conclusion, PML methanolic extract exhibit hypoglycaemic effect without affecting sperm quality in male mice.

INTRODUCTION

Physalis minima Linn (PML) belongs to the family Solanaceae and commonly known as "Leletup" in Malaysia. This herb is found throughout India, Afghanistan, Africa, Indonesia, Malaysia and Australia (Patel *et al*., 2011). The flowers are hermaphrodite (have both male and female organs) and pollinated by insects. The fruit is edible, yellowish and encapsulated in papery cover. The infusion of PML is said to relieve pain, lower fever, relieve indigestion, relieve cough with phlegm, be diuretic and relieve oral thrush (Nathiya and Dorcus, 2012). Herbal medicine practitioners in Malaysia have used the plant in combination with other local plants to treat hypertension, diabetes and also as an appetizer. Although the beneficial effects of Malaysian PML have been heavily exploited, little scientific research has been conducted on its hypoglycaemic and reproductive toxicity activities. Diabetes mellitus (DM) is characterised by chronic hyperglycaemia caused by defects in insulin secretion, insulin action, or both resulting in impaired function in carbohydrate, lipid and protein metabolism. World Health Organization (WHO) estimates that currently more than 180 million people worldwide have diabetes and it is likely to double by 2030 (Bisla *et al*., 2014). The complications associated with DM are severe. The illness is one of the main causes of blindness, kidney disease, atherosclerosis, liver disease and a variety of debilitating neuropathies that diminish the quality of life and life expectancy of the patients (Ghate *et al*., 2014). In the past decade, rigorous research had been conducted to find out the best way to treat DM and to overcome the implications as observed in DM, either by modern or alternative medicine (Sani *et al*., 2014). Male reproductive toxicology has recently become a rapidly extending area of research and testing (Ajayi and Jegede, 2013).

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Since herbal medicines have recently become a widespread form of therapy, male reproductive toxicity studies should be considered as part of the safety evaluation process of medicinal plants (Kimmel *et al*., 1995). The male reproductive system is very sensitive to the action of harmful factors, and the exposure to the certain agent can interfere with sexual maturation, the production and transportation of sperm, the spermatogenic cycle, the sexual behaviour and male fertility (Goncalves *et al*., 2014). As far as our literature survey could ascertain, no attempts have been made to investigate the effect of Malaysian PML on the male reproductive system.

The purposes of the present study were (i) to ascertain the scientific basis for the use of this plant in the management of blood glucose/diabetic (ii) to elucidate whether PML methanolic extract implies reproductive hazards to male reproductive system.

MATERIALS AND METHODS

Plant materials and methanolic extract preparation

Fresh wild plants of *Physalis minima* Linn (PML) were collected from Gomali Estate, Segamat, Johor. The collected plant was authenticated by taxonomist of Herbarium, Universiti Kebangsaan Malaysia and voucher sample number PML-01-MNJ was deposited in our research laboratory. The whole plants (leaves, stems, roots and fruits) were washed, cut into pieces and shade dried at room temperature. The dried plants were subjected to size reduction to a coarse powder using a mechanical grinder (Steel Thomas Wiley Mini Mill 3383L40, USA) and passed through a 150 µm metal sieve using Vibrator Sieve Shaker (Retsch, Germany). This powder (10 g) was packed into soxhlet apparatus (Advantec, Japan) and extracted successively with 250 ml methanol at 63 ℃. The extracts were evaporated to dryness under reduced pressure untila semisolid mass was obtained and were stored in airtight containers (Norizzah *et al*., 2012). The suspensions of the extract were prepared by using physiological saline as a solvent for the experiment.

Animals and experimental design

Twenty four healthy adult ICR strain male mice, 10 weeks old and weighing about 25-30 g were used for the study. Housed in polypropylene cages and maintained under standard conditions (12 h lights and 12 h dark cycle). All animals were fed with commercial rat chow (5 g/head/day) and water *ad libitum*. The animals were randomly divided into four groups of six animals in each group, and received the following treatment daily for four weeks by oral gavage; Group A was administered with 2 ml/kg bwt of distilled water and served as a control, Group B was supplemented with 50 mg/kg bwt of PML, Group C was supplemented with 100 mg/kg bwt of PML and Group D was supplemented with 200 mg/kg bwt of PML. The use of animals in this study was monitored by the Research Ethics Committee of the Faculty of Applied Sciences, Universiti Teknologi MARA and approved by the UiTM Research Committee on the Ethical Use of Animals (UiTM Care No. 111/2015). At the end of the experimental period, all mice were sacrificed by cervical dislocation and the carcasses were collected by a commercial company appointed by the Faculty of Applied Sciences, Universiti Teknologi MARA for disposal.

Bodyweight measurement and estimation of blood glucose

Changes in bodyweight of all mice were recorded on a weekly basis using a precision scale (Kern & Sohn, Germany) to the nearest gram. Meanwhile, blood sample was collected from overnight fasting mouse by tail vein puncture method. Blood glucose level was estimated using an Accu-Check Advantage II Electronic Glucometer (Roche Diagnostics, Germany) and was made weekly throughout the period of study.

Sperm quality analysis

At the end of the experimental duration, all mice were sacrificed by cervical dislocation. Cauda epididymis was separated and minced using a scissors in a petri dish containing 1 ml phosphate buffer saline (Abu *et al*., 2013). The debris was removed and the sperm suspension was incubated in a CO_2 incubator (Memmert, Germany) for 15 minutes at 37^0C and 5% $CO_2$to allow the motile sperm to swim up (Liu *et al*., 2012). The sperm suspension was subjected to the determination of sperm count, sperm motility and sperm morphology according to WHO criteria (NAFA, 2002). Sperm count determination was carried out using the Makler Counting Chamber according to manufacturer's instructions (Sefi Medical Instruments, USA). Meanwhile, sperm motility evaluation was assessed by visual estimation under the light microscope (Olympus CX21, Japan). For the purpose of this study, at least 100 sperm were observed and classified as either motile or non-motile (Daud *et al*., 2015). Sperm morphology determination was carried out by means of the giemsa staining method (Casarett, 1953). At least 100 sperm were classified as either normal or abnormal morphology (head, midpiece and tail anomalies). Head anomalies: detached, tapered, microcephalous, macrocephalous, multiple or absent acrosome. Midpiece anomalies: cytoplasmic droplet, thin or bent. Tail anomalies: absent, coiled or multiple.

Statistical analysis

Results are presented as means ± SEM (n=6). Data being collected at several time intervals were analysed by repeated measures of ANOVA. Probability of $p<0.05$ was considered to be significant.

RESULTS AND DISCUSSION

Bodyweight

Bodyweight of mice in all groups significantly ($p<0.05$) increased over time. However, the bodyweight was not significantly affected ($p>0.05$) by entire dosages of *Physalis minima* Linn (PML) methanolic extract (Figure 1).Bodyweight of normoglycaemic mice supplemented with 50, 100 and 200 mg/kg

bwt of PML methanolic extract increased by24.49, 18.16 and 16.64%, respectively, following four weeks of experimental period. All groups increased their bodyweight during the experimental period indicating that PML supplementation allowed the growth of the animals. In the current study, we do not measure the feed intake but these results convinced us to speculate that feed intake was not affected by PML consumption. Perk *et al*., (2013) reported that the percentage of bodyweight changes did not differ significantly in between control rats and rats supplemented with 1000 mg/kg of *Physalis peruviana*. In addition to, Nabila (2012) documented that rats fed diet contains Physalis extract showed a significant increase in bodyweight gain and food intake.

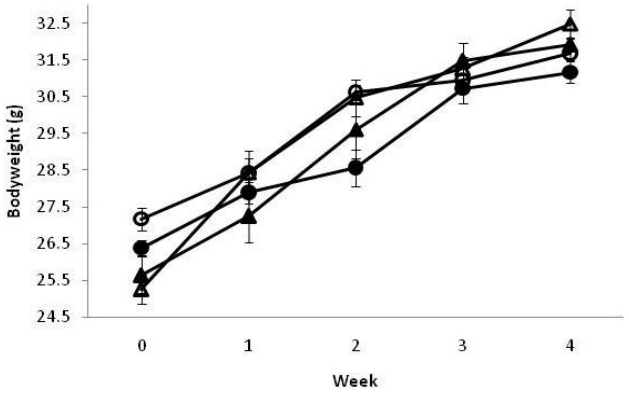

Fig. 1: Bodyweight of mice fed with maintenance diets and supplemented orally with2 ml/kg bwt of distilled water (Group A, Δ), 50 mg/kgbwt of PML (Group B, ▲), 100 mg/kg bwt of PML (Group C, ○) and 200 mg/kg bwt of PML (Group D, ●). Values are presented as mean ± standard error of mean (n=6).

Blood glucose level

Blood glucose level significantly ($p<0.05$) decreased in normoglycaemic mice supplemented with PML methanolic extract in dose dependent manner compared to control (Figure 2). Normoglycaemic mice supplemented with 50, 100 and 200 mg/kg PML methanolic extracts showed 21.54, 32.81 and 33.87% of reduction in blood glucose level, respectively. Previous authors reported that, PML aqueous extract significantly decreased the blood glucose level inalloxan-induced diabetic rats (Sucharita and Estari, 2013). The obtained results also were agreed with Estakhr and Javdan (2011) who recorded that Physalis sp.ethanolic extract normalized blood glucose level in alloxan-induced diabetic rats. Chothani and Vaghasiya (2012) documented that, the PML ethanolic extract possess *in-vitro* inhibitory activity on intestinal alpha glucosidase maltase. Nathiya and Dorcus (2012) demonstrated that saponins was present in PML methanolic extract. It was studied that saponins can exhibit anti-hyperglycaemic properties through modulation of calcium channel and can stimulate insulin secretion by the pancreas (Koneri *et al*., 2014). Another mechanism of saponins as an anti-hyperglycaemic agent is through stimulation of 5-adenosine monophosphate activated protein kinase signals to stimulate glucose uptake in skeletal muscles, reduce hepatic glucose production and reduce fatty acid oxidation in adipose tissues (Coughlan *et al*., 2014). In the current study, there is no attempt was made to ascertain the mechanism of the observed hypoglycaemic activity. The possible mechanisms of PML action may be by promoting the insulin release from the pancreas β-cells (Zhang *et al*., 2012), action by insulin like molecules (Sucharita and Estari, 2013) or enhancing hepatic glycogen formation (Yin *et al*., 2002). Further studies are recommended to find out the possible PML mechanism of action.

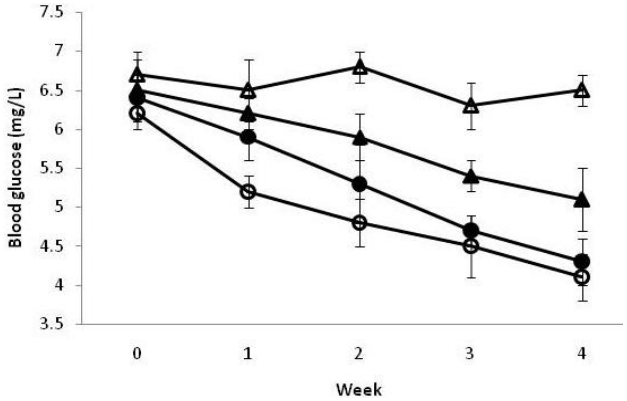

Fig. 2: Blood glucose level in mice fed with maintenance diets and supplemented orally with 2 ml/kg bwt of distilled water (Group A, Δ), 50 mg/kg bwt of PML(Group B, ▲), 100 mg/kg bwt of PML (Group C, ○) and 200 mg/kg bwt of PML (Group D, ●). Values are presented as mean ± standard error of mean (n=6).

Sperm quality

Sperm quality (sperm count, sperm mortality and sperm morphology) was not affected by PML methanolic extract (Table 1).

Table 1: Sperm quality of mice fed with maintenance diets and supplemented orally with 2 ml/kg bwt of distilled water (Group A), supplemented with 50 mg/kg bwt of PML (Group B), supplemented with 100 mg/kg bwt of PML (Group C) and supplemented with 200 mg/kg bwt of PML (Group D). Values are presented as mean ± standard error of mean (n=6).

AB	CD
Sperm count (10^6/ml)	34.3 ± 1.3^a 34.7 ± 2.4^a 33.5 ± 1.5^a 35.2 ± 3.1^a
Non-motile sperm (%)	28.6 ± 2.6^a 31.4 ± 2.3^a 30.3 ± 3.1^a 30.9 ± 1.9^a
Abnormal sperm (%)	35.7 ± 1.5^a 34.6 ± 1.7^a 37.3 ± 2.3^a 36.9 ± 0.9^a

[a]Superscript in the same row shows no significant difference ($p>0.05$)

Taken together, these results showed that the oral treatment with different dosages of PML methanolic extract (50, 100 and 200 mg/kg) for four weeks induces no toxic effects in the male reproductive system. It is well established that motile sperm in sufficient concentration and free from abnormalities are highly correlated with fertility because sluggishly motile or immotile sperm are not likely to penetrate the cervical mucus and fertilize the ova (Abu *et al*., 2013). In contrast, Mohana and Purushotaman (1981) demonstrated that Physaline-B, an active compound isolated from PML, shows anti-fertility activity. Perhaps, this can be explained by the different types of extract used in the experiments. The current study used methanolic crude extract meanwhile Mohana and Purushotaman (1981) utilised pure active compound. Interestingly, Chothani and Vaghasiya (2012) reported that quercetin that have positive effects on sperm was present in PML. The effects of quercetin on sperm quality depend on the

dose and duration of treatment. According to Taepongsorat *et al*., (2008), positive effects of quercetin on sperm quality were found only at a dose of 270 mg/kg of bodyweight and for the duration of at least 14 days. The current study suggest that the amount of quercetin and Physaline-B in PML methanolic extract (50, 100 and 200 mg/kg of crude extract) insufficient to alter sperm quality of normoglycaemic mice.

CONCLUSIONS

In conclusion, the present study provided scientific validation of the folklore use of the *Physalis minima* Linn (PML) and suggested that this plant has promising therapeutic activity for the management of blood glucose. Current data also suggested that, administration of PML does not have an evident effect on sperm quality of normoglycaemic mice.

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CONFLICT OF INTEREST AND AUTHOR'S CONTRIBUTION

The authors declare that there is no conflict of interests regarding the publication of this paper. All authors were involved in the writing, revision and final approval of the paper.

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