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Phytochemical Analysis of *Phaleria macrocarpa* Leaves Methanol Extraction and Its Medicinal Effects on Diabetic Rats.

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ABSTRACT

The methanol extraction of *Phaleria macrocarpa* leaves has studied for its antidiabetogenic effects on diabetic rats. STZ-diabetic rats were orally force-fed daily with (500 mg/kg bw) of the extract for 14 days. Blood glucose levels have determined by evaluating levels of RBG, FBG, and IPGTT within the period of the treatment. Reductions in blood glucose measurements indicated significantly in the diabetic treated rats (9.83±4.98 mmol/L) compared to diabetic non-treated (21.05±1.78 mmol/L). The extract did not cause any acute toxicity or allergic reactions, as been observed in non-diabetic treated group compared to non-diabetic control. Qualitative phytochemical analysis of leaves extract showed presence of alkaloid, saponin, flavonoid, tannins, reducing sugar, terpenoids, cardiac glycosides, and phenolic, which can be accounted for the observed antihyperglycemic activity. Results indicated that this extract can be used with safety to improve glucose tolerance in glucose-impaired cases, and suggest that this extract can serve as a candidate for developing safe, complaisance, and promising nutraceutical product for the management of diabetes mellitus.

Keywords: *Phaleria macrocarpa*, Leaves Extract, Diabetes Mellitus, antihyperglycemic

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INTRODUCTION

Diabetes mellitus is a complex heterogeneous metabolic disorder affecting nearly 4% of the population worldwide. Its prevalence is expected to increase by 5.4% in 2025 [1], and up to 7.7% (439 million adults) by 2030 [2]. Antidiabetic medicinal plants are in general known to exert their rational means for the treatment of diabetes. Though, the action modes shall depended on the phytochemicals components endowed in the plant [3]. Additionally, medicinal plants have the advantage over synthetic drugs due to the presence of antioxidant compounds which are important to modulate the level of oxidative stress [4]. Free radical scavenging molecules such as terpenoids, tannins, lignins, flavonoids, alkaloids, phenols, and other metabolites are rich in antioxidant activity [5]. *Phaleria macrocarpa,* commonly known as "God's Crown" or Mahkota Dewa, is indigenous to Indonesia and Malaysia. Traditionally, it contributes to human healthcare system, whereas, its extracts reported for numbers of valuable medicinal properties, such as anti-cancer, anti-diabetic, anti-inflammatory, anti-fungal, anti-oxidant, anti-bacterial, and vasorelaxant activities [6]. Recent studies on animal models have confirmed the antidiabetic efficacy of the fruits and some other parts of this plant [6-10]. Therefore, this study has designed to investigate the protective effects of *P. macrocarpa* leaves methanol extract on STZ-diabetic rats.

MATERIALS AND METHODS

Plant Extract

Fresh leaves of *P. macrocarpa* were collected from the northwestern part of Malaysia (Kedah), and were taxonomically identified. The selected plant parts dried, crushed in an electric grinder, and pulverized into a coarse powder form, out of this powder, 100 g were weighed. The methanolic extraction was prepared by soaking 100 g of the coarse powder in a conical flask with mixture solvent, consisting of 240 ml distilled water and 320 ml absolute methanol. The mixture kept in an incubator at 37ºC for 36hr and stirred intermittently at 4hr intervals. It was then filtered, filtrated and dried under low pressure and low temperature rotary evaporator fitted with vacuum pump. A final of 23.75 g of the powder was collected at the end of the process. The samples were dissolved in normal saline at fixed dose for the treatment [11].

Qualitative Phytochemical Analysis

The qualitative phytochemical screening of the extract, to reveal the presence of alkaloids, saponins, flavonoids, tannins, reducing sugar, terpenoids, steroids, cardiac glycosides, and phenolic, were conducted as described before [9,10].

Selection of Animal and Animal Care

Male, matured, normoglycemic, Sprague Dawley rats, weighed 250-300 g, were used in present study. The animals were acclimated for a period of 7 days prior to actual experiments, under laboratory standard conditions of 12:12 hour light/dark cycle and fed on standard laboratory pellets and water *ad libitum*. The principles of Laboratory Animal throughout the study were supervised by the internal Animal Ethical Committee.

Induction of Diabetes

Animal were induced diabetes by combination of Streptozotocin (STZ) (65 mg/kg bw) and Nicotinamide acid (NA) (230 mg/kg, bw). NA injected in overnight fasted rats 15 minutes prior to STZ administration, which dissolved in normal saline and administered by a single intravenous (iv) injection in order to develop moderate and stable non-fasting hyperglycemia [12-14]. Induced diabetes validated by elevated glucose levels in plasma, determined for 14 days after STZ administration. The rats with uniform Diabetes (FBG>170 mg/dl) for at least a week were forwarded for the proposed study.

Acute Oral Toxicity Study

Acute toxicity test was conducted as previously describe [15]. The rats (n=28) were divided into two main groups, diabetic and non-diabetic. Each of these groups further divided into treated and non-treated

groups of 7 animals per group. Plant extract has prepared at a dose of 500 mg/kg and feed orally to the respective groups. Animals then observed at time intervals of 4, 6, 24, and 48 hours after administration of the extract. Furthermore, the LD_{50} was calculated to be greater than 2000 mg/kg. Therefore, the dose of 500 mg/kg of the extract was subjected to be used in this experimental study.

Draize Skin Irritation Test

Acute skin irritation is evaluated *in vivo* on experimental animals. Plant extract was applied onto a shaved area on rat's back to access the appearance, oedema or erythema, which evaluated at 1, 24, 48 and 72 hours after applied. There were no irritation recorded, and thus, the extract was considered as safe to be used.

Blood Glucose Measurement

Glucose measurements have done using an electronic glucometer (AccuCheck Advantage Blood Glucometer-US, Roche Diagnostics). Blood samples obtained via tail snip. Fasting blood glucose (FBG) measured after one week of STZ induction to confirm the diabetic-like state in animals. A constant reading of 15mmol/L has considered as diabetic. The diabetic rats were monitored, for the following two weeks constantly, by random blood glucose (RBG) and fasting blood glucose (FBG) measurements to ensure their chronic stable hyperglycemia.

Body Weight Measurement

Total body weight measurements (using laboratory electrical balance) were determined daily, right from the day after inducing diabetes and subsequently for the next 14 days.

Statistical Analysis

Rresults expressed as mean±SEM, the significance of differences of P value were confirmed by one-way ANOVA and multiple Dunnett t-tests, where $P<0.05$ considered statistically significant, and $P<0.001$ highly significant differences in all cases.

RESULTS

Toxicity evaluation of the extract did not show any toxicity in rats, even at the highest dose tested. There were no changes in behavioral pattern, and mortality was not observed.

The phytochemical analysis of *P. macrocarpa* leaves methanolic extraction indicated the secondary metabolites of the extract. Results confirmed the presence of alkaloid, saponin, flavonoid, tannin, reducing sugar, terpenoids, cardiac glycosides and phenolic compounds. The main constituent that present may contribute to the expose the bioactivity of this extract toward maintaining blood glucose levels in diabetic rats (Table 1).

Table 1: The phytochemical constitutes of *P. macrocarpa* leaves methanolic extraction

Phytochemical Assay	Aqueous-Methanol Extract
Alkaloid (Mayer's test)	+
Alkaloid (Wagner's test)	+
Saponin	+
Flavanoid	+
Tannin	+
Reducing sugar	+
Terpenoids (Salkwoski test)	+
Steroids (Lieberman- burchard test)	-
Cardiac glycosides	+
Phenolic	+

(+) Present (–) Absent

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The antidiabetic properties of the extract have investigated by 24 hours of glucose measurement (Figure 1). The results indicated highly significant reductions (P<0.001) in blood glucose level of treated diabetic rats, throughout the 14 days of treatment, compared to diabetic controls that showed a constant increase of their blood glucose levels throughout the same duration. Interestingly, the 24 hours blood glucose levels of non–diabetic rats did not show any signs of hypoglycaemia with the same oral dose used, compared to non-diabetic controls (Table 2).

Table 2: 24 hour glucose measurement of four groups studied for 14 days (Mean±SD)

Groups	24hr Glucose (mmol/L) for 14 days
Diabetic treated with *P. macrocarpa*	9.83±4.98
Diabetic control treated with normal saline	21.05±1.78 *
Non-Diabetic treated with *P. macrocarpa*	4.56±0.22
Non-Diabetic control treated normal saline	4.65±0.24

** highly significant (P<0.001)*

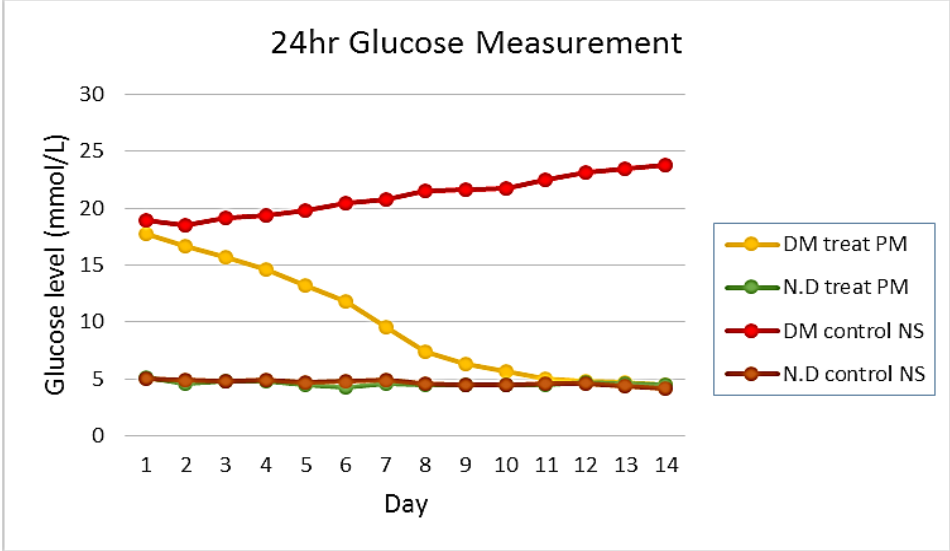

Figure 1: Mesrearment of 24hr blood glucose levels in the four groups studies; (DM: Diabetes mellitus, N.D: non-diabetic)

Intraperitoneal glucose tolerance test (IPGTT) used to measure the effect of insulin production on glucose tolerance (Figure 2). Diabetic group treated with the extract showed rapidly decrease of glucose levels (P<0.05) after 60 minutes (19.90±1.36 mmol/L), and after 120 minutes (10.90±1.52 mmol/L), as compared to control diabetic group (non-treated) which indicated an increased hyperglycemia after 60 and 120 minutes, (25.20±1.89 mmol/L) and (26.75±1.89 mmol/L) respectively. These IPGTT results, whereby glucose reduction has recorded in treated diabetic rats, highlights the sugar lowering ability of the extract at statistically significant levels (Table 3).

Table 3: IPGTT of diabetic and non-diabetic groups (Mean±SD)

Groups	Minutes (Min)				
	0	30	60	90	120
Diabetic treated with *P. macrocarpa*	18.20±1.50	22.60±1.95	19.90±1.36	15.75±1.71	10.90±1.52*
Diabetic Control with Normal saline	18.50±2.15	23.60±2.22	25.20±1.89	25.70±1.89	26.75±1.89*
Non-Diabetic Control with Normal saline	4.74±0.36	7.74±0.34	6.96±0.59	6.18±0.50	5.20±0.45

** highly significant (P<0.05)*

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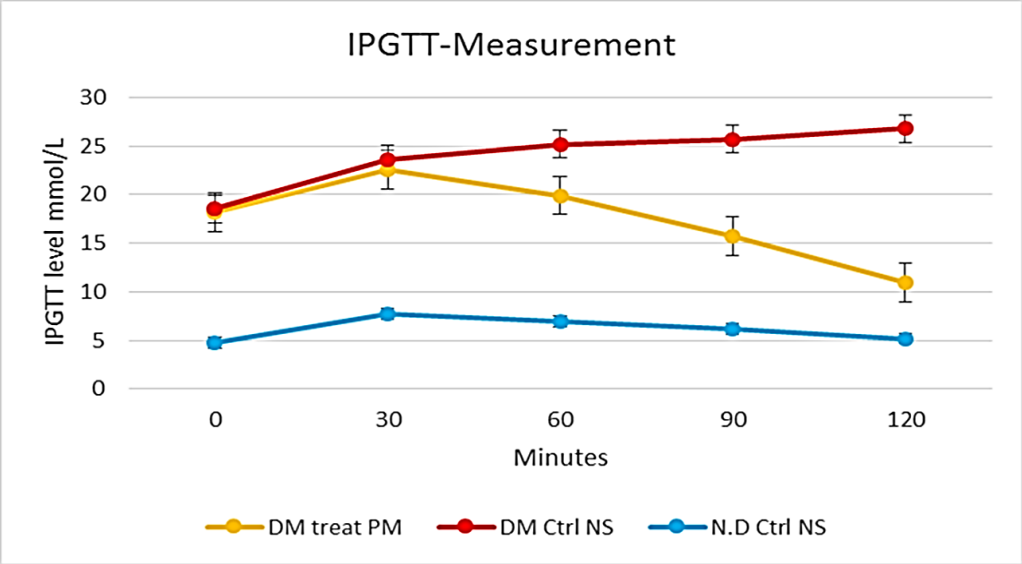

Figure 2: Measurements of IPGTT of the diabetic and non-diabetic rats; (DM: Diabetes mellitus, N.D: non-diabetic)

DISCUSSION

There are more than 80% of the world population still depends on herbal and traditional medications for their ailments, especially in diabetes mellitus. A comparative model of screening experiments was applied in this study to evaluate the antidiabetic properties of *P. macrocarpa* leaves extract towards induced diabetes in animals. *P. macrocarpa* is a native efficacious as traditional medication in Indonesia and Malysia, its fruits empirically been used to treat various types of diseases, such as cancer, liver disorders, heart disease, diabetes, arthritis, kidney disorders, stroke, and high blood pressure [13]. *P. macrocarpa* leaves extract has successfully carried out with methanol, at a ratio 1:3, which found to be a good solvent for the separation of the active constituents of the leaves. The phytochemical analysis showed variety of secondary metabolites, which are synthesized as secondary metabolites as the plant grows, including alkaloid, saponin, flavonoid, tannins, reducing sugar, terpenoids, cardiac glycosides and phenolic compound. The presence of alkaloids is interesting, hence significant quantities were reported to be used as anti-malarials, analgesics and stimulants [12]. The flavonoids are known to inhibit tumor growth, and also serve to protect against gastrointestinal infections [7]. Some of these bioactive compounds serve to protect the plant against microbial attacks and predation by animals, and thus, the plant has been traced to the extraction and development of several drugs and chemotherapeutic agents for dug industry, as well as, for traditional use of herbal remedies [14]. The presence of tannins, flavonoids and glycosides in the methanol extract may be responsible for the antidiabetogenic activities of the leaves extraction. The Antidiabetic effects of *P. macrocarpa*, has managed to lower blood glucose levels in diabetic animals, and at the same time, it does not cause hypoglycemia in non-diabetic treated group. On the other hand, to confirm the antidiabetic properties of the extract, IPGTT has obtained. It indicates that after 120 minutes of glucose administration, glucose levels dropped near to normal compared to diabetic control, which increased in 120 minutes. The administration of the extract to diabetic rats showed significant increases in the total body weight of animals. In contrast, control diabetic rats (receiving oral treatment of saline) showed rapid weight loss and decreased in muscle mass, as usually seen in human uncontrolled diabetic mellitus. Interestingly, leaves extract of *P. macrocarpa* has shown, otherwise, that diabetic rats have increased their body mass after seven days of treatment, and continued increasing the fourteen days period. Presumably, these findings suggest the extract's ability in sugar correction may contribute to the effects which hypothetically suggest that the extract may possess insulin secretary or insulin activity in sugar correction, leads to prevention of weight loss.

CONCLUSION

The experimental results suggest that the methanolic extract of *P. macrocarpa* leaves has an ability to possess significant antidiabetic properties toward high blood sugar corrections, in addition, comparable improvement for the physical appearance and body mass, as seen on experimental animals. Thus, these

findings temptingly suggest *P. macrocarpa* leaves extract can possess 24 hours blood glucose lowering effects for diabetics, in addition, euglycemic maintaining ability for diabetics and non-diabetics, without altering glucose levels toward hypoglycemia. These potent medical benefits may authenticate its used in treatment of ailments and the possible potential for the development of promising nutraceutical product for treatment of diabetes. However, the mechanisms of action of this extract still warrant further investigations.

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