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Effect of ethanolic extract of *Pericampylus glaucus* (Lam) Merr on biochemical parameters in Sprague-Dawley rats with high fats diets

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ABSTRACT

Objective: To evaluate the effect of ethanolic extract of *Pericampylus glaucus* (Lam) Merr (*P. glaucus*) on biochemical parameters in high fats diets treated rats.
Methods: The effect of *P. glaucus* extract on biochemical parameters was studied in male Sprague Dawley rats. The high-fat diets along with standard normal laboratory rat's diets were given for a period of 28 days along with ethanolic extract of *P. glaucus* and standard drug. The standard group was treated with simvastatin at dose of 80 mg/kg and the tested groups were treated with ethanol extract of *P. glaucus* at 400, 600 and 800 mg/kg daily for 28 days of the experiment. The weight of animals in each group was measured weekly and the effect of extract and standard on serum lipids profile was evaluated at the end of the study.
Results: The results showed that the ethanolic extract of *P. glaucus* at different doses of 400, 600 and 800 mg/kg and standard drug induced a significant reduction in the body weight ($P < 0.001$), total cholesterol, triglycerides (TG), low density lipoproteins (LDL) and uric acid levels and increase ($P < 0.001$) in high density lipoproteins (HDL) as compared to untreated hyperlipidemic group.
Conclusions: The present work indicates that the ethanolic extract significantly suppressed the biochemical parameters in high fats diet treated animals, suggesting the antihyperlipidemic potential of *P. glaucus*.

1. Introduction

Accumulation of fats in body results in high risk for various chronic diseases such as hyperlipidemia, diabetes mellitus, arthritis, arteriosclerosis and certain types of cancer[1].

Hyperlipidemia is connected with type II diabetes mellitus which is also known as non-insulin dependent diabetes mellitus (NIDDM). In addition to their association with diabetes, high serum levels of cholesterol, triglycerides (TG), and low density lipoprotein (LDL) are the key factors responsible for the development of coronary heart disease, cardiovascular disease and high blood pressure[2]. The primary type of hyperlipidemia is treated with synthetic anti-hyperlipidemic drugs, but for the secondary type that results from diabetes mellitus, treatments needed are to cure original disease rather than hyperlipidemia. A person is said to be hyperlipidemic as his/her total cholesterol level becomes greater than or equal to 240 mg/dL or 6.21 mmol/L[3]. A large number of synthetic drugs are available for treating hyperlipidemia; however, their usage are limited because of their harmful side effects like gastric irritation, diarrhea, myositis, hyperuricemia, flushing and abnormal liver function[4]. Worldwide 250 000 to 500 000 species of the nature are existing and only 1% of the natural products are studied scientifically[5]. The use of natural remedies for the treatment of hyperlipidemia and other related disorders has long history, and plays a vital role in treatment of various types of chronic diseases[6]. The high cost, low availability and undesirable side effects of synthetic drugs have been some of the factors leading to a preference of plant origin drugs, which are believed to be more suitable for treatments of chronic diseases such as hyperlipidemia[6-8].

The species *Pericampylus glaucus* (Lam) Merr (*P. glaucus*) belongs to the family of Menispermaceae and is widely distributed

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throughout Thailand, India, China, Indonesia, Myanmar, Taiwan, Philippine and Vietnam[9]. In traditional system of medicine various parts of the plant are claimed to be used as a therapeutic and pharmacological agent. The roots are effectively used for lowering high lipid levels and blood glucose level traditionally in Malaysia[10]. In Taiwan, the plant is used to stop bleeding, abdominal pain, productive cough, and treat inflammation, arthritis, sore throat, colds, headache and abdominal pain[11]. The leaves and stem of the plant are used traditionally by Vietnamese against the snake bite[12]. In China the plant is used for the treatment of laryngitis, cough and various fractures[13]. Despite of their traditional uses, some of their *in vitro* and *in vivo* activities against hepatitis B, hepatitis C, and HIV virus and cancer have been published[13,14]. The plant was reported to have significant hypoglycemic effect in STZ induced diabetic rats at low dose[15]. The researcher scientifically approved the positive effect of compounds from the active fraction of *P. glaucus* on high blood glucose and lipid profiles in STZ induced diabetic rats[16]. The free radical scavenging activity was also reported for the plant *P. glaucus*[17]. The compound isolated from the plant was reported to have significant hypoglycemic effect in STZ induced diabetic rats[18]. However, to date no studies have reported the antihyperlipidemic activity of the plant. Therefore, the present study aimed to evaluate the anti-hyperlipidemic effect of *P. glaucus* extract in high fats diets treated male Sprague-Dawley rats.

2. Materials and methods

2.1. Drugs and chemicals

Simvastatin, solvents, serum cholesterol kit, serum HDL kit, serum triglyceride kit, cholesterol kit and serum LDL kit were provided by DNA Bio Sciences SDN BHD, Malaysia. All the chemical reagents used were from Merck (Darmstadt, Germany), Astral Laboratory Chemicals R/M Chemicals, Loba Chemicals, Alpha Chemika and Sigma Aldrich Co. (UK). The biochemical parameter was determined through biochemistry analyzer (model BA- D200A) that was from China.

2.2. Test animal

Male and female Sprague-Dawley rats weighing 90–110 g were used and kept in the animal house of the Faculty of Pharmacy, Lincoln University College, Malaysia. The animals selected for the study were kept in the plastic cages (34 cm × 47 cm × 18 cm) at animal house in an air conditioned environment with five animals in each cage and maintained at room temperature of (25 ± 2) ˚C with relative humidity of 60% ± 10% under 12 h night and light cycle. The experiments using animals were approved by Animal Ethical Committees, Lincoln University College with reference number of LUC-AEC number PHARM/2013/MSM/02-August/11/ September 2014-August 2016.

2.3. Collection of plant material

The plant *P. glaucus* was collected from village "Kampong Jeram Kedah", Negri Sembilan State of Malaysia in 2014.

2.4. Identification of plant material

The plant was authenticated by Ms. Tan Ai Lee at Forest Research Institute of Malaysia (FRIM), and the voucher specimen herbarium number [FRIM/394/490/5/18(118)] was submitted to the Faculty of Pharmacy for the source of reference in future.

2.5. Preparation of plant extract

The collected *P. glaucus* was dried in shade for a period of 21 days. After shade drying the leaves were removed and ground into coarse powder through mechanical blender (Sieves No. 20). The powder of the plant material was extracted by continuous hot extraction using the Soxhlet apparatus at a temperature of 78 ˚C for 48 h using 95% absolute ethanol. The extract collected was then concentrated under reduced pressure through rotary evaporator at temperature of 40–45 ˚C and was preserved in desiccator until used for further studies.

2.6. Phytochemical screening of ethanolic extract

The presence of phytochemicals in *P. glaucus* ethanolic extract was screened via different chemical identification tests[16] indicating the presence of alkaloids, reducing sugar, terpenoids, tannins, saponin, and absence of anthraquinone and fixed oil in the extract before determining their effects on the biochemical parameters in high fat diets (HFD) treated animals.

2.7. Acute toxicity studies

The oral acute toxicity of ethanolic extract of *P. glaucus* was evaluated according to OECD guidelines 423 on female Sprague-Dawley rats weighing 90–110 g[19], where the limited test dose of 2 000 mg/kg was used. The animals were kept fasting overnight (14 h) before the experiment with only free access to water. The investigated animals were divided into 4 groups with each comprising 3 animals. The 1st group served as negative control, whereas the 2nd, 3rd, and 4th groups were considered as tested groups that received orally the ethanolic extract at a dose of 300, 1 000 and 2 000 mg/kg that was diluted in normal saline. Before extract administration, body weight of each animal was determined and the dose was calculated according to the average body weight of each animal group. The animals were observed for any toxic effect for first 4 h after the treatment period, then 3 days and finally 14 days for any toxic effect[20]. Behavioral changes and other parameters such as body weight, urination, food intake, water intake, respiration, convulsion, tremor, temperature, constipations, and changes in eye and skin colors were also under observation.

2.8. Induction of obesity and hyperlipidemia

Obesity and hyperlipidemia were induced in male Sprague-Dawley rats by giving high fats diet (HFD) that was prepared by mixing cholesterol (2%), sodium cholate (1%), yellow corn (15%), milk powder (15%), coconut oil (11%) and butter (11%), caseins (6%) and one multivitamin capsule with powdered standard animal food[21].

2.9. Dose selection

Three doses of ethanolic plant extract were selected (400, 600, and 800 mg/kg) *p.o*. The doses were selected on the basis of acute toxicity studies.

2.10. Experimental design

The effect of the ethanolic extract was determined in male Sprague Dawley rats (90–110 g) treated with normal and high-fat diets (HFD)[22]. The animals were divided into 6 groups containing 6 animals in each group. The high-fats diets were given to all groups except normal diet group along with standard simvastatin and ethanolic extract of *P. glaucus* consecutively for 28 days. Group I fed on normal diet (normal control group); Group II fed on only high-fats diet (HFD) (hyperlipidemic group); Group III was given simvastatin (80 mg/kg, *p.o.*) and high fats diet (HFD); Group IV wad given high-fat diet (HFD) and 400 mg/kg *P. glaucus* extract; Group V was given high-fat diet (HFD) and 600 mg/kg *P. glaucus* extract; Group VI was given high-fat diet (HFD) and 800 mg/kg *P. glaucus* extract.

2.11. Estimation of biochemical parameters

The tested animals were sacrificed under anesthesia (ether) on 29th day of experiment after keeping them fasting overnight (12–14 h). The blood sample from each animal group was collected separately in test tube without containing anticoagulant respectively for the analysis of the biochemical parameters. The blood was allowed to clot after centrifugation at 2500 r/min for 15 min to obtain the serum and stored at –20 ℃ until analysis[23]. Various parameters like total cholesterol (TC), high density lipoproteins (HDL), triglycerides (TG), low density lipoproteins (LDL) and very low density lipoprotein (VLDL) were determined through biochemical analyzer (model BA-D200A) using specific kits.

2.12. Statistical analysis

Results were expressed as mean ± SEM. The statistically significant differences between control and treated groups were evaluated using Two-way ANOVA. For multiple comparisons among the groups, Bonferroni test was performed. A probability level of $P < 0.05$, $P < 0.01$ and $P < 0.001$ was accepted for statistical significance.

3. Results

3.1. Acute toxicity studies

The acute toxic effect of ethanolic extract of *P. glaucus* was determined as per the OECD guidelines 423. No treatment related toxic symptoms or mortality were observed after the oral administration of the tested ethanolic plant extract (*P. glaucus*) at doses of 300, 1 000 and 2 000 mg/kg. The extract treated group, standard treated group and control group observed for 4 h, followed by 72 h and then 14 days did not display any drug related changes in behavior, breathing, skin, water intake, food intake and body temperature. Therefore, the ethanolic extract seems to be safe at dose level of 2000 mg/kg, and the lethal dose was considered to be > 2000 mg/kg. However, there were signs of drowsiness in groups treated with extract at 1000 and 2000 mg/kg compared to control group, but the animals became normal 10 h after receiving the extract. The parameters observed for acute toxicity after the administration of the test plant extract compared with normal group are presented in Table 1.

Table 1
General behavioral observations of acute toxicity study.

Observation	Control	300 mg/kg	1000 mg/kg	2000 mg/kg
Body weight	Normal	No change	No change	No change
Temperature	Normal	Normal	Normal	Normal
Food intake	NO	NO	NO	NO
Change in skin	No effect	No effect	No effect	No effect
Drowsiness	Not present	Not present	Present	Present
Sedation	No effect	No effect	Not present	Not present
Diarrhea	Not present	Not present	Not present	Not present
Coma	Not present	Not present	Not present	Not present
Death	Alive	Alive	Alive	Alive

NO: Not observed.

3.2. Effect of ethanolic extract of P. glaucus on body weight

The body weight of the high-fat diet treated animals which were given plant extract at 400, 600 and 800 mg/kg was recorded on Day 0 and then weekly for consecutively 28 days using digital weighing balance. The ethanolic plant extract showed significant reduction in body weight compared to high-fat diets group. The animals fed with high-fat diet showed significant increase in body weight compared to those fed with normal food diet and extract (*P. glaucus*). In all animal groups, reduction in body weight was started from the 2nd week of receiving the tested drugs and became significant ($P < 0.01$, $P < 0.001$) on 21st and 28th days of treatment. Treatment with ethanol extract of *P. glaucus* (400 mg/kg/day and 600 mg/kg/day) showed slight decrease in body weight and up to 14th day became significant at 400 mg/kg and 600 mg/kg as compared to hyperlipidemic group. However, the tested ethanolic plant extract at 800 mg/kg was found to significantly ($P < 0.001$) decrease body weight of animals compared to high-fats diet group (Table 2).

3.3. Effect of ethanolic extract on biochemical parameter in Sprague-Dawley rats

The present study showed the dyslipidemic changes with increased total level of cholesterols (TC), triglycerides (TG), low density lipoprotein (LDL) and a decrease in serum level of high-density lipoprotein (HDL) in hyperlipidemic group. The anti-hyperlipidemic potential of *P. glaucus* ethanolic extract was evaluated at three doses of 400, 600 and 800 mg/kg and was compared with untreated high fats diet group (hyperlipidemic group). The results indicated that oral administration of ethanolic extract of *P. glaucus* at 400 mg/kg produced a non significant decrease ($P > 0.05$) in uric acid, total cholesterol (TC), low density lipoprotein (LDL) and slight decrease ($P < 0.05$) in triglycerides (TG) level when compared to high-fat diet group (HFD). However, the ethanolic extract at 600 mg/kg exhibited a significant reduction ($P < 0.01$) in total cholesterol (TC), triglycerides (TG), low density lipoprotein (LDL) and a significant increase ($P < 0.01$) in high-density lipoprotein (HDL) level compared to untreated hyperlipidemic group. The effect of ethanolic extract at 800 mg/kg in reducing levels of total cholesterol (TC), triglycerides (TG), low density lipoprotein (LDL) was more significant ($P < 0.001$) when compared to hyperlipidemic group and other extract treated groups (400 and 600 mg/kg). Similarly, the extract induced a significant ($P < 0.001$) increase in high density lipoprotein (HDL) at 800 mg/kg as compared to

Table 2

Effect of ethanolic extract on body weight in male Sprague-Dawley rats

Treatment	0 Day	7 Days	14 Days	21 Days	28 Days
Hyperlipidemic group	100.00 ± 2.19	120.13 ± 2.27	130.29 ± 1.47	141.00 ± 1.29	154.75 ± 1.70
400 mg/kg extract	100.15 ± 3.87	112.00 ± 2.54	121.20 ± 2.78[a]	129.00 ± 1.47[b]	136.20 ± 1.70[c]
600 mg/kg extract	94.00 ± 2.54	113.70 ± 2.65	119.20 ± 2.52[b]	124.70 ± 2.00[c]	130.70 ± 1.93[c]
800 mg/kg extract	98.00 ± 2.85	111.00 ± 1.68	116.25 ± 1.03[c]	121.75 ± 0.85[c]	126.25 ± 1.03[c]
STD 80 mg/kg	93.00 ± 2.48	112.25 ± 2.32	116.25 ± 1.65	120.00 ± 1.58	124.75 ± 1.97
Normal group	96.50 ± 3.06	101.00 ± 3.16	102.50 ± 2.78	102.75 ± 1.18	107.50 ± 1.55

Data are expressed as mean ± SEM. $N = 6$ in each group. [a]: $P < 0.05$, [b]: $P < 0.01$, [c]: $P < 0.001$ as compared to hyperlipidemic control. Statistical test employed was Two-way ANOVA followed by Bonferroni test.

Table 3

Effect of ethanol extract on biochemical parameters in Sprague Dawley rats.

Treatment	Uric (mmol/L)	Total cholesterol (mmol/L)	HDL (mmol/L)	LDL (mmol/L)	Triglycerides (mmol/L)
Hyperlipidemic group	0.25 ± 0.01	6.80 ± 0.24	0.63 ± 0.09	4.86 ± 0.08	3.93 ± 0.14
400 mg/kg	0.22 ± 0.01	5.33 ± 0.18[a]	1.09 ± 0.06	3.86 ± 0.11	3.45 ± 0.06[a]
600 mg/kg	0.21± 0.03	4.47 ± 0.06[b]	2.23 ± 0.03[b]	3.25 ± 0.12[b]	3.00 ± 0.12[b]
800 mg/kg	0.20 ± 0.02	4.00 ± 0.05[c]	3.10 ± 0.05[c]	3.03 ± 0.05[c]	2.90 ± 0.03[c]
STD	0.16 ± 0.00	3.56 ± 0.03	3.30 ± 0.11	2.90 ± 0.05	2.25 ± 0.05
Normal group	0.18 ± 0.01	2.70 ± 0.11	1.43 ± 0.14	1.96 ± 0.09	1.76 ± 0.14

Values are expressed as mean ± SEM. $N = 6$. [a]: $P < 0.05$, [b]: $P < 0.01$, [c]: $P < 0.001$ as compared to hyperlipidemic control. Statistical test employed was Two-way ANOVA followed by Bonferroni test.

hyperlipidemic group (Table 3).

4. Discussion

The present research was aimed to validate the effect of *P. glaucus* against high biochemical parameters in male Sprague-Dawley rats. Hyperlipidemia is a medical abnormality that results in the accumulation of excessive fats in body and causes negative effect on health, leading to increased health problems and reduced the life expectancy[24]. Hyperlipidemia increases the risk factor for various diseases, particularly type II diabetes, heart disease, and certain types of cancer, obstructive sleep apnea and osteoarthritis[25]. The acute toxicity study of *P. glaucus* indicated that the ethanolic extract was safe and nontoxic up to dose of 2000 mg/kg (body weight). High fats diets induced obesity rats have been used as a model among researchers due to its high similarity of mimicking the usual route of obesity episodes in human and considered as consistently good tool for studying obesity as they will readily gain weight when feed high-fat diets (HFD)[26]. A decrease in body weight was observed in animals that were fed with ethanolic plant extract of *P. glaucus* for a period of 28 days when compared to hyperlipidemic rats. The reduction and loss in body weight with crude extract of *P. glaucus* might be due to the presence of flavonoid in the extract, which has been confirmed by researcher to play significant roles in inhibiting pancreatic lipase enzyme, metabolism of lipids, amylase and glycosidase, reducing the uptake of energy by pressing the appetite, increasing energy spending, inhibiting adipocyte proliferation, and differentiation[27]. There was a significant reduction in the levels of serum total cholesterol (TC), triglycerides (TG), low density lipoprotein (LDL) and increase in high density lipoprotein HDL levels in animal feed with high fats diets (HFD) with tested plant extract *P. glaucus* at a dose at dose of 400 and 800 mg/kg body weight, that might be involved in inhibiting of pancreatic cholesterol esterase enzyme, cholesterol micellization, binding of beta cells and decrease the absorption of dietary cholesterol[28,29]. Significant reduction in serum total cholesterol (TC), triglycerides (TG), low density lipoprotein (LDL) levels, and a rise in low density lipoprotein (HD) levels was found in high-fats diet (HFD) rats treated with simvastatin. The positive lowering effect of the crude extract on cholesterol might be due to the inhibition of dietary cholesterol esterification or absorption, because two enzymes such as intestinal acyl CO-A-cholesterol acyl transferase enzyme 20 and pancreatic cholesterol esterase 19 are involved in esterification or absorption of cholesterol[30], which might be suggested that the crude extract contain such constituents that are responsible for inhibiting enzymes activity. The active fraction of *P. glaucus* indicates the presence of 10 compounds through GC-MS[31] analysis that might be responsible for attenuation of lipids profiles in high fats diets Sprague-Dawley rats.

It is concluded from the present research work that *P. glaucus* plant extract produced hypolipidemic and weight loss effects in dose dependent manners. The present research work confirmed significant effect of *P. glaucus* on biochemical parameters and justified the traditional used of plant *P. glaucus* against hyperlipidemia and weight. However, further experiments are required to find out the particular component(s) present in the ethanolic extract, possible mechanism that is responsible for above activities and advantage of *P. glaucus* extracts over other drugs.

Conflict of interest statement

We declare that we have no conflict of interest.

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