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Aqueous Extracts of *Andrographis paniculata* Improve Lipid Profiles of Rats Fed with High Cholesterol Diet

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Abstract: The aim of present study was to investigate the potential roles of aqueous extracts of *A. paniculata* in lowering the plasma lipid parameter which is responsible for hyperlipidemia and its damaging consequences and also to determine the kidney and liver functions of rats. Plasma Total Cholesterol (TC), Low Density Lipoproteins (LDL) cholesterol and Triglycerides (TG) had progressively increased in cholesterol-fed rats up to 4 weeks of cholesterol-feeding. Both 100 and 200 mg kg⁻¹ concentrations of *A. paniculata* extracts had kept TC, LDL and TG values within the normal range even after 4 weeks of feeding. No significant enhancement was found in the amount of High Density Lipoproteins (HDL) cholesterol and the kidney and liver enzymes of the rats, i.e. Blood Urea Nitrogen (BUN), total creatinine and Lactate Dehydrogenase (LDH) and Aspartate Amino Transferase (AST) and Alanine Aminotransferase (ALT), respectively indicating normal kidney and liver functions. From the current study, it can be concluded that 100 and 200 mg kg⁻¹ aqueous extract of *A. paniculata* appeared to possess great potentials as anti-hyperlipidemic agent in rats.

Key words: *Andrographis paniculata*, hyperlipidemia, rats, liver, kidney functions

INTRODUCTION

Andrographis paniculata (AP), also known commonly as King of Bitters, is a member of the plant family *Acanthaceae* and has been used for centuries in Asia to treat not only one, but several types of illnesses. The herb can be located in most of the tropical Asian countries: India (and Sri Lanka), Pakistan, Malaysia and Indonesia-but is cultivated extensively in China and Thailand, the East and West Indies and Mauritius^[1]. The leaves contain the highest amount of andrographolide (2.39%), the most medicinally active phytochemical in the plant, while the seeds contain the lowest^[2]. The other medicinal chemicals are also bitter principles: Diterpenoids viz., deoxyandrographolide, -19 β -D-glucoside and neo-andrographolide, all of which have been isolated from the leaves^[3].

Hyperlipidemia is well known to play a main role in the development of atherosclerosis and is widely recognized as a major risk factor in the development of other cardiovascular diseases. Chronic elevation of blood lipid may also lead to the development of fatty liver^[4] and renal damage^[5] indicated by raised liver and kidney enzymes. *A. paniculata* has been reported to exhibit its

hypoglycemic^[6] and anti-hypertensive^[7] potentials in rats and has been used as traditional medicine as painkiller and to treat fever and ulcers^[8]. Its cardiovascular benefits include preventing repeated narrowing of vessels after coronary angioplasty^[9]. It is also an anti-clotting agent^[10] and has been reported to reduce the damage to heart muscles during myocardial infarction^[11]. When given to rabbits fed with cholesterol-rich diet, *A. paniculata* preserved their endothelial functions and acted as an antioxidant as well as maintaining the balance of nitric oxide/endothelin^[12].

It is the aim of the present study to investigate the potential roles of aqueous extracts of *A. paniculata* in lowering the plasma lipid parameters such as Total Cholesterol (TC), Low Density Lipoproteins (LDL) cholesterol and Triglycerides (TG) responsible for hyperlipidemia and its damaging consequences and also to determine the kidney and liver functions of rats during and after 4 weeks of cholesterol-feeding.

MATERIALS AND METHODS

Plant material: *Andrographis paniculata* plants were purchased from the Malaysian Agricultural Research and

Development Institute (MARDI) and cultivated until they achieved 0.5 to 1.0 m height. The leaves were then harvested and identified for their correct botanical identity at the Phytomedicinal Herbarium, Institute of Bioscience, Universiti Putra Malaysia, Selangor (Voucher No. SK965/04 *Andrographis paniculata*).

Preparation of extract: The leaves were oven-dried at 50°C for two days and ground into powder-form with a grinder. For the preparation of aqueous extracts of this plant, a total of 200 g powder of the plant was extracted using water at the ratio of 1:5. They were left in the water bath at 55°C for 3 h. The liquid obtained was then filtered and freeze-dried to calculate a final concentration of 100 and 200 mg kg⁻¹ body weight.

Experimental animals: All experiments were performed in male Sprague-Dawley (SD) rats weighing 125-185 g placed in the animal house of the Faculty of Medicine and Health Sciences, Universiti Putra Malaysia. The animals (four per group) were maintained under standard laboratory conditions (light period of 12 h/day and temperature 28±2°C) with access to food and water *ad libitum*. All rats received approximately 20 g of standard rat pellets per day, with or without cholesterol added. All experimental procedures were carried out in strict compliance with the Institutional Animal Ethics Committee regulations.

Induction of hypercholesterolemia: The effects of *A. paniculata* leaf extracts on normal and cholesterol-fed rats were studied for 27 days (4 weeks). All rats were subjected to a trial period (week 0) prior to cholesterol feeding to estimate the amount of pellet taken daily and to let them adapt to confinement in cages. They were given a basal diet during this period. They were divided into four groups with 4 rats per group; the normal control Group (N), the cholesterol-control Group I, cholesterol-fed groups treated with *A. paniculata* leaf extract at concentrations of 100 and 200 mg/kg/day orally (T₁ and T₂), respectively. Group N continued to be fed a basal diet (cholesterol-free) for the next 4 weeks whereas Group C, T₁ and T₂ were given cholesterol-enriched diet (normal pellet+3% cholesterol). The extracts of *A. paniculata* leaf were fed to Group T₁ and T₂ daily via oral gavage for 4 weeks.

Blood collection and storage: Approximately 2 mL of blood was collected from each animal at the end of week 0 (day 0) and at day 9, day 18 and day 27 for the analysis of plasma lipids, kidney and liver functions. Blood was taken via cardiac puncture using 23G needles and 3 mL syringes and collected into EDTA tubes

(Sigma Chemicals, UK). The plasma were immediately separated by centrifugation at 3000 rpm for 10 min. They were then transferred into eppendorf tubes and stored under -80°C for further analysis.

Analysis of plasma lipids, kidney and liver enzymes: The plasma lipids measured were Total Cholesterol (TC), Low Density Lipoprotein (LDL) cholesterol, High Density Lipoprotein (HDL) cholesterol and Triglycerides (TG). They were determined enzymatically using Hitachi 902 automated analyzer. Kidney and liver enzymes, namely Blood Urea Nitrogen (BUN), total creatinine and Lactate Dehydrogenase (LDH), Aspartate amino Transferase (AST) and Alanine aminotransferase (ALT) were determined in the same manner on the Hitachi 902 automated analyzer. All reagents used for the biochemical analysis were purchased from Roche Diagnostics, USA.

Statistical analysis: Data are expressed in means (±SEM) where appropriate and analyzed statistically by using two-way ANOVA and those at p<0.05 are accepted as significant. Duncan post test is employed to further evaluate the differences among the groups.

RESULTS

Plasma lipids: Plasma TC of group C had increased significantly beginning from day 9 and continued to increase another 5 fold at day 27 compared to day 0 (p<0.05) (Fig. 1). These changes were also significantly higher than the other groups at day 9, 18 and 27. The administration of 100 and 200 mg kg⁻¹ aqueous extracts of *A. paniculata* to cholesterol-fed rats had kept TC within the normal range throughout the study period. The changes in LDL level in Group C showed the same trend as TC (Fig. 2). LDL started to increase beginning at day 9 but the changes were significant only at day 18 and day 27 compared to day 0 and all the other groups (p<0.05). LDL was not raised in *A. paniculata*-treated rats at both doses even after 4 weeks of cholesterol feeding.

There was no significant changes in the level of HDL in all the animal groups throughout the study period (Figure not shown). Plasma TG increased significantly at day 9, 18 and 27 in Group C, compared to day 0 (p<0.05). The changes in TG were also significant when compared to all the other groups throughout the study (p<0.05) (Fig. 3). The level of TG remained unchanged in *A. paniculata*-treated rats (Group T₁ and T₂) throughout the 4 weeks of cholesterol feeding.

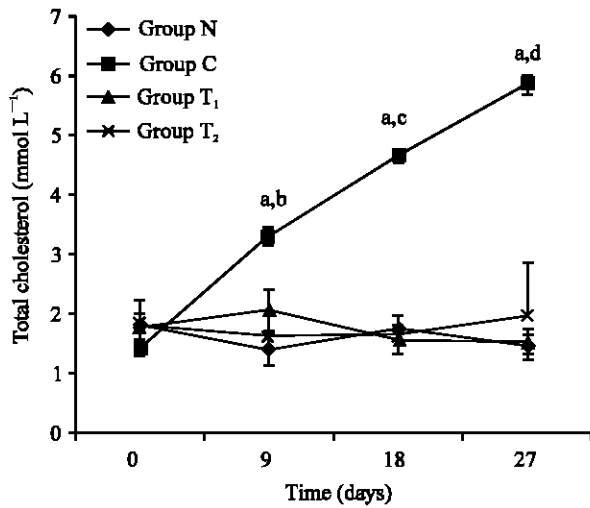


Fig. 1: Changes in plasma total cholesterol concentrations in control and *A. paniculata*-treated rats, a: significant difference vs. day 0 ($p < 0.05$), b: significant difference vs. all other groups at day 9 ($p < 0.05$), c: significant difference vs. all other groups at day 18 ($p < 0.05$), d: significant difference vs. all other groups at day 27 ($p < 0.05$)

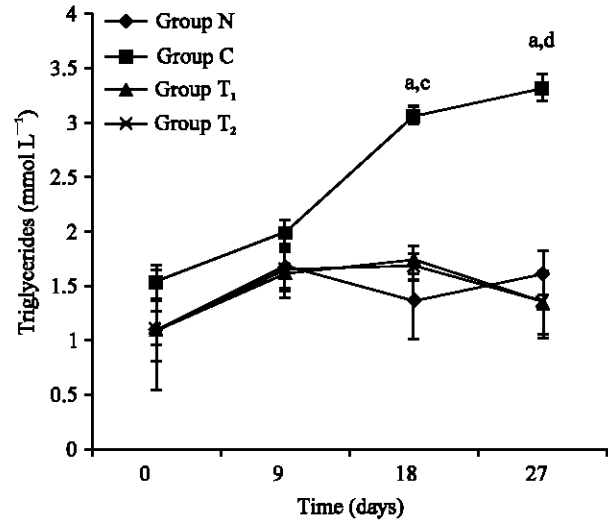


Fig. 3: Changes in plasma triglyceride (TG) concentrations in control and *A. paniculata*-treated rats, a: significant difference vs. day 0 ($p < 0.05$), b: significant difference vs. all other groups at day 9 ($p < 0.05$), c: significant difference vs. all other groups at day 18 ($p < 0.05$), d: significant difference vs. all other groups at day 27 ($p < 0.05$)

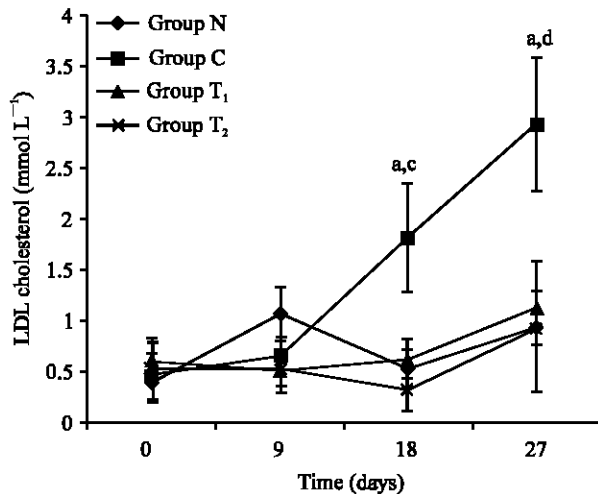


Fig. 2: Changes in plasma low density lipoprotein (LDL) cholesterol concentrations in control and *A. paniculata*-treated rats, a: significant difference vs. day 0 ($p < 0.05$), b: significant difference vs. all other groups at day 9 ($p < 0.05$), c: significant difference vs. all other groups at day 18 ($p < 0.05$), d: significant difference vs. all other groups at day 27 ($p < 0.05$)

Kidney and liver functions: The kidney and liver functions indicated by the kidney and liver enzymes,

respectively, were unaffected even for cholesterol-control group (Group C) throughout the study duration of 4 weeks. The levels of creatinine, BUN, LDH, AST and ALT had remained within the normal range in all groups (Table 1 and 2).

DISCUSSION

The ability of dietary cholesterol to induce hyperlipidemia has been regularly verified^[13], mostly by observing during their intake the rise in the levels of cholesterol or other lipids such as triglyceride in blood. In the present study, the rats fed with hypercholesterolemic diet showed a steady rise in plasma concentrations of total cholesterol as compared to rats given normal diet. A simultaneous increase was observed in another plasma cholesterol, i.e. the LDL cholesterol and also TG. These changes were time-dependent and the values obtained were the highest at the end of the study duration (day 27). When the aqueous extracts of *Andrographis paniculata*, at the concentrations of 100 and 200 mg kg⁻¹ body wt. day⁻¹ were administered to two separate groups of cholesterol-fed rats (T₁ and T₂), no such increments in the values of total cholesterol, LDL and triglyceride were seen, but no enhancement of HDL was noted either. It is most interesting to see that in the

Table 1: Mean±SEM of kidney enzymes in control and *A. paniculata*-treated rats

	Time (days)			
	0	9	18	27
Lactate dehydrogenase (IU L ⁻¹)				
Normal	418.00±58.5	483.00±91.00	434.00±60.00	1232.00±945.5
Cholesterol	447.00±3.0	509.00±59.00	408.00±21.50	1220.50±115.5
<i>A. paniculata</i> 100 mg kg ⁻¹	313.00±78.0	625.50±139.50	1063.50±8.50	1339.00±751.00
<i>A. paniculata</i> 200 mg kg ⁻¹	296.00±3.00	354.50±29.50	505.00±238.50	584.50±155.50
Blood Urea Nitrogen (mmol L ⁻¹)				
Normal	8.25±2.65	6.60±0.30	6.60±0.30	5.60±0.40
Cholesterol	7.15±0.85	5.20±1.30	5.20±1.30	6.25±0.15
<i>A. paniculata</i> 100 mg kg ⁻¹	6.00±0.11	4.60±0.20	6.15±0.35	6.60±0.15
<i>A. paniculata</i> 200 mg kg ⁻¹	6.20±0.10	4.30±0.43	4.90±0.20	6.70±0.20
Creatinine (μmol L ⁻¹)				
Normal	32.74±2.26	35.84±0.29	34.43±0.57	44.87±1.98
Cholesterol	33.02±4.24	37.25±1.13	31.04±0.56	33.02±3.11
<i>A. paniculata</i> 100 mg kg ⁻¹	31.89±1.79	38.37±1.69	35.55±2.25	44.02±14.11
<i>A. paniculata</i> 200 mg kg ⁻¹	29.35±3.95	32.17±0.56	35.84±2.54	36.96±4.79

Table 2: Mean±SEM of liver enzymes in control and *A. paniculata*-treated rats

	Time (days)			
	0	9	18	27
Aspartate aminotransferase (IU L ⁻¹)				
Normal	95.55±3.35	116.65±10.85	129.20±2.00	145.10±34.20
Cholesterol	101.65±17.35	114.85±3.15	106.05±7.35	144.05±3.55
<i>A. paniculata</i> 100 mg kg ⁻¹	89.20±8.20	100.00±1.00	121.80±6.10	118.70±26.60
<i>A. paniculata</i> 200 mg kg ⁻¹	97.45±11.15	98.80±3.50	130.30±38.10	108.25±12.55
Alanine aminotransferase (IU L ⁻¹)				
Normal	54.50±7.50	53.50±7.50	129.20±6.50	77.0±6.00
Cholesterol	50.50±8.50	59.50±0.39	106.50±7.00	78.5±5.50
<i>A. paniculata</i> 100 mg kg ⁻¹	59.50±2.59	46.50±0.50	67.50±6.50	58.50±9.50
<i>A. paniculata</i> 200 mg kg ⁻¹	64.50±8.50	51.50±9.50	59.50±11.50	70.00±13.00

cholesterol control group (Group C) the kidney and liver functions of the rats remained unaffected even after 4 weeks of cholesterol-feeding. These findings were indicated by the normal kidney and liver enzymes measured in the cholesterol-fed rats. Similar results were seen in rats treated with *A. paniculata* extracts. These results indicated that both 100 and 200 mg kg⁻¹ of *A. paniculata* extracts had excellent lipid-lowering capabilities even during a continuous and prolonged feeding of dietary cholesterol but their nephro- and hepatoprotective ability could not be determined in this study due to the failure in inducing liver and kidney injuries within the short study duration implemented.

A strong association between certain types of dyslipidemia including hypercholesterolemia, hypertriglyceridemia and combined hyperlipidemia and development of atherosclerotic lesions has been documented by a number of clinical trials, epidemiological and experimental studies^[14]. To achieve this dyslipidemic condition in animal models, it has been concluded that dietary cholesterol is needed^[15]. Characteristics of the animal model used in the present study were similar to previous studies: animals fed a hypercholesterolemic diet showed higher serum cholesterol levels. Similar rise were seen in LDL-cholesterol and triglyceride levels of rats

from the same group. Treatment with *A. paniculata* extract successfully prevented dyslipidemia from occurring in separate sets of cholesterol-fed rats. Even though this is still a preliminary study, it is among the earliest documented and reported work regarding the anti-hyperlipidemic activity of *A. paniculata*. *A. paniculata* has been used traditionally among the Asians to cure many illnesses. The result of the present study is to add to its many other beneficial effects.

Hypercholesterolemia if not treated may lead to atherosclerosis, a chronic, multifactorial, inflammatory disease characterized by the focal accumulation of inflammatory cells. In fact, local inflammatory processes are present from the early stage and responsible for the formation of plaque. It is possible that in addition to *A. paniculata*'s anti-hyperlipidemic activity that consequently reverse all the effects of hyperlipidemia including inflammation and atherogenesis, its anti-inflammatory property could also play a role in suppressing this deleterious inflammatory process. It has been reported by Madav *et al.*^[16] that androglapholide, one of the active compounds of this plant, possesses an anti-inflammatory activity.

Analysis of plasma for kidney and liver enzymes revealed, first, all parameters were within the normal range.

These suggest that *A. paniculata* did not affect the normal function of liver and kidneys in the cholesterol-fed rats. Even though previous studies had shown that hypercholesterolemia itself might induce liver or kidney damage^[4,5], this could not be seen in this study may be due to its short duration.

This study showed that 4 weeks of feeding 3% cholesterol to rats had successfully raised plasma total cholesterol, low-density lipoproteins and triglycerides. Administrations of aqueous extracts of *Andrographis paniculata* at both 100 and 200 mg kg⁻¹ in cholesterol-fed rats managed to prevent these three parameters from rising during the study duration. The normal values of creatinine, BUN, LDH, AST and ALT in cholesterol-fed rats indicated that the kidney and liver functions of the rats had remained unaffected within the four weeks of feeding high cholesterol diets or with the addition of the *A. paniculata* extract itself.

Collectively, results from this present study suggest that the aqueous extract of *A. paniculata* has an anti-hyperlipidemic activity. Future study using the active phytochemical constituent andrographolide, must be performed to elucidate the mechanism of antihyperlipidemic activity of *A. paniculata*.

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