A PRELIMINARY STUDY ON THE EFFECT OF METHANOLIC EXTRACTS OF BARKS OF *Phyllanthus reticulatus* AND *Bridelia retusa* ON SERUM LIPID PROFILES IN HYPERCHOLESTEROLEMIC RATS (*Mus norvegicus albinus*)

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INTRODUCTION

Hypercholesterolemia is one of the serious disorders affecting a large population of the world today. There is substantial evidence documenting the relationship between coronary heart disease and atherosclerosis with increased serum cholesterol levels. A relatively constant level of cholesterol in blood is maintained primarily by controlling the level of *de novo* cholesterol synthesis. It is regulated in part by the dietary intake of cholesterol. Medications currently used to reduce body cholesterol levels are associated with unwanted side effects. Therefore, there is a growing interest in search of hypocholesterolemic metabolites from herbal medicines (Maruthappan and Sakthi, 2010; Yadav *et al*, 2014). Biological investigations of family Euphorbiaceae revealed that many members of this family consist of various medicinal properties such as hepatoprotective activity (Asha *et al*., 2006), lipid lowering activity (Maruthappan and Sakthi, 2010), anti-inflammatory activity (Rao *et al*, 2005), antidiabetic activity (Chauhan *et al*., 2010) and other activities (Mwine and Damme, 2011). *Bridelia retusa* and *Phyllanthus reticulatus* are such medicinally important two plant species available in Sri Lanka (Lalith *et al*, 2003). Very few studies have been conducted so far to investigate the effect of methanolic bark extracts of these two plant species on lipid lowering activity. The objective of the present study was to screen the methanolic crude extracts of *P. reticulatus* and *B. retusa* in a rat model for hypocholesterolemic activity.

METHODOLOGY

Plant material

Fresh barks of *P. reticulatus* and *B. retusa* were collected from Dambulla and Polonnaruwa districts respectively. Plant specimens were identified by comparing herbarium specimens deposited in Botanical Garden, Peradeniya, Sri Lanka and referring related literature. The bark samples were cut into pieces and subjected into shade drying. Dried pieces were powdered mechanically and stored in air- tight containers at room temperature for future use.

Preparation of bark extracts

A quantity of the ground samples (approximately 50 g) was weighed and subjected to soxhlet extraction with 400 ml methanol at 75°C for 6-8 hr. Extracts were slowly evaporated to dryness at 40°C using a rotary evaporator. The yield of *P. reticulatus* and *B. retusa* were 16.06% and 31.99% respectively. The extracts were stored between 2-8°C for further studies.

Experimental animals and cholesterol induction

Twelve male Wistar albino rats (*Mus norvegicus albinus*) weighing 180-200 g purchased from Medical Research Institute, Borella, Sri Lanka were used. They were kept under standardized animal house conditions (Photoperiod: approximately 12 h natural light per day, temperature: 28-30°C, humidity: 55%-60%) with water and standard diet *ad libitum.*

The experimental animals were acclimatized for 7 days before the commencement of the study and labeled appropriately. Cholesterol was induced in rats using a mixture of cheese (50 g), butter (50 g) and cow ghee (20 ml). The equal amount of this mixture (~2 ml) was given to all the rats expect the control group (Group 1) for three weeks to induce hypercholesterolemia and then the first 7 days treatment period one time per day orally.

Experimental design

In this preliminary study, twelve rats were divided into four groups of three rats each and were given the following treatments.

Group 01- Control group Group 02- Hypercholesterolemic control group

Group 03- Crude extract of *P. reticulatus* given at 2000 mg/kg body weight

Group 04- Crude extract of *B. retusa* given at 2000 mg/kg body weight

Treatment period for all these groups were 7 days and the extract was given orally once a day.

Measurements of body weight and biochemical parameters

At the beginning and on the 7^{th} day at the end of treatments, and 14^{th} and 28^{th} days the animals were deprived of food overnight and anesthetized using diethyl ether to collect bloodsamples from the tail vein. Samples were centrifuged at 2000 rpm for 30 minutes to obtain serum. Total cholesterol (TC), LDL Cholesterol (LDL-C), Triglyceride (TG), HDL Cholesterol (HDL-C) and Glucose were measured using standard kits.

Statistical analysis

Summary statistics were expressed as mean and standard deviation. The significance of difference between the hypercholessterolemic control and treated groups were determined using one-way analysis of variance (ANOVA) using SPSS Ver. 20. The acceptable level of significance was $p < 0.05$.

RESULTS AND DISCUSSION

Feeding rats with a high fat diet significantly elevated serum total cholesterol levels ($p<0.05$) in comparison with normal rats received standard diet during the period of three weeks. These results indicate that the hypercholesterolemic diets increased cholesterol levels in rats successfully (Table 1).

Table 1.Cholesterol levels of normal and induced rats with the percentage of increment. Mean follows the standard deviation within parenthesis.

Group	**No. of rats**	**Initial cholesterol level** (mg/dl)	**Induced cholesterol level** (mg/dl)	**Percentage of cholesterolincrease** (%)
01	3	73.73 (6.12)	80.56 (1.87)	9.2
02	3	73.22 (19.54)	128.83 (6.49)	75.94

Table 2. Lipid profile of the rats at the 7^{th}, $14^{th}$and 28^{th}day of the experiment. Mean follows the standard deviation within parenthesis. * indicated that the difference is statistically significant at $p < 0.05$).

Treatment	**Day**	**01(Normal diet control)**	**02(Hypercholester-olemic control)**	**03 (*P. reticulatus* treated)**	**04 (*B. retusa* treated)**
Total cholesterol (mg/dl (SD)	**07**	82.76 (4.35)	129.37 (4.26)	99.15 (1.20)*	96.83 (3.55)*
	14	81.76 (1.41)	129.18 (1.80)	90.61 (0.94)*	92.80 (3.47)*
	28	81.76 (1.41)	131.58 (1.75)	91.65 (3.11)*	88.89 (0.44)*
Triglyceride (mg/dl (SD)	**07**	53.06 (5.65)	103.24 (10.03)	82.06 (0.38)*	88.92 (1.53)*
	14	52.82 (3.55)	105.88 (4.12)	70.38 (0.24)*	80.69 (0.41)*
	28	52.96(2.91)	107.09 (5.38)	68.95 (0.24)*	79.36 (0.83)*
LDL-C (mg/dl (SD)	**07**	40.64 (3.11)	116.22 (10.41)	100.91 (0.50)*	102.36 (7.36)*
	14	40.28 (0.80)	115.59 (10.39)	100.78 0.76)	104.47 (10.40)
	28	40.49 (0.31)	116.77 (10.55)	98.15 (0.84)	102.36 (11.15)
HDL-C (mg/dl (SD)	**07**	51.37 (4.07)	78.47 (2.46)	93.60 (2.91)*	92.39 (1.39)*
	14	53.11 (3.22)	77.44 (2.43)	86.99 (3 .93)*	83.93 (2.93)*
	28	53.36 (2.04)	74.38 (2.32)	83.70 (2.17)*	81.93 (3.66)*

Results given in Table 2 revealed that administration of *P. reticulatus* (Group 03) and *B. retusa* (Group 04) significantly decreased the total cholesterol and triglyceride levels and significantly increased HDL-Cholesterol levels compared to hypercholesterolemic control group (Group 02). The increase of LDL-Cholesterol levels was significant only on the 7th day of the experiment. Blood parameters measured on the 14th day and 28th day of the experiment (without treatment of crude extracts) revealed that there was a continuous decrease of total cholesterol, LDL-Cholesterol, triglyceride and increase of HDL-Cholesterol levels when compared with the parameters of 7th day of the experiment.

According to literature (Tatiya *et al*., 2011), *B. retusa* bark extract acts as hypoglycemic agent in rat models. Similarly our results of fasting blood glucose level (Table 3) showed that there was a decrease in blood glucose level of rats supplemented with the crude extract of *B. retusa* (Group 04) when compared with the initial blood glucose level of rats, but it was not statistically significant ($p > 0.05$). The crude methanolic extracts of the two plants showed no harmful elevation or reduction of glucose levels in blood levels of Wistar albino rats when considering the normal blood glucose level of them (50-135 mg/dl, Braslasu, 2007).

Table 3. Fating blood glucose levels of rats

Group	Glucose (mg/dl (SD))	
	0th day	7th day
01	79.04 (16.27)	72.91 (2.92)
02	79.99 (36.18)	88.29 (27.32)
03	89.52 (22.89)	97.99 (16.07)
04	82.85 (19.81)	71.27 (10.88)

The atherogenic index (TC/HDL-C ratio) is used to predict the risk of coronary heart disease and as a marker of small dense LDL-Cholesterol (Maruthappan and Sakthi, 2010). These results indicated that the reduction of atherogenic index of treated groups when comparing the cholesterol induced group showing the beneficial effect of extracts in cardiovascular disease (Table 4).

Table 4. Atherogenic index in all groups

Group	Atherogenic index
01	1.611
02	1.648
03	1.059
04	1.048

Previous studies reported that the ability of some phytochemicals such as flavonoids to lower high cholesterol in hyperlipidemic rat models (Kurowska *et al*., 2000). Therefore, high amount of saponins, phytosterols, tannins and flavonoids present in the metahnolic extracts of *P. reticulatus* and *B. retusa* may be responsible for this hypolipidemic effect in rats (Sarin *et a*l., 2014).

Reduction of the induced cholesterol level by pretreatment of crude methanolic extracts of *P. reticulatus* and *B. retusa* could be due to interfering with metabolism or biosynthesis of lipids in the body. However, additional studies are being done to investigate the exact phytochemicals of crude extractsof the plants responsible for lowering cholesterol in the lipid profile and the mechanism of action.

CONCLUSIONS

The results of this preliminary study show a potentially beneficial effect of crude methanolic extracts of barks of *B. retusa* and *P. reticulatus* on hypercholesterolemic conditions in Wistar rats due to the reduction of total cholesterol, LDL cholesterol and triglyceride levels and increase in HDL cholesterol levels in rats. However, dose dependent and time dependent studies should be carried out to confirm these results.

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