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Der Pharmacia Lettre, 2018, 10 [12]: 1-8
[http://scholarsresearchlibrary.com/archive.html]

ISSN 0975-5071
USA CODEN: DPLEB4

Effects of *Luffa acutangula L.* (Cucurbitaceae) Fruit Extract on the Blood Lipid Profile of Albino Rats

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ABSTRACT

Controlling hyperlipidemia is one of the fundamental preventions of cardiovascular diseases. In this study, the lipid lowering activity of ethanolic extract of Luffa acutangula with treatment concentrations of 50%, 75%, and 100% were tested in hypercholesterolemic-induced albino rats. Six albino rats in triplicates were acclimatized for a week and were given buttered pellets as their high-fat diet. The test organisms were divided into three groups and after a four-week treatment, the weights were recorded, collection and analysis of blood for the lipid profile – triglycerides, high-density lipoprotein (HDL), low-density lipoprotein (LDL) and total cholesterol (TC) took place. At the end of the study, the extract was proven to be ineffective for weight loss but has lowered the lipid profile. In respect to the baseline values, the highest reduction of 4.9 mg/dl, 16.92 mg/dl, and 21.79 mg/dl for HDL, LDL and TC, respectively, were observed. Statistically, results showed that there is no significant

difference among the three concentrations. The study reveals that the fruit extract does not have a lowering effect on the body weight but possesses a lipid lowering activity due to the presence of flavonoids and saponins.

Keywords: Hyperlipidemia, High-density lipoprotein, Low-density lipoprotein, Total cholesterol, Flavonoids, Saponins.

INTRODUCTION

Hypertension and diseases of the heart are among the ten leading causes of illnesses each year [1]. In the Philippines, the Department of Health said that cardiovascular diseases (CVD) are among the top killers in the Philippines, causing more than half of all deaths annually [1]. Hypercholesterolemia, which is characterized by increase of low-density lipoprotein cholesterol (LDL) and triglycerides (TG) serum, are one of the main determinants of a known CVD called atherosclerosis [2]. Furthermore, various clinical studies have shown strong correlations between elevated circulating TG, total cholesterol (TC) and reduced high-density lipoprotein (HDL) levels as major predictors of obesity, diabetes, and hyperlipidemia [3]. Many medications have been used to manage CVD over the years, but prolonged used of these medications may lead to various side effects such as liver and muscle toxicity, rhabdomyolysis, myopathy, and acute renal failure that may interrupt treatment. On the other hand, plants have been proven to be very useful to man as a source of medicine for the past decades [3]. As estimated by the World Health Organization, 80% of the global populations in developing countries depend on traditional medicines mainly of plant origin. Therefore, according to Lee et al. [3] researches nowadays on natural products with nutraceutical effects received considerable interest in several chronic diseases such as hypertension, hypercholesterolemia, and cancer. Recent studies by Sutrisna et al. [4] and Ezechukwu et al. [5] revealed that *Tamarindus india* L. (tamarind) and *Solanum macrocarpum* (African eggplant) contain compounds, that possess bioactivities that may help in reducing total cholesterol and triglycerides, namely flavonoids and saponins [4]. In addition, *Opuntia ficus–indica* (Barbary fig) which is rich in phenols, an inhibitor pancreatic lipase enzyme, was also proven to lower TC levels in the blood which may be critical therapeutic target to control hyperlipidemia and obesity [6]. Hence, there is a need of further studies on natural remedies to provide a more attainable treatment for the marginalized community. *Luffa acutangula* L. or commonly known as patola is one of the plants widely cultivated in the Philippines and according to Pimple et al. [7], it is proven to be effective in treatment of hyperglycemic, gastric complications, and respiratory disorders. As mentioned by Ananthalakshmi et al. [8]. It was revealed to have secondary metabolites which include the flavonoids and saponins but since there are limited studies on this area, its anti-hypercholesterolemic potential is yet to be discovered. Thus, this experimental study aimed to determine if the different concentrations of *L. acutangula* fruit extract had significant lowering effects on the weight and levels of TG, HDL, LDL, and TC on the blood of the test organisms.

RESEARCH METHODOLOGY

Sample collection and preparation

Five kilograms of fresh and matured patola fruits were purchased from a public market and were washed with tap water to eliminate unwanted dirt. The peel and seeds were retained and was cut into smaller pieces. Samples were then soaked in 90% ethanol for 48 hours at a room temperature, and then extracted and filtered. The filtrate was subjected to rotary evaporation at 45°C for purification. The 100% concentration of the fruit extract was collected after rotary evaporation and from this other concentrations, i.e., 50% and 75% were obtained through adding the required amount of distilled water [9].

Collection and acclimatization of Albino rats

Eighteen male albino rats weighing from 250 g -300 g were acquired from the Animal Breeding of the University of the Philippines in Diliman, Quezon City. The rats had no history of drug consumption. The test organisms were housed separately in a metal cage with the dimension of 30 cm × 28 cm, under hygienic conditions and were acclimatized for one week with normal feeds and ad libitum water [5].

Preparation and administration of high fat diet

After one week of acclimatization, the albino rats were fed with buttered pellets that served as the high fat diet (HFD). The HFD were prepared by mixing 10% high fat butter with commercial pellet feeds. The buttered pellets were fed, 10 g/300 g body weight, along with ad libitum water to the test organisms for two weeks. After which, the albino rats were divided randomly into three groups [2].

Blood extractions before and after induction of hyper-lipidemic condition

Blood samples were extracted from each of the test organisms before and after the induction of hyperlipidemic condition. The blood was extracted from the lateral tail of the albino rat using a sterilized syringe under the supervision of a licensed veterinary medicine doctor. The blood lipid profile (TG, HDL, LDL, and TC) was analysed at Sto. Domingo Diagnostic and Medical Center [10].

Administration of treatment

After the hyper-lipidemic condition was induced, different concentrations of 50% 75% and 100% of patola fruit extract were administered orally through gavage method for four weeks. Each test organism was given with 500 mg/kg body weight of patola fruit extract with the desired concentration for each treatment based on a volume of 100 ml [6,9].

Data gathering and statistical analysis

After four weeks of treatments with the patola extract, final blood extraction was performed for the analysis of final TG, HDL, LDL, and TC. The extracted blood samples of the test organisms were stored in a thermal bag with ice and were brought at Sto. Domingo Diagnostic and Medical Center for analysis of TG, TC, HDL, and LDL.

The results that were gathered prior and after the treatment were compared statistically. Paired t-test was used to compare between the blood lipid profile before and after induction of hyper-lipidemic condition and before and after treatments. On the other hand, one-way analysis of variance (ANOVA) was used to compare the significant difference between the blood lipid profiles of different treatment groups. All comparisons were done at 0.05 significant levels.

RESULTS AND DISCUSSION

Effects of various concentrations of patola fruit extracts on the weight and blood lipid profile of albino rats

After four weeks of treatment, results obtained show that the patola fruit extracts (T1 - 50%, T2 - 75% and T3 - 100%) had no significant difference on the weight of the albino rats in reference to the increased weight after high fat diet (Table 1).

Table 1: Comparison of weights of albino rats from different treatment groups.

Treatments	Average weights (g)			
	Baseline	**After high fat diet**	**After treatment**	**Difference (after treatment – after high fat diet)**
T1 (50%)	249.17^{A}	271.67^{B}	283.67^{B}	12.00^{X}
T2 (75%)	261.50^{A}	291.00^{B}	286.00^{B}	-5.00^{X}
T3 (100%)	243.67^{A}	267.67^{B}	267.00^{AB}	-0.67^{X}
Note: Letters ABC indicate significant difference (SD) between baseline, after high fat diet (AHFD) and after treatment for each group. Letter X indicates the SD between the difference of after treatment and AHFD. Different letters indicate SD at 0:05 level.				

The data indicates that the fruit extract was not able to induce lowering effects on the body weight of the test organisms. In fact, after inducing the extracts for 4 weeks those that were induced with the 50% treatment concentration had an average increase in weight from 271.67 g to 283.67 g or about 4.42% increase in reference to the average weight of the group after high fat diet. For the blood lipid profile, the following were observed, the triglyceride (TG) showed significant difference in the given treatments while in high density lipoprotein (HDL), low density lipoprotein (LDL), and total cholesterol (TC) the values showed only a significant difference on treatment 3 (Table 2).

Table 2: Comparison of the blood lipid profile of the albino rats from different treatment groups.

Treatments	**Triglycerides (mg/dl)**			
	Baseline	**After high fat diet**	**After treatment**	**Difference (after treatment – after high fat diet)**
T1 (50%)	49.71^{A}	124.34^{B}	45.73^{A}	-78.62^{X}
T2 (75%)	37.17^{A}	123.17^{B}	31.42^{A}	-91.75^{X}
T3 (100%)	46.17^{A}	167.41^{B}	37.47^{A}	-129.95^{X}
Treatments	**High Density Lipoprotein (mg/dl)**			
	Baseline	**After high fat diet**	**After treatment**	**Difference (after treatment – after high fat diet)**
T1 (50%)	13.20^{A}	30.90^{B}	8.91^{C}	-21.99^{X}
T2 (75%)	10.77^{A}	27.89^{B}	9.04^{C}	-18.85^{X}
T3 (100%)	12.69^{A}	47.12^{B}	9.75^{A}	-37.37^{X}
Treatments	**Low Density Lipoprotein (mg/dl)**			
	Baseline	**After high fat diet**	**After treatment**	**Difference (after treatment – after high fat diet)**
T1 (50%)	42.37^{A}	93.98^{B}	25.45^{C}	-68.53^{X}
T2 (75%)	34.68^{A}	84.63^{B}	29.10^{C}	-55.53^{X}
T3 (100%)	40.58^{A}	90.96^{B}	30.64^{A}	-60.32^{X}
Treatments	Total Cholesterol (mg/dl)			
	Baseline	**After high fat diet**	**After treatment**	**Difference (after treatment – after high fat diet)**
T1 (50%)	65.32^{A}	157.57^{B}	43.53^{C}	-114.04^{X}
T2 (75%)	52.82^{A}	137.69^{B}	44.49^{C}	-93.20^{X}
T3 (100%)	62.56^{A}	155.39^{B}	47.82^{A}	-107.57^{X}

Note: Letters ABC indicate significant difference (SD) between baseline, after high fat diet (AHFD) and after treatment for each group. Letter X indicates the SD between the difference of after treatment and AHFD. Different letters indicate SD at 0:05 level.

The parameters of blood lipid profile shown on Table 2 indicates that the extract with concentrations of 50%, 75%, and 100% were able to decrease the levels of TG, HDL, LDL, and TC. In addition, the results after treatment are observed to be significantly lower than the values of the baseline but statistically only treatment 1 and 2 induced a significant difference on the blood lipid profile except for the TG that shows no significant difference in all concentrations. However, in comparison to the values on the baseline, the HDL, LDL, and TC levels of the groups treated with 50% concentration were observed to have the highest reduction of 4.9 mg/dl, 16.92 mg/dl, and 21.79 mg/dl respectively. This suggests that the extract is effective on lowering hyperlipidemia which coincides with the previous studies stating that elevated TG, TC, LDL and reduced HDL promotes hyperlipidemia [2,3].

Furthermore, effectivity of its lipid – lowering activity can be attributed to the presence of bioactive constituents in the extract which have been reported to possess such activity [5]. As mentioned by Ananthalakshmi et al. [8], patola contains the secondary metabolites which are flavonoids, saponins, terpenoids and tannins. These metabolites were proven to be effective in lowering the TG and TC on the previous study of Sutrisna et al. [4], about the effect of tamarind fruit extract on rats induced with hyperlipidemia. In addition, studies of Jia et al. [11] and Leontowicz et al. [12] proved that flavonoids positively affect the plasma lipid profile.

Significant difference between the weight and blood lipid profiles (TG, HDL, LDL, TC) of albino rats treated with various concentrations of patola fruit extracts.

The difference of the weights and blood lipid profile that were compared using one-way analysis of variant (ANOVA) shows that the treatment was able to decrease the levels of TG, HDL, LDL, and TC. In addition, it was observed on the results that the highest mean difference between after treatment and after high fat diet on the TG and HDL were induced by 100% concentration giving them the values of 129.95 mg/dl and 37.37 mg/dl respectively. On the other hand, LDL and TC had the highest mean difference of 68.53 mg/dl and 114.04 mg/dl after being treated with the concentration of 50%. However, in spite the following observation, it was found statistically that the three treatment groups have no significant difference with each other, hence making the groups to have an equal effect on the blood lipid profile of the albino rats.

Similar results were reported by Ezechukwu et al. [5], wherein the extracts they used are observed to be not dose-dependent. It was concluded in their study that the lipid lowering effect activity of *Solanum macrocarpum* is due to the flavonoids and saponins it possesses that are also present in *Luffa acutangula* as mentioned above. However, this is contradicted by Lee et al. [3], wherein the study of *Camellia japonica* fruit extract showed that the higher concentrations of 40% and 80% were able to

lower the body weight of the test organisms and the latter which is the highest concentration was able to increase the levels of HDL and lower the TC and TG levels [13].

CONCLUSION

Based on the results that were obtained, the study therefore concludes that patola fruit extract induced in concentrations of 50%, 75% and 100% have no lowering effect on the body weight of the albino rats. On the other hand, comparing the three treatment groups in terms of the blood lipid profiles, results indicate that the extract with concentrations of 50%, 75%, and 100% were able to significantly decrease the levels of TG, HDL, LDL, and TC. The lowering effect of the fruit extract can be attributed to the presence of bioactive constituents in the extract which have been reported to possess such activity. In addition, there is no significant difference between the treatment groups in terms of their lowering effects in the levels of the blood lipid profile; hence it is more appropriate to use the one with the lowest concentration to minimize any possible side effects.

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