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CHOLESTEROL LOWERING EFFECT OF *Aporosa lindleyana* IN MALE WISTAR RATS

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Abstract

***Aporosa lindleyana** is a green leafy vegetable of family Euphorbiaceae grown in tropical countries. In this study the effect of **Aporosa lindleyana** on the serum cholesterol level of hypercholesterolaemic rats and normocholesterolaemic Wistar rats fed on a cholesterol enriched diet was investigated. The results indicate that feeding of plant extract for two weeks reduces the total cholesterol in hypercholesterolaemic Wistar rats by 25.2% and normocholesterolaemic rats fed with cholesterol enriched diet by 37.7%. In addition the test group had significantly lower ($p<0.05$) total/HDL than the controls indicating that **Aporosa lindleyana** extract possesses hypochlosterolaemic activity.*

keywords: : *Aporosa lindleyana*, Total cholesterol, High Density Lipoprotein (HDL)

1 Introduction

Hypercholesterolaemia is a risk factor for coronary heart disease and atherosclerosis, which are regarded as some of the main causes of death in the modern world. If a drug is to be effective for combating hypercholesterolaemia, it should act to reduce the total serum cholesterol especially the low-density lipoprotein (LDL) cholesterol, the major cholesterol carrying fraction (70%) in human and also to increase the serum high density lipoprotein (HDL). In addition to the properties mentioned above, the drug should also not have any side effects or toxicity. The currently available hypocholesterolaemic agents lack the desired properties of an ideal drug[1] and

often result in patient non-compliance. Efforts to develop new and better hypocholesterolaemic drugs have led to the discovery of many natural and synthetic agents. Many of the Sri-Lankan plants such as *Myristica fragrans*[1], *Allium sativum*[2], *Emblica officinalis*[3], *Murraya Koonengi*, mustard seeds[4], *Croton cajuccara*[5], *Terminalia arjuna*[6] have been identified to possess, cholesterol lowering activity.
Aporosa lindleyana (known as "Kabella" in Sinhala) is used as a vegetable in the Sri-Lankan diet; that has been known to possess hypocholesterolaemic activity according to folklore[7].
To date no scientific investigations have been carried out to confirm the validity of the belief that *Aporosa lindleyana* possesses hypocholesterolaemic activity. Therefore the study on the effect of *Aporosa lindleyana* on the serum cholesterol level of Wistar rats was undertaken.

2 Materials and methods

2.1 Study design

The study was an analytical interventional study, in which the test group was administered the plant extract and the control was administered water.

2.2 Animal model

The animal model used in this study were male Wistar rats purchased from the Medical Research Institute Colombo. Rats were housed under standard conditions and water *ad libitum.*

2.3 Plant material

Aporosa lindleyana was purchased from a house in Colombo district and identified by Dr. P. Tissera of the Dept. of Botany, Faculty of Applied Sciences, University of Sri Jayewardenepura, Sri Lanka.

2.4 Preparation of plant material and dose

The edible portion of the tender leaves was chopped and immersed in boiling water ($12.5g$ plant material in 25 ml water) for 3 min and finely blended using a motor and a pestle for 5 minutes. The whole extract ($2\ ml/rat/day$, equivalent to a dose of $3.3\ g/kg$ body weight) was used for administration in both experiments.

2.5 Administration

The plant extract and distilled water were administered orally via a sondi needle. In all the experiments, the extracts were administered once a day between 10:00 and 11:00 *hour*.

2.6 Experiments

2.7 Development of the hypercholesterolaemic rat model

Male rats (n =10, mean body weight 231±21 *g*, 8 weeks old) were purchased from the Medical research Institute, Colombo and were kept under standard conditions with access to a standard diet, and water *ad libitum*. Rats were fed with 0.2 *g* of 95% cholesterol(ALDRICH brand) in 1 *ml* "Palm olein" vegetable cooking oil (Turkey brand, purchased from a grocery shop) daily for 2 weeks in addition to the normal standard diet to induce hypercholesterolaemia.

2.8 Effect of *Aporosa lindleyana* on hypercholeterolaemic Wistar rats

The hypercholesterolaemic rats n=10 were divided randomly into two groups the test and the control. The hypercholesterolaemic diet was substituted with a normal diet and, the test group received 2 *ml* of the plant extract while the control received 2 *ml* of distilled water once a day in addition to the normal standard diet. Water was provided *ad libitum* and blood samples (2 *ml*) were collected on day0, day7, and day14, for determination of total and HDL cholesterol levels.

2.9 Effects of *Aporosa lindleyana* on normal Wistar rats fed on a cholesterol-enriched diet

Male rats (n =12, mean body weight of 360 *g*, four months old) were divided into two groups (test and control) with six rats in each. Both groups were given 1% cholesterol-enriched diet for one week, and 0.5% cholesterol enriched diet in the second week. The use of a Cholesterol concentration of 0.5% in the second week was based on our previous studies which indicated that 0.5% Cholesterol-enrichment was sufficient to maintain the high cholesterol levels in Wistar rats[8]. The test group received 2 *ml* of the plant extract while the control received 2 *ml* of distilled water once a day. Both groups were given limited feed of 25 *g/rat* that was 6 *g* more than the initial average intake. Water was provided ad libitum in both groups. Blood samples (2 *ml*) were collected on day0, day7 and day14 for determination of total and HDL cholesterol levels.

2.10 Blood sampling

2 *ml* venous blood was collected from the lateral tail vein under anaesthesia with diethyl ether in all the experiments. The blood samples were centrifuged at 3000 *rpm* for 7 *min* to separate the serum.

2.11 Determination of serum Total and HDL cholesterol levels

The serum samples were separated and analyzed for Total and HDL cholesterol levels on the same day using DMA reagent kits (USA). HDL cholesterol was separated by adding a precipitating reagent (phosphotungstic acid, buffer, and a preservative) provided in the kit that precipitates the LDL and VLDL fractions. The absorbance of the coloured product produced was measured at 500 *nm* using an ELICO SL 150 spectrophotometer.

2.12 Statistical Analysis

All the results are presented as mean ± S.D. The significance was tested using students t-test in Microsoft excel. $P < 0.05$ was considered as significant.

3 RESULTS AND DISCUSSION

Table 1: Development of the hypercholesterolaemic rat model

Total cholesterol mg/dl			HDL Cholesterol mg/dl	Total/HDL Ratio
Day 0	Day 7	Day14	Day 0	Day 0
71.73 ± 12.13	116.86 ± 22.81	134.41 ± 13.77	40.934 ± 5.04	1.73 ± 0.17

Rats were fed with a hypercholesterolaemic diet (0.2 *g* cholesterol enriched) from day 0-14. n=10

Table 2: Development of the hypercholesterolaemic rat model

	Total cholesterol mg/dl			HDL Cholesterol mg/dl	Total/HDL Ratio
	Day 0	Day 7	Day14	Day 14	Day 14
Control n=5	134.41 ± 4.74	72.00 ± 8.82	71.62 ± 7.55	40.84 ± 4.10	1.75 ± 0.16
Test n=5	134.41 ± 20.09	59.3 ± 23.60	*53.59 ± 3.23	35.11 ± 3.18	*1.53 ± 0.03

Rats were treated with the plant material (test group, equivalent to 3.3 *g* of the original plant material */kg* body weight) or distilled water (control group) from day 1-14. 2 *ml* of the whole plant extract was used for administration. On comparison of control test groups (t-test) * $p < 0.05$ considered as significant. The values given are the Mean value ± Standard deviation, n=5 per group.

The total cholesterol level decreased significantly (* $p < 0.05$) in the test group (53.59±3.23 mg/dl) compared to that of the control (71.62±7.55 mg/dl) after two weeks of treatment with the plant extract. The total/HDL was significantly decreased even though the HDL cholesterol was not significantly increased. Feeding with the plant extract for 7 days decreased the total cholesterol level by 12.7 mg/dl. However that was not statistically significant.

Table 3: Development of the hypercholesterolaemic rat model

	Total cholesterol mg/dl			HDL Cholesterol mg/dl			Total/HDL Ratio		
	Day 0	Day 7	Day14	Day 0	Day 7	Day 14	Day 0	Day 7	Day 14
Control n=6	70.06 ± 16.89	193.003 ± 20.25	124.15 ± 30.21	45.90 ± 10.38	86.27 ± 7.52	66.83 ± 13.95	1.53 ± 0.25	2.26 ± 0.401	1.87 ± 0.31
Test n=6	71.01 ± 16.24	*144.86 ± 12.61	*77.35 ± 19.66	42.46 ± 8.78	*95.74 ± 4.91	56.92 ± 13.17	1.71 ± 0.11	*1.51 ± 0.12	*1.35 ± 0.07

The rats were treated with 1% cholesterol-enriched diet from day 0-7 and 0.5% cholesterol enriched diet from day 7-14.

Rats were treated with the plant material (test group, equivalent to 3.3 g of the original plant material $/kg$ body weight) or 2 ml distilled water (control group) from day 1-14. 2 ml of the whole plant extract was used for administration. On comparison of control treated group (t-test) *$p < 0.05$ considered as significant. The values given are the Mean value ± Standard deviation n=6 per group.

The test group had a significantly lower (*$p<0.05$) level of total cholesterol on day 7 and 14 (144.86 ± 12.61, 77.35± 19.66 mg/dl respectively) than that of the control (193.003±20.25, 124.15±30.21 mg/dl) indicating that *Aporosa lindleyana* possesses a cholesterol lowering activity.

Since there was no significant difference between the average feed intake of the test (23 ± 2 g/day) and control groups (22 ± 2 g/day), the observed difference in the total cholesterol levels could not be attributed to a difference in feed intake.

The plant extract also decreased the total/HDL cholesterol in the test group on day 7 and 14 (1.51± 0.12, 1.35± 0.07 mg/dl respectively) significantly ($p<0.05\%$) as compared to that of the control group (2.26± 0.401, 1.87± 0.31 respectively).

The dose of plant extract given is comparable to double the amount of this vegetable consumed by a human in an average lunch packet. This dose was selected because the basal metabolic rate of rat is higher than that of humans[9]. The double dose extract was sufficient to decrease the total cholesterol level in diet induced hypercholesterolaemic male Wistar rats and normocholesterolaemic rats fed with a 0.5% cholesterol enriched diet.

When 1%cholesterol enriched diet was given for one week the total cholestesterol level increased in both test and control but the test group had a significantly lower

value than that of the control.
The plant material did not increase the HDL cholesterol level significantly but there was a significant decrease in the total cholesterol and the total/HDL.
These data indicate that *Aporosa lindleyana* extract reduces the blood cholesterol level of Wistar rats. The mechanism by which this plant extract reduces the blood cholesterol level of Wistar rats is not clear. Since dietary fibre has been known to reduce cholesterol absorption from the gastrointestinal tract, fibre present in the whole extract may be responsible for the hypocholesterolaemic activity of *Aporosa lindleyana*.
Experiments to determine the identity of the active principle are underway.

Conclusion

The studies provide evidence that *Aporosa lindleyana* possesses hypocholesterolaemic activity.

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