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EVALUATION OF HYPOLIPIDEMIC ACTIVITY OF LEAF JUICE OF *CATHARANTHUS ROSEUS* (LINN.) G. DONN. IN GUINEA PIGS

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Abstract: Our aim of the study was to evaluate the hypolipidemic activity of leaf juice of *Catharanthus roseus* (Linn.) G. Donn. in guinea pigs. Adult guinea pigs of either sex were divided into seven groups: group 1 – normal diet; group 2 – high fat diet; group 3 and 4 – normal diet plus leaf juice of *Catharanthus roseus* (Linn.) G. Donn. in the dose of 0.5 and 1 mL/kg, respectively; group 5 and 6 – high fat diet with leaf juice of *Catharanthus roseus* (Linn.) G. Donn. in the dose of 0.5 and 1 mL/kg, respectively; group 7 – high fat diet plus atorvastatin (3 mg/kg). Above diet treatment was given for six weeks and drug was given during last three weeks. Serum lipid profile (total cholesterol, triglycerides, LDL-c, VLDL-c, HDL-c) was performed in each group of animals before and at the end of six weeks. Histological study of aorta, liver and kidney was done in group 1, 2, 6 and 7 and blood cell count was done in animals that were treated juice of *C. roseus* (Linn.) G. Donn. before and after juice administration. Simultaneous administration of leaf juice of *C. roseus* (Linn.) G. Donn. in the dose of 0.5 mL/kg prevents the rise of serum lipid parameters and decreases the fatty changes in the tissue induced by high fat diet, whereas in the dose of 1 mL/kg not only counteracts the elevation, but also significantly ($p < 0.05$) reduces the serum level LDL-c and the ratio of total cholesterol and HDL-c. Leaf juice of *C. roseus* (Linn.) G. Donn. possesses significant lipid lowering and anti atherosclerotic activity.

Keywords: *Catharanthus roseus*, hypolipidemia, atorvastatin

Cardiovascular disease (CVD) is now the most common cause of death worldwide. Today CVD accounts for ~30% of deaths worldwide, including nearly 40% in high-income countries and about 28% in low- and middle-income countries (1). In India, CVD accounted for 32% of all deaths in 2000, and the World Health Organization (WHO) estimates that 60% of the world's cardiac patients will be Indian by 2010. It is well established that increased levels of blood cholesterol, especially low density lipoprotein cholesterol (LDL-c), is the single most important risk factor for the development of CVD (2, 3). Risk of development of CVD and subsequent morbidity and mortality increase when elevated cholesterol level is associated with other risk factors like obesity, hypertension, diabetes mellitus, a constellation of syndromes known as metabolic syndrome (4). It is well established that atherosclerosis is the most commonly implicated pathology in the development of coronary heart disease and abnormalities in plasma lipoproteins and derangements in lipid metabolism rank among the most firmly established and best understood risk factors for atherosclerosis (5).

Plants and herbs are mines of large number of bioactive phytochemicals that might serve as lead for the development of effective, safe, cheap novel drugs. A number of medicinal plants have shown their beneficial effect on the cardiovascular disease (CVD) by virtue of their lipid lowering, antianginal, antioxidant and cardioprotective effects (6, 7).

Among the several herbs *Catharanths roseus* (Linn.) G. Donn. (*vinca rosea*) is the one, which possesses antihyperglycemic and hypotensive activities (8). Fresh leaf juice of *Catharanths roseus* (Linn.) G. Donn. has been reported to reduce blood glucose in normal and alloxan diabetic rabbits (9). Recent study also has shown hypolipidemic activity of leaf juice of *Catharanthus roseus* (Linn.) G. Donn. in normolipidemic rats (10). Based on the above

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actions of leaf juice of *Catharanthus roseus* (Linn.) G. Donn., we wanted to evaluate the hypolipidemic activity of the juice in normal and hyperlipidemic guinea pigs and its possible implication in the management of metabolic syndrome (hyperlipidemia, diabetes mellitus and hypertension).

MATERIALS AND METHODS

Animals

Forty two guinea pigs of either sex weighing 450–625 g were used for the experiments. The animals were procured from the Central Animal House of Government Medical College, Bhavnagar, Gujarat.

Preparation of leaf juice of *Catharanthus roseus* (Linn.) G. Donn.

Fresh leaves of the *Catharanthus roseus* (Linn.) G. Donn. (Pink flower) were collected from the garden of the CSMCRI (Central Salt and Marine Chemicals Research Institute), Bhavnagar and authenticated at Botany Department, Bhavnagar University, Bhavnagar, Gujarat. A day before the collecting the leaves, water was sprinkled over the plants of the *Catharanthus roseus* (Linn.) G. Donn. to washout the dust from all over the plants. On next day morning leaves were collected and no further washing was done to avoid dilution of the juice. The leaves were crushed by electric grinder without adding water. Juice was separated from the crushed leaves by squeezing with the fine cloth and stored in a refrigerator at 4^{o}C until used for the experiment.

Methodology

The study was conducted at the Department of Pharmacology, Government Medical College, Bhavnagar, Gujarat. The study protocol was approved by the Institutional Animal Ethics Committee of the same institute. The study was conducted according to CPCSEA (Committee for the Purpose of Control and Supervision of Experiments on Animals) guidelines for animal experiments.

Guinea pigs were housed in a stainless steel cages and kept under controlled room temperature (24 ± 2^{o}C; relative humidity 60–70%) in a 12 h light/dark cycle and provided standard laboratory feed and water *ad libitum* throughout the experiments. After proper acclimatization for 10 days, they were divided into seven groups (n = 6) as follows:

1. Normal diet; 2. High fat diet; 3. Normal diet plus leaf juice of *Catharanthus roseus* (Linn.) G. Donn. (0.5 mL/kg); 4. Normal diet plus leaf juice of *Catharanthus roseus* (Linn.) G. Donn. (1 mL/kg); 5. High fat diet plus leaf juice of *Catharanthus roseus* (Linn.) G. Donn. (0.5 mL/kg); 6. High fat diet plus leaf juice of *Catharanthus roseus* (Linn.) G. Donn. (1 mL/kg) 7. High fat diet plus atorvastatin (3 mg/kg).

Diet composition

Normal diet: in the morning – mixtures of cereals and pulses (60% wheat plus 35% green gram plus 15% peanuts), total 50 g/animal. In the evening – green leafy vegetables, 30 g/ animal.

High fat diet: in the morning – **c**holesterol powder (Central Drug House (P) Ltd., New Delhi) 500 mg/kg mixed in 10 g of wheat and green gram flour followed by 40 g of the above mixtures of the normal diet/animal. In the evening – green leafy vegetables, 30 g/animal.

After dividing the animals, baseline blood sample (1 mL) was collected from lateral saphenous vein of hind paw of each animal after 12 h fasting and sent to the Clinical Biochemistry Laboratory (NABL accredited), of the institute for the serum lipid profile analysis. After blood collection, the animals were provided diet as mentioned in the respective groups. The above diet plan was continued for next six weeks in groups 1 and 2. In groups 3, 4, 5 and 6, blood samples were collected from the lateral saphenous vein of hind paw after overnight fast and sent for analysis of blood for hemoglobin, red blood cells, platelets and WBC to the Central Pathology laboratory (NABL accredited) of the institute. After that, leaf juice of *Catharanthus roseus* (Linn.) G. Donn. in the dose of 0.5 mL/kg, 1 mL/kg, 0.5 mL/kg and 1 mL/kg, respectively, in group 3, 4, 5, 6, whereas group 7 animals were given atorvastatin calcium (Vista Life Lab., Ahmedabad) in the dose of 3 mg/kg for the next three weeks while continuing the respective diet plan. Both of the above drugs were given orally by gavages feeding tube daily in the morning in the fasting state to ensure maximum absorption. At the end of six weeks, animals of group1, 2, 6 and 7 were sacrificed after the overnight fast. Blood samples were collected from the carotid artery and sent for the analysis of lipid profile for all the four groups and blood count analysis for the group 5 and 6. Also, the liver, kidney and part of thoracic aorta were removed from each animal of above four groups for the histopathological analysis. Blood samples from the saphenous vein were collected from the animals of group 3, 4 and 5 without sacrificing them and sent for the analysis of lipid profile and blood cell count for group 3 and 4.

Table 1. Effect of each treatment strategy on serum lipid profile in guinea pigs (values are expressed as the median and interquartile range, n = 6 in each group).

Treatment groups (n = 6 in each group)	Time period	Total cholesterol (mg/dL)	Triglycerides (mg/dL)	LDL cholesterol (mg/dL)	VLDL cholesterol (mg/dL)	HDL cholesterol (mg/dL)	Total cholesterol/ HDL cholesterol
Normal control (group 1)	Baseline	55.45 49.73, 68.65	91.30 82.12, 98.55	26.48 18.92, 17.96	18.26 16.42, 19.71	15.15 12.05, 18.15	4.035 3.337, 4.442
	Six weeks	57.65 49.25, 67.53	88.40 83.30, 92.85	26.24 31.19, 32.51	17.68 16.66,18.57	14.95 14.08,17.03	3.870 3.586, 4.002
High fat control	Baseline	30.00 27.00, 35.75	64.75 52.88, 76.00	16.45 10.75, 18.50	6.000 5.400, 7.150	8.000 2.625, 17.75	4.705 2.135, 11.75
	Six weeks	68.62* 60.14, 73.14	95.50* 76.00, 107.8	36.10* 27.55, 48.29	13.72* 12.02,14.63	16.80 11.10, 24.85	4.110 2.635, 6.858
Normal diet + *C. roseus* (0.5 mL/kg)	Baseline	59.95 51.30, 69.25 8	8.60 79.95, 95.33	27.71 16.94, 32.33	17.72 15.99, 19.07	17.05 13.70, 19.80	3.346 3.185, 4.303
	Six weeks	49.35 36.40, 57.58	77.65* 72.55, 83.30	17.35 3.245, 22.50	15.53* 14.51, 16.66	17.95 14.35, 20.68	2.621 2.207, 3.378
Normal diet + *C. roseus* (1 mL/kg)	Baseline	61.05 52.60, 67.98	83.90 72.13, 87.68	31.38 21.09, 36.57	16.78 14.43, 17.54	16.20 11.75, 17.63	4.046 3.582, 4.759
	Six weeks	38.05* 30.70, 39.80	60.70* 53.88, 64.08	5.820* 2.520, 8.045	12.14* 10.78, 12.82	19.45 14.58, 21.40	2.001* 1.774, 2.173
High fat + *C. roseus* (0.5 mL/kg)	Baseline	54.00 46.25, 60.13	84.90 77.18, 107.2	23.61 18.43, 31.61	16.98 15.44, 21.45	8.100 5.243, 18.23	6.011 3.196, 11.25
	Six weeks	52.05 44.03, 59.30	84.75 75.48, 104.2	21.08 15.62, 29.50	16.95 15.10, 20.84	9.300 5.865, 19.30	5.193 2.939, 9.529
High fat + *C. roseus* (1 mL/kg)	Baseline	64.55 56.08, 68.98	101.9 96.83, 111.4	28.66 25.17, 33.29	20.37 19.37, 22.27	13.70 10.30, 15.45	4.978 3.704, 5.721
	Six weeks	54.45 44.28, 60.45	94.85 84.68, 100.5	20.06* 12.43, 23.88	18.97 16.94, 20.10	15.00 12.75, 17.48	3.361* 2.878, 4.363
High fat + Atorvastatin (3 mg/kg)	Baseline	60.59 56.66, 64.46	82.58 77.64, 95.41	29.60 20.11, 31.41	16.52 15.53, 19.08	17.05 13.83, 19.40	3.617 3.261, 4.466
	Six weeks	58.15 55.93, 64.85	75.40 71.20, 87.08	24.27 16.58, 27.46	15.08 14.24, 17.42	21.20 18.48, 25.28	2.875 2.497, 3.212

LDL = low density lipoprotein, VLDL = very low density lipoprotein, HDL = high density lipoprotein; Statistical analysis was done by Wilcoxon signed ranked test: * $p < 0.05$ compared with baseline.

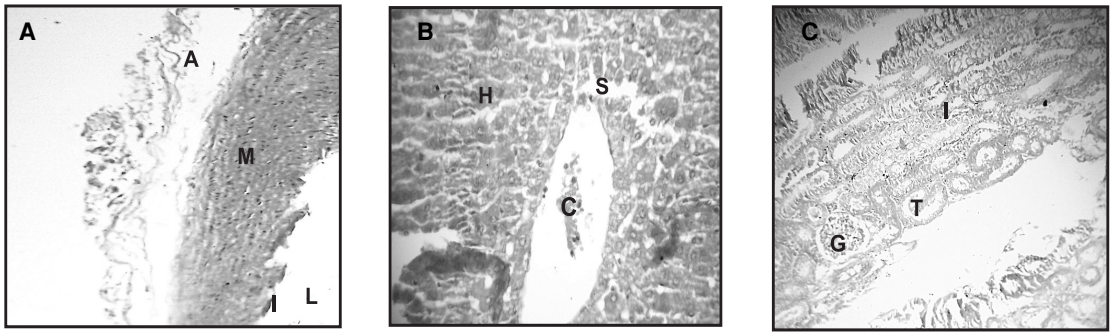

Figure 1. Group 1: Normal diet; H & E stain; 40×.
(A) Guinea pig normal aorta. A. Adventitia tunica, M: Media tunica, I: Intima tunica, L: Lumen
(B) Guinea pig normal liver. C: Central vein, H: Hepatocyte, S: Sinusoid
(C) Guinea pig normal kidney. T: Tubule, G: Glomeruli, I: Interstitum

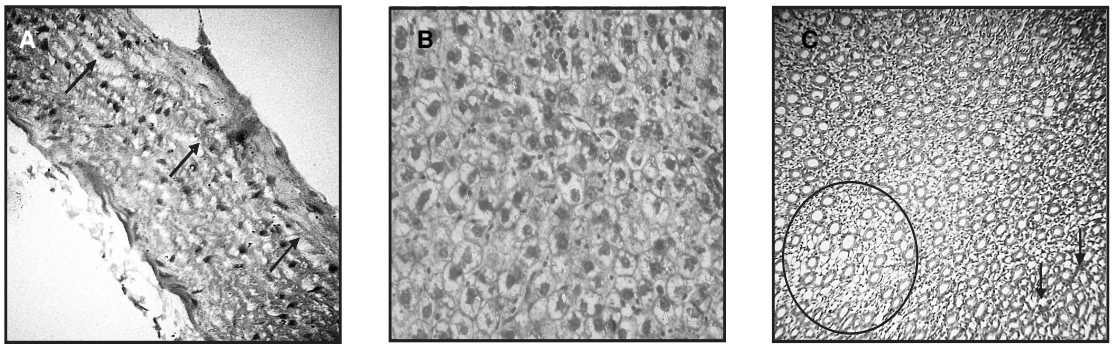

Figure 2. Group 2: High fat diet; H & E stain; 40×.
(A) Guinea pig aorta. Arrows indicates foamy changes in intima and media (Grade 3+).
(B) Guinea pig liver. Diffuse areas of ballooning degeneration (Grade 4+).
(C) Guinea pig kidney. Circle: Interstitial fatty infiltration. Arrows: Eosinophilic cast

Serum lipid profile

Serum samples were analyzed for total cholesterol, HDL-c, and triglycerides (11–13). LDL-c and VLDL-c were calculated by the method of Friedwald (14). Serum total cholesterol: HDL-c was also calculated to access the atherogenic risk.

Toxicity assessment

Blood count analysis

Hemoglobin was estimated by spectrophotometric method. Red blood cells, white blood cells and platelet count estimations were done using electrical cell counter before and after the start of *leaf juice of Catharanthus roseus* (Linn.) G. Donn. Administration to check the effect of juice on hematopoesis.

Daily food intake measurements

Weighing of the food given and left out at the end of day was done for each animal daily throughout the study period to check the effect of juice on appetite.

Weighing of the animals

Weighing of the each animal in all groups was done before the start of the study, at 3 weeks interval and at the end of six weeks to rule out any effect of juice on the weight of the animal.

Histological analysis

Specimens from the organs: aorta, liver and kidney were fixed in 10% neutral buffer formalin. Sections were processed for the light microscopy with formalin fixation, embedded in paraffin and stained with hematoxylin-eosin dye. All sections were coded and analyzed blindly by the pathologist without knowledge of diet or treatment plan. Histological results were graded from grade 0 to 4 according to severity of the changes. 0 implies no change, 1+ slight, 2+ mild, 3+ moderate and 4 + severe histopathological changes.

Statistical analysis

Statistical analysis was done using Graph Pad Prism demo version 5. The values are expressed as

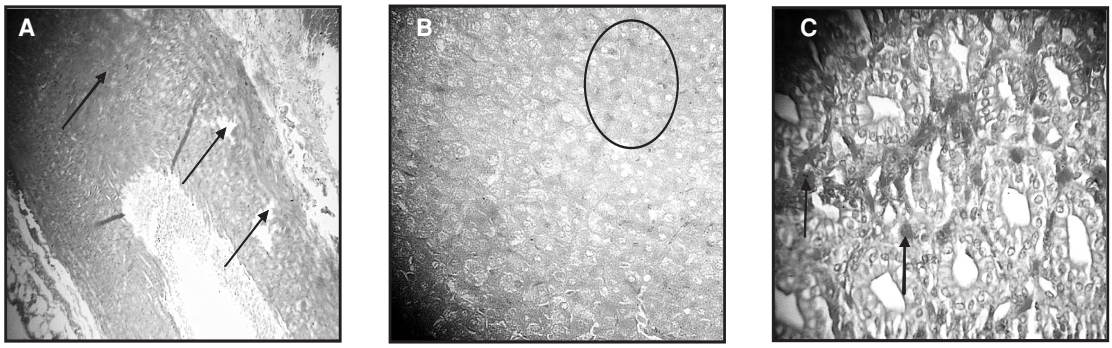

Figure 3. Group 6: High fat diet plus leaf juice of *C. roseus* (Linn.) G. Donn. (1 mL/kg); H & E stain; 40 ×.
(A) Guinea pig aorta. Arrows indicates muscular hypertrophy and foamy changes in media tunica (grade 1+ to 2+).
(B) Guinea pig liver. Circle indicates areas of ballooning degeneration (Grade 1+).
(C) Guinea pig kidney. Arrows indicates eosinophilic cast

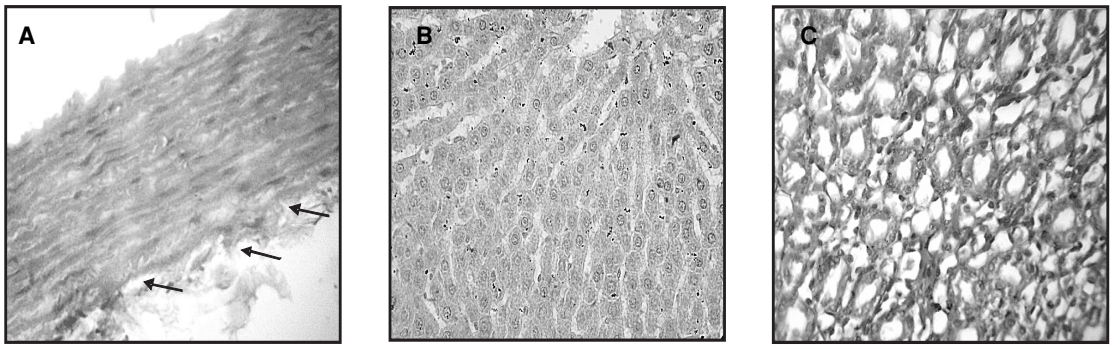

Figure 4. Group 7: High fat diet plus atorvastatin (3 mg/kg); H & E stain; 40×.
(A) Guinea pig aorta. White spaces suggests foamy changes (Grade 1+ to 2+).
(B) Guinea pig liver. No histological changes.
(C) Guinea pig kidney. No histological changes seen

the median and interquartile range. Wilcoxon signed ranked test was used to compare the intra group differences of lipid values and blood cell count from baseline value to six weeks values. Mean increases of weight at the end of six weeks in each group of animals were compared by paired *t*-test and an extent of weight gain among the groups was compared by one way analysis of variance (ANOVA). Value of $p < 0.05$ was considered significant.

RESULTS

Serum lipid profile

Table 1 shows the baseline and six weeks values of serum lipid profile in each diet treatment group. In high fat diet group, there is a significant increase in the serum total cholesterol, LDL-c, VLDL-c and serum triglyceride level ($p < 0.05$) at the end of six weeks compared with baseline. In the normal diet group, leaf juice of *Catharanthus roseus* (Linn.) G. Donn. in the dose of 0.5 mL/kg, significantly reduced the level of serum triglycerides and VLDL-c, while in the dose of 1 mL/kg of b.w. significantly reduced the level of serum cholesterol, LDL-c, VLDL-c, triglycerides and ratio of total cholesterol: HDL-c at the end of six weeks. In high fat treated group, there wasn't significant change in the lipid parameters with the leaf juice of *Catharanthus roseus* (Linn.) G. Donn. in the dose of 0.5 mL/kg b.w., whereas in the dose of 1 mL/kg, the juice was effective in reducing the level of LDL-c and ratio of total cholesterol: HDL-c. There wasn't significant change in the serum lipid profile with atrovastatin in the dose of 3 mg/kg in spite of treatment with high fat diet.

Histopathological studies

In normal diet group animals (group 1), histopathological examination of aorta, liver and kidney showed normal histology of the organs (Fig. 1A-C).

In high fat diet group (group 2), histological study of aorta shows focal areas of foamy changes of varying degrees (grade 3+ to 4+) in tunica media and tunica intima and separation of muscle fibers in

all six animals (Fig. 2A). Histopathological examination of the liver showed diffuse areas ballooning degeneration of hepatocytes of varying degrees (grade 3+ to 4+) and congestion of central vein and hepatic sinusoids (Fig. 2B) in all animals. Histology of the kidney showed fatty infiltration in the interstitium and eosinophilic cast in lumen of renal tubules (Fig. 2C).

In group 5 and 6 (high fat diet plus leaf juice *of Catharanthus roseus* (Linn.) G.Donn.) histopathology of aorta showed lesser degree of foamy changes and muscular thickening compared to group 2 animals (grade 1+ to 2+; Fig. 3A). Liver histology showed lesser degree of ballooning degeneration (grade 1+ to 2+; Fig. 3B) and kidney examination of these groups animals showed no fatty infiltration in the interstitium but only occasional eosinophilic cast (Fig. 3C).

In group 7 (high fat diet plus atorvastatin 3 mg/kg), aortas of the animals showed nil or minimal degree (grade 1+; Fig. 4A) of foamy changes with no muscular wall thickening while liver (Fig. 4B)

Table 2. Effect of leaf juice of *Catharanthus roseus* (Linn.) G. Donn. on blood cell counts in guinea pigs (Values are expressed as the median and interquartile range).

Treatment group	Time period	Hb (g %)	RBCs (mm^3)	WBCs (mm^3)	Platelets (mm^3)
Normal diet + *C. roseus* (0.5 mL/kg)	Baseline	12.35 11.18, 13.20	5.35×10^6 4.87, 5.55	10.50×10^3 9.82, 11.15	45.45×10^4 39.58, 53.23
	Six weeks	12.05 11.40, 12.33	4.90×10^6 4.55, 5.30	10.00×10^3 9.52, 11.10	45.60×10^4 42.05, 51.08
Normal diet + *C. roseus* (1 mL/kg)	Baseline	12.75 11.75, 13.85	5.00×10^6 4.62, 5.35	11.25×10^3 10.58, 12.03	46.20×10^4 40.80, 54.48
	Six weeks	12.60 11.45, 13.25	5.05×10^6 4.67, 5.30	11.10×10^3 10.43, 11.50	43.50×10^4 42.05, 52.95
High fat + *C. roseus* (0.5 mL/kg)	Baseline	12.00 11.25, 12.33	4.70×10^6 4.25, 5.37	10.00×10^3 8.97, 11.35	48.45×10^4 41.63, 56.80
	Six weeks	12.10 11.35, 12.65	5.00×10^6 4.65, 5.55	10.90×10^3 9.20, 13.00	49.50×10^4 42.13, 58.78
High fat + *C. roseus* (1 mL/kg)	Baseline	12.55 12.10, 13.13	5.15×10^6 4.97, 5.70	9.85×10^3 9.35, 10.78	46.65×10^4 32.08, 64.48
	Six weeks	12.50 11.93, 13.63	5.40×10^6 5.15, 5.80	10.90×10^3 9.85, 12.63	45.65×10^4 30.85, 64.70

Statistical analysis of data was carried by Wilcoxon signed ranked test. Hb = hemoglobin, RBCs = red blood cells, WBCs = white blood cells, n = 6 in each group.

Table 3. Daily food intake and weight of the leaf juice of *Catharanthus roseus* (Linn.) G. Donn. treated guinea pigs. (Values are expressed as the mean ± SEM). ±

Treatment group	Daily food intake in grams/animal		Weight of the animal in grams	
	Baseline	At six weeks	Baseline	At six weeks
Normal diet	42.8 ± 2.31	43.7 ±3.21	540.0 ± 20.53	564.2 ± 21.27*
High fat diet	41.8 ± 2.75	42.1 ± 2.25	554.2 ± 17.67	582.5 ± 14.87 *
	Before leaf juice of *C. roseus*	After leaf juice of *C. roseus*	Before leaf juice of *C. roseus*	After leaf juice of *C. roseus*
Normal diet + *C. roseus* (0.5 mL/kg)	43.4 ± 3.41	42.5 ± 2.57	529.2 ± 12.41	564 .2± 15.30*
Normal diet + *C. roseus* (1 mL/kg)	43.7 ± 2.57	41.9 ± 3.42	548.3 ± 20.36	580.8 ± 16.95*
High fat + *C. roseus* (0.5 mL/kg)	40.6 ± 2.74	40.3 ± 1.36	533.3 ± 22..05	565.8 ± 23.32*
High fat + *C. roseus* (1 mL/kg)	41.4 ± 3.45	40.5 ± 2.78	548.3 ± 18.15	588.3 ± 23.01*

and kidney (Fig. 4C) examination of these animals showed no morphological alterations.

Blood cell count

Table 2 shows the various blood cell count values before and after treatment of guinea pigs with the leaf juice of *Catharanthus roseus* (Linn.) G. Donn.. There wasn't significant change in the various blood cell counts associated with the juice therapy.

Daily food intake and body weight

Table 3 shows changes in the food intake and body weight of guinea pigs treated with the leaf juice of *Catharanthus roseus* (Linn.) G. Donn. There wasn't any significant change in the food intake in any of the animal groups at the end of six weeks. There was significant increase in the mean body weight of all groups but, the difference in the extent of increase was not significant among the groups at the end of study period.

DISCUSSION

Coronary heart disease resulting from progressive atherosclerosis, remains the most common cause of morbidity and mortality all over the world (15). Apart from elevated level of total and LDL-c and low level of HDL-c, many other factors like diabetes, hypertension, smoking, glucocorticoid, diet and psychological factors are contributing to its etiology (16). Oxidative modification of low density lipoprotein cholesterol (LDL-c) appears to have an important role in initiation and progression of atherogenic changes in aorta (17). The agents, who can lower serum cholesterol and scavenge or inhibit free radicals formation, have gained wide therapeutic value. Apart from reducing LDL-c, which is the primary target in the management of hyperlipidemia (18), modification of other risk factors like diabetes mellitus, hypertension, smoking etc., will decrease the morbidity and mortality from cardiovascular disorders. Various experimental studies have shown that leaf juice of *Catharanthus roseus* (Linn.) G. Donn. improve blood glucose level in normal and alloxan induced diabetic animals (9, 19). There are some evidences of the hypolipidemic activity of leaf juice of *Catharanthus roseus* (Linn.) G. Donn. in normal and hyperlipidemic experimental animals (10, 19). Leaf juice of *Catharanthus roseus* (Linn.) G. Donn. has also shown hypotensive effect in preclinical study (20). Further, patients with hyperlipidemia are at higher risk of having hypertension and diabetes mellitus and all these together, known as metabolic syndrome (Syndrome X), increase the rate of cardiovascular mortality and morbidity. From above information, we decided to evaluate the hypolipidemic activity of leaf juice of *Catharanthus roseus* (Linn.) G. Donn. in guinea pigs as we also wanted to know the possible implication of juice in the management of metabolic syndrome (Syndrome X).

We choose guinea pig as a model for diet induced atherosclerosis because, in addition to the possible advantages in cost, guinea pigs might be a better surrogate to human atherosclerosis than rabbits. Physiologically, guinea pigs present the closest lipid metabolism to humans (21).

The results of our study have shown that, when guinea pigs were given high fat diet for six weeks, there was significant increase ($p < 0.05$) in the serum level of total cholesterol, triglycerides, LDL-c and VLDL-c and also the fatty changes in the aorta, kidney and ballooning degeneration in the liver (Fig. 2 and Tab. 1). This increase and change in the histology of aorta was in accordance with the previous experimental studies with high fat diet (22, 23). Supplementation of cholesterol in diet rapidly results in a marked increase in the production of cholesteryl ester rich-VLDL-c by the liver and intestine and a reduced number as well as rate of cholesterol removal by the hepatic LDL-c receptors (24, 25). Based on study done by Raha R Ahmad-Raus et al., six weeks period was considered sufficient to produce fatty changes in serum and tissues.

Administration of the leaf juice of *Catharanthus roseus* (Linn.) G. Donn. in the animals of normal diet groups results in dose dependent decrease in serum level of total cholesterol, triglycerides, LDL-c and VLDL-c suggesting beneficial influence on cholesterol metabolism and turnover (Tab. 1). Elevated serum triglyceride is considered as independent risk factor for cardiovascular disease (26). A significant decline in the serum triglycerides level observed in leaf juice treated guinea pigs supports the cardiovascular protective influence. In a previous study, there was dose dependent decrease in lipid parameters in normolipidemic Wistar rats when exposed to leaf juice of *Catharanthus roseus* (Linn.) G. Donn. only for seven days (10). Administration of leaf juice of *Catharanthus roseus* (Linn.) G. Donn. in the dose of 0.5 mL/kg for three weeks in high fat diet guinea pigs, counteract the serum elevations of the lipid parameters, while in the dose of 1 mL/kg not only counteract the elevation, but also significantly ($p < 0.05$) reduced the serum level LDL-c and ratio of total cholesterol and HDL-c and also decrease the fatty changes in liver,

aorta and kidney (Fig. 3 and Tab. 1). This result suggests protective effect of the leaf juice of *Catharanthus roseus* (Linn.) G. Donn. against the rise of lipid levels.

Atorvastatin in the dose of 3 mg/kg for three weeks in high fat group animals also counteracts the rise in serum level of total cholesterol, triglycerides, LDL-c, VLDL-c and ratio of total cholesterol : HDL-c and resist the fatty changes in the aorta and liver induced by fatty diet (Fig. 4 and Tab. 1). These results are consistent with the previous findings (27). Thus, protection against raised lipid parameters by leaf juice of *Catharanthus roseus* (Linn.) G. Donn in the dose of 0.5 mL/kg was comparable with the atorvastain (3 mg/kg), while in the dose of 1 mL/kg it has greater efficacy in reducing the LDL-c and total cholesterol/HDL-c ratio. LDL-c is the primary target of treatment in clinical lipid management (18). Thus, significant decrease in LDL-c and total cholesterol / HDL-c at higher dose of leaf juice is an important finding of our study.

This reduction in lipid profile could have resulted from the antioxidant effect of the leaf juice of Catharanthus *roseus* (Linn.) G. Dinn. whose photochemical components include flavonoid, which is known for antioxidant effect (28, 29). Atherosclerosis is no longer simply viewed as a disease of cholesterol accumulation in the arterial wall, but rather as a process that involves low-grade vascular inflammation in all stages. Vinpocetine, a naturally occurring compound, present in minor periwinkle, decreases inflammation by targeting the activity of a specific enzyme, known as IKK (IκB kinase). The presence of vinpocetine like substance in leaf juice could be the explanation of anti-atherosclerotic activity (30).

CONCLUSION

In our study, significant anti atherosclerotic activity as suggested by reduction in the serum levels of total cholesterol, triglycerides, LDL-c, VLDL-c and histology of aorta, liver and kidney with the leaf juice of *Catharanthus roseus* (Linn.) G. Donn. could have resulted from the antioxidant effect of flavonoid, and probably, vinpocetine like compound present in leaf juice of *Catharanthus roseus* (Linn.) G. Donn. Further investigations are warranted to identify the hypolipidemic active principles and to elucidate their mechanism of action.

The implication of this result is that the use of leaf juice of *Catharanthus roseus* (Linn.) G. Donn. along with statin (atorvastatin) therapy in the treatment of hyperlipidemia will also ameliorate the occurrence of CHD in patient with metabolic syndrome (Syndrome X) by its additional glucose and blood pressure lowering action.

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