EVALUATION OF ACUTE HYPOLIPIDEMIC ACTIVITY OF DIFFERENT PLANT EXTRACTS IN TRITON WR-1339 INDUCED HYPERLIPIDEMIA IN ALBINO RATS

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Summary

The lipid lowering activity of *Randia dumetorum* fruit extract & *Paederia foetida* aerial part extract has been studied in Triton WR-1339 induced hyperlipidemia in male albino rats. The dried and ground parts of fruit of *Randia dumetorum* and aerial part of *Paederia foetida* were subjected to extraction with methanol using a soxhlet apparatus for 72 Hrs. The obtained methanolic extracts were suspended in distilled water and administered orally to Swiss albino rats through oral feeding tube till a period of 2 days. Prior to that male albino rats were randomly divided into five groups. Groups I and II serves as vehicle control (demineralized water) and triton control (Triton WR-1339 - 200 mg/kg; i.p.), respectively. Group III was treated with atorvastatin (7.2 mg/kg).Groups IV and V were treated with test substance *Paederia foetida* and *Randia dumetorum* extract, at the dose of 400 mg/kg/day respectively as single dose for two days. The Blood samples of each animal were collected at 0, 18, 24,40and 48 Hr post treatment and the results were analyzed. All the data were analyzed by using one way ANOVA followed by Dunnett's t test to observe any significant difference. The statistical significance of results was tested at two confidence levels viz. $p<0.05$ and $p<0.01$.The mean bodyweight of animals of each group was calculated daily till the end of the experiment. In this experiment *Paederia* extract resulted in lowering of serum total cholesterol from 157.47±17.75mg/dl (18^{th} Hr) to 133.15±16.52mg/dl (24^{th} Hr), *Randia* extract lowers it from 121.38±21.14mg/dl (18^{th} Hr) to133.44±19.64mg/dl (24^{th} Hr) and atorvastatin reduces it from 125.80±15.46mg/dl (18^{th} Hr) to 112.80±18.27mg/dl (24^{th} Hr). In case of serum triglycerides, atorvastatin treatment resulted in lowering from 661.72±153.31mg/dl (18^{th} Hr) to337.34±105.01mg/dl (24^{th}Hr), *Paederia* extract non-significantly lowers it from 865.47±134.87mg/dl (18^{th} Hr) to 457.03±96.84mg/dl (24^{th} Hr), and incase of randia extract there was non-significant decrease from 557.13±197.66mg/dl to 345.99±118.45mg/dl.The results of the present study demonstrated lipid lowering activity in fruit extract of *Randia dumetorum*.

KEY WORDS: - Anti-hyperlipidimic activity, *Randia dumetorum*, Atorvastatin, *Paederia foetida*.

Introduction

Hyperlipidemia is defined as an elevation of one or more of the following: cholesterol, cholesterol esters, phospholipids, or triglycerides. Abnormalities of plasma lipids can result in predisposition to coronary, cerebrovascular, and peripheral vascular arterial diseases [1]. Keys to prevention and treatment are the elimination or modification of risk factors, if possible, in conjunction with treatment of the specific lipid disorders [2]. Disorders of lipid metabolism, hyperlipidemia, hypertension and obesity are associated with increased oxidative stress and overproduction of oxygen free radicals [3]. Moreover, hyperlipidemia following oxidative stress may cause oxidative modifications in low-density lipoproteins, which play an important role in the initiation and progression of atherosclerosis and related cardiovascular diseases [4]. Furthermore, there have been reports that the lipid lowering drugs: fibrates, statins and bile acid sequestrants used for the treatment of hyperlipidemia and associated disorders do not possess antioxidant property and they are also not free from toxic side effects [5]. World ethno botanical information reported that a number of herbal medicines from plants and vegetables are used for controlling hyperlipidemia and related complications in patients [6]. *Randia dumetorum* commonly known as indigo berry(family Rubiaceae, Hindi name; Mainphal, Madan) is one such ayurvedic remedy that has been mentioned in many Indian medical literatures for the treatment of many diseases including its role as antioxidant and liver protectant. Oleonolic acid 3-glucoside, isolated from the seed, exhibited this property as found from the previous studies. *Paederia foetida* commonly known as stinkvin (Family Rubiaceae, Hindi name: Prasarini) is an ayurvedic remedy & the antioxidant activity of fresh and dried extract of this plant has been reported earlier. The current research work is to compare the antihyperlipidemic activity of *Randia dumetorum* fruit extract and *Paederia foetida* aerial part extract in Triton induced hyperlipidemia model.

Materials and Methods

Materials

Tyloxapol, Sodium chloride and Triton WR 1339 were purchased from HiMedia Laboratories Pvt. Ltd.; India. Atorvastatin was a gift from Ranbaxy Laboratories Ltd., India. Total Cholesterol assay kit and Triglycerides assay kit was purchased from Span Diagnostics Ltd., Surat, India.

Extraction of plant material

The fresh fruits of *Randia dumetorum* & aerial part of *Paederia foetida* were obtained from the local village in Berhampur, India. The plants were authenticated by Prof.S. K. Das (Head of the department Biotechnology, College of Pharmaceutical Sciences, Mohuda, Berhampur, Odisha (India). Dried and ground fruit parts of *Randia dumetorum* & aerial parts of *Paederia foetida* (approx.100 g) were subjected to extraction with 300 mL methanol (80 %) in a soxhlet apparatus for 72 hours. After extraction, the solvent was filtered and evaporated using rotavapor (Heidolph, UK).The obtained alcoholic extract was stored at -20^{o}C for further use.

Animal studies

Male adult albino rats (200–225 g) bred in the animal house of the institute were used. A group of six animals in a cage were kept in controlled conditions, temperature 25–26°C, relative humidity 60–80% and 12/12 h light/dark cycle (light from 08:00 a.m. to 08:00 p.m.). The identification of animals has been done by cage card and corresponding colour body markings using picric acid. The animals were kept in polypropylene cages with stainless steel grill top, facilities for feed and water bottle and bedding of clean paddy husk, fed on standard pellet diet and U.V. purified and filtered water will be provided ad libitum in polypropylene bottles with stainless steel sipper tubes. The study design was in compliance with guidelines of Institutional Animal Ethical Committee (IAECRegd No: 926/ab/06/CPCSEA). The animals will be randomly divided into five groups. Groups I and II will serve as vehicle control (demineralized water) and triton control (Triton WR-1339 - 200 mg/kg; i.p.), respectively [7]. Group III will be treated with atorvastatin (7.2 mg/kg). Groups IV and V, will be treated with the test substance *Paederia foetida* aerial part extract and *Randia dumetorum* fruit extract, at the dose of 400 mg/kg respectively after the intra-peritoneal administration of Triton WR-1339 at the dose of 200 mg/kg. The vehicle, reference standard or test substance will be administered orally as a single dose. The blood samples of each animal will be collected at 0, 18, 24,40and 48 h post treatments and serum will be separated for estimation of cholesterol and triglycerides.

Acute toxicity study [8]

Albino mice of 10 animals per group weighing 20 to 25g were administered graded dose (100 to 3000 mg/kg, i.p.) of methanolic extract of *Randia dumetorum* and *Paederia foetida*. After administration of the extract the mice were observed for toxic effects if any after 48 hour of treatment. The toxicological effects were observed in terms of mortality expressed as LD 50.The number of animals dying during a period was noted. No mortality was observed therefore the extract is safe to use even at the doses of 3000 mg/kg of body weight orally.

Sampling and measurement of lipid profile parameter from serum

Blood sample (0.5 ml) was withdrawn from the sublingual vein under ether anesthesia and was collected in micro tubes previously filled with 10% EDTA solution (20 µl of 10% EDTA/ ml of blood). The micro tubes were centrifuged at 4000 rpm at 4°C for 20 min to obtain clear plasma. The plasma was then analyzed by using autoanalyzer (3000 Evolution, BSI Italy) for triglyceride and total cholesterol content by using commercially available biochemical kits.

Statistical analysis

All the data were analyzed by using one way ANOVA followed by Dunnett's t test using Prism® software to observe any significant difference. The statistical significance of results was tested at two confidence levels viz. $p<0.05$ and $p<0.01$.

Results and Discussion

Body Weight

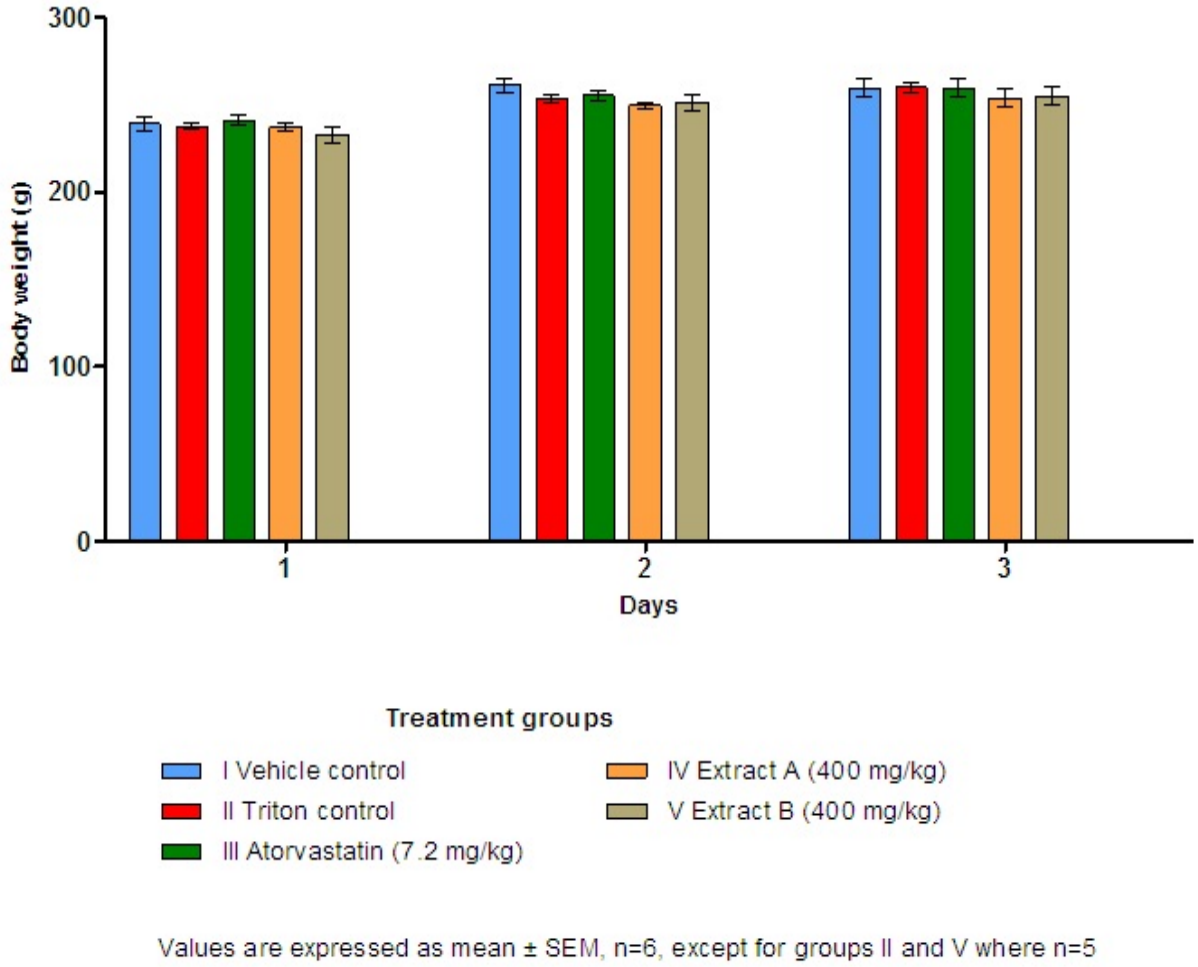
The mean body weight of each group is presented in Table 1.There is no significant difference in the mean body weight of vehicle control and triton control group through out the experiment period. Also, the treated groups did not show any significant body weight change when compared to triton control.

Table 1: Effect of different extracts on body weight in Triton-induced hyperlipidaemic rats

Treatment groups	Body weight (g)		
	Day 0	Day 1	Day 2
I Vehicle control (Demineralised water; 10 ml/kg)	239.17 ± 4.21	261.33 ± 4.30	259.83 ± 5.52
II Triton control (200 mg/kg; *i.p.*)	237.80 ± 1.88	253.40 ± 2.58	260. 00 ± 3.21
III Atorvastatin (7.2 mg/kg)	241.00 ± 2.86	255.67 ± 3.03	259.67 ± 4.92
IV *Paederia foetida*(400 mg/kg)(Extract A)	237.33 ± 2.76	249.83 ± 1.76	253.67 ± 5.16
V *Randia dumetorum* (400mg/kg)(Extract B)	232.80 ± 5.05	251.20 ± 4.76	255.20 ± 5.02

Values are expressed as mean ± SEM, n = 6, except for groups II and V where n = 5

Figure1:Effect of different extracts on body weight in Triton-induced hyperlipidaemic rats (*Paederia foetida* is extract A & *Randia dumetorum* is extract B) .



Total cholesterol content

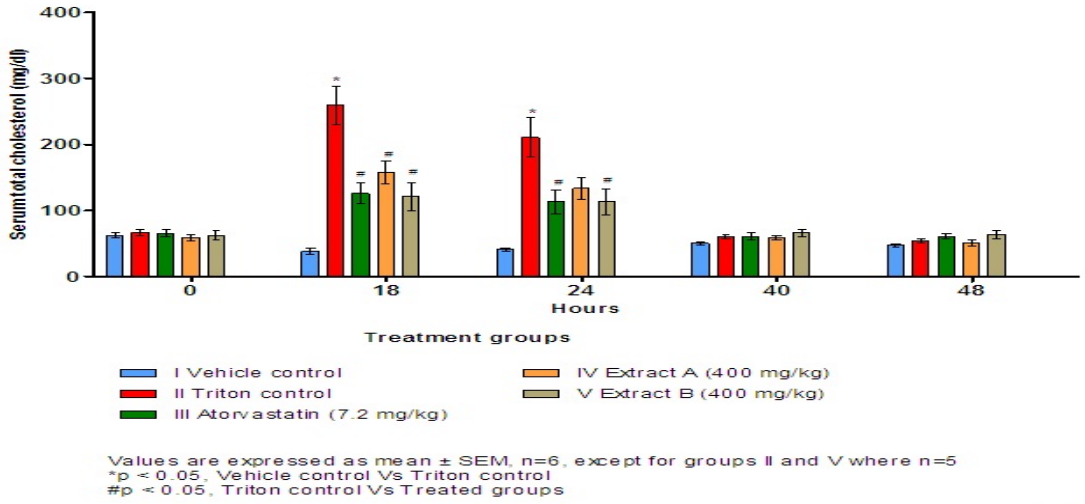
The mean serum total cholesterol level of each group is presented in Table 2. At 0 hour, there is no significant change in the serum total cholesterol levels between all the groups. After induction of triton, the vehicle control group showed a significant increase in serum total cholesterol level at 18 and 24 hour when compared with triton control group. Treatment with atorvastatin showed a significant decrease in serum total cholesterol level when compared with Triton control group. The groups treated with *Paederia foetida* and *Randia dumetorum* extract showed significant decrease in total cholesterol level in serum at 18 hour and 24 hour, except for a non-significant decrease at 24 hour in *paederia foetida* treated group were shown when compared to Triton control group.

Table 2: Effect of different extracts on serum total cholesterol in Triton-induced hyperlipidemic rats

Treatment groups	Serum total cholesterol (mg/dl)				
	Hour 0	**Hour 18**	**Hour 24**	**Hour 40**	**Hour 48**
I Vehicle control (Demineralised water; 10 ml/kg)	62.48 ± 3.45	38.12 ± 4.88	40.91± 2.25	50.70± 2.28	47.22± 1.93
II Triton control (200 mg/kg; *i.p.*)	66.44 ± 5.21	258.85 ± 29.47*	210.72 ± 29.81*	60.79± 3.33	54.14± 3.16
III Atorvastatin (7.2 mg/kg)	65.44 ± 5.22	125.80 ± 15.46$^{\#}$	112.80± 18.27$^{\#}$	60.85± 5.06	60.86± 4.27
IV *Paederia Foetida* Extract(400 mg/kg)	59.25 ± 4.91	157.47 ± 17.75$^{\#}$	133.15± 16.52	58.61± 3.28	51.14± 4.65
V *Randia dumetorm* Extract(400 mg/kg)	62.62 ± 7.82	121.38 ± 21.14$^{\#}$	113.44± 19.64$^{\#}$	65.88± 5.19	63.55± 5.95

Values are expressed as mean ± SEM, n=6, except for groups II and V where n=5, $^{*}p \leq 0.05$, Vehicle control Vs Triton control, $^{\#}p \leq 0.05$, Triton control Vs Treated groups

Figure 2: Effect of different extracts on serum total cholesterol in Triton-induced hyperlipidemic rats(*Paederia foetida* is extract A & *Randia dumetorum* is extract B) .



Serum triglyceride

The mean serum triglycerides level of each group is presented in Table 3. At 0 hour, there is no significant change in the serum triglycerides level between all the groups. After induction of triton, the vehicle control group showed a non-significant increase in serum triglycerides level at 18 and 24 hour when compared with triton control group. Treatment with atorvastatin showed a non-significant decrease in serum triglycerides level when compared with triton control group. The groups treated with extract of *Paederia foetida* and *Randia dumetorum* showed non-significant decrease in serum triglycerides level at 18 hour and 24 hour when compared with triton control group. Triton-WR1339 is a non ionic detergent with surface tension reducing properties. Systemic administration of the Triton-WR1339 is reported to inhibit lipoprotein lipase and thereby elevates serum cholesterol and a triglyceride level in rats [9,10]. Triton induced hyperlipidemia is biphasic. Initially there will be a steep increase in serum cholesterol levels (phase I) due to an initial accumulation of excess non-cholesterol lipids in plasma which then mobilize cholesterol not only from hepatic but also, and perhaps chiefly, from extra hepatic sources. Triton WR-1339 causes hyperlipidemia *via* inhibition of lipolysis of triglyceride (TG)-rich lipoprotein [11, 12] and increase in hepatic cholesterol synthesis by enhancing the activity of 3-hydroxy-3-methylglutaryl coenzyme A (HMG CoA) reductase as the rate-limiting enzyme in cholesterol synthesis [13]. Previous studies have shown that Triton WR-1339 could produce rapid dose-related increases in plasma lipids and fibrinogen [14]. This will be followed by a decrease in serum cholesterol near to normal levels (phase II) within next 24 hours [15]. Substances that reduce triton-induced elevated levels of serum lipids can be considered as potential anti-hyperlipidemic agents. The results of our study demonstrated that *Randia dumetorum* fruit extract caused significant decrease in the plasma lipid levels in triton induced hyperlipidemic rats in

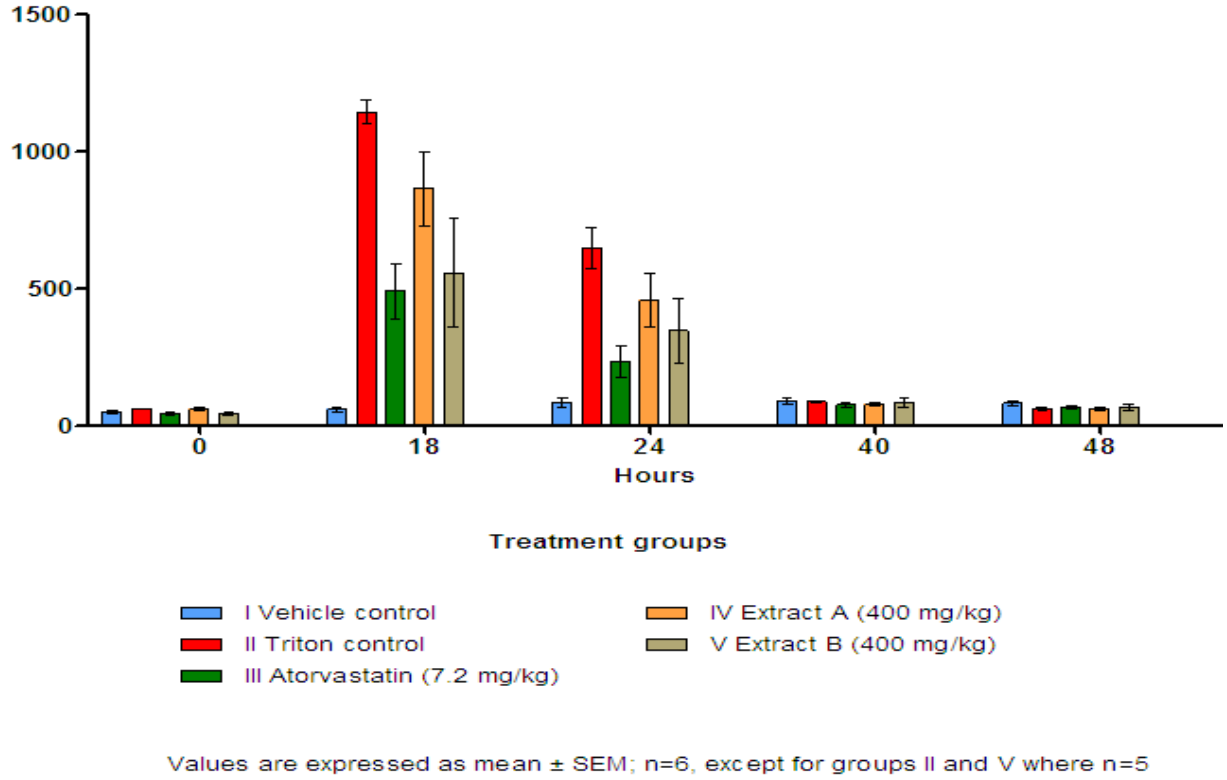
comparison to extract of aerial part of *Paederia foetida*. Triton WR-1339 acts as surfactant and cause structural modifications in circulatory lipoproteins suppress the action of lipases and as a consequence block the uptake of circulating lipids by extra hepatic tissues, resulting in increased blood lipid concentration [12]. The extract may have interfered with substrate modifications and stimulated the activity of lipases and reduces the formation of harmful lipids. Chemical investigations of *Randia dumetorum* fruit extract have shown that it contains oleonolic acid 3-glucoside. The above-mentioned compound of *Randia dumetorum* may be responsible for exerting beneficial effects. However, not much work has so far been done to investigate the biological activities of this compound.

Table 3: Effect of different extracts on serum triglycerides in Triton-induced hyperlipidemic rats

Treatment groups	Serum triglycerides (mg/dl)				
	Hour 0	**Hour 18**	**Hour 24**	**Hour 40**	**Hour 48**
I Vehicle control (Demineralised water; 10 ml/kg)	51.14± 5.05	59.67± 10.56	86.65± 16.65	91.33±13.27	81.91±6.99
II Triton control (200 mg/kg; *i.p.*)	62.42±2.16	1144.36± 45.85	648.78±75.40	87.89± 4.29	63.02±4.51
III Atorvastatin (7.2 mg/kg)	42.68± 5.75	661.72± 153.31	337.34±105.01	75.86± 7.18	67.50±5.95
IV *Paederia foetida* Extract(400 mg/kg)	60.71± 6.25	865.47± 134.87	457.03±96.84	78.68± 6.44	62.32±4.57
V *Randia dumetorum* Extract(400 mg/kg)	43.77±4.54	557.13± 197.66	345.99±118.45	84.80±16.77	68.10±11.82

Values are expressed as mean ± SEM; n=6, except for groups II and V where n=5

Figure 3: Effect of different extracts on serum triglycerides in Triton-induced hyperlipidemic rats *Paederia foetida* is extract A & *Randia dumetorum* is extract B) .



Conclusions

The results of the present study demonstrated new properties of *Randia dumetorum* fruit extract as a potent lipid lowering agent. Moreover currently available hypolipidemic drugs have been associated with a number of side effects [16]. Therefore, *Randia dumetorum* when compared with *Paederia foetida* showed comparatively better anti-hyperlipidemic activity and hence, *Randia dumetorum* can be further subjected to dose response study and chronic study because there is need to elucidate the exact mechanism by which such plant products provides lowering of bad cholesterol. The studies on hyperlipidmia have immense potential for exhaustive research.

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References

1. Wells GB, Dipiro J, Schwinghammer T, Hamilton C. Pharmacotherapy Handbook, 7th Edn, The Mcgraw Hill Companies, USA, 2007; 98-108.
2. Diaz MN, Frei B, Vita JA, John F , Keaney Jr. Antioxidants and atherosclerotic heart disease. N Engl J Med 1997; 337: 408-416.
3. Parthasarthy S, Steinberg D, Seitztum JL. The role of oxidized low-density lipoprotein in the pathogenesis of atherosclerosis. Ann Rev Med 1992; 43: 219–225.
4. Dahanukar SA, Kulkarni RA, Rege NN. Pharmacology of medicinal plants and natural products. Indian J Pharmacol 2000; 32: S81–S118.
5. Sircar NON. Pharmacological basis of Ayurvedic therapy. In: Atal CK, Kapoor BM, editors. Cultivation and Utilization of Medicinal Plants. New Delhi, India: Publication and Information Directorate, CSIR; 1992: 507–518.
6. Sklar IV, Kakkar KK, Chakre OJ. Glossary of Indian Medicinal Plants With Active Principles. New Delhi: CSIR; 1992: 75.
7. Umachigi SP, Kumar GS, Jayaveera KN, Kishore Kumar DV, Ashok Kumar CK, Dhanapal R. Antimicrobial, wound healing and antioxidant activities of Anthocephalus Cadamba. Afr J Trad Comp Alt Med 2007; 4: 481–487.
8. Litchfield JT, Jr. and Wilcoxon F. A simplified method of evaluating dose effect. J Pharmac exp Ther 1949; 96(2): 99-113.
9. Schurr PE, Schultz JR, Parkinson TM. Triton-induced hyperlipidemia in rats as an animal model for screening hypolipidemic drugs. Lipids 1972; 7(1): 68-74.
10. Levine S, Saltzman AA. Procedure for inducing sustained hyperlipemia in rats by administration of a surfactant. J Pharmacol Toxicol Methods 2007; 55(2): 224-226.
11. Borensztajn J, Rone MS, Kotlar TJ. The inhibition *in vivo* of lipoprotein lipase (clearing-factor lipase) activity by TritonWR-1339. Biochem J 1976; 156: 539-543.
12. Hayashi H, Ninobe S, Matsumoto Y. Effects of Triton WR-1339 on lipoprotein lipolytic activity and lipid content of rat liver lysosomes. J Biochem 1981; 89: 573-579.
13. Kuroda M, Tanzawa K, Tsujita Y, Endo A. Mechanism for elevation of hepatic cholesterol synthesis and serum cholesterol levels in Triton WR-1339-induced hyperlipidemia. Biochemicaet Biophysica Acta 1997; 489: 119-125.
14. Okazaki M, Suzuki M, Oguchi K. Changes in coagulative and fibrinolytic activities in Triton WR-1339-inducedhyperlipidemia in rats. Japan J Pharmacol 1990; 52: 353-361.
15. Vogel G, Vogel WH. Drug discovery and evaluation-Pharmacological Assays. Spriger-Verlag, Berlin, 1997;598.
16. S.L. Brown. Lowered serum cholesterol and low mood.Br Med J 1996; 313: 637-638.