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Original Article

Lipid Lowering Activity of *Anthocephalus indicus* Root in Hyperlipidemic Rats

Vishnu Kumar[1], Mohammad Mubin Khan[2], Ashok Kumar Khanna[2], Ranjana Singh[3], Sushma Singh[3], Ramesh Chander[3], Farzana Mahdi[1], Abbas Ali Mahdi[3], Jitendra Kumar Saxena[2] and Raj Kumar Singh[3]

[1]Department of Biochemistry, Era's Lucknow Medical College & Hospital, Lucknow-226003, [2]Division of Biochemistry, Central Drug Research Institute, Lucknow-226001 and [3]Department of Biochemistry, King George's Medical University, Lucknow-226003, India

The lipid lowering activity of *Anthocephalus indicus* (family Rubiaceae; Hindi name Kadamba) root extract has been studied in triton WR-1339 induced hyperlipidemia in rats. In this model, feeding with root extract (500 mg kg^{-1} b.w.) lowered plasma lipids and reactivated post-heparin lipolytic activity in hyperlipidemic rats. Furthermore, the root extract (50–500 μM) inhibited the generation of superoxide anions and hydroxyl radicals, in both enzymic and non-enzymic systems, *in vitro*. The results of the present study demonstrated both lipid lowering and antioxidant activities in root extract of *A. indicus*, which could help prevention of hyperlipidemia and related diseases.

Keywords: *Anthocephalus indicus* – hypolipidemic agent – oxygen free radical scavengers – plant antioxidant – Rubiaceae – triton model of hyperlipidemia

Introduction

Disorders of lipid metabolism, hyperlipidemia, hypertension and obesity are associated with increased oxidative stress and overproduction of oxygen free radicals (1). An excess of superoxide anions (O_2^-) are further converted into other reactive oxygen species and among them hydroxyl radical (OH^-) is more damaging to lipids and lipoproteins (2). 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 (3). Furthermore, there have been reports that the lipid lowering drugs: fibrates, statins and bile acid sequestraints used for the treatment of hyperlipidemia associated disorders do not possess antioxidant property and they are also not free from toxic side effects (4). Therefore, on the basis of above stated facts there is an urgent need to have a drug having the dual property of lowering lipid level and antioxidant activities together and for this natural products are the best option.

World ethnobotanical information reported that a number of herbal medicines from plants and vegetables are used for controlling hyperlipidemia and related complications in patients (5). *Anthocephalus indicus:* the *cadamba Miq* (family Rubiaceae, Hindi name; Kadamba) is one such Ayurvedic remedy that has been mentioned in many Indian medical literatures for the treatment of fever, anemia, uterine complaints, menorrhigia, blood and skin diseases, diarrhea, colitis, stomatitis, dysentery and in improvement of semen quality (6,7). *Anthocephalus indicus* grows through out India, especially at low levels in wet places. In traditional system of remedies warm aqueous extract of *A. indicus* leaves have been used to alleviate the pain, swelling and for cleansing and better wound healing. Recently, *A. indicus* has been reported to posses

For reprints and all correspondence: Dr Raj Kumar Singh, Senior Professor & Head, Department of Biochemistry, King George's Medical University, Lucknow-226003, India. Tel: +91-522-2257449; Fax: +91-522-2257539; E-mail: singhrk23a@hotmail.com

antimicrobial, wound healing, antioxidant, antimalarial and hepatoprotective activity (8–10). The fruit juice of the plant augments the quantity of breast milk of lactating mothers and also works as a lactodepurant (11). Root extract of this plant is salutary in urinary ailments like dysurea, calculi and glycosuria (11). There are reports that heartwood, leaves, flower and seeds of *A. indicus* contain typical alkaloid; Cadambine and its derivatives, some complex polysaccharides and other common constituents (12). However, pharmacological effects of these compounds are not well known. Also much work has not so far been done to explore other biological activities and/or the isolation of active ingredients from the root. Therefore, the present study was designed to investigate the lipid lowering activity of *A. indicus* root in hyperlipidemic rats. Furthermore, to explain the positive role in protection against oxidative degradation of body lipids, the antioxidant potential of natural product was also assessed *in vitro*.

Methods

Preparation of Root Extract

Anthocephalus indicus roots were collected from Lucknow and identified taxonomically by the Department of Pharmacology, Era's Lucknow Medical College, Lucknow. A voucher specimen was also submitted. Roots were dried under shade and made into fine powder using laboratory mill. Powder (100 g) was extracted thrice with 500 ml portions of 95% ethyl alcohol in a laboratory perculator at room temperature. Time allowed for each extraction was 4 h. The root extract obtained after third extraction was colorless. All the extracts were mixed (1400 ml), alcohol was distilled out at reduced temperature (20°C) and reduced pressure (100 psi) in a rotatory evaporator fitted with vacuum pump. This whole mass was taken out in a pre-weight beaker and subjected to vacuum drying for 6 h. Finally this yielded 6.5 g of crude extract, which was used for *in vivo* and *in vitro* studies. Triton WR-1339 and standard lipid lowering drug; gemfibrozil were procured from Sigma Chemical Company, St Luis, MO, USA.

Lipid Lowering Activity in Triton Induced Hyperlipidemia

Animal study was performed with the approval of Animal Care Committee of Division of Laboratory Animals; Central Drug Research Institute, Lucknow, India and confirmed to the guide for the Care and Use of Laboratory Animals (CDRI, Lucknow). Male adult rats of Charles Foster strain (200–225 g) bred in the animal house of the Institute were used. The animals were divided in four groups. 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.) and provided with standard pellet diet (Lipton India, Ltd) and water *ad libitum*. Details about dietary ingredients and nutrients of pellet diet are given in Table 1. Hyperlipidemia in rats was induced by a single injection of triton WR-1339 (400 mg kg^{-1}, b.w.). Triton was diluted with normal saline (400 mg ml^{-1}) and injected 1 ml kg^{-1} b.w. to each rat intraperitoneally (13). The root extract as well as gemfibrozil were macerated (50 mg ml^{-1}) with 2% aqueous gum acacia and fed orally (10 ml kg^{-1}, b.w.) at a dose of 500 mg kg^{-1}, b.w. simultaneously with triton. Control animals received the same amount of vehicle. The experimental design and schedule of treatment was followed as:

Group 1: Control rats fed with aqueous gum acacia.

Group 2: Triton WR-1339 (400 mg kg^{-1}, b.w.) induced hyperlipidemic rats fed with aqueous gum acacia (13,14).

Group 3: Triton induced hyperlipidemic rats treated with *A. indicus* root extract (500 mg kg^{-1}, b.w.) (10).

Group 4: Triton induced hyperlipidemic rats treated with gemfibrozil (500 mg kg^{-1}, b.w.) (15).

Table 1. Ingredient and nutrient composition of the control basal diet fed to rats

Ingredients	Amount (g kg^{-1})
Casein	21
Corn starch	440
Sucrose	100
Maltose dextrin	100
Cellulose	50
Soybean oil	50
Vitamins mix	10
Mineral	35

Other ingredients include choline bitartarate (2 g kg^{-1}) and *t*-butyl hydroquinone (0.008 g kg^{-1}). Proximate analysis 21% crude protein, 5% crude fat, 4% crude fiber, 8% ash.

Blood Collection and Biochemical Analysis in Plasma

At the end of experiment, rats were fasted for 18 h and then injected with heparin solution prepared in normal saline (10 mg ml^{-1}) at the dose of 1ml kg^{-1}, b.w. through tail vain. After 15 min of treatment the animals were anaesthetized with sodium pentothal solution (50 mg kg^{-1}, i.p.) prepared in normal saline. Blood was withdrawn from retro-orbital sinus using glass capillary in EDTA coated tubes (3 mg ml^{-1} blood). The blood was centrifuged and the post-heparin plasma was separated. Plasma was diluted with normal saline in the ratio 1:3 v/v and used for the analysis of lipids and proteins.

Total cholesterol (TC), phospholipids (PL), triglyceride (Tg), protein and post-heparin lipolytic activity (PHLA) were determined according to the methods reported earlier (16).

Estimation of Antioxidant Activity of *A. indicus* Root Extract

Generation of Superoxide Anions

Superoxide anions(O_2^-) were formed in an enzymic system composed of xanthine, xanthine oxidase in the absence or presence of *A. indicus* root extract at different concentrations of 50, 100, 250 and 500 μM. The amount of uric acid formed was assayed spectrophotometrically at 295 nm (17). Another reaction mixture contained xanthine, xanthine oxidase and nitroblue tetrazolium (NBT) with or without addition of root extract (50–500 μM). After incubation the amount of formazone formed, as a result of reduction of NBT by O_2^-, was measured at 560 nm on spectrophotometer(17). The system employed for non-enzymic generation of O_2^- comprised of phenazine methosulphate, NADH and NBT in the absence or presence of root extract (50–500 μM). After incubation the amount of formazone formed was measured as above (18).

Generation of Hydroxyl Radicals

Root extract of *A. indicus*(50–500 μM) was tested against the formation of hydroxyl radicals (OH^-) in an enzymic system composed of hypoxanthine, $FeSO_4.7H_2O$ (Fe^{+2}), sodium ascorbate and xanthine oxidase, assayed for 3,4-dihydroxybenzoate formed by OH^- mediated hydroxylation of salicylate (19). In another set of experiment OH^- were generated non-enzymically by Fe^{+2}, sodium ascorbate, H_2O_2 and deoxyribose. After reaction in the absence or presence of root extract (50–500 μM), the reaction mixture was assayed for malondialdehyde formed (20).

Statistical Analysis

One way analysis of variance (ANOVA-New man's student *t*-test) were performed by comparison of values for triton treated group with control, triton and drug treated groups with triton (21). All hypothesis testing were two-tailed. $P < 0.05$ was considered statistically significant and the results were expressed as mean ± SD. The statistical analysis was carried out by the Graph Pad INSTAT 3.0 software. Similarly, the generations of oxygen free radicals with different concentrations of *A. indicus* root extract were compared with that of their formation without extract. The values were tested for significance at a $P < 0.05$.

Results

Effect of *A. indicus* Root on Triton Induced Hyperlipidemia

The acute administration of triton WR-1339 caused marked increase ($P < 0.001$) in plasma levels of TC (260%), PL (103%), Tg (124%) and protein (81%) following inhibition of PHLA by 24%. Treatment with *A. indicus* root extract caused significant reversal ($P < 0.01$) in these levels of TC, PL, Tg and protein by 22–23% following reactivation of PHLA by 40% (Figs 1, 2, 3). The lipid lowering activity of gemfibrozil (29–37%) was comparatively more than that of *A. indicus*.

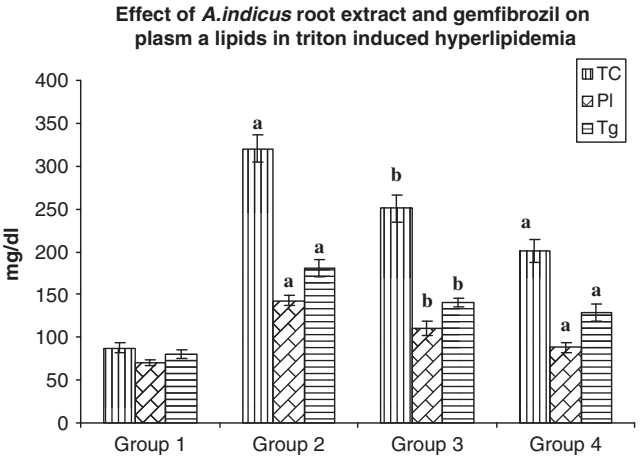

Figure 1. Values are mean ± SD of six rats $^aP < 0.001$, $^bP < 0.01$. Hyperlipidemic (control Group 2) was compared with control (Group 1). Hyperlipidemic + *A. indicus* treated (Group 3) and hyperlipidemic + gemfibrozil (Group 4) was compared with hyperlipidemic control (Group 2).

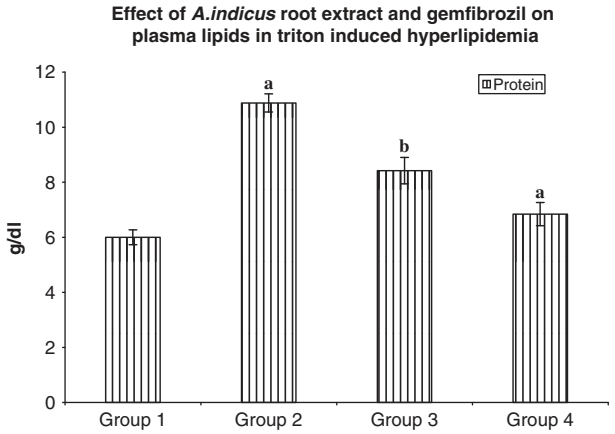

Figure 2. Values are mean ± SD of six rats $^aP < 0.001$, $^bP < 0.01$. Hyperlipidemic (control Group 2) was compared with control (Group 1). Hyperlipidemic + *A. indicus* treated (Group 3) and hyperlipidemic + gemfibrozil (Group 4) was compared with hyperlipidemic control (Group 2).

Effect of *A. indicus* on Generation of O_2^- Anions

Figure 4 shows that enzymic oxidation of xanthine to uric acid by O_2^- (i) as well as the generation of O_2^- anions in xanthine-xanthine oxidase systems, as measured

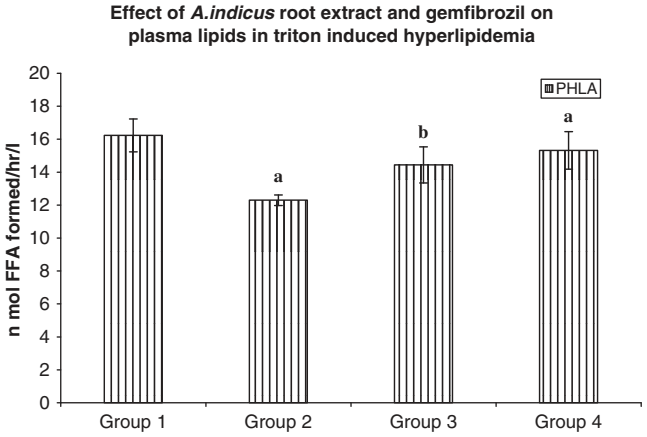

Figure 3. Values are mean ± SD of six rats $^{a}P < 0.001$, $^{b}P < 0.01$. Hyperlipidemic control (Group 2) was compared with control (Group 1). Hyperlipidemic + *A. indicus* treated (Group 3) and hyperlipidemic + gemfibrozil (Group 4) was compared with hyperlipidemic control (Group 2).

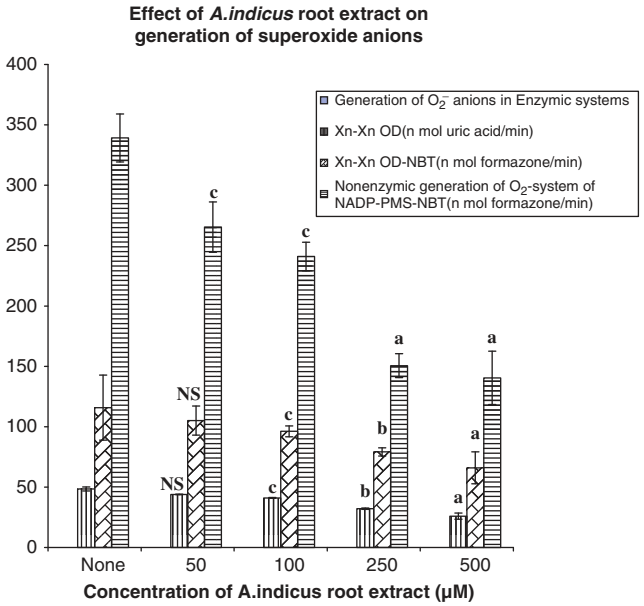

Figure 4. Values are mean ± SD of four separate observations. $^{a}P < 0.001$, $^{b}P < 0.001$, $^{c}P < 0.05$, NS = non significant. The systems added with *A. indicus* were compared with those without adding *A. indicus*.

by reduction of NBT to formazone (ii) was inhibited to varying extents by root extract (50–500 µM) in a concentration dependent manner ($P < 0.05$–0.001) and this effect was maximum by 47% and 43%, respectively, at 500 µM of extract. The root extract also trapped the O_2^- anions generated by non-enzymic system of NADH-phenazine methosulphate as measured by reduction of NBT in the reaction mixture. The effect was dose dependent ($P < 0.05$–0.001) and 59% inhibition was observed at 500 µM of test substance.

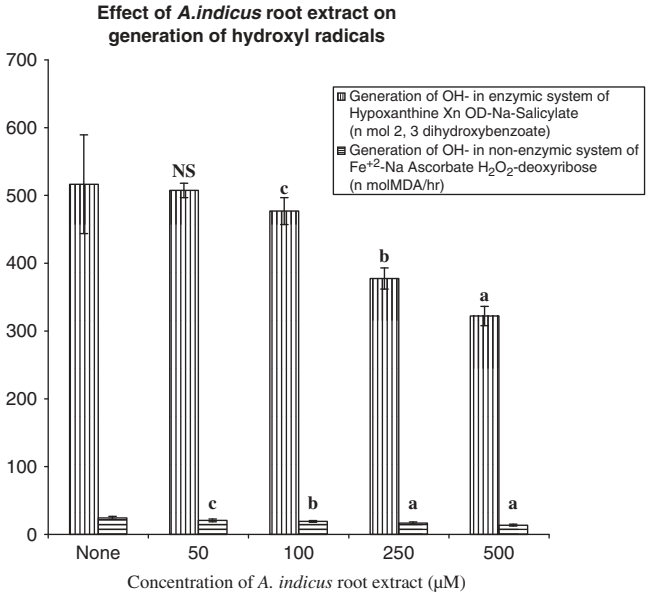

Figure 5. Values are mean ± SD of four separate observations. $^{a}P < 0.001$, $^{b}P < 0.001$, $^{c}P < 0.05$, NS = non significant. The systems added with *A. indicus* were compared with those without adding *A. indicus*.

Effect of *A. indicus* on Generation of OH^- Radicals

The data in Fig. 5 shows that *A. indicus* root extract inhibited the formation of OH^- by enzymic system of hypoxanthine-xanthine oxidase and Fe^{+2}. Addition of extract (50–500 µM) inhibited the OH^- mediated formation of 2,3-dihydroxybenzoate in concentration dependent manner, the significant effects were observed with (100–500 µM) showing 38% inhibition ($P < 0.001$) at 500 µM of extract. Furthermore, this preparation, when added with the reaction mixture containing Fe^{+2}- sodium ascorbate -H_2O_2 employed for non-enzymic generation of OH^-, inhibited OH^- mediated fragmentation of deoxyribose ($P < 0.05$–0.001) into MDA and this was maximum by 44% at 500 µM concentration of root extract.

Discussion

The present study was planned, as there have been reports that various hypolipidemic drugs available in market, like statins, fibrates and bile acid sequestraints have numerous side effects. It has been reported that statins, the inhibitors of cholesterol biosynthesis, regress hypercholesterolemia and also possess antioxidant properties. In our earlier studies we also observed (our unpublished data) that lovastatin at the dose of 20 mg kg^{-1} lowered the blood level of TC, PL and Tg, by 39, 32 and 31%, respectively, in Triton-rat model. Furthermore, we also observed that lovastatin possess potent antioxidant activity as at test concentrations of 5, 10 and 20 µM, it inhibited the generation of OH^- *in vitro* by 62, 80 and 98%, respectively. However, all the statins,

including lovastatin, have many adverse effects. Compactin and mevinolin cause nausea, skin rashes, diarrhea, rise in CPK and transaminases and increases risk of development of premature nuclear cataract. Clinical studies have contraindicated the use of lovastatin in case of pregnant and lactating women (22). Similarly, the bile acid sequestraints, cholestyramine and cholestipol, are reported to cause gastrointestinal problems (23), fibrates cause epigastria, abdominal pain, diarrhoea and also dermatisis, blurred vision and headache (24).Therefore, to overcome these adverse effects there is an urgent need for the development of hypolipidmic drugs from natural resources. In recent year many natural products have been screened for lipid lowering and antioxidant activity (25).

The results of our study demonstrated that *A. indicus* root and gemfibrozil caused significant decrease in the plasma lipid levels in triton induced hyperlipidemic rats. Triton WR-1339 act 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 (13). *Anthocephalus indicus* root extract may have interfered with substrate modifications and stimulated the activity of lipases, and this may be the case as we observed the reactivation of post-heparin lipolytic activity in the above model. Earlier, we have successfully used this model for evaluation of lipid lowering activity of various drugs (14,26,27). The results also showed that *A. indicus* root extract possess antioxidant property as it potentially inhibited the *in vitro* generation of O_2^- and OH^- free radicals in both enzymic and non-enzymic systems. The hydroxyl radical plays a major role in peroxidative damage to lipids and lipoproteins and this, in turn, may be responsible for initiation and progression of atherosclerosis in hyperlipidemic subjects (3). Thus, the antioxidant property of *A. indicus* may help in preventing the oxidative modifications of various vital biomolicules, including lipids and proteins, in hyperlipidemic subjects.

Chemical investigations of *A. indicus* have shown that heartwood and leaves of this plant contain cadambine, 3α and 3β isomers of dihydrocadambine and isodihydrocadambine (28,29). It has been reported that its stem bark contains cadambagic acid along with quinovic acid and β-sitosterol (30). Moreover, a complex polysaccharide from flowers and seeds of *A. indicus* has been isolated (31). The above-mentioned compounds of *A. indicus* may be responsible for exerting beneficial effects. However, not much work has so far been done to investigate the biological activities of these compounds. Chlorogenic acid isolated from *A. indicus* (Cadamba) has been reported to be a potent hepatoprotective agent (10). There are reports that the plant metabolites also possess antimalarial activity (9). Recently, alcoholic and aqueous extracts of *A. indicus* are reported to have significant antimicrobial, wound healing and antioxidant activities (8). It is suggested that the antimicronbial activity may be due to the presence of terpenes (32). It is suggested that *A. indicus* root may have typical saponins, terpenes, glycosides and alkaloids which may be responsible for lipid lowering and antioxidant activities, as observed by us, however, this needs further confirmation.

In conclusion, it may be stated that the results of the present study demonstrated new properties *of A. indicus* root as a potent lipid lowering and antioxidant agent and these beneficial activities may contribute to its cardio protective and antiatherosclerotic role. Moreover, further studies on drug metabolism and to assess the biological activity, *in vivo* and *in vitro*, of *A. indicus* root and its various fractions are under progress to substantiate the present findings.

References

1. Zalba G, San JG, Moreno MU, Fortuno A, Beaumont FJ, Diez J. Oxidative stress in arterial hypertension. Role of NADPH oxidase. *Hypertension* 2001;38:1395–9.
2. Rehman S, Mahdi AA, Hasan M. Trace metal induced lipid peroxidation in biological system. *SFRR (India) Bull* 2003;2:12–18.
3. Parthasarthy S, Steinberg D, Seitztum JL. The role of oxidized low-density lipoprotein in the pathogenesis of atherosclerosis. *Ann Rev Med* 1992;43:219–25.
4. Chattopadhyaya R, Pathak D, Jindal DP. Antihyperlipidemic agents. A review. *Indian drugs* 1996;33:85–97.
5. Dahanukar SA, Kulkarni RA, Rege NN. Pharmacology of medicinal plants and natural products. *Indian J Pharmacol* 2000;32:S81–S118.
6. Sircar NN. Pharmacological basis of Ayurvedic therapy. In: Atal CK, Kapoor BM (eds). *Cultivation and Utilization of Medicinal Plants*. New Delhi, India: Publication and Iinformation Directorate, CSIR; 1992, 507–18.
7. Slkar IV, Kakkar KK, Chakre OJ. *Glossary of Indian Medicinal Plants With Active Principles*. New Delhi: CSIR, 1992, 75.
8. 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;4:481–7.
9. Sianne Schwikkard, FanieR van Heerden. Antimalarial activity of plant metabolite. *Nat Prod Rep* 2002;19:675–92.
10. Kapil A, Kouli B, Suri OP. Antihepatotoxic effects of chlorogenic acid from *Anthocephalus Cadamba*. *Phytother Res* 1995;9:189–93.
11. Paranjpe P. Indian medicinal plants, forgotten healeres. In: Paranjpe P (ed). *A Guide to Ayurvedic Herbal Medicine*. Delhi, India: Chaukhamba Sanskrit Pratishthan, 2001, 112–3.
12. Shaua NP, Koike K, Jia Z, Banerjee S, Mandal NB, Nikaido T. Triterpene glycoides from bark of *Anthocephalus Cadabma*. *J. Chem Res* 2000;1:22–3.
13. Schurr PE, Schultz JR, Parkinson TM. Triton induced hyperlipidemia in rats as an animal model for screening of hypoliperimic drugs. *Lipids* 1972;7:68–74.
14. Nityanand S, Kapoor NK. Cholestrol lowering activity of the various fractions of the guggul. *Indian J Exp Biol* 1973; 11:395–6.
15. Schweitzer M, Tessier D, Vlahos WD, Leiter L, Collet JP, McQueen MJ, et al. A comparison of pravastatin and gemfibrozil in the treatment of dyslipoproteinemia in patients with non-insulin-dependent diabetes mellitus. *Atherosclerosis* 2002;162:201–10.
16. Rizvi F, Puri A, Bhatia G, Khanna AK, Wulff EM, Rastogi AK, et al. Antidyslipidmic action of fenofibret in dyslipidemic diebetic hamstermodel. *Biochem Biophys Res Commun* 2003;305:215–22.
17. Bindoli A, Valente M, Cavallim L. Inhibition of xanthine oxidase and xanthine dehydrogenase activity. *Pharmacol Res Commun* 1985;17:831–9.

18. McCord JM, Fridovich I. Superoxide dismutase, an enzyme function for erythrocuprin (hemocuprin). *J Biol Chem* 1969;244:6049–55.
19. Richmond R, Halliwell R, Chauhan J, Darbre A. Superoxide dependent formation of hydroxyl radicals: detection of hydroxyl radicals by hydroxylation of aromatic compounds. *Anal Biochem* 1981;118:320–35.
20. Halliwell B, Gutteridge JMC, Arouma OI. The deoxyribose method: a simple test tube assay for determination of rate constants for reaction of OH- radicals. *Anal Biochem* 1987;165:215–9.
21. Woodson RF. *Statistical Methods for the Analysis of Biochemical Data*. Chichester: Wiley, 1987, 315.
22. Mabuchmet H, Sakai T, Sakai Y, Yoshimura A, Watanbe A, Wakasugi T, et al. Reduction of serum cholesterol in heterozygous of familial hypercholesterolemia additive effects of compactin and cholestyramine. *New Engl J Med* 1983;308:609–13.
23. Casdorph HR. Treatment of hypercholesteremia with cholestyramine, a bile acid sequestering resin. *Vasc Dis* 1976;4:305–8.
24. Frick MH, Nikkila EA. Helsinki Heart Study: primary prevention trail with gemfibrozil in middle aged men with dyslipidemia. *New Engl J Med* 1987;317:1237–45.
25. Emami SA, Asili J, Mohagheghi Z, Hassanzadeh MK. Antioxidant activity of leaves and fruits of *Iranian conifers*. *Evid Based Complement Alternat Med* 2007;4:313–9.
26. Khanna AK, Chander R, Kapoor NK, Dhawan BN. Hypolipidemic activity of picroliv in albino rats. *Phytother Res* 1994;8:403–7.
27. Khanna AK, Rizvi F, Chander R. Lipid lowering activity of *Phyllanthus niruri* in hyperlipidemic rats. *J Ethnopharmacol* 2002;82:19–22.
28. Brown RT, Fraser SB, Banerji J. Heart wood of cadamba contains glucoalkaloids of isodihydrocadambine. *Tetrahedron Lett* 1974;29:3335.
29. Brown RT, Chapple CL. *Anthocephalus* alkaoids: 3α- dihydrocadambine and 3β- isodihydrocadambine. *Tetrahedron Lett* 1976;31:2723–4.
30. Sahu NP, Mahto SB, Banerji N, Chakravarti RN. Cadamibic acid. A new triterpenic acid from *Anthocephalus cadamb*. *Indian J Chem* 1974;17:284–6.
31. Chandra O, Gupta D. A complex polysaccharide from the seeds of *Anthocephalus indicus*. *Carbohydr Res* 1980;83:85–92.
32. Toyota M, Asakawa Y. Sesquiterpenoids and cyclic bis (bibenzyls) from the Pakistani Liverwort *Plagiochasma appendiculatum*. *J Hattori Bot Lab* 1999;86:161–7.

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