HYPOGLYCEMIC, LIPID LOWERING AND ANTIOXIDANT ACTIVITIES IN ROOT EXTRACT OF *ANTHOCEPHALUS INDICUS* IN ALLOXAN INDUCED DIABETIC RATS

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ABSTRACT

The present study was carried out to evaluate the hypoglycemic, lipid lowering and antioxidant activities in root extract of *Anthocephalus indicus* (*A indicus*) in alloxan induced diabetic rats. Oral administration of ethanol extract of root (500mg/ kg body weight) for 21 days resulted in significant decrease in the levels of blood glucose, triglycerides, total cholesterol, phospholipid and free fatty acids. Furthermore, the root extract (100-400µg) inhibited the generation of superoxide anions and hydroxyl radicals, in both enzymic and non-enzymic systems, in vitro. The result of the present study demonstrated hypoglycemic, lipid lowering and antioxidant activities in root extract of *A indicus*, which could help in prevention of diabetic dyslipidemia and related diseases.

KEY WORDS

Anthocephalus indicus, Hypoglycemic agent, Oxygen free radical scavengers, Lipid lowering agent, Plant antioxidant.

INTRODUCTION

Anthocephalus indicus (family, Rubiaceae: Hindi name Kadam) is one such Ayurvedic remedy that has been mentioned in many ancient Indian medical literatures to possess antidiarrhoeal, detoxifier, analgesic and aphrodisiac properties (1,2). The plant is found in plenty throughout India specially at low levels in wet places. In traditional system of remedies, warm aqueous extract of Kadam leaves have been used to alleviate pain, swelling and for cleansing and better healing of wounds as well as for the treatment of menorrhagia. The decoction of bark of this plant is effective in diarrhoea, dysentery and colitis. The root extract of Kadam is salutary in urinary ailments like dysurea, calculi and glycosuria. Chemical investigation of Kadam has shown that heartwood and leaves of this plant contain cadambine, 3α and 3β isomers of

dihydrocadambine and isodihydrocadambine (3,4). Stem bark contains cadambagic acid along with quinovic acid and β sitosterol (5). A complex polysaccharide from flower and seeds of *A. indicus* has also been isolated. The above mentioned compound of this plant may be responsible for exerting beneficial effects. Furthermore fruit juice of the plant augments the quality of breast milk of lactating mothers and also works as a lactodepurant (7,8). Recently we have reported that alcoholic extract of *A. indicus* root exert lipid lowering activity in triton induced hyperlipidemic rats and *in vitro* experiments showed that it also possess antioxidant activity (9). Furthermore, fruit extract of this plant possesses hypolipidemic activity in triton and high fat diet induced hyperlipidemia probably due to reactivation of post heparin lipolytic activity in above models (10).

Diabetes mellitus is a major public health problem in the developed as well as developing countries (11). It is ranked seventh among the leading cause of death and third when its fatal complications are taken in to account (12). Diabetes is a syndrome, initially characterized by a loss of glucose homeostasis. The disease is progressive and is associated with oxidative stress with high risk of diabetic dyslipidemia which is responsible for micro and macro vascular

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complications of diabetes mellitus (13). Currently available therapy for diabetes and diabetic dyslipoproteinemia include insulin and various oral antidiabetic agents such as sulfonylurea, metformin, α -glucosidase inhibitors, troglitazone (14) and antidyslipoproteinemic agents as gemfibrozil and flavastatin are used (15), but they cause a number of serious adverse effects in patients. On the other hand it is considered that natural products are free from side effects and safe. This study was, therefore, planned to explore antidiabetic, antioxidant and lipid-lowering activity of *A. indicus* root in alloxan induced diabetic rats.

MATERIALS AND METHODS

Preparation of root extract: *A. indicus* roots (secondary and tertiary) were collected from local area of 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 (100g) was extracted thrice with 500ml portions of 95% ethyl alcohol in a laboratory perculator at room temperature. Time allowed for each extraction was 4 hrs. The root extract obtained after third extraction was colour less. All the extracts were mixed (1400ml), alcohol was distilled out at reduced temperature (20°C) and pressure (100psi) in a rotatory evaporator. This whole mass was taken out in a pre-weight beaker and subjected to vacuum drying for 6 hrs. Finally this yielded 6.5g of crude extract which was used for *in vivo* and *in vitro* studies. Alloxan monohydrate and standard drug glibenclamide were procured from Sigma Chemical Company St. Louis, MO, USA.

Hypolipidemic activity in Alloxan induced diabetic rats:

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 (200-225g) 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 AM to 08:00 PM) and provided with standard pellet diet (Lipton India, Ltd) and water *ad libitum*. Diabetes was induced by intraperitoneal injection of alloxan monohydrate 120mg/Kg b.w in animals (11). After three days of injection, diabetes was confirmed by glucometer (16). Rats with serum glucose level 280-367mg/dL were taken for the study.

Experimental Design: The rats were divided in four groups

having six animals in each as follows: Group 1, Control rats (on normal saline); Group 2, Alloxan treated diabetic rats (on normal saline); Group 3, Alloxan treated diabetic rats + *A. indicus* (500mg/kg b.w); Group 4, Alloxan treated diabetic rats + glibenclamide (600µg/kg b.w). After 21 days of feeding rats were fasted overnight and blood was withdrawn from retro-orbital plexus. The blood was used for the estimation of glucose level (17) simultaneously serum was separated and used for the estimation of total cholesterol: TC (18), phospholipid: PL (19), triglyceride: TG (20) and free fatty acids: FFA (21).

In vitro Antioxidant activity:

Enzymic and Nonenzymic generation of superoxides:

The effect of *A. indicus* root extract on the generation of superoxide anions (O_2^-) *in vitro*, in an enzymic system of xanthine-xanthine oxidase was investigated (22). Xanthine oxidase activity in a system (A) containing Xanthine and different concentrations of root extract (100-400µg) added with 0.03 U/ml of Xanthine oxidase in phosphate buffer, was assayed spectrophotometrically at 295nm. The change in optical density corresponding to amount of uric acid formed was compared with reaction mixture which did not include with their test substance. The influence of root extract on nitroblue tetrazolium (NBT) reduction by O_2^- anions, was measured in a reaction mixture (B) containing Xanthine-xanthine oxidase and NBT in absence or presence of extract (100-400µg). After incubation, the amount of formazone formed was measured at 560nm on spectrophotometer. Another system employed for non-enzymic generation of O_2^- anion was comprised of phenazine methosulphate, NADH and NBT (23). After 90 sec incubation in absence or presence of test extract 100-400µg, the amount of formazone formed was read at 560nm against respective reagent blank.

Enzymic and non-enzymic generation of hydroxyl radical:

A. indicus root extract (100-400µg) was tested against the formation of hydroxyl radical ($OH^\cdot$) *in vitro* in an enzymic system composed of sodium salicylate, $FeSO_4$, Hypoxanthine and Xanthine oxidase, assayed for 2,3 dihydroxybenzoate formed by $OH^\cdot$ radical mediated hydroxylation of salicylate on spectrophotometer at 510nm (24). In another set of experiment, $OH^\cdot$ were generated non-enzymatically by $FeSO_4$, sodium ascorbate, H_2O_2 and deoxyribose. After reaction in the absence or presence of root extract (100-400µg), incubation mixture was assayed for malondialdehyde formed (25).

One way analysis of variance (ANOVA-New man's student *t*-test) was performed by comparison of values for alloxan treated group with control, alloxan and drug treated groups

Table 1: Effect of *A. indicus* root extract on biochemical parameters in blood of alloxan induced diabetic rats

Groups	Blood Glucose (mg/dl)	Serum Total Cholesterol (mg/dl)	Serum Triglyceride (mg/dl)	Serum Phospholipid (mg/dl)	Serum Free Fatty acid (μ mol/L)
Control	87.00 $\pm$ 9.67	87.48 $\pm$ 13.31	80.00 $\pm$ 7.77	70.09 $\pm$ 8.23	1.68 $\pm$ 0.17
Alloxan treated	316.79 $\pm$ 24.90** (+3.60 fold)	129.83 $\pm$ 11.90** (+1.5 fold)	157.42 $\pm$ 8.17** (+2.0 fold)	92.00 $\pm$ 8.20** (+ 1.3 fold)	2.56 $\pm$ 0.30** (+ 1.52 fold)
Alloxan + <i>A. indicus</i> (500mg/Kg b.w)	248.63 $\pm$ 23.13** (-22 %)	97.96 $\pm$ 10.05** (-24 %)	123.06 $\pm$ 10.51* (-22 %)	72.93 $\pm$ 5.84* (-22 %)	2.32 $\pm$ 0.38 (- 9.0 %)
Alloxan + glibenclamide (500 ig/Kg b.w)	175.38 $\pm$ 16.67** (-45 %)	105.36 $\pm$ 17.05** (-19 %)	116.51 $\pm$ 12.35** (-25 %)	89.04 $\pm$ 7.86 (- 3.1 %)	1.88 $\pm$ 0.29** (-27 %)

Values are expressed as mean $\pm$ SD of six rats * $p < 0.05$, ** $p < 0.001$, rest not significant.

Alloxan treated diabetic group is compared with normal rats and alloxan + drug treated groups with diabetic group.

with alloxan (26). All hypothesis testing were two-tailed. $P < 0.05$ was considered statistically significant and the results were expressed as mean $\pm$ SD. The statistical analysis was carried out by the Graph Pad INSTAT 3.0 software. Similarly, the generation 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 $P < 0.05$.

RESULTS

Effect of *A. indicus* in alloxan induced hyperglycemia:

The acute administration of alloxan caused marked increase in their plasma levels of blood glucose 3.6 fold, TC 1.50 fold, TG 2.0 fold, PL 1.3 fold and FFA 1.52 fold. However, treatment with *A. indicus* root extract caused reversal in these levels of

blood glucose by 22.0%, TC by 24.0%, PL by 22.0%, Tg by 22.0% and FFA by 9.0%. The hypoglycemic and lipid lowering activity of test extract was comparatively less to that of glibenclamide (Table 1).

Effect of *A. indicus* on generation of superoxide anions:

The data in Table 2 showed that enzymic oxidation of xanthine to uric acid (A) as well as the generation of $O_2^{\cdot -}$ anions in xanthine-xanthine oxidase system, as measured by reduction of NBT to Formazone (B) were inhibited to varying extents by root extract in a concentration dependent manner and this effect was maximum by 46% and 40% respectively at 400 μ g/ml of test sample. The root extract also trapped the $O_2^{\cdot -}$ anions generated by nonenzymic system of NADH-phenazine-methosulphate and were responsible for reduction of NBT in the reaction mixture. The effect was dose dependant and was

Table 2: Effect of *A. indicus* root extract on generation of oxygen free radical *in vitro*

Concentration of <i>A. indicus</i> root extract (μ g/ml)	Generation of $O_2^{\cdot -}$ anions			Generation of OH $^{\cdot}$ radicals	
	Enzymatic System A (Xn-XnOD system) a	B (Xn-XnOD-NBT system) b	Non Enzymatic System (NADH-PMS-NBT System) b	Enzymatic System (Sodium salicylate-FeSO $_4$ HypoXn-XnOD System) c	Non Enzymatic System (FeSO $_4$ -EDTA-H $_2$ O $_2$ -Sod .ascorbate-Deoxy ribose) d
None	48.44 $\pm$ 1.48	116.05 $\pm$ 22.40	334.76 $\pm$ 17.72	549.17 $\pm$ 46.41	25.54 $\pm$ 2.33
100	41.03 $\pm$ 1.04* (-15 %)	98.16 $\pm$ 6.40* (-15)	241.67 $\pm$ 7.35** (-28)	505.66 $\pm$ 11.17 ^{NS} (-8 %)	20.29 $\pm$ 1.29* (-21)
200	35.18 $\pm$ 0.75** (-27)	82.43 $\pm$ 3.64** (-29 %)	163.86 $\pm$ 5.18** (-51)	411.87 $\pm$ 23.20** (-25)	17.10 $\pm$ 0.87** (-33)
300	30.33 $\pm$ 0.75** (-37)	73.86 $\pm$ 3.42** (-36)	156.72 $\pm$ 10.38** (-53)	348.98 $\pm$ 16.09** (-36)	15.53 $\pm$ 2.83** (-39)
400	26.33 $\pm$ 0.52** (-46)	69.88 $\pm$ 0.96** (-40)	148.02 $\pm$ 13.88** (-56)	305.94 $\pm$ 12.73** (-44)	14.92 $\pm$ 0.72** (-42)

Units: a; n mol uric acid formed/min, b; n mol formazon formed/min, c; n mol 2,3 dihydroxy benzoate formed/hr, d; n mol Malondialdehyde formed/hr. Values are mean $\pm$ SD of four separate of observations, * $P < 0.05$, ** $P < 0.01$ NS= Non significant as compared to respective controls in which no test extract was added. Values in the parenthesis are % inhibition.

highest by 56% at 400µg/ml of test substance.

Effect of *A. indicus* on generation of OH⁻ radicals: The data in Table 2 also showed that *A. indicus* root extract inhibited the formation of OH⁻ by enzymic system of hypoxanthine-xanthine oxidase and Fe⁺². Addition of extract (100-400µg) inhibited the OH⁻ mediated formation of 2,3 dihydroxybenzoate in concentration dependent manner which was 44% at 400µg/ml of test extract. Furthermore, this preparation, when added with the reaction mixture containing Fe⁺²-sodium ascorbate-H₂O₂ employed for nonenzymic generation of OH⁻, inhibited fragmentation of deoxyribose into MDA and this effect was maximum by 42% at peak concentration (400µg/ml) of root extract.

DISCUSSION

A. indicus root and glibenclamide both caused a significant decrease in the plasma levels of blood glucose and serum lipids in alloxan induced hyperglycemia. Although the precise mechanism of alloxan induced diabetes remains unclear, there is increasing evidence that it involves the degeneration of islet β- cells by accumulation of cytotoxic free radicals (12). In our experiment, we have observed higher levels of serum lipids in alloxan treated diabetic rats. The level of serum lipids is usually raised in diabetes and such an elevation represents a risk factor for cardiovascular disease (27). Lowering of serum lipid levels through dietary or drug therapy seems to be associated with a decrease in risk of vascular disease (28). The abnormal high concentration of serum lipids in diabetes is mainly due to the increase in the mobilization of free fatty acids from the peripheral depots, since insulin inhibits the hormone sensitive lipase. On the other hand glucagon, catecholamines and other hormones enhance lipolysis. The marked hyper lipidemia that characterizes the diabetic state may, therefore, be regarded as a consequence of the irregular actions of lipolytic hormones on the fat depots (27). In our study we have also observed higher levels of serum TC, PL, FFA in alloxan induced diabetic rats. Similar results have been reported by other workers in alloxan induced diabetic rats (29). There is no mention in available literature regarding the ability of *A. indicus* root extract to reduce the levels of serum lipids in alloxan induced diabetic rats. First time we have showed that an alcoholic extract of *A. indicus* root possess hypoglycemic and hyperlipidemic activity in alloxan induced diabetic rats.

Furthermore, due to hyperglycemia, increase in non enzymic glycosylation occurs, accompanied with glucose oxidation and these reactions are catalysed by Cu⁺² and Fe⁺² resulting in

the formation of O₂⁻ and OH⁻ radicals which further accelerates the risk of cardiac disease and dyslipoproteinemia (30,31). Traditional medicines derived from medicinal plants are used by about 60% of the world's population. Diabetes is an important human ailment afflicting many from various walks of life in different countries. In India it is proving to be a major health problem, especially in the urban areas. Though there are various approaches to reduce the ill effects of diabetes and its secondary complications, herbal formulations are preferred due lesser side effects and its low cost. One of the etiologic factors implicated in the development of diabetes and its complications is the damage induced by free radicals. To overcome these ailments, a drug having multifold properties such as hypoglycemic, lipid lowering and antioxidant activities together is in great demand. An attempt has been made for the first time to describe new properties of *A. indicus* root as a potent sugar, lipid lowering and antioxidant agent and these beneficial activities may contribute to antglycosuric activities of the natural products (8). Further work on drug metabolism and to assess the biological activity *in vivo* and *in vitro* of *A. indicus* root and its fraction is under progress to substantiate the present findings.

REFERENCES:

1. Jain SK, De Fillips RA. *Anthocephalus indicus*: In Medicinal plants of India. Reference publication Inc.; Algonac, Michigan, 1991; 2: 515-6.
2. Sircar NN. Pharmacological basis of Ayurvedic therapy. In cultivation and utilization of medicinal plants. Atal CK, Kapoor BM eds. Publication and information Directorate, CSIR, New Delhi, India, 1992: 507-18.
3. Brown RT, Fraser SB, Banerji J. Heart wood of Cadamba contains glucoalkaloids of isodihydrocadambine. Tetrahedron Letters 1974, 29: 3335L.
4. Brown RT, Chapple CL. Anthocephalus alkaloids: 3α – dihydrocadambine and 3β – isodihydrocadambine. Tetrahedron Letters 1976,31: 2723-24L.
5. Sahu NP, Mahto SB, Banerji N, Chakravarti RN. Cadambic acid. A new triterpenic acid from Anthocephalus cadam. Ind J Chem 1974; 17: 284-6.
6. Chandra O, Gupta D. A complex polysaccharide from the seeds of Anthocephalus indicus. Carbohydrate Res 1980; 83: 85-92.
7. Chopra RN, Nair SL, Chopra IC. *Anthocephalus indicus*. In Glossary of Indian Medicinal Plants. Eds; Publication and Information Directorate, CSIR, New Delhi, India, 1956; p 20.
8. Paranjpe P. Indian Medicinal Plants, Forgotten Heales, A guide to Ayurvedic Herbal Medicine, Chaukhamba Sanskrit pratishtan, Delhi, India, 2001:112-3.

9. Kumar V, Singh S, Khanna AK, Khan MM, Chander R, Mahdi F, Saxena JK, Singh R, Singh RK. Hypolipidemic activity of *Anthocephalus indicus* (Kadam) in hyperlipidemic rats. *Medicinal Chemistry Research* 2008; 17: 152-8.
10. Kumar V, Khan MM, Khanna AK, Singh R, Singh S, Chander R, Mahdi F, Mahdi AA, Saxena JK, Singh RK. eCAM 2008, DOI L:10.1093/eCAM /nen001.
11. Ragavan B, Krishna Kumari S. Antidiabetic effect of *T. Arjuna* Bark extract in alloxan induced diabetic rats. *Ind J Clin Biochem* 2006, 21 (2): 123-8.
12. Trivedi NA, Mazumdar B, Bhatt JD, Hemavathi KG. Effect of Shilajit on blood glucose and lipid profile in alloxan induced diabetic rats. *Ind J Pharmacol* 2004, 36 (6): 373-6.
13. Pavana P, Sethupathy S, Manoharan S. Antihyperglycemic and Anti lipid peroxidative effect of *Tephrosia purpurea* seed extract in streptozotocin induced diabetic rats. *Ind J Clin Biochem* 2007; 22(1): 77-83.
14. Rao BK, Kesavulu MM, Apparao C. Evolution of antidiabetic effect of *Momordica cynobalaria* Fruit in alloxan diabetic rats. *J Fitoterapia* 2003; 74:7-13.
15. Schweitzer M, Tessir D, Valhos WD, Leiter L, Collet JP, Moqueen MJ, Harvey L, Alaupovic FA Comparison of pravastatin and gemfibrozil in the treatment of dyslipoproteinemia in patients with non insulin dependent diabetes mellitus. *J Atherosclerosis* 2002; 162: 201-10.
16. Al-Awadi FM, Kattar MK, Gumma KA. On the mechanism of the hypoglycemic effect of a plant extract. *Diabetologia* 1985; 28: 432-4.
17. Trinder P. Determination of glucose in blood using glucose oxidase with an alternative oxygen acceptor. *Ann Clin Biochem* 1969; 6: 24-30.
18. Zlatkis A, Zak B, Boyle AJ. A new method for the direct determination of serum cholesterol. *J Lab Clin Med* 1953;41: 486-92.
19. Zilversmit DB, Davis AK, Memphis B, Tenn N. Estimation of phospholipids in biological fluids. *J Lab Clin Med* 1950; 35: 155-60.
20. Van Handel E, Zilversmit DB. Micromethod for direct determination of serum triglycerides. *J Lab Clin Med* 1957; 50(1): 152-7.
21. Mosinger F. Photometric adaptation of Dole's microdetermination of free fatty acids. *J lipid Res* 1965; 6:157-9.
22. Bindoli A, Valante M, Cavallin L. Inhibition of Xanthine oxidase and xanthine dehydrogenase activity. *Pharmacol Res Communication* 1985;17: 831-9.
23. McCord JM, Fridovich I. Superoxide dismutase, an enzyme function for erythrocyte (hemocuprin). *J Biol Chem* 1969;244: 6049-55.
24. Richmond R, Halliwell R, Chauhan J, Darbre A. Superoxide dependent formation of hydroxyl radicals by hydroxylation of aromatic compounds. *Anal Biochem* 1981; 118: 320-35.
25. 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.
26. Woodson RF. In: Statistical Methods for the Analysis of Biochemical Data. Chichester: Wiley. 1987; 315.
27. A1- shamaony L, A1- Kharaji SM, Twaij HA. Hypoglycemic effect of *Artemisia herba alba*. 11. Effect of a valuable extract on some blood parameters in diabetic animals. *J Ethnopharmacol* 1994; 43:167-71.
28. Rhoads GG, Gulbrandse CL, Kagan A. Serum lipoproteins and coronary artery disease in a population study of Hawaiian Japanese men. *New Eng J Med* 1976; 294: 293-8.
29. Stanley P, Prince M, Menon VP. Hypoglycemic and hypolipidemic action of alcohol extract of *Tinospora cordifolia* roots in chemical induced diabetes in rats. *Phytother Res* 2003; 17: 410-13.
30. Ceriello A. New insights on oxidative stress and diabetic complications may lead to a "Causal" antioxidant therapy. *Diabetes care* 2003; 26:1589-96.
31. Bloomgarden ZT. Aspect of blood pressure, lipid and glycemic treatment *Diabetes Care* 2004; 27: 264-9.