Hypolipidemic and antioxidant activity of *Anthocephalus indicus* (Kadam) root extract

Vishnu Kumar¹, Farzana Mahdi¹, Ramesh Chander¹, Ranjana Singh², Abbas Ali Mahdi³, Ashok Kumar Khanna³, Sanjay Bhatt⁴, Rajeev Singh Kushwaha⁴, Kalbe Jawad⁵, Jitendra Kumar Saxena¹ and Raj Kumar Singh⁴

¹Department of Biochemistry, Era’s Lucknow Medical College & Hospital, Lucknow 226001
²Department of Biochemistry, CSM Medical University, Lucknow 226003
³Division of Biochemistry, Central Drug Research Institute, Lucknow 226003
⁴Department of Biochemistry, Shri Guru Ram Rai Institute of Medical & Health Sciences, Patel Nagar, Dehradun 248001
⁵Department of Biochemistry, UP Rural Institute of Medical Sciences & Research, Saifai, Etawah, UP 206301

Received 06 November 2009; revised 03 March 2010

The present study was carried out to explore the anti-diabetic, anti-dyslipoproteinemic and anti-oxidant activities of *Anthocephalus indicus* root extract in alloxan-induced (150 mg/kg body wt.) diabetic rats. A marked increase in plasma levels of glucose and lipid peroxides accompanied with an elevation in the lipids and apoprotein levels of serum very low density lipoprotein (VLDL) and low density lipoprotein (LDL) following decrease in lipid and protein constituents of high density lipoprotein (HDL) were observed. The alterations in lipoprotein pattern was associated with inhibition of lipolytic and antioxidant enzymes. Oral administration of root extract (500 mg/kg body wt.) for 30 days in dyslipidemic animals resulted in significant decrease in plasma glucose, total cholesterol, phospholipids, triglyceride and lipid peroxides. The decrease of lipids and apoprotein levels of VLDL and LDL were followed by stimulation of plasma post-heparin lipolytic activity and lecithin cholesterol acyltransferase as well as hepatic superoxide dismutase and catalase activities. Lipid and apoprotein levels of HDL were also recovered partially on treatment with root extract.

**Keywords:** Cholic acid, Deoxycholic acid, Lipid peroxides, Superoxide dismutase, Catalase, Lipoprotein lipase, Triglyceride lipase, Lecithin cholesterol acyltransferase.

*Anthocephalus indicus* (Family: Rubiaceae) is commonly known as Kadam and its roots, bark, leaves and fruits have been used in Ayurvedic remedy for skin diseases and metabolic disorders and also mentioned in many other ancient Indian medical texts to possess antidiarrheal, detoxifier, analgesic and aphrodisiac properties.³,⁴ The plant is found abundantly throughout India, especially at low altitude and in humid places. In traditional system of medicine, warm aqueous extract of leaves has been used to alleviate the pain, swelling and for cleansing and healing of wounds and in treatment of menorrhagia. The decoction of bark is effective in diarrhea, dysentery and colitis. The root extract is helpful in urinary ailments like dysuria, calculi and glycosuria.

The heartwood and leaves contain 3 α and 3 β isomers of dihydrocadambine³,⁴ and stem bark cadambagic acid, quinovic acid and β sitosterol⁵. A complex polysaccharide from flower and seeds has also been isolated⁶. The above-mentioned compound (cadambagic acid, quinovic acid, β sitosterol, 3 α and 3 β isomers of dihydrocadambine) of the plant may be responsible for its antidiarrheal, detoxifier, analgesic and aphrodisiac properties. Furthermore, fruit juice augments the quality of breast milk of lactating mothers and act as a lactodepurant⁷. Recently, we reported that alcoholic extract of *A. indicus* root exerts hypoglycemic activity in alloxan-induced diabetic rats and hypolipidemic activity as well as anti-oxidant activity⁸,⁹,¹⁰.

Diabetes mellitus (DM) is a chronic disease caused by the inherited and/or acquired deficiency in production of insulin by pancreas or by the
ineffectiveness of the insulin produced, which leads to hyperglycemia and at later stages lipid metabolism is also affected\textsuperscript{11,12}. DM in human and experimental models also increased oxidative stress due to persistent and chronic hyperglycemia\textsuperscript{13}, and protein glycation\textsuperscript{14} due to depletion of antioxidant defense system\textsuperscript{15}, thus promotes \textit{de novo} free radical generation\textsuperscript{16}. Current therapies used for controlling diabetic complications are associated with several side effects\textsuperscript{17}. Moreover, as synthetic antioxidants are suspected to be carcinogenic\textsuperscript{18}, there is a need for effective, safe and better oral hypoglycemic agents.

Herbal formulations are preferred due to lesser side effects and their low cost. One of the etiologic factors implicated in the development of diabetes and its complications is the damage induced by free radicals. Thus, a drug having multi-fold properties such as anti-diabetic, lipid lowering and antioxidant activities is in great demand. Therefore, in the present study, an attempt has been made to explore hypolipidemic and antioxidant activities of \textit{Anthocephalus indicus} (Kadam) root extract.

**Material and Methods**

Alloxan monohydrate and standard drug glibenclamide were procured from Sigma Chemical Co., St. Louis, MO, USA.

**Preparation of root extract**

\textit{A. indicus} (secondary and tertiary) roots were collected from local area of Lucknow and identified taxonomically by the Department of Pharmacology, Era’s Lucknow Medical College & Hospital, Lucknow and a voucher specimen was also submitted to Department of Pharmacology, Era’s Lucknow Medical College & Hospital, Lucknow (AI-001/06). Roots were dried under shade and made into fine powder using laboratory mill and powder (250 g) and extracted thrice with 1500 ml portions of 95% ethyl alcohol in a laboratory percolator at room temperature\textsuperscript{9}. Time allowed for each extraction was 8 h. The root extract obtained after third extraction was colorless. All the extracts were mixed (total amount of solvent of 3 extractions were 1500 ml), alcohol was distilled out at reduced temperature (20°C) and reduced pressure (100 psi) in a rotor evaporator. The whole mass was then taken out in a pre-weighed beaker and subjected to vacuum drying for 6 h which yielded 16 g of crude extract, which was used for \textit{in vivo} and \textit{in vitro} studies.

**Animals and treatment**

Animal study was performed with the approval of Animal Care Committee of Division of Laboratory Animal, Central Drug Research Institute, Lucknow, India and confirmed to the guidelines for Care and Use of Laboratory Animals of the Institute. Male adult rats of \textit{Charles Foster} strain weighing 200-225 g bred in the animal house of the Institute were used. The rats were divided into four groups having six animals each as follows: Group 1: Control rats; Group 2: Diabetic rats (on normal saline); Group 3: Diabetic rats + \textit{A. indicus} (500 mg/kg b.w.)\textsuperscript{8-10}; Group 4: Diabetic rats + Glibenclamide (600 µg/kg b.w.).\textsuperscript{20} After 30 days of feeding rats were fasted overnight and blood was withdrawn from the retro-orbital plexus. A group of normal rats without treatment with alloxan were also included to serve as control. These animals were housed in polypropylene cages and kept in uniform hygienic conditions, temperature 25-26°C, relative humidity 60-70% and 12/12 h light/dark cycle (light from 08:00 to 20:00) and provided with standard pellet diet (Lipton India Ltd.), and water \textit{ad libitum}. Diabetes was induced in rats by a single intraperitoneal injection of alloxan monohydrate 150 mg/kg b.w. in 18 animals. After 2 weeks, rats with serum glucose level 280-367 mg/dl were taken for the study\textsuperscript{19}.

**Biochemical analysis**

Serum from above rats was fractionated into very low density lipoprotein (VLDL), low density lipoprotein (LDL) and high density lipoprotein (HDL) by polyanionic precipitation method\textsuperscript{7}. Serum as well as lipoproteins were analyzed for their total cholesterol (TC)\textsuperscript{22}, triglyceride (TG)\textsuperscript{23}, phospholipids (PL)\textsuperscript{24} and apoprotein\textsuperscript{25}. Serum lipid peroxides (LPO)\textsuperscript{26}, free fatty acid (FFA)\textsuperscript{27}, plasma protein\textsuperscript{28}, plasma lecithin cholesterol acyl transferase (LCAT) activity\textsuperscript{29} and PHLA\textsuperscript{30} were also estimated. Liver homogenized (10% w/v) in cold 1 M phosphate buffer (pH 7.2) was used for the assay of lipoprotein lipase (LPL)\textsuperscript{30} and triglyceride lipase (TGL)\textsuperscript{31} activities. Liver homogenate 10% w/v in 0.15 M KCl was also used for the estimation of superoxide dismutase (SOD)\textsuperscript{32} and catalase (CAT)\textsuperscript{33}. The lipid extract of each homogenate was used for the estimation of TC, PL and TG by above-mentioned methods. The rats faeces spilling from all groups over 30 days were collected and processed for the quantification of cholic and deoxycholic acid\textsuperscript{34}.
Statistical analyses
One-way-analysis of variance (ANOVA-Newman’s student test) was performed by comparison of values for alloxan-treated group with control, alloxan and drug-treated with alloxan only. All hypothesis testing were two-tailed. P<0.05 was considered statistically significant and the results were expressed as mean ± SD. The Graph pad INSTAT 3.0 software was used to carried out the statistical analysis.15

Results

Effect of root extract on serum lipid, FFA, LPO and plasma protein in alloxan induced diabetic rats
Table 1 shows that acute administration of alloxan markedly increased in the plasma TC, TG and PL levels (79% ± 12.16, 29% ± 12.04, P<0.001), FFA (52% ± 4.09, P<0.001), LPO, (23% ± 4.67, P<0.001), and decreased plasma protein (31% ± 4.89, P<0.001). However, treatment with A. indicus root extract caused reversal in the levels of TC (21% ± 3.17), PL (20% ± 8.70, P<0.05), TG (29% ± 9.80, P<0.001), FFA (9% ± 1.60), LPO (35% ± 12.35, P<0.001) and protein by 4% ± 1.00. Glibenclamide reversed the levels; TC (18.0% ± 3.13), TG (24.0% ± 9.270, P<0.05), PL (4.0% ± 2.18), FFA (27% ± 3.62, P<0.001), LPO (38% ± 12.28, P<0.001) and protein (8.0% ± 1.45).

Effect of root extract on serum lipoprotein profile in alloxan-induced diabetic rats

Analysis of hyperglycemic serum (Table 2) showed marked increase in the levels of lipids and apoprotein constituting β-lipoproteins (VLDL and LDL) in alloxan-treated group and these effects were more pronounced for VLDL-TC, PL and TG (50% ± 14.25, 28 ± 11.43 and 90% ± 13.30, P<0.001 respectively) and apoprotein (17% ± 6.36, P<0.05). There was decrease in LDL-TC, PL, TG (300% ± 88.82, 45% ± 10.49, 90% ± 13.26, P<0.001 respectively) and apoprotein (20% ± 4.80, P<0.05). The decrease in

Table 1—Effect of A. indicus root extract on serum lipid, protein, lipid peroxides and FFA levels in alloxan-induced diabetic rats

<table>
<thead>
<tr>
<th>Experimental schedule</th>
<th>Total cholesterol (mg/dl)</th>
<th>Triglycerides (mg/dl)</th>
<th>Phospholipids (mg/dl)</th>
<th>Protein (g/dl)</th>
<th>Serum lipid peroxides (n mol MDA/ml plasma)</th>
<th>Serum free fatty acid (µ mol/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>84.23 ± 10.66</td>
<td>87.72 ± 7.54</td>
<td>96.76 ± 11.30</td>
<td>7.13 ± 0.45</td>
<td>2.73 ± 0.49</td>
<td>1.68 ± 0.17</td>
</tr>
<tr>
<td>Alloxan-treated</td>
<td>148.15 ± 26.80 **</td>
<td>173.81 ± 13.60 **</td>
<td>124.88 ± 14.68 **</td>
<td>4.94 ± 0.36 **</td>
<td>8.98 ± 1.40 **</td>
<td>2.56 ± 0.30 **</td>
</tr>
<tr>
<td>Alloxan + A. indicus  (500 mg/kg b.w.)</td>
<td>117.13 ± 20.29 *</td>
<td>123.46 ± 9.60 **</td>
<td>100.25 ± 19.57 *</td>
<td>5.48 ± 0.39 NS</td>
<td>5.80 ± 0.99 **</td>
<td>2.32 ± 0.38 NS</td>
</tr>
<tr>
<td>Alloxan + glibenclamide (600 µg/kg b.w.)</td>
<td>122.18 ± 18.86*</td>
<td>131.78 ± 11.24*</td>
<td>119.45 ± 11.67 NS</td>
<td>5.67 ± 0.69 NS</td>
<td>5.55 ± 0.99 NS</td>
<td>1.88 ± 0.29 NS</td>
</tr>
</tbody>
</table>

Alloxan-treated group was compared with control, alloxan and drug-treated group with alloxan. *P<0.05, **P<0.001, NS = Non-significant.

Table 2—Effect of A. indicus root extract on lipoprotein profile in alloxan-induced diabetic rats

<table>
<thead>
<tr>
<th>Experimental schedule</th>
<th>VLDL</th>
<th>LDL</th>
<th>HDL</th>
</tr>
</thead>
<tbody>
<tr>
<td>TC (mg/dl)</td>
<td>PL (mg/dl)</td>
<td>TG (mg/dl)</td>
<td>Apo-protein (mg/dl)</td>
</tr>
<tr>
<td>Control</td>
<td>8.08 ± 1.99</td>
<td>39.10 ± 3.95</td>
<td>6.85 ± 1.04</td>
</tr>
<tr>
<td>Alloxan-treated</td>
<td>12.09***</td>
<td>20.92***</td>
<td>73.76***</td>
</tr>
<tr>
<td>Alloxan + A. indicus  (500 mg/kg b.w.)</td>
<td>20.92***</td>
<td>73.76***</td>
<td>8.00*</td>
</tr>
<tr>
<td>Alloxan + glibenclamide (600 µg/kg b.w.)</td>
<td>10.04</td>
<td>52.02***</td>
<td>7.20*</td>
</tr>
</tbody>
</table>

Alloxan-treated group was compared with control, alloxan and drug-treated groups with alloxan. ***P<0.001, **P<0.01, *P<0.05, NS = Non-significant.
Effect of root extract on hepatic SOD, CAT, TGL and LPL in alloxan-induced diabetic rats

The data in Table 3 show that administration of alloxan in rats inhibited the activities of SOD (24% ±7.71, \( P<0.001 \)), CAT (25% ±12.76, \( P<0.001 \)), TGL (20% ± 1.83, \( P<0.001 \)) and LPL (25% ± 3.17, \( P<0.001 \)) respectively. The treatment with \( A. \text{indicus} \) roots extract for 30 days increased SOD (25% ± 8.43, \( P<0.001 \)), CAT (25% ± 12.76, \( P<0.001 \)) respectively. The treatment with alloxan + glibenclamide decreased the levels of cholic acid (17% ± 3.93, \( P<0.01 \)) and deoxycholic acid (38% ± 2.19) in feces, as well as LCAT (33% ± 1.27, \( P<0.01 \)) and PHLA (28% ± 1.27, \( P<0.01 \)) in plasma. The treatment with \( A. \text{indicus} \) roots extract for 30 days increased the levels of cholic acid (17% ± 3.93, \( P<0.01 \)) and deoxycholic acid (17% ± 2.17, \( P<0.01 \)) in feces and LCAT (14% ± 1.64, \( P<0.01 \)) and PHLA (13% ± 1.47, \( P<0.01 \)) in plasma of alloxan-induced diabetic rats. Glibenclamide decreased the levels of cholic acid (28% ± 1.22, \( P<0.01 \)) and deoxycholic acid (28%±3.02) in feces also and LCAT (29% ± 8.29, \( P<0.01 \)) and PHLA (25% ± 7.47, \( P<0.01 \)) in plasma (Table 4).

Table 3—Effect of \( A. \text{indicus} \) root extract on hepatic SOD, CAT, TGL and LPL in alloxan-induced diabetic rats

<table>
<thead>
<tr>
<th>Experimental schedule</th>
<th>SOD (Unit/min/mg protein)</th>
<th>CAT (Unit/min/mg protein)</th>
<th>TGL (n mol FFA released/h/mg protein)</th>
<th>LPL (n mol FFA released/h/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>2.80 ± 0.20</td>
<td>3847 ± 248.17</td>
<td>74.11 ± 5.78</td>
<td>85.69 ± 7.71</td>
</tr>
<tr>
<td>Alloxan-treated</td>
<td>2.14 ± 0.16***</td>
<td>2873 ± 402.08***</td>
<td>59.63 ± 4.62*</td>
<td>66.36 ± 5.46**</td>
</tr>
<tr>
<td>Alloxan + ( A. \text{indicus} )</td>
<td>2.69 ± 0.10**</td>
<td>3403 ± 499.69*</td>
<td>69.74 ± 5.18*</td>
<td>80.02 ± 9.81**</td>
</tr>
<tr>
<td>Alloxan + glibenclamide</td>
<td>2.70 ± 0.13***</td>
<td>2625 ± 482.02**</td>
<td>69.85 ± 7.08*</td>
<td>81.89 ± 8.68*</td>
</tr>
</tbody>
</table>

Alloxan-treated group was compared with control, alloxan and drug treated group with alloxan. *\( P<0.05 \), ** \( P<0.01 \), *** \( P<0.001 \).

Table 4—Effect of \( A. \text{indicus} \) root extract on faecal bile acids, plasma lecithin cholesterol acyltransferase and plasma post-heparin lipolytic activities in alloxan-induced diabetic rats

<table>
<thead>
<tr>
<th>Experimental schedule</th>
<th>Faecal bile acids</th>
<th>Plasma lecithin cholesterol acyltransferase activity (n mol cholesterol released/h/l)</th>
<th>Plasma post-heparin lipolytic activity (n mol FFA formed/h/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cholic acid (µg/g feces)</td>
<td>Deoxycholic acid (µg/g feces)</td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>76.31 ± 6.86</td>
<td>56.76 ± 11.36</td>
<td>61.57 ± 4.92</td>
</tr>
<tr>
<td>Alloxan-treated</td>
<td>53.68 ± 6.49**</td>
<td>35.34 ± 7.83**</td>
<td>41.04 ± 3.76**</td>
</tr>
<tr>
<td>Alloxan + ( A. \text{indicus} ) root extract (500 mg/kg b.w.)</td>
<td>62.70 ± 1.90**</td>
<td>41.40 ± 3.81**</td>
<td>46.75 ± 4.92**</td>
</tr>
<tr>
<td>Alloxan + glibenclamide (600 µg/kg b.w.)</td>
<td>68.69 ± 3.81**</td>
<td>44.81 ± 2.08**</td>
<td>53.00 ± 1.23**</td>
</tr>
</tbody>
</table>

Alloxan-treated group was compared with control, alloxan and drug-treated group with alloxan. *\( P<0.05 \), ** \( P<0.01 \).
Discussion
Alloxan selectively damages the β-cells and suppresses the production of insulin in pancreas, thus is used to induce diabetes in laboratory animals. This occurs most likely because of selective uptake of the alloxan, due to its structural similarity to glucose as well as highly efficient uptake mechanism in β-cells. However, alloxan is not toxic to human β-cells, even in very high doses, probably due to differing glucose uptake mechanisms in human and rodents.

In our experiment, we 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 diseases. Lipases play a significant role in lipoprotein metabolism and decreased lipoprotein lipases activities in diabetes is main cause of atherosclerosis. In case of DM, PHLA, serum LPL, HTGL, sciatic nerve LPL and adipose tissue LPL activities have been found to decrease. On the other hand, pancreatic lipase and co-lipase activities increase in pancreatic acinar tissues of diabetic rats. In the present study, activities of SOD and CAT were found to decrease in alloxan-induced diabetic rats and similar observations were reported by other workers.

The treatment with A. indicus recovered the activity of these antioxidant enzymes in alloxan-induced diabetic rats.

The abnormal high concentration of serum lipid in diabetes is mainly due to the increase in the mobilization of free fatty acid from the peripheral depots, since insulin inhibits the hormone sensitive lipase. On the other hand, glucagon, catecholamine and other hormones enhance lipolysis. The marked hyperlipidemia that characterizes the diabetic state may, therefore, be regarded because of the unregulated actions of lipolytic hormones on the fat depots. In the present study, we also observed higher levels of blood glucose, TC, TG, PL and FFA in alloxan-induced diabetic rats. Similar results also have been reported by other workers in alloxan-induced diabetic rats.

A. indicus root and glibenclamide both caused a significant decrease in the plasma levels of TC, TG, PL and FFA in alloxan-induced hyperglycemia. In alloxan-induced diabetic rats, A. indicus increased the level of HDL by stimulating the activity of LCAT, which might contribute to the regulation of blood lipids. LCAT play a key role in lipoprotein metabolism and most of the lipoprotein changes are the outcome of primary abnormality owing to the diseases related with lipid metabolism. A. indicus enhanced the excretion of bile acids through feces and this contributed to regress the cholestesteosis in liver damage.

In conclusion, the lipid lowering activity of A. indicus might be due to inhibition of hepatic cholesterol biosynthesis, activation of tissue lipases, SOD, and CAT. These beneficial effects might be due to bioactive compounds like typical alkaloids, quinovich acid, cadambine and its derivatives present in the root.

Acknowledgement
One of us (V K) is grateful to Director, CDRI, Lucknow for experimental support and Era’s Lucknow Medical college, Lucknow for financial support.

References
KUMAR et al.: HYPOLIPIDEMIC AND ANTI-OXIDATIVE EFFECT OF ANTHOEPhALUS INDICUS ROOT

22 Zlatkis A Zak B & Boyle A J (1953) J Lab Clin Med 41, 486-492
26 Ohkawa H, Ohisha N & Yagi K(1979) Anal Biochem 95, 351-360
27 Mosinger F (1965) J Lipid Res 6, 157-159
30 Wing D R & Robinson D (1968) Biochem J 109, 841-849
38 Nikila E A (1971) Progr Biochem Pharmacol. 6, 102-129
39 Elkeles R S and Hambley J (1977) Diabetes, 26, 58-60