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Lowering of blood sugar by water extract of *Azadirachta indica* and *Abroma augusta* in diabetes rats

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Combination (1:1) of water extract of dried powder of root and leaves (200 mg/kg body wt) of *A. augusta* and *A. indica* respectively was administered orally to alloxan diabetic rats once a day for 8 weeks. This treatment caused significant lowering of blood sugar in fasted as estimated by glucose tolerance test. The treatment resulted in a significant reduction in serum lipids. Aqueous extract also decreased the formation of lipid peroxides estimated as thiobarbituric acid reactive substance, (TBARS), and increased antioxidants (superoxide dismutase, catalase, glutathione peroxidase and glutathione transferase) in erythrocytes. There was reduction in LPO as TBARS in heart, liver, kidney, and muscles. It also prevented decrease in body weight. Present study showed that *Abroma augusta* roots and *A. indica* leaves when given together as water extract had hypoglycaemic action and had better effect than given alone.

Keywords: *Abroma augusta*, *Azadirachta indica*, Blood sugar, Diabetes, Hypoglycaemia

Many herbal products have been described for the care of diabetes mellitus in ancient literature of Ayurveda in India. Hypoglycaemic action in animals and humans by herbs have also been reviewed[1,2]. *Artemisia herba alba* is reported to have hypoglycemic effect[3]. Extracts of ripe leaves, tender leaves, fruits and flowers of *Azadirachta indica* (neem) have been reported to possess antidiabetic and antiviral activity[4,5]. Effect of extract of leaves has been studied on cardiovascular system of anaesthetized monkeys and rabbits[4]. Neem has been shown to possess a number of pharmacological effects like cardiovascular, antimicrobial and immunomodulatory action[6]. Isolated studies have reported anti-inflammatory, immunostimulant and hypoglycaemic effects of *A. indica* leaf extract. One well studied property of neem is its hypoglycaemic effect[7,11]. The plant *Abroma augusta* is effective in the treatment of diabetes and in amenorrhoea. Abromine, the active constituent, has been identified as betaine[12,13]. The leaves contain octacosanol, tarasxerol, β-sitosterol acetate and mixture of long chain fatty diols[13,14]. Recently we have demonstrated the antidiabetic activity of *A. augusta* water extract[15]. In Ayurveda extracts of more than one plant are also used in combination for treatment. Therefore, in the present study, we studied the combined effect of *A. augusta* and *A. indica* in alloxan induced diabetic rats.

Materials and Methods

Plant material—The root bark of *Abroma augusta* was obtained from Khasia Hills, Assam, India and was identified by National Botanical Research Institute, Lucknow. Root bark (25 g) was air-dried and ground in an electrical mill. Freshly collected neem leaves were air dried and powdered as mentioned above. Equal quantities of root and leaves powder of *Abroma augusta* and *Azadirachta indica* respectively were mixed. Powder (25g) of individual plant or mixture of plants was well extracted by soaking in distilled water (100ml) for two days at 4°C with frequent stirring. The extract was concentrated in a lyophilizer and kept separately in airtight containers in a deep freeze until use.

Animals—Healthy adult male albino rats (25) of Wistar strain (100-200 g) originally obtained from Center for Cellular and Molecular Biology, Hyderabad India, were fed on a pellet diet (Hindustan Lever Ltd, Mumbai) and water was provided *ad libitum.*

Induction of diabetes—The method of Sochor *et al.*[16] was followed. Rats were starved overnight and each rat received a single subcutaneous injection of freshly prepared solution of alloxan monohydrate (20 mg/100 g body wt) in sodium-acetate buffer (0.15 *M; p*H 4.5). The volume to be injected was kept

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between 100-150 µl. The same volume of acetate buffer was given to each control rat. Next day a single injection of 2 units of protamine-Zn insulin prepared in normal saline was given to each alloxan treated rat. This procedure was continued for 6 days. It decreased the mortality of the animals. Controls were given the same volume of normal saline instead of insulin. Fasting blood sugar was checked every week from 7th day. Animals became diabetic after one month. Animals with fasting blood sugar >150 mg/dl were used as diabetic rats with or without treatment. Treatment was started after one month when the diabetes was well established.

Blood collection—The blood was collected retro orbitally from the inner canthus of the eye using micro hematocrit capillaries, Mucaps. The blood was collected in oxalate- sodium fluoride in Eppendorf tubes.

Estimation of plasma glucose—It was estimated by glucose oxidase method using the Kit from Ranbaxy Labs, New Delhi, India.

Glucose tolerance test—The standard oral glucose tolerance test was performed on all animals before and after 8 weeks of experiment.

Lipid peroxidation products and antioxidants—Lipid peroxidation (LPO) products were estimated as thiobarbituric acid reactive substance (TBARS) in plasma and tissues[17]. Among the antioxidants, reduced glutathione was determined by the method of Poliodoro *et al*[18]. Superoxide dismutase and catalase was estimated by the method of Wendel[19, 17].

Serum lipid profile—Total cholesterol (TC), HDL cholesterol (HDLC) and triacylglycerols (TG) were estimated using standard kits of Randox, Mumbai . LDL cholesterol (LDLC) was calculated from the above measurement by using Friedwalds formula[20]. LDLC = TC − (HDLC + TG).

Experimental design—Animals were divided into 5 groups of 5 each. Group 1 served as healthy controls. Group 2 were untreated diabetic rats. Group 3 diabetic rats were given water extract of *A. augusta*, Group 4 diabetic rats received *A. indica* and Group 5 diabetic rats were treated with combination of *A. augusta* and *A. indica*. The dose of the extract was 200mg in ml/day for each animal. Rats of Groups 1 and 2 were given 4 ml of saline daily in place of plant extracts.

Results and Discussion

It can be seen from Table 1 that treatment for 8 weeks with the extracts of the plants brought down fasting plasma glucose (FPG) to normal range. The reduction was more (25%) when treated with combination of two plants than *A. indica* (43%) or *A. augusta* (37%) alone. Similar improvement was seen in glucose tolerance studies also (Table2). In *A. augusta* plus *A. indica* treated animals, the peak values (0.5 hr) were in the normal range and by 2 hr the plasma glucose values were not only normal, but even lower than the initial value. The values in the animals treated with only one plant were in between diabetic untreated and diabetic animals treated with both the plants.

Results in Table 3 show that the treatment with water extract of *A. augusta* and *A.indica* for 8 weeks

Table 1 — Effect of water extract of (200 mg / kg body wt) *Abroma augusta* and *Azadirachta indica* alone or in combination on the fasting plasma glucose level in rats*

[Values are mean ± SD of 5 animals]

	Plasma glucose (mg/dl)	
Treatment	0 weeks	8 weeks
Normal	99.4 ± 19.5	89.8 ± 5.2
Diabetic Untreated	172.2 ± 15.4[b]	285.6 ± 42.6[a]
A. augusta	165.6 ± 26.0c	105.4 ± 26.6
A. indica	168.9 ± 28.2[c]	95.8 ± 3.7
A.augusta + *A. indica*	167.9± 98.5[a]	80.1± 2.0

*Initial values were not shown.
[a]$P < 0.001$, [b]$P < 0.01$, [c]$P < 0.05$

Table 2 — Plasma glucose level in glucose tolerance test in normal, diabetic untreated and diabetic treated after 8 weeks

[Values are mean ± SD of 5 animals]

	Plasma glucose (mg/dl)				
Treatment	0 hr	0.5 hr	1 hr	1.5 hr	2 hr
Normal	83.0 ± 6.5	160.5 ± 4.2	145.2 ± 5.3	110.5 ± 6.7	86.3 ± 5.2
Diabetic untreated	160.7 ± 14.3[a]	240.5 ± 77.2[b]	270.0 ± 90.0[b]	282.5 ± 81.4[a]	273.1 ± 83.2[a]
A. augusta	89.8 ± 25.2	115.0 ± 21.1[b]	116.4 ± 16.0	104.0 ± 11.4	90.5 ± 25.0
A. indica	87.8 ± 24.5	111.2 ± 19.6[b]	114.3 ± 15.2[b]	102.0 ± 10.8	82.3 ± 2.5
A.augusta + *A indica*	80.1 ± 2.0	92.0 ± 2.5[a]	86.3 ± 3.0[a]	84.7 ± 2.3[a]	72.5 ± 3.6[c]

[a]$P < 0.001$, [b]$P < 0.01$, [c]$P < 0.02$

improved serum lipid profile, while HDLC was unaffected. However, after treatment with the extract of the two plants, the values of TC and LDLC/HDLC returned to even below the values of normal group and HDLC values were considerably higher only in the group treated with both the plants. This showed that treatment with *A.augusta* and *A. indica* improved lipid profile better than with either plant alone in diabetic animals (Tables 4 and 5). There was increase in LPO and decrease in antioxidant enzymes in diabetic untreated animals. After treatment with plant extracts there was an improvement in treated animals i.e. reduction in LPO and increase antioxidant enzymes. All the three treatments (in combination or individual plant) were more or less equally effective. The diabetic untreated animals showed loss in body weight, reduction in total hemoglobin and sugar in urine (Table 6). After treatment with water extract of combination of two plants, total hemoglobin was nearly normal. The body weight also increased by 15-20% in the treated animals. Experiments will be carried out in future by treating the animals for a longer time to find out obesity in treated animals. The diabetic untreated rats showed retinopathy damage, muscles and skin injury on tail and feet, and paw edema (Fig. 1). All these symptoms recovered after treatment for 8 weeks with the water extract of combination of two plants, *A. augusta* and *A. indica.* These observations on reversal of retinopathy and tissue injury effects by the extract needs experimental evidence and diagnostic documentation. From the improvement in FPG and glucose tolerance in the

Table 3 — Effect of treatment for 8 weeks with water extract (200 mg/kg b.w) of *A. augusta* and *A. indica* alone or in combination on plasma lipid profile of normal, diabetic untreated and diabetic treated rats

[Values are mean ± SD of 5 animals]

Treatment	TC (mg/dl)	LDLC (mg/dl)	HDLC (mg/dl)	LDLC/ HDLC	TG (mg/dl)
Normal	160.0 ± 14.4	82.2 ± 8.79	46.87 ± 8.6	1.77 ± 0.13	110.6 ± 5.2
Diabetic untreated	235.0 ± 16.0[a]	165.8 ± 6.7[a]	48.3 ± 2.5	3.83 ± 0.19[a]	188.5 ± 55.30[c]
A. augusta	185.0 ± 14.8	99.8 ± 16.6	51.3 ± 8.4	1.9 ± 0.4	175.0 ± 15.7[b]
A. indica	175 0 ± 1.2	95.6 ± 16.2	48.2 ± 7.3	2.0 ± 0.4	135.0 ± 13.2[c]
A.augusta + *A. indica*	138.1 ± 35.8	91.1± 13.2	64.6 ± 2.9	1.56 ± 0.03	125.2.± 22.1[b]

[a]$P < 0.001$, [b]$P < 0.01$, [c]$P < 0.02$

Table 4 — Effect of treatment with water extract (200mg/kg b.w) of *A. augusta and A. indica* alone or in combination for 8 weeks on lipid peroxidation product as TBARS (LPO) , SOD , CAT glutathione peroxidase (GPX) and glutathione transferase (GST) activities in erythrocytes in normal , diabetic untreated and diabetic treated rats

[Values are mean ± SD of animals]

Treatment	LPO	SOD	CAT	GPX	GST
Control	286.9 ± 4.5	380.4 ± 44.3	271.8 ± .4.1	27.7 ± 2.0	27.7 ± 2.0
Diabetic untreated	406.8 ± 6.2[a]	298.2 ± 6.3[a]	205.8 ± 3.0[a]	20.2.± 1.7[a]	15.0 ± 1.09[a]
A. augusta	173.4 ± 6.5[a]	332.6 ± 2.4	210.6 ± 12.5[a]	30.6 ± 12.2[a]	35.2 ± 146[a]
A. indica	130.1 ± 40.1[a]	323.5 ± 1.8	218.25 ± 45.2[a]	27.08 ± 8.2[a]	33.2 ± 5.4[a]
A. augusta + *A. indica*	200.4 ± 10.11[a]	325.6 ± 1.5	268.3 ± 11.0[a]	32.2 ± 3.5[a]	26.6 ± 1.8[a]

Values are expressed as — SOD — Units /mg protein; CAT — nmole of H_2O_2/min/mg protein; GPx — nmole of glutathione oxidized/min/mg protein; LPO — nmole of malondialdehyde/mg protein; and GST — µmole of H_2O_2 utilized/min/mg protein.

[a]$P < 0.001$.

Table 5 — Effect of treatment with water extract of *A. augusta* and *A. indica* for 8 weeks on lipid peroxidation product as TBARS in normal, diabetic and diabetic treated rats

[Values are mean ± SD of 5 animals]

Treatment	Concentration of TBARS (m*M*/100g wet tissue)			
	Heart	Liver	Kidney	Muscle
Control	0.45 ± 0.004	0.90 ± 0.006	1.25 ± 0.06	124.30 ± 4.2
Diabetic untreated	2.12 ± 0.02	1.46 ± 0.05	1.78 ± 0.05	200.2 ± 6
A. augusta + *A. indica*	0.38 ± 0.07[a]	0.93 ± 0.04	0.81 ± 0.14[b]	125.3 ± 13.2[b]

[a]$P * < 0.05$; [b]$P < 0.001$

Table 6 — Effect of treatment with water extract of *A. augusta* and *A. indica* alone or in combination on total haemoglobin, body weight and urine sugar of normal, diabetic untreated and diabetic treated rats

[Values are mean ± SD of 5 animals]

Treatment	Haemoglobin (g/100ml)	Body weight (g)	Urine sugar
Normal	16.0 ± 4.2	132.9 ± 15.8	–
Diabetic untreated	10.5 ± 1.2	120.1 ± 2.1	+++
A.augusta	13.6 ± 0.2	155.0 ± 3.1[a] (+ 16%)	–
A. indica	12.9 ± 1.3	153.2 ± 4.2[a] (+ 15%)	–
A. augusta + *A. indica*	14.9 ± 0.3	160.6 ± 20.5[b] (+ 20%)	–

[a]P *< 0.05; [b]$P < 0.001$

Fig. 1 — Diabetic rat suffering from retinopathy damage, muscle and skin injury on tail and feet, and paw edema.

present study, one can presume that either blood insulin levels are elevated or sensitivity to insulin increased during glucose tolerance test. It is also quite possible that the extract directly stimulates the activity of enzymes of pathways of glucose utilization in the tissues. Since the treatment with water extract of the two plants brought down TC and LDLC to normal range and decreased TG levels (Table 3) and increased HDLC, it indicated antihyperglycemic and hypolipidemic effect of the treatment. It is known that in diabetic animals there is an increase in the free radicals and lipid peroxidation products and decrease in the concentration of the antioxidants as seen in the untreated diabetic animals (Tables 4, 5) that was recovered by treatment with plant extract. This shows that the extract of the two plants has antioxidant activity. The untreated diabetic animals showed signs of retinopathy, damage to skin of tail, injury to legs and edema of paws that recovered after treatment of water extract of two plants in combination. In conclusion it can be stated that the water extract of the two plants *A. augusta* and *A. indica*, has a synergistic effect when given in combination. They have a strong antihyperglycaemic, antihyperlipidemic effect and are capable of reversing the free radical and lipid peroxidation mediated tissue damage and other complications like retinopathy, swelling of feet and damage to skin of tail.

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