STUDIES ON THE ANTIHYPERTENSIVE EFFECT OF ABANA IN RATS

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Summary
Treatment with Abana (3 g/kg/day, p.o) for 3 weeks, reduced the blood pressure of unilaterally nephrectomized DOCA-salt-treated hypertensive rats. Similar reduction in blood pressure was also observed in the CdCl₂ (1 mg/kg/day, i.p. for two weeks) treated hypertensive rats. The pressor response to adrenaline and noradrenaline (1 and 2 µg/kg) was reduced by chronic administration of Abana in these hypertensive animals. However, Abana (3 g/kg/day, p.o) administered to normotensive female albino rats for 3 weeks did not alter the mean blood pressure and the pressor response to adrenaline and noradrenaline (1 and 2 µg/kg). The vascular reactivity to noradrenaline in isolated aortic strip and portal vein was also reduced by the chronic administration of Abana in the hypertensive rats. It is concluded that the antihypertensive effect of Abana might be due to its alteration of cation transport into the cell.

Key words
Abana  hypertension  rats

Abana is a herbomineral compound commonly advocated for the prevention and management of various heart diseases. In a series of comprehensive clinical and laboratory investigations, the antihypertensive as well as antianginal significance of this compound has been already proved.

Each Abana tablet contains Terminalia arjuna, withania somnifera, tinospora cordifolia and many other useful plant extracts in their optimum concentrations.

The present study is aimed at determining the antihypertensive effect of Abana in hypertensive animal models to substantiate the clinical findings.

MATERIALS AND METHODS

1. In vivo studies
Experiments on chronic administration of Abana on rat blood pressure and pressor response to adrenaline and noradrenaline: For the first series of experiments, pulverized Abana (3 g/kg/day, p.o) suspension (in water) was administered to normal female albino rats (Haffkine strain, 200-250 g), while the control rats received the same volume of water alone for 3 weeks.

After the treatment schedule, the treated and control rats were anaesthetized with pentobarbitone sodium (40 mg/kg, i.p) and tracheostomy was performed. The mean blood pressure was measured directly through the left common carotid artery by a pressure transducer and recorded on a calibrated polyrite (Medicare) recorder. The femoral vein was cannulated with a needle (24 No. stylus removed) connected to a fine polyethylene catheter for injecting the drugs. Heparinised saline (100 U/ml) was filled in the dome of the transducer and also in the fine polyethylene catheter cannulated to the carotid artery to prevent clotting.

The effects of intravenous administration of adrenaline (1 and 2 µg/kg) and noradrenaline (1 and 2 µg/kg) were recorded in the control and Abana treated normotensive animals.

Experiments on chronic administration of Abana on unilaterally nephrectomized deoxycorticosterone acetate (DOCA) treated hypertensive rats and pressor response to agonists: In the second series of experiments, unilateral nephrectomy was performed in male rats.¹ Though female rats appear to be more susceptible to develop hypertension, literature suggests the use of male rats for the development of hypertension in nephrectomised DOCA-salt animals.¹,² A week after unilateral nephrectomy or Sham operation, the animals were divided into 4 groups of 5-6 rats each.

Group A: Control, sham-operated animals received daily injections of 0.5 ml of sterilised cotton seed oil subcutaneously for 5 weeks and 1% saline ad libitum as drinking water.

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Table 1. Effect of 3 weeks treatment with Abana on mean blood pressure (mm Hg) and pressor response to adrenaline and noradrenaline in normotensive rats (Mean ± SEM; n=5 to 6). The values in brackets show the % change from the basal blood pressure.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Basal blood pressure (mm Hg)</th>
<th>Change in B.P. (mm Hg)</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td>Adrenaline (µg/kg)</td>
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<tr>
<td></td>
<td></td>
<td>1</td>
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<tr>
<td>Control</td>
<td>133.45 ± 2.65</td>
<td>13.230 ± 2.680 (9.91)</td>
</tr>
</tbody>
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Figure 1. Mean blood pressure of sham-operated controls (sterilised cotton 'seed oil, S.C. for 5 weeks and 1% saline ad libitum), sham-operated animals treated with Abana (3 g/kg/day, p.o. 3 weeks), unilaterally nephrectomised rats treated with DOCA (25 mg/kg/week, S.C. 5 weeks) and unilaterally nephrectomised animals treated with DOCA + Abana. Vertical lines indicate SEM (n = 5-6).

* P < 0.001 as compared with control.
@ P < 0.001 as compared with unilaterally nephrectomised DOCA hypertensive rats.

Group B: Sham-operated animals received Abana (3 g/kg/day, p.o) for 3 weeks and 1% saline ad libitum as drinking water.

Group C: Nephrectomised animals received DOCA injection (25 mg/kg/week, s.c.) for 5 weeks, dissolved in sterilised cotton seed oil and 1% saline ad libitum as drinking water.

Group D: Nephrectomised animals received DOCA injection (25 mg/kg/week, s.c.) for 5 weeks, Abana (3 g/kg/day, p.o) for the last 3 weeks and 1% saline ad libitum as drinking water.

After completion of the treatment schedule, rats from each group were anaesthetised with pentobarbital sodium (40 mg/kg, i.p) and the blood pressure was measured as mentioned previously. The effects of intravenous administration of adrenaline (1 and 2 µg/kg) and nor-adrenaline (1 and 2 µg/kg) were also recorded in these groups of rats.

Experiments on chronic administration of Abana on CdCl2-induced hypertensive rats and pressor response to adrenaline and nor-adrenaline: In the third series of experiments performed in female rats, CdCl2 (1 mg/kg, i.p) dissolved in 0.9% saline was injected daily for 2 weeks, whereas the control group of animals received daily an i.p. injection of saline (0.1 ml) for a similar period. To another group of animals, Abana alone (3 g/kg, p.o) was administered daily for the 3 weeks. In the fourth group Abana was given for first week followed by Abana + CdCl2 (1 mg/kg, p.o) for another two weeks (second and third weeks). After the completion of treatment schedule the animals were anaesthetised and the blood pressure was measured. The pressor effects of intravenous administration of adrenaline (1 and 2 µg/kg) and noradrenaline (1 and 2 µg/kg) were also recorded in these groups of rats.

II. In vitro studies

The dose-response curve of noradrenaline was studied on the isolated aortic strips and portal vein of unilaterally nephrectomized rats treated with DOCA + Abana, or DOCA alone.

Rats (250-300 g) were sacrificed by a blow on the head and bled to death by cutting the neck vessels. A helically cut aortic strip was prepared from the thoracic aorta and mounted in an organ bath of 35 ml capacity. The bathing medium contained modified Krebs Henseleit solution of the following composition (mM): NaCl, 118.0; KCl, 4.5; NaH2PO4, 1.1; MgSO4, 7H2O, 0.58; CaCl2, 2.5; NaHCO3, 25.0 and dextrose, 11.1. The solution in the organ bath was maintained at 37± 0.5°C, pH 7.4 and gased with carbogen. During 2 hours of stabilisation the tissue was washed every 15 minutes. Contractile responses to cumulative addition of agonist was recorded on a smoked Kymograph paper using an isotonic frontal writing lever (X 15 and 2 g tension). Cumula-
Figure 2. Change in blood pressure (mm Hg) produced by intravenous administration of (a) adrenaline (1 and 2 µg/kg) and (b) noradrenaline (1 and 2 µg/kg) in sham-operated controls (0.5 ml sterilised cotton seed oil, S.C. for 5 weeks), sham-operated animals treated with Abana (3 g/kg/day, p.o. 3 weeks), unilaterally nephrectomised rats treated with DOCA (25 mg/kg/week, S.C. 5 weeks) and unilaterally nephrectomised rats treated with DOCA + Abana. Vertical lines indicate SEM (n = 5-6).

* P < 0.001 as compared with control; †P < 0.001 as compared with DOCA salt nephrectomised animals.

Figure 3. Mean blood pressure of saline-treated controls (0.1 ml of 0.9% NaCl, i.p. 2 weeks), animals treated with Abana (3 g/kg/day, p.o. 3 weeks), CdCl2-treated rats (1 mg/kg/day, i.p. 2 weeks) and rats treated with CdCl2 + Abana. Vertical lines indicate SEM (n = 5-6).

* P < 0.001 as compared with control; †P < 0.001 as compared with CdCl2 treated hypertensive rats.

Rat portal mesenteric vein: The rat portal vein was cleaned and the longitudinal vascular tissue was mounted in a 35 ml organ bath containing modified Krebs Henseleit solution maintained at 37 ± 0.5°C and gased with carbogen. Contraction due to the cumulative addition of agonist was recorded on a smoked Kymograph paper using an isotonic frontal writing lever (X 15 and 1 g tension). The tissue was allowed to stabilise for 1 h during which the bathing medium was changed every 15 minutes.

Cumulative dose-response curve of non-adrenaline (8.47 x 10^-8 to 6.50 x 10^-5) was recorded in nephrectomised animals treated with DOCA + Abana, or DOCA alone.

Statistical analysis: Students ‘t’ test was applied to determine the level of significance. P < 0.05 was considered as statistically significant.

Drug and chemicals used: Abana (The Himalaya Drug Co., Bombay); adrenaline (BDH, London); Cadmium chloride (Sarabhai Chemicals, Baroda); deoxycorticosterone acetate (DOCA) lnfar India Ltd., Calcutta); noradrenaline bitartarate (Sigma Chemicals, St. Louis, USA); pentobarbitone sodium (Loba-Chemie, Bombay).

RESULTS

In vivo studies: The chronic administration of Abana (3 g/kg/day, p.o) for 3 weeks did not produce any significant change in the mean blood pressure in normotensive animals. The pressor responses to all the doses of adrenaline (1 and 2 µg/kg) and noradrenaline (1 and 2 µg/kg) were not significantly altered after the chronic administration of Abana as compared to those in the controls (Table 1).
In the sham-operated rats, chronic administration of Abana (3 g/kg/day, p.o) for 3 weeks did not alter the mean blood pressure as compared to controls. Unilateral nephrectomy with DOCA injection (25 mg/kg/week, s.c.) for 5 weeks produced a significant (P < 0.001) elevation of mean blood pressure as compared to controls. However, administration of Abana (3 g/kg/day, p.o) for 3 weeks significantly (P < 0.001) reduced the mean blood pressure in the nephrectomized DOCA-salt-treated animals (Figure 1), implying an antihypertensive effect.

The pressor responses to all the doses of adrenaline (1 and 2 µg/kg) and noradrenaline (1 and 2 µg/kg) were not significantly altered in the sham-operated animals which received Abana (3 g/kg/day, p.o) for 3 weeks as compared to sham controls. There was a significant increase (P < 0.001) in the pressor response to adrenaline (1 and 2 µg/kg) and noradrenaline (1 and 2 µg/kg) in DOCA salt-treated hypertensive rats as compared to sham-operated controls. However, the pressor responses to adrenaline and noradrenaline were significantly reduced (P < 0.001) in nephrectomised DOCA salt-treated rats which received Abana (3 g/kg/day, p.o) for 3 weeks (Figures 2a and b), as compared to the nephrectomised DOCA salt-treated hypertensive rats.

Female rats treated chronically with CdCl₂ (1 mg/kg/day, i.p) for 2 weeks exhibited significant elevation (P < 0.001) of mean blood pressure as compared to controls. The administration of Abana alone in normotensive female rats did not show any significant change in the mean blood pressure. However, administration of Abana (3 g/kg/day, p.o) for 3 weeks significantly reduced (P < 0.001) the mean blood pressure of the CdCl₂ treated animals as compared to the animals treated with CdCl₂ alone, implying an antihypertensive effect of Abana (Figure 3).

The pressor response to all the doses of adrenaline (1 and 2 µg/kg) and noradrenaline (1 and 2 µg/kg) were not significantly altered in the female rats treated chronically with CdCl₂ (1 mg/kg/day, i.p) for 2 weeks as compared to controls. However, administration of Abana for 3 weeks significantly (P < 0.001) reduced the pressor response to adrenaline and noradrenaline in CdCl₂ treated rats (Figures 4a and b).

**In vitro studies:*** Treatment with Abana (3 g/kg/day, p.o for 3 weeks) in the unilaterally nephrectomised DOCA salt-treated rats significantly (P < 0.001) shifted the dose-response curve of noradrenaline to the right in isolated aortic strip and portal vein, as compared to the dose-response curve of the nephrectomised DOCA salt-treated hypertensive animals (Figures 5 and 6).

**DISCUSSION**

The present study shows that Abana has a significant antihypertensive effect in experimentally
induced hypertensive models. This is in line with the clinical findings that Abana exerts an antihypertensive effect in mild to moderate hypertensive patients.

The hypertension induced by DOCA is due to retention of sodium and water. It has been further shown that the altered membrane permeability in the unilaterally nephrectomised DOCA salt-treated hypertensive models causes abnormal cation turnover. This abnormal cation turnover leads to vasoconstriction and finally to increased arterial blood pressure. The increased vascular sensitivity to noradrenaline in DOCA salt-treated hypertensive rats is also due to increased mobilisation of calcium ion into the vascular smooth muscle. It is possible that the alteration in voltage operative calcium channels or calcium permeability are the main reasons for the maintenance of hypertension in the DOCA salt-treated hypertensive model.

Cadmium-induced hypertension and vascular lesion in the kidney of rats fed with subtoxic levels were similar to those found in rats and dogs due to partial constriction of one renal artery or some other procedure. It was shown that the cadmium-induced pressor response as well as hypertension were prevented by calcium channel blockers like verapamil and nifedipine. It was further suggested that cadmium might mimic calcium ion as a partial agonist or the metal might alter the calcium transport across the cell membrane.

In both the forms of hypertension, it seems that there is alteration in the cation transport which leads to increased blood pressure.

The present in vivo and in vitro studies have shown that there is increased vascular reactivity to noradrenaline and adrenaline in DOCA salt-treated hypertensive models. This reduction in vascular reactivity by Abana in DOCA salt-treated hypertensive rats suggests that there is alteration in the sensitivity of the adrenoceptor to noradrenaline and adrenaline.

Based on the mechanism of hypertension in the unilaterally nephrectomised DOCA salt-treated and CdCl₂ hypertensive models, it is suggested that the antihypertensive effects of Abana might be due to its alteration in the transport of cations across the cell membrane.

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REFERENCES


