Effect of Abana, an Ayurvedic Preparation on Ethinyl Estradiol-induced Hypertension in Rats

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SUMMARY:
Objectives: To study the effect of Abana, an Ayurvedic herbomineral preparation on blood pressure and vascular reactivity to noradrenaline (NA), acetylcholine (Ach) and isoprenaline (Iso) in normotensive control and ethinyl estradiol (EE)-induced hypertensive rats.

Methods: Animals were divided into six groups receiving different treatments consisting of vehicle, EE (1.5 mg/kg/day i.m.) and Abana (3 g/kg/day orally) either alone or in combination for 3 or 6 weeks. After completion of the treatment schedules, effect of Ach, NA and Iso on the blood pressure vasopressor effects of NA on the hindquarter preparation was studied. Dopamine-β-hydroxylase (DBH) activity was also measured in adrenal glands after completion of the different treatments.

Results: Abana treatment for 3 weeks to normotensive rats produced significant lowering of blood pressure, enhancement of vasopressor response to low dose of NA (0.5 µg/kg IV). However, DBH activity of the adrenal glands and vasodepressor responses to Ach and Iso were not altered in Abana-treated rats. Combined treatment with Abana and EE for 3 weeks or pretreatment with Abana for 3 weeks followed by the combined treatment prevented EE-induced hypertension and increased the DBH activity of the adrenal glands. Also the altered vascular reactivity to NA (0.5 µg/kg IV) observed in EE-treated and Abana-treated groups were abolished in the groups receiving the combined treatments.

Conclusions: It is suggested that Abana produced protective effects against EE-induced hypertension in rats probably by its sympathetic blocking property.

Key Words: Ethinyl estradiol; hypertension; Abana; rats

INTRODUCTION
An elevated arterial pressure is an important public health issue in developed countries. Although it is common, asymptomatic and readily detectable it can often lead to lethal complications, if left untreated. Because of its high incidence and morbidity, various drugs and regimes have been advocated for the control of hypertension. Many new drugs have been introduced which may demonstrate better efficacy but possess side effects. Recently, attention has been focused towards herbal and mineral preparations, which are traditionally used as potential therapeutic agents in the prevention and management of cardiovascular disorders. Keeping the above facts in view, the antihypertensive efficacy of a herbal and
mineral preparation, Abana, was evaluated in estrogen-induced hypertensive rats. Abana is a compound preparation of indigenous drugs considered to be useful in the management of angina and hypertension.

The exact mechanism by which Abana produces antihypertensive effect is not known. Down-regulation of β-adrenoceptors, however, was suggested by the results reported with isolated atrium and intestine from Abana-fed rabbit. Recently, Bhattacharya et al., reported that chronic treatment of guinea-pigs with Abana does not affect the β-adrenoceptor mediated tracheal relaxation. Also Balaraman et al., suggested that the antihypertensive effects of Abana might be due to its alteration in the transport of cations across the cell membrane. In the present study, we attempted to evaluate the antihypertensive effect of Abana in experimental hypertensive model induced by chronic treatment with estrogen and to delineate its probable mechanism of antihypertensive action.

MATERIALS AND METHODS

Animals and treatment schedule: Healthy female albino rats (200-260 g) were divided into six groups each containing eight rats. Group 1 receiving water (vehicle for Abana) orally served as a control group. Group 2 received arachis oil (0.2 ml of vehicle for ethinyl estradiol) intramuscularly daily for 3 weeks. Group 3 received ethinyl estradiol (1.5 mg/kg/day, i.m.) for 3 weeks. Finely pulverized powder form of Abana was suspended in water for oral administration by gastric intubation. Abana (3 g/kg/day) was administered orally for 3 weeks in Group 4, while Abana (3 g/kg/day) was administered orally along with EE-treatment (1.5 mg/kg/day i.m.) for 3 weeks in group 5. Group 6 received Abana (3 g/kg/day) orally for 3 weeks followed by combined treatment consisting of Abana (3 g/kg/day) orally and EE (1.5 mg/kg/day, i.m.) for another 3 weeks.

The animals were used for experimental study 24 h after the completion of treatment schedules.

Body weights and blood pressure: Body weight of all the rats were recorded before starting the treatment and then after completion of treatments.

The mean blood pressure of anaesthetized (pentobarbitone sodium 40 mg/kg i.p.) rats was recorded directly from the common carotid artery by means of a Statham Pressure transducer coupled to a calibrated Twin Viso Recorder (Sanborn model). The femoral vein was cannulated with a fine polyethylene catheter for the administration of drugs. Noradrenaline (NA; 0.5 µg, 1 µg and 2 µg per kg), (ACh; 0.5 µg, 1 µg and 2 µg per kg) and isoprenaline (Iso; 0.5 µg, 1 µg and 2 µg per kg) were administered intravenously in a volume of 0.1 ml followed by 0.2 ml of 0.9% sodium chloride solution.

Hindquarter perfusion: Female rats (225-250 g) were anaesthetized with pentobarbitone (30 mg/kg, i.p.) and hindquarter perfusion was set up as described by Bhatt and Gulati. After 30 min of equilibration period, NA was injected intra-arterially at various doses in volumes
of 0.03-0.05 ml with a Hamilton syringe inserted into the rubber tubing connected to the abdominal aorta through the three-way cannula.

**Dopamine-β-hydroxylase (DBH) activity:** DBH activity with tyramine as substrate was measured spectrophotometrically in adrenal glands of the control as well as the treated groups.

**Statistical analysis:** Student ‘t’ test was applied to determine the significant differences between groups. \( p<0.05 \) was considered statistically significant.

**RESULTS**

Effects of Abana and EE-treatment on body weight: There was a significant reduction in the body weights of rats after chronic EE treatment. However, there was a significant increase in the body weights of rats after chronic Abana treatment. Combined treatment with EE and Abana did not significantly alter the body weights (Table 1).

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>n</th>
<th>Body weight (g) Before</th>
<th>Body weight (g) After</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Control</td>
<td>8</td>
<td>235 ± 8.2</td>
<td>253 ± 4.5</td>
</tr>
<tr>
<td>2</td>
<td>Vehicle</td>
<td>8</td>
<td>225 ± 7.7</td>
<td>246 ± 6.8</td>
</tr>
<tr>
<td>3</td>
<td>EE (3 weeks)</td>
<td>8</td>
<td>224 ± 3.5</td>
<td>175 ± 3.6**</td>
</tr>
<tr>
<td>4</td>
<td>Abana (3 weeks)</td>
<td>8</td>
<td>233 ± 4.2</td>
<td>263 ± 6.9*</td>
</tr>
<tr>
<td>5</td>
<td>Combined EE + Abana (3 weeks)</td>
<td>8</td>
<td>227 ± 5.4</td>
<td>217 ± 6.8</td>
</tr>
<tr>
<td>6</td>
<td>Abana (3 weeks) followed by Abana + EE (3 weeks)</td>
<td>8</td>
<td>232 ± 5.6</td>
<td>241 ± 4.0</td>
</tr>
</tbody>
</table>

*\( p<0.05 \) and **\( p<0.01 \) significant from its corresponding value before treatment. Values are mean ± SEM.

EE=Ethinyl estradiol

Effects of Abana and EE-treatment on blood pressure: Chronic treatment with EE produced a significant elevation of blood pressure; however, chronic treatment with Abana produced a significant reduction of blood pressure (Figure 1). Abana prevented EE-induced elevation of blood pressure when administered along with ethinyl estradiol (Figure 1).

Effects of Abana and EE-treatment on vascular reactivity blood pressure response: NA produced a dose-related vasopressor response in control as well as vehicle-treated rats. However, there was a significant enhancement of vasopressor response to the lowest dose (0.5 \( \mu \)g/kg) of NA in both the EE-treated and Abana treated rats (Table 2). Combined
treatment with Abana and EE for three weeks or pretreatment with Abana and EE for 3 weeks significantly reduced the enhancement of vasopressor response to the lower dose of NA as compared to that observed in the EE-treated rats (Table 2).

### Table 2: Effects of various treatments on the blood pressure responses of anaesthetized rats to i.v. noradrenaline

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Increase (change) in mean blood pressure (mmHg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0.5 µg/kg</td>
</tr>
<tr>
<td>1</td>
<td>Control</td>
<td>14 ± 1.4</td>
</tr>
<tr>
<td>2</td>
<td>Vehicle</td>
<td>13 ± 1.5</td>
</tr>
<tr>
<td>3</td>
<td>EE (3 weeks)</td>
<td>29 ± 2.6**</td>
</tr>
<tr>
<td>4</td>
<td>Abana (3 weeks)</td>
<td>22 ± 2.5*</td>
</tr>
<tr>
<td>5</td>
<td>Abana + EE (3 weeks)</td>
<td>20 ± 4.0</td>
</tr>
<tr>
<td>6</td>
<td>Abana (3 weeks) followed by Abana + EE (3 weeks)</td>
<td>18 ± 3.7*</td>
</tr>
</tbody>
</table>

* *p*<0.05 significantly different from control group.  
** *p*<0.01 significantly different from vehicle-treated groups.  
* *p*<0.05 significantly different from EE-treated group.  
n=8; values are mean ± SEM.

ACh and Iso produced a dose-related vasodepressor response in control, vehicle-treated and EE-treated rats. Neither chronic treatment with Abana nor combined treatment with Abana and EE-altered the vasodepressor responses to ACh or Iso.

**Hindquarter perfusion:** NA produced a graded dose-related increase in perfusion pressure in control and vehicle-treated rats. Chronic treatment with EE produced a significant increase in the perfusion pressure in the hindquarter preparation, reflected in a parallel leftward shift of the dose response of curve of NA with leftward shift of the dose response of curve of NA with increase in the maximal response (Figure 2); however, there was no change in the sensitivity of the rat hindquarter to NA in other groups (Figure 2).

**Effect of Abana and EE treatments on DBH activity of adrenal glands:** There was a significant increase in DBH activity of adrenal glands after chronic EE treatment; however, DBH activity was not altered significantly after chronic Abana treatment. Combined treatment with Abana and EE of pretreatment with Abana followed by combined treatment with Abana and EE prevented the EE-
induced increase in DBH activity of adrenal glands (Table 3).

DISCUSSION

Abana a compound preparation of various indigenous drugs is useful in the management of hypertension. The mechanism by which it lowers the blood pressure is not known. Differential effects on the β-adrenoceptor function by Abana in differential tissues and species have been reported. Down-regulation of the β-receptors of the myocardium and intestine of rabbits\(^1\) and lack of effects on the β-adrenoceptor functions of the isolated trachea of guinea-pigs\(^3\), treated chronically with Abana has been reported.

There was a significant decrease in the body weight of all the rats treated chronically with EE as reported earlier\(^6\). However, chronic Abana treatment produced significant weight gain in all the rats suggesting its anabolic action. Also, combined treatment with Abana and EE prevented EE-induced decrease in the body weights of the rats suggesting anticatabolic action of Abana.

Chronic Abana treatment produced significant decrease in the blood pressure of rats. Furthermore, combined treatment with Abana and EE prevented EE-induced hypertension in rats. A note of sympathetic nervous system in EE-induced hypertension has been suggested. It is thus possible that the antihypertensive effect of Abana may be related to its effect on the sympathetic nervous system.

Increased vascular reactivity to the lower dose of NA in EE-induced hypertension may either be due to an increase in the affinity of the α-adrenoceptors or to their upgradation\(^4\). The combined effect of Abana and EE abolished the increase in vascular reactivity to the lower dose of NA due to EE treatment. This suggests that Abana treatment may either decrease the affinity of the α-adrenoceptors to NA and/or cause down-regulation of α-adrenoceptor. In agreement with this contention, Abana treatment significantly antagonized the increased vascular sensitivity of perfused hindquarter of the rat to NA observed after chronic EE treatment.

It is also interesting to note that in Abana-treated animals, the blood pressure response to the lower dose of NA was enhanced, while vasoconstrictor responses of hindquarter preparation to all the doses of NA were unaltered. This could be explained on the premise that in the whole animal the presence of cardiovascular reflexes may enhance the blood pressure response to the lower dose of NA while in an isolated perfused hindquarter preparation such reflexes are absent.

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>DBH activity (nmol/min/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Control</td>
<td>33.5 ± 6.2</td>
</tr>
<tr>
<td>2</td>
<td>Vehicle</td>
<td>34.3 ± 5.1</td>
</tr>
<tr>
<td>3</td>
<td>EE (3 weeks)</td>
<td>270.0 ± 62.2*</td>
</tr>
<tr>
<td>4</td>
<td>Abana (3 weeks)</td>
<td>37.3 ± 8.1</td>
</tr>
<tr>
<td>5</td>
<td>Abana + EE (3 weeks)</td>
<td>43.4 ± 7.1a</td>
</tr>
<tr>
<td>6</td>
<td>Abana (3 weeks) followed by combined Abana + EE (3 weeks)</td>
<td>36.6 ± 4.5a</td>
</tr>
</tbody>
</table>

Values are mean ± SEM; n=8, *p<0.05 significantly from vehicle-treated group, **p<0.05 different from EE-treated group.
Another explanation for the enhancement of vasopressor response to the lower doses of NA could be due to the lower level of the basal blood pressure in the chronic Abana treated animals.

It is unlikely that the enhancement in responsiveness to NA after Abana treatment could be due to alteration in the structural component of the resistant vessels\(^7\), as there was no change in the blood pressure responses to the other agonists such as ACh and Iso.

Women taking oral contraceptive pill have been shown to have higher serum DBH activity\(^8\). Also, increase in the DBH activity of the mesenteric vessel wall of young SH rats has been reported\(^9,10\). Similarly, chronic EE treatment of rats produces significant elevation of blood pressure and increase in DBH activity of adrenal glands\(^4\). Chronic Abana treatment of rats did not significantly alter the DBH activity of the adrenal glands; however, in the combined Abana and EE-induced increase in DBH activity in adrenal glands was prevented. This observation further supports the hypothesis regarding the sympathetic blocking effect of Abana in hypertensive animals.

REFERENCES


