SHORT COMMUNICATION
Protection by Abana, a Herbomineral Preparation, against Myocardial Necrosis in Rats induced by Isoproterenol

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SUMMARY
Abana, a herbomineral preparation, showed significant protection against the biochemical changes induced by isoproterenol in rats. In myocardial necrosis, the increased levels of serum creatine phosphokinase, glutamate oxaloacetate transaminase, glutamate pyruvate transaminase and γ-glutamyl transpeptidase were found to be reversed by Abana treatment. In addition the decreased levels of glycogen, γ-glutamyl transpeptidase, succinate dehydrogenase and mitochondrial oxygen uptake in heart were also significantly protected. This shows that Abana treatment could contribute to restoring myocardial integrity and cardiac function disturbed by isoproterenol-induced ischaemia.

Keywords: Abana isoproterenol; ischaemia; myocardial necrosis

INTRODUCTION
It has been recently reported (Khanna et al., 1991) that Abana, a herbomineral preparation, showed significant hypolipidaemic activity in rats. It is well known that abnormalities of lipid and lipoprotein metabolism are positively correlated with heart disease (Seidel, 1987). We have also reported that some natural products such as gugulip, guggulsterone and coleonol which exert a lipid lowering action, also showed significant protection against changes in creatine phosphokinase (CPK), glutamate oxaloacetate transaminase (GOT), glutamate pyruvate transaminase (GPT) in serum and heart glycogen and mitochondrial oxygen uptake in myocardial necrosis products was comparable with the standard drugs propranolol and nifedipine (Dhawan et al., 1978; Kapoor et al., 1979; Kalu and Kapoor, 1989a; 1989b; 1991; Kaul et al., 1990). Therefore, the aim of the present study was to investigate the action of Abana on biochemical changes in ischaemic rats treated with isoproterenol. The results are communicated in this paper.

MATERIALS AND METHODS
Male albino rats (150 ± 10 g, Charles-Foster strain) obtained from the CDRI Animal House were used in this study. The animals were fed a standard pellet diet ad libitum and had free access to water.

Induction of ischaemia and treatment with Abana. Abana tablets were the gift of The Himalaya Drug Co., Bangalore, India. Each tablet consists of Terminalia arjuna 30 mg, Withania somnifera (Ashwagandha) 20 mg, Tinospora cordifolia (Giloe) 10 mg, Nepeta hindostana (Billilotan) 20 mg, Phyllanthus emblica (Amla) 10 mg, Terminalia chebula (Hirda) 10 mg, Dashamoola 20 mg (a mixture of ten herbs containing equal proportions of...
Aegle marmelos, Premna integrifolia, Oroxylum indicum, Stereospermum suaveolens, Gmelina arborea, Desmodium gangeticum, Uraria picta, Solanum indicum, Solanum xanthocarpum and Tribulus terrestris) Eclipta alba (Bhrangraj) 10 mg, Glycyrrhiza glabra (Yashtimadhu) 10 mg, Asparagus racemosus (Shankpushpi) 10 mg, Ocimum sanctum (Tulsi) 10 mg, Nardostachys jatamansi (Jatamansi) 10 mg, Cyperus rotundus (Motha) 5 mg, Acorus calamus (Vach) 5 mg, Embelia ribes (Vidang) 5 mg, Piper longum (Pipali) 10 mg, Carum coticum (Ajwain) 10 mg, Zingiber officianale (Sonth) 10 mg, Syzygium aromaticum (Lavang) 5 mg, Celastrus paniculatus (Malkangni) 5 mg, Santalum album (Chandan) 5 mg, Elettaria cardamomum (Chotti elaichi) 5 mg, Foeniculum vulgare (Sonf) 5 mg, Rosa damascena (Gulat ka pool) 5 mg, Cinnamomum cassia (Taja) 5 mg, Crocus sativus (Keshar) 2 mg, Asphaltum (Shilajeet) 20 mg, Serpent stone, the silicate of magnesium and iron (Jaharmohra) 10 mg, conch (Shankha bhasma) 10 mg, sulphide of mercury (Makar dhwaj) 10 mg, mica (Abrak bhasma) 5 mg, Mytilus magaritiferus (Pearl pishiti) 5 mg, Agate (Akit pishiti) 5 mg, Jade (yeshab pishiti) 5 mg, Ruby (Yakut pishiti) 5mg and Corallium rubrum (coral pishiti). Bhasma and Pishiti are the typical Ayurvedic preparations from the said raw materials. Before feeding, Abana tablets were macerated with gum-acacia and suspended in water.

The rats were divided into four groups each containing six animals. Group I served as a control, Group II was Abana treated, group III was ischaemic and group IV was treated with Abana and was ischaemic. Abana (50 mg/kg, once daily) was orally administered for 7 consecutive days to the animals of groups II and IV, while the animals of groups I and III received an equivalent amount of gum-acacia solution. After 5 days of Abana/gum acacia treatment, ischaemia was induced by an i. p. injection of isoproterenol (85 mg/kg) for 2 consecutive days. Abana/gum-acacia treatment was continued on days 6 and 7 along with isoproterenol treatment. On day 8 i.e. 48 h after the first injection of isoproterenol the animals were killed and blood and heart were taken for analysis of biochemical parameters.

Estimations of biochemical parameters. Levels of serum CPK, GOT and GPT were estimated by standard methods as described earlier (Kaul and Kapoor, 1989b). Cardiac acid phosphatase (Wright et al., 1972) was measured in heart homogenates (20% w/v in 5 mM Tris-HCl buffer pH 7.2 containing 250 mM sucrose) without submitting them to freezing and thawing to assess the magnitude of rupture of lysosomes and leakage of enzymes in situ resulting from damage to the integrity of cardiac lysosomes by isoproterenol. Heart mitochondria were prepared by the method described by Kaul and Kapoor (1989b) and used for the oxygen uptake studies by the method of Estabrook (1967) using a Gilson-Oxygraph (Gilson Medical Electronics, 3000W Beltline HWY, Middleton WI. 53562, USA, Model 5/64 oxygraph). Mitochondrial succinate dehydrogenase: γGT (both in mitochondria and serum) as well as levels of glycogen and protein in heart were estimated by standard methods as described earlier (Dwivedi et al., 1993). Statistical analysis p values were calculated using Student’s t-test.
RESULTS AND DISCUSSION

Treatment with Abana alone to control rats did not result in any significant change in the biochemical parameters of serum and heart. Therefore, only the control values of various biochemical parameters have been reported in Tables 1, 2 and 3. Serum CPK, GOT, GPT and γ-GT were significantly reversed by treatment with Abana in ischaemic rats (Table 1). The increased levels of serum enzymes in myocardial ischaemia may be due to the leakage of the enzymes into blood (Kaul and Kapoor, 1989; Kumari and Menon, 1987). Abana treatment offers partial protection by protecting the membrane and by preventing the release of membrane bound enzymes. Abana treatment showed 90% protection against decreased glycogen level in ischaemic rats (Table 2).

Table 1: Protective effect of Abana on isoproterenol-induced changes in serum enzymes

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control</th>
<th>Ischaemic (% change)</th>
<th>Ischaemic + Abana (% protection)</th>
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<tr>
<td>CPK&lt;sup&gt;a&lt;/sup&gt;</td>
<td>50.2 ± 3.85</td>
<td>90.6 ± 4.80&lt;sup&gt;d&lt;/sup&gt; (82)</td>
<td>72.5 ± 5.80&lt;sup&gt;d&lt;/sup&gt; (45)</td>
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<tr>
<td>GOT&lt;sup&gt;b&lt;/sup&gt;</td>
<td>80.9 ± 5.02</td>
<td>146 ± 8.64&lt;sup&gt;d&lt;/sup&gt; (80)</td>
<td>95.4 ± 11.3&lt;sup&gt;d&lt;/sup&gt; (77)</td>
</tr>
<tr>
<td>GPT&lt;sup&gt;b&lt;/sup&gt;</td>
<td>50.6 ± 2.98</td>
<td>78.9 ± 6.24&lt;sup&gt;d&lt;/sup&gt; (56)</td>
<td>67.9 ± 4.20&lt;sup&gt;a&lt;/sup&gt; (39)</td>
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<tr>
<td>γ-GT&lt;sup&gt;c&lt;/sup&gt;</td>
<td>7.29 ± 0.73</td>
<td>11.2 ± 0.49&lt;sup&gt;d&lt;/sup&gt; (53)</td>
<td>8.60 ± 0.32&lt;sup&gt;d&lt;/sup&gt; (66)</td>
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Units expressed as:
<sup>a</sup> n moles of Pi released/min/mg serum protein.
<sup>b</sup> µ moles of sodium pyruvate formed/min/L.
<sup>c</sup> µ moles of p-nitroaniline liberated/min/L.
Values are mean ± SD of six rats.
<sup>d</sup> p<0.01, <sup>e</sup> p<0.05.
Ischaemic group compared with control.
Ischaemic + Abana treated group compared with ischaemic group.

Table 2: Protective effect of Abana on isoproterenol-induced biochemical changes

<table>
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<tr>
<th>Parameter</th>
<th>Control</th>
<th>Ischaemic (% change)</th>
<th>Ischaemic + Abana (% protection)</th>
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<td>Heart homogenate glycogen&lt;sup&gt;a&lt;/sup&gt;</td>
<td>14.7 ± 0.45</td>
<td>8.53 ± 0.30&lt;sup&gt;e&lt;/sup&gt; (42)</td>
<td>14.1 ± 0.48&lt;sup&gt;e&lt;/sup&gt; (90)</td>
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<td>Acid phosphatase&lt;sup&gt;b&lt;/sup&gt;</td>
<td>17.1 ± 1.21</td>
<td>23.9 ± 1.30&lt;sup&gt;e&lt;/sup&gt; (42)</td>
<td>16.5 ± 1.20&lt;sup&gt;e&lt;/sup&gt; (100)</td>
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<td>Heart mitochondria γ−GT&lt;sup&gt;c&lt;/sup&gt;</td>
<td>4.39 ± 0.50</td>
<td>2.46 ± 0.04&lt;sup&gt;e&lt;/sup&gt; (44)</td>
<td>4.58 ± 0.08&lt;sup&gt;e&lt;/sup&gt; (100)</td>
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<td>SDH&lt;sup&gt;d&lt;/sup&gt;</td>
<td>0.421 ± 0.033</td>
<td>0.221 ± 0.028&lt;sup&gt;e&lt;/sup&gt; (48)</td>
<td>0.380 ± 0.030&lt;sup&gt;e&lt;/sup&gt; (80)</td>
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Units expressed as:
<sup>a</sup> µg/mg protein.
<sup>b</sup> n moles of p-nitrophenol formed/min/mg protein.
<sup>c</sup> n moles of p-nitroaniline liberated/min/mg protein.
<sup>d</sup> Change in OD/min/mg protein.
Values are mean ± SD of six rats.
<sup>e</sup> p<0.01.
Ischaemic group compared with control.
Ischaemic + Abana treated group compared with ischaemic group.
The decreased level of glycogen in ischaemic heart may be due to impaired energy metabolism which leads to glycogen breakdown, an important source for the production of ATP (Kaul et al., 1990). Cardiac acid phosphatase was enhanced by 42% in ischaemic rats and found to be reversed completely in Abana treated rats (Table 2). Wildenthal et al., (1978) showed that in cardiovascular disorders, phospholipase and acid phosphatase levels were elevated due to lysosomal membrane destruction. The results suggest that Abana prevents the damage to lysosomes induced by isoproterenol and hence avoids leakage of acid phosphatase. Ogawa et al., (1988) also reported that ischaemia results in the disruption of mitochondria along with leakage of lysosomal enzymes. Heart mitochondrial enzymes such as γ-GT and SDH were reduced by 44% and 48%, respectively (Table 2). In Abana treated ischaemic rats, the activity of γ-GT was completely reversed and SDH by 80%. The improved activities of SDH and γ-GT after Abana treatment suggest a recovery of mitochondrial damage and energy metabolism which is further confirmed by studying the changes in the oxygen uptake of heart mitochondria (Table 3).

Ischaemia resulted in markedly reduced endogenous oxygen uptake. Oxygen uptake of metabolic substrates such as succinate, ADP and glucose were also found to be reduced in ischaemic heart mitochondria. Treatment with Abana showed an increase in the endogenous as well as exogenous mitochondrial oxygen uptake. The decreased oxygen uptake in ischaemia may be due to an impairment of myocardial energy production (Jarmakani et al., 1978). The improved oxygen uptake suggests that Abana may act by stabilizing the structure of biological membranes.

Thus Abana provides significant protection to myocardium against ischaemia in rats and its cardioprotective activity is comparable to other natural products such as gugulip, guggulsterone, coleonol and standard drugs of propranolol and nifedipine reported earlier (Kaul and Kapoor, 1989a; 1989b; 1991; Kaul et al., 1991). The study also reveals that Abana, like other natural products namely, gugulip, guggulsterone and coleonol, could be employed as a potential cardioprotective drug.
ACKNOWLEDGEMENTS

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REFERENCES


