Preliminary studies on acute and subacute toxicity of an antidiabetic herbal preparation, Dianex

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Dianex, a polyherbal formulation intended to use for diabetic patients, has been screened for toxic effects. For acute toxicity studies, Dianex was administered orally in graded doses of 0.75-10 g/kg to the mice. For subacute toxicity studies, different doses of Dianex (1.0, 1.5 and 2.5 g/kg) were administered orally to the rats once daily for 30 days. Animals were observed for physiological and behavioural responses, mortality, food and water intake and body weight changes. Hematological evaluation was performed weekly. All the animals were sacrificed on 31st day and changes in organ weights and histology were examined. Biochemical studies were done in liver and serum. No mortality was observed up to 10 g/kg of Dianex in acute toxicity study. Daily administration of as high as 2.5 g/kg dose of Dianex did not result in any mortality or changes in gross behaviour, body weight, weight and histology of different organs or serum and liver biochemistry. However, significant increase in RBC count and hemoglobin level was observed in the treated animals at all doses. Other peripheral blood constituents were in the normal range. The dose of Dianex to produce significant antidiabetic activity in mouse, 0.25-0.5 g/kg, is much lower than the doses used in the present study. Therefore such doses may be safe for daily administration without causing any serious side effects.

Diabetes mellitus is a heterogeneous metabolic disorder characterised by altered carbohydrate, lipid and protein metabolism. The management of diabetes mellitus is considered a global problem and successful treatment is yet to be discovered. The modern drugs, including insulin and oral hypoglycemic agents, control the blood sugar level as long as they are regularly administered and they also produce a number of undesirable effects. The treatment of diabetes has been attempted with different indigenous plants and polyherbal formulations. Based on the antidiabetic activity and other medicinal properties of selected plants like Aegle marmelose, Gymnema sylvestre, Eugenia jambolana, Momordica charantia, Azadirachta indica, Cassia auriculata, Withania somnifera and Curcuma longa, a polyherbal formulation, Dianex, has been prepared by the Apex Laboratories, Chennai for application in humans. The preliminary screening of this formulation in our laboratory has shown significant hypoglycemic activity in mice. However, preclinical toxicity studies are essential for determining a safe dose for human trials. Therefore, the present study was initiated to determine the acute and subacute toxicity of Dianex in laboratory animals.

Materials and Methods

Animals

Acute and subacute toxicity studies were carried out in Swiss albino mice and Wistar rats, respectively. Adult mice (6-8 weeks old) of either sex, weighing 25-30 g, were obtained from the Department of Radiobiology, Kasturba Medical College, Manipal. They were housed in polypropylene cages, 4 animals per cage, with free access to food and water. Six to eight weeks old Wistar rats of either sex, weighing 150-200 g, were obtained from the Department of Pharmacology, Kasturba Medical College, Manipal. The rats were housed, two per cage, in elevated wire mesh cages with free access to food and water.

Chemicals

Deoxycholate, diphenylamine, orcinol, 4-nitro phenyl phosphate, 2-amino-2-methyl-1-propanol, DNA, RNA and bovine serum albumin were purchased from Sigma, USA. Trichloroacetic acid was purchased from Qualigens, India. All the other chemicals used were of analytical grade. The Dianex mixture containing

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the aqueous extracts of *Gynemna sylvestre* (Asclepiadaceae, leaf, 15 mg), *Eugenia jambolana* (Myrtaceae, seed, 19 mg), *Monordica charantia* (Cucurbitaceae, fruit, 30 mg), *Azadirachta indica* (Meliaceae, leaf, 7.5 mg), *Cassia auriculata* (Caesalpiniaceae, flower, 25 mg), *Aegle marmelos* (Rutaceae, fruit, 12.5 mg), *Withania somnifera* (Solanaceae, root, 20 mg) and *Curcuma longa* (Zingiberaceae, rhizome, 4 mg) was supplied by the Apex Laboratories, Chennai.

**Acute toxicity studies**

Eighty mice were used for the study. Food and water was withdrawn 18 hr before administration of Dianex. The mice were divided into 8 groups and each group contained 10 animals. Group 1 received 0.2 ml of 0.5% sodium carboxymethylcellulose (CMC) orally and served as control. Groups 2-8 received 0.75-10 g/kg of Dianex orally. The animals were observed continuously for the first 2 hr, then occasionally up to 6 hr and then daily up to 14 days post-treatment for any toxic symptoms and mortality.

**Subacute toxicity studies**

The rats were divided into 4 groups of 10 each (5 males and 5 females). One group was given 0.5 ml of 0.5% CMC (control) orally and other 3 groups were administered with 1.0, 1.5 and 2.5 g/kg of Dianex daily for 30 days. All the rats were observed for any physiological and behavioural changes and mortality. Food and water consumption was checked daily. Body weight was recorded at the beginning and twice weekly throughout the study. Hematological parameters, total RBC, WBC, differential leukocyte counts and hemoglobin were estimated weekly in blood collected from the orbital sinus into sterilized heparinized tubes. Twenty-four hours after the last administration (on the 31st day of the experiment) blood samples were collected from each rat individually into non-heparinized tubes and were allowed to coagulate. Serum was separated by centrifugation and alkaline and acid phosphatases were analysed by spectrophotometric method and alanine transaminase (ALT), aspartate transaminase (AST), urea and creatinine were analysed using Autoanlyser (Hitachi 911, Japan). The liver, lungs, heart, thymus, spleen, adrenals, testes, uterus and kidneys were removed and weighed immediately. A part of the liver was processed for estimation of DNA, RNA and total protein. Acid and alkaline phosphatases were also estimated as above. Pieces of organs, other than lung and heart, were fixed in Bouin's fixative and processed routinely for histological examination. The slides were stained with haematoxyline and eosin and observed under low power microscope for any pathological changes. The Student's t-test was employed to analyse the results. Difference below the probability level 0.05 was considered statistically significant. The experimental protocol was approved by the Institutional Animal Ethics Committee, Kasturba Medical College, Manipal.

**Results and Discussion**

No acute toxicity was observed even at 10 g/kg of Dianex on oral administration and all animals were found to be normal during and at the end of the observation period (14 days). In the subacute toxicity study, there were no deaths during the treatment period either in the control or in the treated groups. Food and water consumption also did not differ significantly. There was no change in general behaviour or other physiological activities of the animals. The control and drug treated groups showed normal increase in the body weight (22.63±0.61% (control) and 23.45±0.82% (for 1 g/kg), 24.65±0.61% (for 1.5 g/kg) and 25.11±1.51% (for 2.5 g/kg), which were not significantly different from each other). None of the organs in the treated rats showed any significant change in weight. Hematological analysis showed a significant increase in RBC count and hemoglobin percentage compared to control (Table 1). But no change in the serum alkaline phosphatase, acid phosphatase, ALT, AST, urea or creatinine levels was observed compared to the control (Table 2). Similarly, none of the biochemical parameters analysed in the liver showed any significant variation from the control values (Table 3). Histological examination of the organs also did not reveal any pathological changes. The observations were similar in the male and female rats.

The results showed that a very high oral dose (10 g/kg) is tolerated by the mice without producing any acute toxicity symptoms. Since some of the plants present in Dianex like *Withania somnifera*, *Aegle marmelos*, *Azadirachta indica* and *Cassia auriculata* contain heterogeneous alkaloids and tannins, toxic effects were expected. However, components like reducing sugars, flavonoids, amino acids and other compounds present in *Azadirachta indica* and *Withania somnifera* and *Aegle marmelos* may have helped in reducing the toxicity. Some compounds like saponinsides of *Withania somnifera* have been reported to reduce stress related changes in the
Table 1 — Peripheral blood changes in rats observed during and after treatment with Dianex for 30 days

[Values are the mean ± SE of 10 animals]

<table>
<thead>
<tr>
<th>Time (Week)</th>
<th>Treatment</th>
<th>RBC (10^6/mm³)</th>
<th>Hb (g/dl)</th>
<th>WBC (10^3/mm³)</th>
<th>Differential leukocyte count (%)</th>
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</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>N</td>
</tr>
<tr>
<td>I</td>
<td>Control</td>
<td>5.93 ± 0.1</td>
<td>12.82 ± 0.20</td>
<td>6.5 ± 0.25</td>
<td>23.7 ± 0.81</td>
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<tr>
<td></td>
<td>Dianex</td>
<td>6.77 ± 0.1*</td>
<td>14.6 ± 0.14*</td>
<td>6.05 ± 0.15</td>
<td>23.9 ± 0.64</td>
</tr>
<tr>
<td></td>
<td>Dianex</td>
<td>6.87 ± 0.1*</td>
<td>15.6 ± 0.15*</td>
<td>6.15 ± 0.18</td>
<td>24.1 ± 0.44</td>
</tr>
<tr>
<td></td>
<td>Dianex</td>
<td>6.91 ± 0.1*</td>
<td>14.8 ± 0.14*</td>
<td>6.54 ± 0.21</td>
<td>23.7 ± 0.51</td>
</tr>
<tr>
<td>II</td>
<td>Control</td>
<td>6.05 ± 0.05</td>
<td>13.17 ± 0.16</td>
<td>6.77 ± 0.17</td>
<td>21.6 ± 1.34</td>
</tr>
<tr>
<td></td>
<td>Dianex</td>
<td>7.08 ± 0.09*</td>
<td>14.86 ± 0.10*</td>
<td>6.69 ± 0.13</td>
<td>22.6 ± 1.07</td>
</tr>
<tr>
<td></td>
<td>Dianex</td>
<td>7.18 ± 0.08*</td>
<td>14.75 ± 0.12*</td>
<td>6.61 ± 0.11</td>
<td>21.8 ± 1.41</td>
</tr>
<tr>
<td></td>
<td>Dianex</td>
<td>7.11 ± 0.09*</td>
<td>14.91 ± 0.11*</td>
<td>6.71 ± 0.11</td>
<td>22.7 ± 1.54</td>
</tr>
<tr>
<td>IV</td>
<td>Control</td>
<td>6.42 ± 0.05</td>
<td>13.91 ± 0.32</td>
<td>7.14 ± 0.09</td>
<td>22.8 ± 1.12</td>
</tr>
<tr>
<td></td>
<td>Dianex</td>
<td>7.23 ± 0.08*</td>
<td>15.51 ± 0.13*</td>
<td>6.91 ± 0.21</td>
<td>23.4 ± 0.94</td>
</tr>
<tr>
<td></td>
<td>Dianex</td>
<td>7.32 ± 0.05*</td>
<td>15.32 ± 0.05*</td>
<td>7.11 ± 0.11</td>
<td>22.4 ± 0.84</td>
</tr>
<tr>
<td></td>
<td>Dianex</td>
<td>7.35 ± 0.04*</td>
<td>15.64 ± 0.11*</td>
<td>7.24 ± 0.22</td>
<td>23.1 ± 0.45</td>
</tr>
</tbody>
</table>

* P < 0.05 compared to Control
N= Neutrophil, L=Lymphocyte, M=Monocyte, E=Eosinophil, B=Basophil

Table 2 — Serum activities of different enzymes in rats treated with Dianex for 30 days

[Values are the mean ± SE of 10 animals]

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Alkaline phosphatase (U/L)</th>
<th>Acid phosphatase (U/L)</th>
<th>AST (U/L)</th>
<th>ALT (U/L)</th>
<th>Urea (mg/dL)</th>
<th>Creatinine (mg/dL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>62.71 ± 4.29</td>
<td>28.43 ± 4.03</td>
<td>90.03 ± 3.74</td>
<td>57.06 ± 2.85</td>
<td>45.25 ± 2.12</td>
<td>0.15 ± 0.12</td>
</tr>
<tr>
<td>Dianex</td>
<td>63.67 ± 5.26</td>
<td>32.95 ± 5.93</td>
<td>68.64 ± 4.48</td>
<td>54.23 ± 5.15</td>
<td>46.23 ± 1.25</td>
<td>0.52 ± 0.22</td>
</tr>
<tr>
<td>Dianex</td>
<td>64.11 ± 4.21</td>
<td>30.19 ± 2.93</td>
<td>67.16 ± 2.48</td>
<td>55.12 ± 3.12</td>
<td>45.11 ± 2.23</td>
<td>0.48 ± 0.14</td>
</tr>
<tr>
<td>Dianex</td>
<td>65.61 ± 3.21</td>
<td>31.19 ± 3.91</td>
<td>68.14 ± 3.41</td>
<td>55.11 ± 2.11</td>
<td>47.22 ± 3.25</td>
<td>0.50 ± 0.11</td>
</tr>
</tbody>
</table>
alimentary system [20]. This is a basic principle in the use of crude plant products or polyherbal preparations in traditional medicine, where the adverse effects of one component will be nullified by the protective effect of the other components, without interfering with their therapeutic properties.

The finding that daily administration of Dianex at different doses like 1.0, 1.5 and 2.5 g/kg for 30 days (total doses of 30, 45 and 75 g/kg) was well tolerated by the rats without any mortality and morbidity symptoms, indicates that the drug tolerance can be enhanced by fractionated dose administration over an extended period, which is desirable for the treatment of chronic diseases like diabetes mellitus.

The formulation did not have any adverse effects on the normal growth of the animals. On the contrary, a small increase in the body weight and a significant increase in the hemoglobin level and RBC count were observed in the treated animals, suggesting an anabolic effect of the preparation. Some of the plants in this formulation like Withania somnifera [19], Momordica charantia [21] and Azadirachta indica [18] contain vitamins, carbohydrates and free amino acids, which may be responsible for the observed anabolic effect. Withania somnifera has been reported to produce anabolic effects, enhancing the synthesis of certain modulator proteins in rat liver and increasing the body weight in humans [22]. The blood enriching property of Eugenia jambolana [23] and high iron content of Withania somnifera [24], Azadirachta indica [18] and Aegle marmelose [17] have been reported.

In another study, Dianex has shown significant hypoglycemic activity at a dose of 0.25 g/kg in mice [25]. The present study shows that such a dose of Dianex may be safe for daily administration, as required in controlling the elevated blood glucose levels in diabetic patients. However, more studies are needed on the chronic toxicity and teratogenic effects before the preparation can be recommended for long-term treatment of diabetes patients, especially in the younger age groups.

Acknowledgement
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References
18 Indian Council of Medical Research, Medicinal plants of India-I (ICMR, New Delhi) 1987, 112.