Evaluation of the hepatotoxicity of the anti-diabetic drug Diarid: An ayurvedic formulation in white swiss albino mice

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ABSTRACT
Diarid is an Ayurvedic antidiabetic drug. Questions and concerns are being raised nowadays on such Ayurveda formulations with their composition for safety aspects. Though, these are being used safely in without any noticeable untoward effects; there is a need to generate scientific evidence that these are safe and non-toxic. The drugs can usually be detoxified, but some of them can bio activated become more toxic. The liver often the target organ, most toxicants enter the body through the gastrointestinal tract and after absorption they are carried by the hepatic portal vein in the liver. The toxicology of the liver is complicated by the variety of liver injuries and by the different mechanisms through which the injuries are induced. In the present study, the safety profile of Diarid is tested for acute toxicity, Diarid was administered at a maximal dose of 600 mg/kg to overnight fasted rats and observed closely for behavioural changes, signs of toxicity and mortality if any, continuously for the first six hours and thereafter periodically up to 45 days. Animals were sacrificed on the 46th day. Biochemical parameters and were studied in both serum and liver tissue. In acute toxicity, Diarid at the dose of 600 mg/kg did not produce any observable toxic effects or mortality. No pathological changes on different biochemical parameters in serum and liver homogenate. Based on these observations, it can be concluded that Diarid is safe at therapeutic dose levels.

INTRODUCTION
Diabetes mellitus Presents with a group of metabolic disorders and there relative or absolute lack of production of insulin by the body which results in hyperglycemia. This is a chronic condition. Among the two major types of diabetes mellitus Type 1 and Type 2, type 1 generally seen in children and prevalence is 5% to 10% in diabetic population, previously known as juvenile diabetes, is the result of the body’s failure to produce enough insulin (Centers for Disease Control and Prevention. National Diabetes Fact Sheet. United States., 2005). Type 2 diabetes is a result of insulin resistance; the body cannot have used the insulin appropriately produced. Prevalence is more, and generally, this condition is diagnosed in adults According to the World Health Organization, recent figures suggest that there are more than 180 million people with diabetes worldwide and this number will double by 2030 (World Health Organization. Diabetes fact sheet, No. 312., 2006). Many millions of people are unaware that they have this disease and remains untreated. Variety of clinical complications such as cardiopathy, nephropathy, and retinopathy, neuropathy is associated with diabetes due to chronic hyperglycemia. If the condition is not managed properly which can lead to life-
threatening complications such as heart disease, stroke, blindness, and kidney failure. Diabetes is the sixth leading cause of death in the U.S. (Danaei et al., 2011). Much attention was not received for hepatotoxicity unlike other complications prevalent in diabetes until the clinical complications emerged due to the anti-diabetic drug therapy in modern medicine. Patients who take anti-diabetic drugs have a high incidence of hepatotoxicity. Underlying mechanisms or predisposing factors for hepatotoxicity in diabetes still unclear. It is recommended that liver enzymes such as alanine aminotransferase should be monitored in patients with diabetes receiving anti-diabetic drugs for which incidences of hepatotoxicity are already reported (Vagula M & Devi SS, 2008).

The liver is one of the most important organs in the body with diverse functions such as metabolism, secretion, storage, and detoxification of endogenous and exogenous substances. The metabolic activities of the liver are by means of various biochemical pathways including hydrolysis. Any disorders in these pathways will lead to the injury of the liver tissue that we call as hepatotoxicity which in turn leads to many life-threatening diseases which results in high mortality worldwide (Madrigal-Santillán E et al, 2014). Hepatotoxicity can be due to medicines, chemicals, dietary disturbances or herb-induced liver damage via hepatotoxins. Hepatic diseases exist as main threats, and this remains unsolved problems worldwide in public health. In spite of enormous advances in modern medicine, hepatic-toxicity exists with the patient, who takes anti-diabetic drugs. Thus, it is needed to identify pharmaceutical alternatives for the treatment of diabetes. The alternatives should be more effective and less toxic. The use of some plants in herbal medicines have played basic roles in human health care, and several scientific researchers have identified these beneficial effects are attributed to the presence of some chemical compounds called phytochemicals. According to WHO, 80% of the world population in the developing countries adopts herbal medicines for their basic health care. Modern medicine can offer only a very limited range of drugs and therapies. So all over the world use of herbal remedies is gaining popularity. Herbal medicines are easy to procure and easy to make formulation. These therapies are fortunately very efficacious and cost-effective. The research world for the constant search of better drug, more potent active principles of the plant, and more palatable formulations for diabetes (Srivastava R & Srivastava P, 2018)

In this view, every effort has been taken to collect and compile the details regarding a drug Diarid, which will be useful to society to venture into an alternative system of medicine. In order to assess the safety or a drug, various studies were carried out in animal-like, mice, rats, guinea pigs, dogs and monkeys under the varying condition of drug administration. After the establishment of pharmacological activity in experimental animals, toxicity studies are carried out in human models. Thus it is normally done to evaluate the safety of a new found plant material (extract, active factor or a compound) or a newer combination of herbal medicine. Ayurveda utilises natural resources of plant, animal, metal and mineral origin in therapeutics of different pathologies. These resources are converted into formulations based upon the need by following specified classical guidelines. Herbo-mineral and metallic formulations are an important part of Ayurveda that is attributed to be safe and efficacious when manufactured and used judiciously. Diarid, is an anti-diabetic drug which contains 0.375 gms of Ekanayakam (Salacia reticulata), marmanjal, (Coscinium fenestratum), manjal (Curcuma longa), venga (Pterocarpus marsupium), paachotti (Symplocos Racemosa), thechi (Ixora coccinea), raamacham (Vetiveria zizanoides), Nellika (Embla officinalis) Importance of following traditional pharmaceutical procedures in preparation of Ayurvedic formulations have been well-established. So, pharmaceutical researchers screening the herbal products and other natural products for the treatment of diabetes mellitus which is less in toxicity and also cost-effective. Considering this, safety profiles Diarid was evaluated in the current study. The current study was conducted to evaluate the hepatotoxic effect of Diarid biochemically by administering it to experimental animals.

MATERIALS & METHODS

Animals: 24 adult young, healthy, nulliparous, virgin female Swiss albino mice weighted 150-190 g were selected and acclimatised for 120 days, maintained under the standard laboratory conditions with the proper food and water for the experiment. The animals were divided into 4 groups of six mice in each group. For acute toxicity study was carried out by following Organization for Economic Cooperation and Development (OECD) 425 guidelines in 2001.

Group 1 (n=6): Normal-Normal diet & distilled water was given.

Group 2 (n=6): drug Control-Normal diet & diarid single dose (60mg/kg body weight per day) was given.

Group 3 (n=6): Sub-acute Group-Normal diet & diarid double dose (120mg/kg body weight per day) was given.

Group 4 (n=6): Acute Group-Normal diet & an acute dose of diarid (600mg/kg body weight per
day) was given. After 7-15 days if there was no LD50 a new dose of 1.2gm/kg body weight is administered to the 50% of the group.

MATERIALS

Dose and schedule: The therapeutic dose is 60 mg/kg body weight. Rat dose was calculated by referring to a table of Paget and Barnes. As classics advocate using diarid capsule content were mixed with the distilled water was administered orally with the help of oral cannula. Contents of the diarid capsule along with water were orally administered at the limit dose of 600 mg/kg to overnight fasted rats. The rats were observed closely for behavioral changes, signs of toxicity, and mortality, if any continuously for the first six hours’ drug administered for 45 days. Animals were fasted overnight, weighed.

Blood collection: Supraorbital plexus was punctured, and blood was collected using capillaries in two different tubes, one containing anticoagulant fluid for hematological parameters and another plain tube for serum biochemical investigations. Then the rats were sacrificed with an overdose of diethyl ether, and the abdomen was opened through midline incision to observe the autopsy changes followed by dissecting out the important organs.

Preparation of Liver homogenate: Liver tissues were removed to ice–cold containers for extraction and for estimations. Accurately weighed 100mg liver tissue was ground in a mortar with a pestle under cold condition. A 10% homogenate was prepared. By adding 9 volumes, i.e. 0.9 ml of sodium carbonate for ALP (sodium bicarbonate buffer, pH 10), for AST&ALT, 0.9 ml of phosphate buffer pH 7.4, FOR glutathione reductase 0.9 ml of phosphate buffer (0.1 m) pH 7.4, TBARS 0.9 ml, 1 ml of homogenate used of TRIS-HCL BUFFER. Centrifuged at 2000rpm. The supernatant was used for the assay of enzymes.

Biochemical parameters: Biochemical parameters in serum and liver were analysed by using the semi-automated biochemical analyser. The studied parameters were serum glutamate pyruvate transaminase (SGPT), Serum glutamate oxaloacetate transaminase (SGOT), alkaline phosphatase (ALP), Glutathione reductase, TBARS.

Study setting: The present study was conducted at the Department of Biochemistry, School of Medical Education, Kottayam, Kerala, India.

Ethical consideration: The study was approved by the Institutional animal ethical committee, school of medical education, Kottayam, India.

Data analysis: Data were analyzed using SPSS.15 versions. To observe the differences between the two groups students’ t-test was used. A p value less than 0.05 considered as significant.

RESULTS

Table 1: Aspartate aminotransferase activity in serum (IU/L) and liver (IU/100gm wet tissue)

<table>
<thead>
<tr>
<th>Groups</th>
<th>Serum</th>
<th>Liver</th>
</tr>
</thead>
<tbody>
<tr>
<td>I. Normal control</td>
<td>23.84±2.84</td>
<td>39.7±2.43</td>
</tr>
<tr>
<td>II. single dose</td>
<td>20.95±1.826</td>
<td>41.8±2.404</td>
</tr>
<tr>
<td>III. double dose</td>
<td>26.7±2.17</td>
<td>42.4±2.505</td>
</tr>
<tr>
<td>IV. acute dose</td>
<td>27.05±2.23</td>
<td>42.75±2.425</td>
</tr>
</tbody>
</table>

Group II, Group III and Group IV compared with group I. Values expressed as mean ± SD of 6 rats.

Table 1 presents the mean values of Aspartate aminotransferase activity in serum (IU/L) and liver (IU/100gm wet tissue) in the control and experimental group.

Table 2: Aspartate aminotransferase activity in serum (IU/L) and liver (IU/100gm wet tissue)

<table>
<thead>
<tr>
<th>Comparison between groups</th>
<th>t values</th>
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</thead>
<tbody>
<tr>
<td>I&amp;II</td>
<td>Serum</td>
</tr>
<tr>
<td>I&amp;II</td>
<td>1.77</td>
</tr>
<tr>
<td>I&amp;IV</td>
<td>1.90</td>
</tr>
</tbody>
</table>

Table 2 presents a comparison of Aspartate aminotransferase activity in serum (IU/L) and liver (IU/100gm wet tissue) in the control and experimental group. P values >0.05 so no statistical difference.

Table 3 presents the mean values of Alanine transaminase activity in serum (IU/L) and Liver (IU/100gm wet tissue) in the control and experimental group.

Table 3: Alanine transaminase activity in serum (IU/L) and Liver (IU/100gm wet tissue)

<table>
<thead>
<tr>
<th>Groups</th>
<th>Serum</th>
<th>Liver</th>
</tr>
</thead>
<tbody>
<tr>
<td>I. Normal control</td>
<td>20.0±2.39</td>
<td>62.27±1.32</td>
</tr>
<tr>
<td>II. Single Dose</td>
<td>18.35±2.01</td>
<td>61.96±2.41</td>
</tr>
<tr>
<td>III. Double Dose</td>
<td>23.05±2.425</td>
<td>63.1±3.80</td>
</tr>
<tr>
<td>IV. Acute dose</td>
<td>23.15±2.525</td>
<td>63.9±4.71</td>
</tr>
</tbody>
</table>

Group II, Group III and Group IV compared with group I. Values expressed as mean ± SD of 6 rats, p-value >0.05 not statistically significant value ≤ 0.05 is statistically significant.

Table 4: Alanine transaminase activity in serum (IU/L) and Liver (IU/100gm wet tissue) Comparison between controls and study group
Table 4 presents a comparison of Alanine transaminase activity in serum (IU/L) and Liver (IU/100gm wet tissue) in control and experimental group p values >0.05 so no statistical difference.

Table 5: Alkaline phosphatase activity in liver (IU/mg protein) and serum (IU/L) in the control and experimental group.

Table 6 presents a comparison of Alkaline phosphatase activity in liver (IU/mg protein) and serum (IU/L) in control and experimental group p values >0.05 so no statistical difference.

Table 7: Activity of Glutathione reductase in liver (10 µmols NADPH oxidized /mt and blood (IU/gm).

Table 8: Activity of Glutathione reductase in liver (10 µmols NADPH oxidized /mt and blood (IU/gm). Comparison between controls and study group.

Table 9: Activity of TBARS in liver (mmols/mg protein) and serum (µ mols/ ml).

Table 10: Activity of TBARS in liver (mmols/mg protein) and serum (µ mols/ ml). Comparison between controls and study group.

All values p value ≤ 0.05 is statistically significant value > 0.05 is not statistically significant

In this study, there is no significant elevation or change in levels of liver enzymes in both the con-
Ayurvedic drugs is a safer alternative to the side effects of chemical medicines. Some adverse effects of modern drugs result in search of natural herbs and thus bringing the traditional medicine systems into the limelight. Still, there are questions regarding the efficacy and toxicity of medicines, and there is a perception that Ayurveda does not have protocols to test the toxicology. But this may be the earliest system with well-developed have developed the discipline of toxicology. Toxicology or *Agada Tantra* is one among the eight clinical specialities of Ayurveda for thousands of years. We can say that Ayurvedic physicians must develop a clear perspective of the safety of Ayurvedic medicines and treatments and find solutions from within. The Ayurvedic toxicologist well trained in *Agada Tantra* seems to be the answer. All potential hazards associated with Ayurvedic treatments should be listed, guidance on how to anticipate them and also manage them would be the first step in this direction (P. Ram Manohar, 2014)

**REFERENCES**


