Effect of *Jatropha curcas* Leaf Extract on Pathological Changes in Experimental Diabetes in Rats

Josh Kumar¹, S.P. Singh¹ and Munish Batra*²

¹Department of Veterinary Pharmacology and Toxicology, ²Department of Veterinary Pathology, College of Veterinary and Animal Sciences, GBPUAT, Pantnagar (UK), India.

Abstract

Twenty five rats were divided into five groups of five rats each. Group I served as healthy control and group II as negative control. The study was conducted for the antidiabetic property evaluation after single injection of alloxan drug at 100 mg/kg body weight intraperitoneally followed after 48 hours by the treatment with metformin (300 mg/kg) in group III, hydroethanolic extract of *Jatropha curcas* leaves (HEJC) (200 mg/kg) in group IV and hydroethanolic extract of *Jatropha curcas* leaves (400 mg/kg) in group V. Gross lesions could only be recorded in liver and kidneys of group II rats. Microscopic examination revealed vacuolar degeneration and swelling of hepatocytes in liver and enlarged glomeruli in kidneys of diabetic rats. Improvement in metformin and high dose extract treated groups (group V) was observed in the form as evident by mild to moderate degeneration of hepatocytes in liver and moderate swelling of glomeruli. Electron microscopic changes revealed reduction in number of secretory granules, degenerative changes in nucleus and severe destruction of nuclear membrane in alloxan induced diabetic group. Treatment groups, however, showed apparently normal nucleus, mitochondria and moderate increase in number of secretory granules. It is concluded from this investigation that HEJC at 200 mg/kg and 400 mg/kg daily for 14 days produced protective effects on tissue changes and ultrastructural changes in alloxan induced diabetic rats. Thus, HEJC could further be investigated to develop a strategy for herbal remedy for the prevention and treatment of diabetes in man and animals.

Key words: Diabetes, *Jatropha curcas*, Pathology, Rats.

1. Introduction

Diabetes mellitus is one of the common endocrine disorders (WHO, 2004). It is expected to affect 5.4% of the global population by 2025. From 2010 and 2030, an increase of 69% is expected in numbers of adults with diabetes in developing countries and a 20% increase in developed countries (Shaw et al., 2010). Diabetes mellitus is characterized by hyperglycemia, defects in reactive oxygen species scavenging enzymes and high oxidative stress induced damage to pancreatic beta cells. In diabetic animals, free radicals are rapidly accumulated and cause oxidative stress, which may impair function of liver and kidney characterized by suppressed antioxidative activities and enhanced lipid peroxidation. Diabetes is associated with oxidative stress leading to an increased production of superoxide radical, hydrogen peroxide and hydroxyl radical or a reduction in the antioxidant defense system (Dahchea et al., 2011).

Currently available therapeutic strategies for diabetes include insulin and various oral antidiabetic agents such as sulfonylureas, biguanids, glucosidase inhibitors and glinides which are used as monotherapy or in combination to achieve better glycaemic regulation (Sy et al., 2005). The treatment of disease in the modern system of medicine is only a regulatory mode with devastating side and after effects including severe hypertension and likelihood of brain hemorrhage. Regardless of enormous advances in medical care, alternative therapies such as medicinal herbs have become popular over the past several years. The challenges posed by diabetes demands a research agenda, which maximizes the prospects of preventing, more effectively treating and curing the disease. Plants used in traditional medicine represent a valuable alternative for the control of the disease. Hence, there is an increasing demand of natural antidiabetic products by patients to avoid side effects of insulin and oral hypoglycemic agents (Prout, 1974; Holman and Turner, 1991; Kameshwar Rao et al., 1997). According to the World Health Organization, there are more than 1200 plant species worldwide used in the treatment of diabetes mellitus. The most effective antidiabetic Indian medicinal plants are *Acacia Arabica* (Babul), *Allium cepa* (Onion), *Allium sativum* (Garlic).
2. Materials and Methods

2.1 Collection of Plant Material and Preparation of Hydroalcoholic Extract

The *Jatropha curcas* leaves were collected from Medicinal Plant Research and Developmental Centre (MRDC), GB Pant University of Agriculture and Technology, Pantnagar. Leaves were washed, chopped, dried at 40°C for 72 to 96 hours and ground. A 50% hydroethanolic extract from *Jatropha curcas* leaves (1:1) was prepared by taking 10 g of leaves. Then 50 ml of water and 50 ml of ethanol was added to it. It was mixed gently after closing the mouth with parafilm.

This solution was stirred with the help of magnetic stirrer for 1 hour and kept in incubator shaker at 37°C with gentle swirling at 120 rpm per minute for overnight. The solution was then filtered. The filtrate thus obtained was centrifuged at 5000 rpm at 37°C for 10 min. The supernatant was collected and ethanol was evaporated with the help of Rota Vapour. Semisolid extract obtained after rota vaporization was lyophilized in order to obtain extract as dry powder. The yield of sample was calculated and it came out to be 5.8%.

2.2 Experimental Design

Twenty five Sprague Dawley albino rats of 2 to 2.5 month of age, weighing between 150 to 170 gm were procured from Laboratory Animal Resource Centre, Indian Veterinary Research Institute, Izatnagar. These were divided randomly and equally into five groups. Group I was kept as healthy control and group II as negative control. Alloxan was administered at 100 mg/kg body weight in groups II, III, IV and V. Group III was administered metformin at 300 mg/kg body weight once orally for 14 days and it served as positive control. HEJC was given at 200 and 400 mg/kg body weight orally daily for 14 days in groups IV and V, respectively. Treatment with the metformin and HEJC commenced 48 hours post administration of alloxan i.e. 0 day of diabetes in groups II, III, IV and V till completion of study i.e. 14 days. The experiment was conducted as per the schedule mentioned in Table 1.

Blood glucose level was observed at weekly intervals. After 14 days, rats were sacrificed humanely. All the sacrificed rats were subjected to detailed post mortem examination and the representative samples were collected from liver and kidney were collected in 10% buffered formalin and processed for histopathological examination as per standard protocol (Luna, 1968) and pancreas for electron microscopy.

The pancreas was collected from all the rats from all the groups sacrificed after completion of 14 days and fixed with 2.5% glutaraldehyde (EM) grade. Secondary fixation was done with osmium tetroxide. Pancreas was washed with ascending grades of ethanol (30%, 50%, 70%, 90%) for 5-7 minutes in each grade and finally with absolute alcohol for 20 minutes. For clearing, the pancreas was kept in tobin for 1 hour. Infiltrated sample was embedded in resin and blocks were made for further processing following the standard protocol.

3. Results

3.1 Gross Pathology

No gross lesion could be seen in any organ of groups I, III, IV and V rats. Gross lesions could only be recorded in the organs of group II rats. Liver of group II rats was yellowish in appearance and slightly enlarged. Kidneys in this group were swollen and enlarged.

3.2 Histopathology

3.2.1 Liver

No microscopic lesions could be recorded in liver of group I rats. Microscopic examination of liver of group II revealed severe vacuolar degeneration of hepatocytes and swelling of hepatocytes leading to occlusion of sinusoids (Fig 1). In group III rats, liver -

### Table 1: Experimental design for the study

<table>
<thead>
<tr>
<th>Groups</th>
<th>Treatment</th>
<th>No. of rats</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Control</td>
<td>5</td>
</tr>
<tr>
<td>II</td>
<td>Alloxan at 100 mg/Kg body weight, intraperitoneally</td>
<td>5</td>
</tr>
<tr>
<td>III</td>
<td>Alloxan + Metformin at 300 mg/Kg body weight for 14 days</td>
<td>5</td>
</tr>
<tr>
<td>IV</td>
<td>Alloxan + HEJC at 200 mg/Kg body weight for 14 days</td>
<td>5</td>
</tr>
<tr>
<td>V</td>
<td>Alloxan + HEJC at 400 mg/Kg body weight for 14 days</td>
<td>5</td>
</tr>
</tbody>
</table>

*Azadirachta indica* (Neem), *Hibiscus rosasinesi* (Garhal), *Jatropha curcas* etc (Patil et al., 2011). *Jatropha curcas* is regarded as a wonder plant because of its numerous attributes. *Jatropha* is still used as a traditional medicine in India and Africa. The hydroalcoholic and chloroform extract of leaves of *Jatropha curcas* demonstrated antidiabetic property at 250 and 500 mg/kg in a dose dependent manner in alloxan induced diabetic rats (Patil et al., 2011). The 50% ethanolic extract of leaves of *Jatropha curcas* L. produced antidiabetic effect in diabetic rats (Mishra et al., 2010). Alternatives to chemotherapeutic antidiabetic therapy are clearly needed in light of the fact that its inability to control all the pathological aspects of diabetes and the high cost unaffordable by the common people. Considering the commercial, socio-economic, medicinal importance and paucity of scientific investigation on antidiabetic properties of *Jatropha curcas* leaves, the present endeavor was undertaken to study the effect of hydroethanolic extract of *Jatropha curcas* leaves on pathological and ultrastructural changes in various visceral organs of rats.
exhibited mild degeneration of hepatocytes only. Group IV rats manifested swelling of hepatocytes, severe vacuolar degeneration was seen in liver. In group V rats, liver showed only mild to moderate degeneration was observed in hepatocytes.

3.2.2 Kidney

No microscopic lesions could be recorded in kidneys of group I rats. Kidney in group II showed very severe swelling of the glomeruli occupying almost whole of the Bowman’s space and almost all the epithelial cells were swollen leading to occlusion of the lumen (Fig 2). In kidneys of group III rats, glomeruli were normal with almost intact tubular epithelial cells. Group IV rats showed severe swelling of glomeruli and majority of tubular epithelial cells showing swelling of the epithelial cells in kidney. Treatment showed some improvement as in group V kidneys, glomeruli showed moderate swelling and almost intact tubular epithelial cells.

The most marked pathological changes were severe vacuolar degeneration of hepatocytes and swelling of hepatocytes leading to occlusion of sinusoids and kidney showed very severe swelling of the glomeruli occupying almost whole of the Bowman’s space and almost all the epithelial cells leading to occlusion of the lumen in group II. Group IV, showed severe swelling of glomeruli and majority of tubular epithelial cells showing swelling of the epithelial cells. In group III, glomeruli were normal with intact tubular epithelial cells and in liver mild degeneration of hepatocytes were observed. Treatment showed some improvement as in group V, only mild to moderate degeneration of hepatocytes in liver was observed. In the kidneys, glomeruli showed moderate swelling and almost intact tubular epithelial cells. Earlier workers have also observed similar findings while evaluating the protective effects of *Trigonella foenum*
graecum seed powder on histopathological abnormalities in tissues of diabetic rats (Thakran et al., 2004).

3.3 Ultrastructural Changes in Pancreas

Transmission electron micrograph of pancreas in group I rats revealed normal nucleus, mitochondria and secretory granules. In group II rats, pancreas revealed decreased secretory granules, degenerative changes in the nucleus and severe destruction of nuclear membrane (Fig 3). Pancreas in group III rats showed increase in the number of secretory granules, apparently normal mitochondria and nucleus. Group IV rats showed swelling in some mitochondria of pancreatic cells. Electron microscopic changes in group V rats were characterized by apparently normal nucleus, mitochondria and moderate increase in the secretory granules (Fig 4).

Electron microscopic changes in the pancreas of rats in group II revealed decreased secretory granules, degenerative changes in the nucleus and severe destruction of nuclear membrane. In group III, increase in the number of secretory granules, apparently normal mitochondria and nucleus was observed. Group V showed apparently normal nucleus, mitochondria and moderate increase in the secretory granules. Group IV showed swelling in some mitochondria of pancreatic cells. Similar observations were reported earlier also in a study undertaken to evaluate antidiabetic activity of Terminalia chebula (Kumar et al., 2006). Significant decrease in the number of secretory granules was observed in the Streptozotocin induced diabetic rats and these pathological abnormalities were normalized after treatment with Terminalia chebula extract (Kumar et al., 2006).

4. Conclusion

It is concluded from this study that hydroalcoholic extract of Jatropha curcas leaves showed protective effect in experimentally induced diabetic rats at 400 mg/kg body weight. It can be further investigated to develop a strategy for herbal remedy for the prevention and treatment of diabetes in man and animals.

References


