Effects of *eugenia jambolana* extract against streptozotocin induced acute liver damage in rats


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**ABSTRACT**

The aim of the study was to compare the effects of *Eugenia jambolana* (EJ) extract and glibenclamide on streptozotocin (STZ) induced hepatotoxicity in rats. Wistar rats were divided into four groups: untreated control (UC), untreated diabetic (UD), EJ (200 mg/kg) treated diabetic (ED) and glibenclamide (0.6 mg/kg) treated diabetic (GD) orally for 45 days. There was various evidence of hepatotoxicity, including significant increase (P<0.001) in serum transaminases activity and histopathological findings in UD rats. In ED group, cellular injury was minimal and the hepatocytes maintained a better morphology when compared to UD rats. The substantially elevated serum transaminases were restored towards normal by the extracts. Findings indicate that EJ extract may have a preventive effect on STZ induced hepatotoxicity and also has potential hepatoprotective activity when compared to GD rats.

**Key words:** *Eugenia jambolana*, Hepatotoxicity, Liver, Serum transaminases, Streptozotocin.

**INTRODUCTION**

The liver has the critical role of maintaining the body’s metabolic homeostasis. This includes the processing of dietary amino acids, carbohydrates, lipids, and vitamins; removal of microbes and toxins; synthesis of many plasma proteins; and detoxification and excretion into bile of endogenous waste products and pollutant xenobiotics (Kumar *et al.*, 2009). Hence, liver diseases have far-reaching consequences. The liver is vulnerable to a wide variety of metabolic, toxic, microbial, circulatory, and neoplastic insults. More often, hepatic damage is secondary to some of the most common diseases in humans, such as cardiac decompensation, disseminated cancer and extra hepatic infections.

Damaged hepatocytes release enzymes such as alanine aminotransferase (ALT) and aspartate aminotransferase (AST) which are the markers of hepatic injury into the blood. Measurement of the levels of these enzymes in the blood provides a reliable clinical measure of hepatotoxicity (Singer *et al.*, 1995).

Modern medicines have little to offer for alleviation of hepatic diseases and it is chiefly the plant based preparations which are employed for their treatment of liver disorders (Karan *et al.*, 1999). *Eugenia jambolana* (EJ) which belongs to the family Myrtaceae, is a large evergreen tree growing up to 30 m height, found widely in India and the Asian subcontinent. The seeds of this plant have been reported to possess many medicinal properties in the Ayurveda system of medicine. EJ seeds have hypoglycemic (Chopra *et al.*, 1958), neuropsycho-pharmacological (Chakraborty *et al.*, 1985), anti-inflammatory (Chaudhuri *et al.*, 1990), anti-diarrheal (Indira and Mohan, 1993), anti-HIV (Kusumoto *et al.*, 1995) and anti-bacterial (Bhuiyan *et al.*, 1996) effects. But there is not much drug available for the treatment of liver disorders. There has been no current information available regarding the protective activity of EJ and a comparison with a standard hypoglycemic drug, glibenclamide in diabetic rats. Diabetic liver is associated with physiological, biochemical and pathological changes. Hence, the objective of this study was to compare the effects of *Eugenia jambolana* (EJ) extract and glibenclamide on streptozotocin (STZ) induced hepatotoxicity in rats.

**MATERIALS AND METHODS**

**Animals:** Adult male Wistar Albino rats weighing about 180-260 g were obtained from Central Animal Facility, Indian Institute of Science, Bangalore-560 012. All animals fed with standard laboratory feed (Amruth Feeds, Bangalore) and...
water *ad libitum*. The animals were kept for acclimatization in animal house for 2 weeks. All experimental procedures involving animals were approved by the Institutional Animal Ethics Committee, Veterinary College, Bangalore-24.

**Drug and chemicals:** Streptozotocin (Sigma Chemicals, USA), Alcoholic seed extracts of *Eugenia jambolana* (Plantex, Vijayawada, Andhra Pradesh), carboxymethylcellulose (Central Drug House, New Delhi) and glibenclamide (Daonil®, 5 mg) were used in the experiments. The plant was identified by HPTLC finger printing and assayed by gravimetric method.

**Experimental induction of diabetes:** Diabetes mellitus was induced experimentally by single intraperitoneal (i.p.) injection of a freshly prepared STZ solution (Sigma Chemicals, USA) dissolved in 0.1 M ice-cold citrate buffer (pH 4.5) at a dose of 45 mg/kg to overnight-fasted rats (Babu and Prince, 2004). Control rats received an i.p. injection of citrate buffer alone. Seventy two hours after STZ administration, blood samples were drawn from retro orbital plexus and fasting blood glucose level were determined to confirm the onset of diabetes. The diabetic rats that exhibited fasting blood glucose level higher than 300 mg/dl were selected for the study. Treatment was started on the fourth day after STZ administration and continued for 45 days.

**Animal study protocol:** Sixty male rats were used in this study and divided randomly into four groups (10 animals for each). The groups were organised as follows: Group I: Untreated control (UC) rats, Group II: Untreated diabetic (UD) rats (STZ alone), Group III: Diabetic rats given alcoholic seed extract of EJ at 200 mg/kg orally (ED), Groups IV: Diabetic rats given glibenclamide at 600 g/ kg orally (GD).

The animals described as fasted were deprived of food for 12 h but allowed free access to drinking water. During the experimental period, the animals were carefully checked twice daily for the clinical signs and were recorded. Blood was collected from retro-orbital plexus of the rats under light ether anaesthesia at different time points from 3rd, 15th, 30th to 45th days of experiment. The serum obtained after centrifugation at 3000 rpm for 10 minutes was used to estimate the blood glucose.

**Biochemical assessments:** Hepatic injury was assessed using the serum levels of alanine aminotransferase (ALT) and aspartate aminotransferase (AST). The serum ALT and AST levels were determined using commercial biochemical kits (Span Diagnostics, Bangalore). These parameters were estimated from serum sample as per the procedure described by Tietz (1982). The separated serum was collected and subjected for glucose estimation immediately after collection and the remaining serum samples were stored in a deep freezer at -20°C for further analysis.

**Histopathology:** Hepatic injury was also assessed by histopathological changes. Two animals from each group were euthanized on 15th, 30th day and rest on the end of the experiment using an overdose of ether for studying the progressive effects of treatment. Animals were immediately dissected and tissues were visualized and palpated for the evidence of gross pathology. For the light microscopic examinations, hepatic samples from left lateral lobes were fixed in 10 % phosphate buffered formalin and embedded in paraffin. The paraffin blocks were sectioned at 4µm and were stained with Hematoxylin and Eosin (H&E) for the evaluation of hepatocytes injury (Luna, 1968).

**Statistical analysis:** Data were analyzed by two way analysis of variance (ANOVA) using statistical software, Graph pad Prism for Windows Version 5.0. Results were presented as Mean ± SE and P< 0.001 were identified as significantly different.

**RESULTS AND DISCUSSION**

This study was designed to evaluate the effects of alcoholic seed extract of EJ on improving the damages of liver parenchyma in the STZ induced rats. The serum ALT and AST were presented in Table 1. The diabetic rats showed a significant increase (P<0.001) in all these parameters when compared to the normal rats. There was a significant decrease (P<0.001) was noticed in blood glucose level of EJ treated (200mg/kg) rats on 45th day when compared to diabetic control rats. A statistically significant decrease (P<0.001) was detected in the serum ALT and AST levels of EJ treated rats on 15th day onwards.

On gross examination, liver appeared swollen and pale on 15th day and pale, soft and friable from 30th day onwards in UD rats. The liver was normal in colour and consistency on 15th, 30th and 45th day post treatment in ED rats. The gross changes were found to be comparable to those of UD rats in GD rats but of mild to moderate degree.

Light microscopic examination of liver samples was assessed following the procedures of Luna (1968). Hepatocyte plates were normal in UC rats throughout the study (Fig. 1A). UD rats revealed remarkable swollen hepatocytes, marked cyttoplasmic granularity, vacuolations, and sinusoidal narrowing as well as marked centrilobular necrosis with inflammatory cell foci on 15th day. Necrosis of hepatocytes was advanced to bridging type on 30th day and the lesions became more diffused on 45th day (Fig. 1B). Lobular zones were infiltrated with mononuclear and occasionally polymorphonuclear cells in the STZ group.
TABLE 1: Effect of alcoholic extract of *EJ* on ALT (U/L) and AST (U/L) in STZ induced diabetic rats

<table>
<thead>
<tr>
<th>Days</th>
<th>Parameter</th>
<th>Group I</th>
<th>Group II</th>
<th>Group III</th>
<th>Group IV</th>
</tr>
</thead>
<tbody>
<tr>
<td>3rd day</td>
<td>ALT</td>
<td>52.47 ± 1.35</td>
<td>142.96 ± 7.71</td>
<td>150.61 ± 5.07</td>
<td>135.47 ± 5.77</td>
</tr>
<tr>
<td></td>
<td>AST</td>
<td>64.95 ± 1.08</td>
<td>180.50 ± 0.74</td>
<td>178.10 ± 3.28</td>
<td>185.36 ± 1.73</td>
</tr>
<tr>
<td>15th day</td>
<td>ALT</td>
<td>51.92 ± 0.36</td>
<td>206.98 ± 1.87</td>
<td>88.16 ± 1.57</td>
<td>120.97 ± 5.13</td>
</tr>
<tr>
<td></td>
<td>AST</td>
<td>64.86 ± 0.96</td>
<td>215.03 ± 3.84</td>
<td>73.68 ± 1.94</td>
<td>152.88 ± 3.52</td>
</tr>
<tr>
<td>30th day</td>
<td>ALT</td>
<td>52.87 ± 0.49</td>
<td>249.18 ± 2.93</td>
<td>57.28 ± 1.76</td>
<td>108.90 ± 4.95</td>
</tr>
<tr>
<td></td>
<td>AST</td>
<td>64.93 ± 0.99</td>
<td>271.11 ± 1.97</td>
<td>82.83 ± 0.89</td>
<td>123.11 ± 6.59</td>
</tr>
<tr>
<td>45th day</td>
<td>ALT</td>
<td>51.96 ± 0.90</td>
<td>345.34 ± 1.62</td>
<td>57.28 ± 1.84</td>
<td>102.80 ± 7.01</td>
</tr>
<tr>
<td></td>
<td>AST</td>
<td>64.68 ± 1.08</td>
<td>355.68 ± 4.97</td>
<td>70.23 ± 0.85</td>
<td>114.65 ± 7.25</td>
</tr>
</tbody>
</table>

For each parameter, means bearing the same superscript do not differ significantly. *Comparison with Group I, $^b$ Comparison with Group II, $^c$ Comparison with Group IV. Values are statistically significant at $P< 0.001$.

Sections of ED rats showed no abnormality in liver histology. Hepatocyte plates were normal and sinusoidal narrowing with inflammatory cell foci was not observed throughout the study. In addition, hepatocellular vacuolization was not seen and the lobuli were regular in shape (Fig 1C). Glibenclamide treated rats showed sustained mild to moderate degree of hepatic damage throughout the study (Fig. 1D).

Streptozotocin (STZ) is a β-cell toxin isolated from the bacterium *Streptomyces achromogens*. STZ uptake into β cells results in the induction of a variety of intracellular mechanisms such as nitric oxide (NO) donation, poly (ADPribose) polymerase (PARP) induction, DNA alkylation, and free radical generation. STZ effectively induced diabetes in all the rats from group-II to IV and showed hyperglycaemia ranging from 428.50 ± 6.74 mg/dL to 436.50±4.50 mg/dL by 72 hours after STZ administration (Babu and Prince, 2004). Glibenclamide is one of the most widely used medications against hyperglycemia which stimulates insulin secretion from â-cells through inactivation of ATP-sensitive potassium channel (Sakamoto et al., 2006).

In STZ induced diabetes, the free radicals generated induce tissue damage by attacking membranes through peroxidation of unsaturated fatty acids which cause leakage of enzymes accounting for increased serum levels of ALT and AST. We found that the serum levels of both ALT and AST were elevated in the STZ group that was in accordance with Can et al., (2004).

There was a progressive and significant decrease ($P<0.001$) in the mean serum ALT and AST values in the EJ extract treatment when compared to those of diabetic control animals throughout the study. In the present study, ALT and AST values in GD group showed an improvement from 3rd day post- treatment onwards in comparison with that of diabetic control group. However, it was observed that the values were significantly higher compared to normal control group and clearly indicated that glibenclamide treatment did not reverse completely the liver damage caused by STZ. This was substantially supported by histopathological findings like persistence of mild to moderate degree of hepatic damage in GD rats till the end of the study.

Microscopically, cellular swelling, granularity, degeneration, sinusoidal narrowing, inflammatory cell infiltration and necrosis were minimal and hepatocytes

FIG 1A: Section of liver in UC rats showing normal architecture on 45th day (H&E X 200).

FIG 1B: Section of liver in UD rats showing diffused swelling (star), vacuolations (arrow) and necrosis of hepatocytes (asterisk) on 45th day (H&E X 200).
maintained a better morphology in the ED group when compared with normal control group. The liver showed apparently normal architecture from Day 15 onwards in ED group in the present study. There was complete absence of STZ induced effects on 15\textsuperscript{th}, 30\textsuperscript{th} and 45\textsuperscript{th} day of the experiment. The reversion of STZ effect in liver could be attributed to the hepatoprotective effects of EJ seed extract which has been reported to contain flavanoids and other substances like saponins, flavonoids, phenolic compounds, glycosides and triterpenoids (Rupasinghe \textit{et al.}, 2003). The antioxidant effects of flavanoids involve quenching of free radicals induced in STZ diabetes such as superoxide and singlet oxygen (Ravi \textit{et al.}, 2004). It was noticed that EJ treatment protected the liver better in comparison with glibenclamide treatment. This clearly indicated that glibenclamide treatment failed to protect the liver completely, unlike EJ and this observation accounted the superiority of EJ over glibenclamide which is a novel antidiabetic drug. The improved ALT and AST values and attainment of normal architecture of hepatic lobules in the present study substantially supporting hepatoprotective effects of EJ extracts in STZ induced diabetes. This is the first report that suggests the seed extract of EJ has protective effects against acute liver damage caused by STZ.

In the present study, the architectural changes in the liver tissue of the diabetic rats were characteristically absent in the liver of the diabetic animals treated with EJ extract. Based on the above biochemical and histopathological findings, EJ extract could be used in conjunct with any conventional hypoglycemic drugs available in the market in order to protect the liver where conventional hypoglycemic drugs could not.

REFERENCES


