Evaluation of hepatoprotective activity of ethanolic extract of *Cajanus cajan* against paracetamol induced hepatotoxicity in rats

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Abstract

**Objective**: Ethanol extract of *Cajanus cajan* (L.) Millsp. (Pigeonpea) leaves (ECC) was investigated for its hepatoprotective activity.

**Methods**: Different groups of Wistar albino rats were administered with paracetamol (500 mg/kg, p.o., once daily for 7 days) for induction of hepatotoxicity. ECC (100, 200 and 300 mg/kg/day) and silymarin (25 mg/kg/day) were administered to the paracetamol induced hepatotoxic rats for seven days. The effects of ECC and silymarin on serum transaminases (SGOT, SGPT), alkaline phosphatase (ALP), bilirubin (Direct and Total), cholesterol (HDL and Total) were measured. The effects of the extract on lipid peroxidation (LPO), glutathione (GSH), superoxide dismutase (SOD) and catalase (CAT) were estimated. Moreover, the histopathological studies on ECC treated paracetamol induced rat liver were done. **Results**: ECC and silymarin produced significant (P < 0.05) hepatoprotective effect by decreasing the activity of serum enzymes, bilirubin, total cholesterol and *in vivo* lipid peroxidation and significantly (P < 0.05) increasing the levels of GSH, SOD, CAT and HDL cholesterol. Antioxidant effects on FeCl₂-ascorbate-induced lipid peroxidation in rat liver homogenate were also shown by ECC. Histopathological profiles showed that the extract had significant protective effect against paracetamol-induced liver injury, which corroborates the above findings. **Conclusion**: These results show that ECC could protect the liver cells from paracetamol-induced liver damages perhaps, by its antioxidative effect on hepatocytes.

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Key words: *Cajanus cajan*; Hepatoprotective effect; Antioxidants; Histopathological studies; Paracetamol.

1. Introduction

Liver diseases remain one of the serious health problems. It is well known that reactive oxygen species (ROS) cause cell damage through covalent binding and lipid peroxidation with subsequent tissue injury. Antioxidant agents of natural origin have attracted special attention because they can protect human body from free radicals [1]. In India several medicinal plants and their formulations are used for liver disorders in ethnomedical practices as well as in traditional systems of medicine [2]. *Cajanus cajan* (L.) Millsp. (Fabaceae) is a perennial woody shrub with strong stems, freely branching, root system deep and extensive with a taproot, distributed throughout India. The young leaves are applied for the treatment of sores and help to expel bladderstones in India. Leaf juice is used for jaundice [3], toothache, dysentery, heart disease, bronchitis and as anthelmintic [4]. The plant has been reported to show hypoglycaemic activity [5]. The in vitro antioxidant activity of the ethanolic extract of the plant has already been published by the authors. [6] The plant is reported to contain β-sitosterol, stigmasterol, flavanone such as cajaflavanone, lupeol, α and β amyrins, a new anthraquinone viz. cajaquinone [7]. As *Cajanus cajan* is traditionally used in the treatment of jaundice it is thought worthwhile to investigate the hepatoprotective activity of *Cajanus cajan* in a scientific manner.

2. Materials and methods

2.1. Plant Material

The plant was identified by the taxonomists of the Botanical Survey of India, Govt. of India, Shibpur, Howrah. After authentication, fresh leaves were collected in bulk from young matured plants from the rural belt of
Salipur, Orissa, India during early summer, washed under running tap water to remove adhering dirt, shade dried and finally milled in to coarse powder by a mechanical grinder. The powdered plant material (400 g) was defatted with petroleum ether (60-80°C) and then extracted with 1.5 L of ethanol (95%) in a soxhlet apparatus. The solvent was then removed under reduced pressure, which obtained a greenish-black sticky residue (yield: 15.5 % w/w with respect to dried plant material). The dried extract (ECC) was stored in a desiccator till further study.

2.2. Preliminary Phytochemical Studies

The ethonolic extract of the plant was subjected to preliminary phytochemical studies using standard procedures [8, 9] to find out the nature of the phytoconstituents present within it.

2.3. Chemicals Used

All the chemicals and reagents used in the study were of analytical grade.

2.4. Animals

Studies were carried out using Wistar albino rats (150–180 g) of male sex. The animals were grouped and housed in polyacrylic cages (38 × 23 × 10 cm) with not more than six animals per cage and maintained under standard laboratory conditions. They were allowed free access to standard dry pellet diet (Hindustan Lever, Kolkata, India) and water ad libitum. The mice were acclimatized to laboratory condition for 10 days before commencement of experiment. All procedures described were in accordance with the recommendations made by the Institutional Animal Ethical Committee.

2.5. Acute Toxicity Studies

The test was carried out as suggested by Seth et al. (1972) [10].

2.6. Hepatoprotective Activity

2.6.1. Paracetamol – Induced Liver Damage in Rats (Acute Model) [11]

Six groups each comprising of six male Wistar albino rats weighing in the range of 150-180 gm were selected. Group I served as control and fed orally with normal saline 5 ml/kg daily for seven days. Group II rats were similarly treated as Group I. Group III, IV and V were treated with ethanolic extract respectively at a dose of 100, 200 and 300 mg/kg/day orally for seven days, while Group VI was fed silymarin 25 mg/kg [12] as standard daily for seven days. Paracetamol suspension was given by oral route in a dose of 500 mg/kg/day to all rats except rats in Group I for seven days. The biochemical parameters were determined after 18 hours of fasting of the last dose. Daily records of body weight of all groups of animals were maintained during the whole experimental period.

2.6.2. Biochemical Studies

After the treatment period, the animals of all groups were anaesthetized and sacrificed. Blood was drawn from heart and serum was separated for the assay of serum glutamate oxaloacetate transaminase (SGOT), serum glutamate pyruvate transaminase (SGPT), alkaline phosphatase (ALP), bilirubin (Direct and Total) and cholesterol (Total and HDL). Analysis of SGOT, SGPT, ALP, Bilirubin (Direct and Total) and Cholesterol (Total and HDL) was performed using analytical kits from Span Diagnostics Ltd., Surat, India. Serum GOT and GPT were measured according to the method of Rietman and Frankel, (1975) [13], serum ALP was measured according to the method of King, (1965) [14], serum bilirubin was estimated following Malloy and Evelyn method, (1937) [15], serum cholesterol (Total and HDL) was measured according to Warnick et al., (1985) [16].

2.6.3. FeCl2-Ascorbic Acid Stimulated Lipid Peroxidation in Liver Homogenate

The Wister albino rats weighing 175-200 g were sacrificed by decapitation and their liver tissues were quickly removed. A portion of liver tissue (2 g) was sliced and then homogenized with 10 ml of 150 mM KCl Tris-HCl buffer (pH 7.2). The reaction mixture was composed of 0.25 ml of liver homogenate, 0.1 ml of Tris-HCl buffer (pH 7.2), 0.05 ml of 0.1 mM ascorbic acid, 0.05 ml of 4 mM FeCl2 and 0.05 ml of various concentrations of ECC. The products of lipid peroxidation were quantified by the formation of the thiobarbituric acid-reactive material, MDA [17]. 1,1,3,3- tetraethoxypropane was used as a standard for calibration of MDA. Appropriate controls were used to eliminate any possible interference with the thiobarbituric acid assay.

2.6.4. Determination of In Vivo Antioxidant Activity

After collection of blood samples the rats were sacrificed and their livers were excised, rinsed in ice cold normal saline, followed by 0.15 M Tris-HCl (pH 7.4) blotted dry and weighed. A 10 % w/v of homogenate was prepared in 0.15 M Tris-HCl buffer and processed for the estimation of lipid peroxidation by the method of Fraga et al., (1981) [18]. A part of homogenate after precipitating proteins with trichloric acetic acid (TCA) was used for estimation of glutathione by the method of Ellman et al., (1959)[19]. The rest of the homogenate was centrifuged at 15000 rpm for 15 min at 4°C. The supernatant thus obtained was used for the estimation of SOD by the method described by Kakkar et al. (1984) [20] and CAT activity was measured by the method of Maehly et al., (1954) [21]. Protein was estimated according to Lowry et al., (1951) [22].

2.7. Statistical Analysis

Data for hepatoprotective activity and in vivo antioxidant activity were expressed as Mean ± SEM from 6 rats in each group. Hepatoprotective and in vivo antioxidant activity were analysed statistically using one-way analysis of variance (ANOVA), followed by Dunnett’s t-test. The minimum level of significance was fixed at P<0.05.

3. Results

3.1. Acute Toxicity Study

The study reveals that there was no mortality at any of the tested doses till the end of 14 days of observation.

3.2. Serum Analysis

Rats treated with paracetamol only, developed significant (P<0.05) hepatocellular damage as evident from significant increase in serum activities of GOT, GPT, ALP and bilirubin concentration as compared to normal control group, which has been used as dependable markers of hepatotoxicity (Table 1). Oral administration of ECC showed significant reduction (P<0.05) in paracetamol-induced increase in levels of GOT, GPT, ALP and bilirubin (Total and Direct) concentration in a dose dependant manner with 300 mg/kg, p.o. being the most effective dose.
### Table 1. Effect of ethanolic extract of Cajanus cajan aerial parts on paracetamol-induced hepatotoxicity in rats

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>SGOT (U/ml)</th>
<th>SGPT (U/ml)</th>
<th>ALP (KA units)</th>
<th>Bilirubin (mg/dl)</th>
<th>Cholesterol (mg/dl)</th>
<th>Liver weight (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Total</td>
<td>Direct</td>
<td>Total</td>
<td>HDL</td>
<td></td>
<td></td>
</tr>
<tr>
<td>I</td>
<td>Control</td>
<td>51.18 ± 2.38</td>
<td>52.74 ± 1.89</td>
<td>75.26 ± 4.31</td>
<td>0.52 ± 0.04</td>
<td>0.09 ± 0.01</td>
<td>121.49 ± 5.63</td>
</tr>
<tr>
<td></td>
<td></td>
<td>118.72 ± 7.25</td>
<td>109.84 ± 6.38</td>
<td>148.29 ± 8.47</td>
<td>4.89 ± 0.42**</td>
<td>0.86 ± 0.07**</td>
<td>168.72 ± 9.82</td>
</tr>
<tr>
<td></td>
<td></td>
<td>86.38 ± 4.43</td>
<td>83.84 ± 5.69</td>
<td>115.33 ± 2.11**</td>
<td>1.16 ± 0.08**</td>
<td>0.43 ± 0.04**</td>
<td>147.61 ± 8.74**</td>
</tr>
<tr>
<td></td>
<td></td>
<td>79.52 ± 4.16</td>
<td>74.57 ± 4.25</td>
<td>101.70 ± 4.84</td>
<td>0.92 ± 0.06**</td>
<td>0.35 ± 0.04**</td>
<td>139.76 ± 6.42**</td>
</tr>
<tr>
<td></td>
<td></td>
<td>64.67 ± 3.88**</td>
<td>67.35 ± 3.62</td>
<td>93.52 ± 4.21</td>
<td>0.74± 0.05**</td>
<td>0.24 ± 0.02**</td>
<td>132.55 ± 5.06**</td>
</tr>
<tr>
<td></td>
<td></td>
<td>59.84 ± 4.41**</td>
<td>61.86 ± 3.19**</td>
<td>87.46 ± 5.52**</td>
<td>0.67 ± 0.05**</td>
<td>0.17 ± 0.02**</td>
<td>128.63 ± 5.04**</td>
</tr>
</tbody>
</table>

All values are Mean ± SEM, n=6 rats in each group
*P < 0.05 as compared with Group I; †P < 0.05 as compared with Group II

<table>
<thead>
<tr>
<th>Sample</th>
<th>Concentration (μg/ml)</th>
<th>% inhibition</th>
<th>IC₅₀ (μg/ml)</th>
<th>r²</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ethanol Extract</td>
<td>50</td>
<td>19.26 ± 0.57</td>
<td>140.44</td>
<td>0.9659</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>29.42 ± 2.06</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>150</td>
<td>47.18 ± 2.56</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>200</td>
<td>78.73 ± 3.49</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

α-tocopherol
128.37 0.9864

n=3, Values are Mean ± SD; r² = regression co-efficient

### Table 2. Effect of ethanolic extract of Cajanus cajan aerial parts on in vitro lipid peroxidation

<table>
<thead>
<tr>
<th>Sample</th>
<th>Concentration (μg/ml)</th>
<th>% inhibition</th>
<th>IC₅₀ (μg/ml)</th>
<th>r²</th>
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</tr>
<tr>
<td></td>
<td>200</td>
<td>78.73 ± 3.49</td>
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</tr>
</tbody>
</table>

α-tocopherol
128.37 0.9864

### Table 3. Effect of ethanolic extract of Cajanus cajan aerial parts on LPO, antioxidant enzymes and GSH in liver of paracetamol-induced hepatotoxic rats in-vivo

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>LPO*</th>
<th>SOD*</th>
<th>CAT*</th>
<th>GSH*</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Control</td>
<td>1.85 ± 0.31</td>
<td>11.69 ± 0.83</td>
<td>61.49 ± 5.52</td>
<td>63.17 ± 4.38</td>
</tr>
<tr>
<td>II</td>
<td>Paracetamol treated</td>
<td>7.88 ± 0.93**</td>
<td>4.64 ± 0.52**</td>
<td>39.68 ± 3.47**</td>
<td>36.22 ± 2.54**</td>
</tr>
<tr>
<td>III</td>
<td>Paracetamol + Extract (100 mg/kg/day)</td>
<td>3.38 ± 0.38**</td>
<td>7.83 ± 0.48**</td>
<td>43.16 ± 3.28**</td>
<td>42.54 ± 3.74**</td>
</tr>
<tr>
<td>IV</td>
<td>Paracetamol + Extract (200 mg/kg/day)</td>
<td>4.07 ± 0.47**</td>
<td>9.25 ± 0.63**</td>
<td>47.82 ± 4.21**</td>
<td>47.18 ± 4.24**</td>
</tr>
<tr>
<td>V</td>
<td>Paracetamol + Extract (300 mg/kg/day)</td>
<td>4.63 ± 0.53**</td>
<td>10.17 ± 0.72**</td>
<td>52.04 ± 4.79**</td>
<td>49.85 ± 4.03**</td>
</tr>
<tr>
<td>VI</td>
<td>Paracetamol + Silymarin</td>
<td>5.17 ± 0.41**</td>
<td>10.76 ± 0.62**</td>
<td>55.74 ± 3.94**</td>
<td>54.59 ± 4.74**</td>
</tr>
</tbody>
</table>

All values are Mean ± SEM, n=6 rats in each group
*P < 0.05 as compared with Group I; †P < 0.05 as compared with Group II

### Treatment with silymarin also reversed the hepatotoxicity significantly (P<0.05).

### Table 1 also reveals that total cholesterol level of serum of rats treated only with paracetamol increased significantly (p<0.05) while HDL level decreased significantly (p<0.05) with respect to control group. But, ECC was successful in blunting this rise in cholesterol level and decrease in HDL level also in a dose dependent manner, which is comparable with the reference drug silymarin.

#### 3.3. Liver Weight

Table 1 also reveals that the liver weight of rats treated with paracetamol only decreased significantly (p<0.05), which is blunted by ECC and silymarin.

### 3.4. In Vitro Lipid Peroxidation

FeCl₂-ascorbic acid induced in vitro lipid peroxidation study reveals that ECC has significant anti lipid peroxidation potential with IC50 value being 140.44 μg/ml, which is comparable with the reference drug α-tocopherol. (Table 2)

### 3.5. In Vivo Antioxidant Activity

In vivo lipid peroxidation study reveals that paracetamol treated hepatotoxic rats showed significant increase (p<0.05) in malondialdehyde (MDA) when compared with rats of normal control group. ECC was able to significantly blunt (p<0.05) this rise in MDA level in a dose dependent manner which is comparable with that of silymarin (Table 3).
Fig. 1. Section of liver tissue of normal rat showing normal liver architecture with central vein and portal triads (H & E, 400x). (Fig. 1a) Section of liver tissue of rat induced with paracetamol showing extensive centrilobular necrosis, extending to other necrotic areas, ballooning degeneration along with fatty degeneration or steatosis (H & E, 400x). (Fig. 1b) Section of liver tissue of ECC treated (300 mg/kg, p.o.) rat showing almost no necrosis, diffuse steatosis and mild increase in inflammatory cells in portal tract (H & E, 400x). (Fig. 1c) Section of liver tissue of rat treated with silymarin (25 mg/kg, p.o.) 3. There was a marked decrease in the level of GSH and the activities of SOD and CAT in paracetamol treated group when compared with normal control group. The GSH level and activities of SOD and CAT were significantly increased (p<0.05) in ECC in a dose dependant manner which is comparable with silymarin (Table 3).

3.6. Histopathological Studies
Histopathological profiles of paracetamol-induced hepatotoxic liver revealed extensive centrilobular necrosis extending to other necrotic areas, ballooning degeneration with steatosis (Fig. 1b). The protective effect of ECC (300 mg/kg, p.o.) was confirmed by histopathological examination of liver section of control, paracetamol-induced and extract treated groups of rats (Fig. 1a-1c). ECC treated rats exhibited a significant improvement of hepatocellular architecture over paracetamol-induced group as evident from considerable reduction in necrosis and steatosis (Fig. 1c). Liver section of rats treated with silymarin (25mg/kg, p.o.) showed significant signs of amelioration of paracetamol-induced liver injury which is evident from normal liver architecture and absence of necrosis and steatosis (Fig. 1d). The study showed that ECC and silymarin showed significant protective effect against paracetamol-induced liver injury which is evident from their histopathological examination (Fig. 1a-1d).

4. Discussion
Paracetamol, a widely used antipyretic and analgesic, in large doses produces acute liver damage. It is metabolized in liver to excretable glucuronide and sulphate conjugates [23, 24]. However, the hepatotoxicity of paracetamol is due to the formation of toxic metabolites when paracetamol is activated by hepatic cytochrome P450 [25], to a highly reactive metabolite N-acetyl-P-benzoquinone imine (NAPQI) [26]. NAPQI is initially detoxified to form mercapturic acid by conjugation with reduced glutathione (GSH) [27]. However, when the rate of NAPQI formation exceeds the rate of detoxification by GSH, it oxidizes tissue macromolecules such as lipid or SH group of protein and alters the homeostasis of calcium after depleting GSH. Paracetomol produces hepatic necrosis when ingested in very large doses. The hepatoprotective activity of the ethanolic extract was monitored by estimating serum transaminases, serum alkaline phosphatase and bilirubin, which give a good idea about the functional state of the liver [28]. Necrosis or membrane damage releases the enzyme into circulation and therefore, it can be measured in serum. High levels of SGOT indicate liver damage, such as that due to viral hepatitis as well as cardiac infarction and muscle injury. SGPT catalyses the conversion of alanine to pyruvate and glutamate, and is released in a similar manner. Therefore, SGPT is more specific to the liver, and is thus a better parameter for detecting liver injury. Our results demonstrate that the ethanolic extract of Cajanus cajan caused significant inhibition of SGOT and SGPT levels. Serum ALP and bilirubin levels on the other hand, are related to the function of hepatic cell. Increase in serum level of ALP is due to increased synthesis, in presence of increasing biliary pressure [29]. Our results also demonstrate that the ethanolic extract of Cajanus cajan caused significant inhibition of serum ALP and bilirubin.
levels. Effective control of alkaline phosphatase activity and bilirubin level points towards an early improvement in secretory mechanism of hepatic cells. The effect of the extract on liver weights of rats is shown in Table 1. The paracetamol treated rats showed a significant loss in liver weight. But the extract administration significantly prevented this paracetamol-induced weight loss of liver in rats, which is comparable with that of standard drug administration.

Most hepatotoxic chemicals including paracetamol and alcohol damage liver by inducing, directly or indirectly, lipid peroxidation [30]. So, the studies on in vitro and in vivo lipid peroxidation of rats are performed. In vitro lipid peroxidation in liver homogenate can proceed in a non-enzymatic way. The process is induced by ascorbate in the presence of Fe2+/Fe3+, and it has been reported that Fe2+ and ascorbic acid stimulated lipid peroxidation in rat liver microsomes and mitochondria. In order to clarify the mode of action of ethanolic extract, in vitro lipid peroxidation experiments were carried out. According to the result obtained, the extract inhibited FeCl2-ascorbic acid-stimulated lipid peroxidation in liver homogenate (Table 2). Cells have a number of mechanisms to protect themselves from the toxic effects of ROS. SOD removes superoxide (O2-) by converting it to H2O2, which can be rapidly converted to water by CAT [31]. In addition, a large reserve of reduced glutathione is present in hepatocytes and red blood cells for detoxification of xenobiotics or free radicals. However, oxidative stress results in toxicity when the rate at which the ROS are generated exceeds the cell capacity for their removal. Lipid peroxidation is an autocatalytic process, which is a common consequence of cell death. This process may cause peroxidative tissue damage in inflammation, cancer and toxicity of xenobiotics and aging. MDA is one of the end products in the lipid peroxidation process [32]. In our in vivo study elevation in levels of end products of lipid peroxidation in liver of rats treated with paracetamol were observed. The increase in MDA levels in liver suggests enhanced lipid peroxidation leading to tissue damage. Pretreatment with ECC significantly reversed these changes. Hence it may be possible that the mechanism of hepatoprotection of extract is due to its antioxidant effect. GSH is widely distributed in cells. GSH is an intracellular reductant and plays major role in catalysis, metabolism and transport. It protects cells against free radicals, peroxides and other toxic compounds. Indeed, GSH depletion increases the sensitivity of cells to various aggressions and also has several metabolic effects, for example, a decrease in the rate of gluconeogenesis or an increase in glycogenolysis. The concept of a glutathione-SH threshold for drug detoxification was discussed by Jollow [33]. GSH is a naturally occurring substance that is abundant in many living creatures. It is widely known that a deficiency of GSH within living organisms can lead to tissue disorder and injury. For example, liver injury included by consuming alcohol or by taking drugs like acetaminophen, lung injury by smoking and muscle injury by intense physical activity [34], all are known to be correlated with low tissue levels of GSH. From this point of view, exogenous ethanolic extract of Cajanus cajan supplementation might provide a mean of recover reduced GSH levels and to prevent tissue disorders and injuries. In the present study, we have demonstrated the effectiveness of the extract by using paracetamol-induced rats, which is a known model for both hepatic GSH depletion and injury. Therefore, the levels of glutathione are of critical importance in liver injury caused by paracetamol. Our results are in line with this earlier report because we found that after ECC supplementation, elevated GSH level in rats with paracetamol could be blunted to normal level. Biological systems protect themselves against the damaging effects of activated species by several means. These include free radical scavengers and chain reaction terminators, enzymes such as SOD and CAT. [35]. The SOD dismutates superoxide radicals O2− into H2O2 plus O2, thus participating with other antioxidant enzymes, in the enzymatic defense against oxygen toxicity. In this study, SOD plays an important role in the elimination of ROS derived from the peroxidative process of xenobiotics in liver tissues. The observed increase of SOD activity suggests that the ethanolic extract of Cajanus cajan have an efficient protective mechanism in response to ROS. And also, these findings indicate that the extract may be associated with decreased oxidative stress and free radical-mediated tissue damage. CAT is a key component of the antioxidant defense system. Inhibition of these protective mechanisms results in enhanced sensitivity to free radical-induced cellular damage. Excessive production of free radicals may result in alterations in the biological activity of cellular macromolecules. Therefore, the reduction in the activity of these enzymes may result in a number of deleterious effects due to the accumulation of superoxide radicals and hydrogen peroxide. Administration of ethanolic extract of Cajanus cajan increases the activities of catalase in paracetamol-induced liver damage rats to prevent the accumulation of excessive free radicals and protects the liver from paracetamol intoxication. This ability of ECC to protect the liver from paracetamol-induced damage might be attributed to its ability to restore the activity of antioxidative enzymes. Thus, results of these studies together with those of earlier ones, suggest that ECC has an ability to protect the liver from paracetamol-induced damage through its direct antioxidative effect. It is well documented that hepatocellular enzymes (SOD, CAT) serve as biomarkers of hepatocellular injury due to alcohol and drug toxicity [36]. So the studies on antioxidant enzymes (SOD, CAT) have been found to be of great importance in assessment of liver damage.

In summary, we demonstrate that ECC prevents paracetamol-induced oxidative stress and hepatic injury. Since these models of hepatic damage in the rat simulate many of the features of human liver pathology, we suggest that natural antioxidants and scavenging agents in ECC might be effective as plant hepatoprotectors and thus may have some obvious therapeutic implications. Therefore, it seems logical to infer that ECC, because of its antioxidant property, might be capable of protecting the hepatic tissue from paracetamol-induced injury and inflammatory changes. The ethanolic extract of C. cajan is reported to be rich in flavonoids [7]. Presence of flavonoids in the
ethanolic extract was confirmed through our preliminary phytochemical screening also. Flavonoids are natural products, which have been shown to possess antioxidant [37], as well as hepatoprotective activities [37, 38]. Studies have confirmed that indeed oxidative stress plays an important role in the initiation and progression of liver disease [13-15]. As *C. cajan* contains large amounts of flavonoids it may be assumed that the hepatoprotective activity may be due to the presence of flavonoids in the extract. The activity may be attributed to their protective action on lipid peroxidation and at the same time the enhancing effects on cellular antioxidant defense contributing to the protection against oxidative damage in paracetamol-induced hepatotoxicity. Further studies regarding the isolation and characterisation of the active principles responsible for hepatoprotective activity is currently under progress.

**Conflict of interest** We declare that we have no conflict of interest.

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