Hepatoprotective Effect of Ethanol Leaf Extract of *Cnestis ferruginea* on Swiss Albino Mice Induced with Paracetamol

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Abstract

The liver serves a vital function in human system. It has the primary metabolic function of regulating the blood concentration of most metabolites, particularly glucose and amino acids. Its characteristic structure and organization enable it to perform vital roles in regulating, synthesizing, storing, secreting, transforming and breaking down of different substances in the body. The hepatoprotective effect of ethanol leaf extract of *Cnestis ferruginea* was evaluated by administering oral dose of 400-2000 mg/kg to twenty adult Swiss albino mice. Dose related differences in all the parameters assayed were observed. After inducing the mice with Paracetamol (toxicant) and the various extract dose for 72 hours, hepatoprotective effects of the extract was measured through Aspartate Aminotransferrase (AST), Alanine Aminotransferrase (ALT), Alkaline Phosphatise (ALP) activities and histopathological study of the liver. The results of histopathological study suggested that the ethanol leaf extract of *Cnestis ferruginea* possessed some degree of hepatoprotective ability.

Keywords: Ethanol Extract, Paracetamol, Hepatoprotective, Leaf, Swiss Mice

1. Introduction

The liver is a vital organ in man and animals responsible for detoxifying thousands of chemicals from bodies every single day and if it is unable to function optimally, toxins begins to build up in the tissues. It aids in digestion and removes waste products, worn-out cells from the blood and also function in protein synthesis. The liver is our greatest chemical factory, it builds complex molecules from simple substances absorbed from the digestive tract, it manufactures bile which aids fat digestion and removes toxins through the bowels (Maton et al, 1993). But the ability of the liver to perform these functions is often compromised by numerous substances we are exposed to on a daily basis. These substances include certain medicinal agents which when taken in over doses and sometimes when introduced within therapeutic ranges injures the organ (Gagliano et al, 2007). It is imperative to understand that with the present day human activities, so many chemical additives and pollutants are now found in the air, water and food this signals to importantly care after the health of our liver. According to the American Liver Foundation in Cedar Grove, N.J., liver disease ranks as the third leading disease-related cause of death for many people of ages 25 to 59. Particularly common liver disorders include hepatitis and cirrhosis. It is generally believed that liver damage follows a spectrum, progressing from inflammation and swelling to fatty degeneration, cirrhosis and cancer. Because the liver can still function with up to 80% deterioration, symptoms are often vague and may not be noticed until damage gets severe. When symptoms and signs do manifest, they may include dinginess in the whites of the eyes, jaundice, pale stools, pain on the right side or under right shoulder blade, loss of appetite, digestive disturbances, a metallic taste in the mouth, frontal headache, and drowsiness after meals.
intolerance of fatty foods and energy loss (Johnston, 1999). Conventional drugs used in the treatment of liver diseases are sometimes inadequate and can have serious adverse effects. Therefore, it is necessary to search for alternative drugs for the treatment of liver disease in order to replace currently used drugs of doubtful efficacy and safety (Ozbek et al, 2004). In the absence of reliable liver protective drugs in allopathic medical practices, herbs play a role in the management of various liver disorders (Buraimoh et al, 2011).

Hepatoprotective agents are those compounds, which mitigate the liver injury caused by hepatotoxins agents. Hepatoprotective effects of plant drugs and herbal formulations are studied against chemicals (alcohol, CCl\textsubscript{4}, alcohol-CCl\textsubscript{4}, beta-galactosamine, thiacetamide) and drugs (Paracetamol, nimesulide, antitubercular drugs such as isoniazid, rifampicin etc.) induced toxicity in rats and mice as they virtually mimic any form of naturally-occurring liver disease (Kumar et al, 2004).

Herbal medicines have been widely used and form an integral part of primary health care of many countries (De Boer et al, 2005 and Nair et al, 2005).

Nearly all culture and civilizations from ancient times to the present day have depended fully or partially on herbal medicines because of their effectiveness, affordability, low toxicity and acceptability (Akharaiyi & Boboye, 2010).

Cnestic ferruginea has various therapeutic uses in herbal medicine. The extract from the roots is applied to the nostrils for migraines and sinusitis. A root decoction is taken as well known aphrodisiac and used as an enema by women with abortion and ovarian troubles (Irvine, 1961). The root is used for toothache and the powdered bark is rubbed on the gums for pyorrhoea among the Igbo tribe in Nigeria (Oliver-Bever, 1986 and Okwu & Iroabuchi, 2004). The plant is an active ingredient in the decoction used presently by herbalists in Eastern Nigerian for treatment of gonorrhoea, joint and waist pains, arthritis, rheumatism, stroke and syphilis (Okwu & Iroabuchi, 2004). There seems to be no much information on Cnestic ferruginea D.C. ethanol leaf extract on the liver functional indices induced with Paracetamol. Therefore, this work was designed to evaluate the hepatoprotective effect of Cnestic ferruginea D.C. ethanol leaf extract on the liver functional indices of Swiss albino mice induce with Paracetamol.

ALT levels have been found generally to be higher than AST when a liver damage is present (Brondeau et al, 1983). The most commonly used markers of hepatocytic injury are Aspartate Aminotransferase (AST) and Alanine Aminotransferase (ALT). While ALT is cytosolic, AST has both cytosolic and mitochondrial forms (Johnston, 1999). AST is present in large amounts in the liver, heart, skeletal muscle, brain and red blood cells. It is therefore less specific for liver disease because it can be elevated also in myocardial infarction, myopathies muscular disease (muscular dystrophy, rhabdomyolisis) or trauma. While ALT is present in large amounts in liver and in lesser amount in kidney, heart and skeletal muscle. It is therefore more specific for liver disease than AST (Song et al, 2004).

2. Materials and Methods

2.1. Collection of Plant and Extract Preparations

Healthy looking leaf, stem bark and roots of C. ferruginea, were collected from forest in Akure, Ondo State, Nigeria and identified in Department of Forestry and Wood Technology, Federal University of Technology, Akure, Nigeria. The plants parts were dried for 3 weeks and then ground to powder with a mechanical grinder. 1.5 kg each of the powders obtained was extracted with ethanol at room temperature (25±2 °C). The resulting crude extract was filtered with sterile muslin cloth and concentrated using a rotary evaporator (RE -52 A Union Laboratories, England) at 40-45 ºC. The obtained dried crude extract was contained in plastic containers and labelled.

2.2. Experimental Animals

Twenty apparently healthy Swiss albino mice of between 100-150 g were used. The animals were contained in a cage and maintained under standard laboratory conditions. They were giving rodent pellets (Vital feeds) and water ad libitum. They were acclimatized for 2 weeks and were fasted over night with free access to water prior the experiments. The animals were conducted in compliance with NIH Guide for Care and Use of Laboratory Animals. Before the experiment, the mice were divided into four groups of five mice per group.

2.3. Experimental Design

Group I served as the negative control and was accessed to normal rat diet and water ad libitum only. Group II served as the positive control and was given 1 g/kg body weight of Paracetamol. Group, III, IV and V were given 400 mg/kg, 1000 mg/kg and 2000 mg/kg body weight of the leaf extracts respectively. And finally Group VI , VII and VIII were given 400 mg/kg, 1000 mg/kg and 2000 mg/kg body weight of the leaf extract after exposure to Paracetamol respectively. The animals were observed continuously for 72 hours for any gross change in behavioural, neurological, autonomic profiles and mortality in each group.
2.4. Haematological Studies

Red blood cells (RBC) and White Blood Cells (WBC) were counted using Neubauer haemocytometer and haemoglobin was estimated using Sahli’s Hemoglobinometer by standard procedures from the blood obtained intracardially (Wintrobe et al, 1961 and D’Armour et al, 1965).

2.5. Preparation of Serum and Liver Homogenate

At the end of the experiment, the mice were then made unconscious in a jar containing cotton wool soaked in chloroform. Thereafter, their jugular veins were cut; head held downwards and allowed to bleed to clean, dry centrifuge tubes. The tubes were left at room temperature for 10 minutes and thereafter centrifuged at 33.5 x  g for 15 minutes. The sera were aspirated using Pasteur pipettes into clean, dry, sample bottles and were then stored at -20 °C for 24 hours. Immediately after the collection of blood, the mice were quickly dissected. The liver was removed and transferred into 0.25 M sucrose solution. The organ was later blotted with tissue paper, cut very thinly with sterile scalpel blade and homogenized in ice-cold 0.25 M sucrose solution (1:5 v/v) (Akanji et al, 1993). The homogenates were further centrifuged at 1340 x  g for 15 min to obtain the supernatant, which was then carefully collected into sample bottle and used for the various biochemical assays. The Randox and QCA Commercial Enzyme Kits were used for the biochemical assay.

2.6. Aspartate Aminotransferase Activity Assay

The method of Bergmeyer et al (1986a) was adopted with little modifications. Briefly, aspartate aminotransferase activity was measured by monitoring the following information of oxaloacetate hydrazo with 2, 4-dinitrophenylhydrazine. The AST substrate phosphate buffer of 0.5 ml each was pipette into two sets of test tubes labelled B (sample blank) and T (sample test) respectively. The serum sample of 0.1 ml was added to the sample test (T) only and mixed properly was incubated for 30 minutes in a water bath regulated at 37°C. After incubation, 0.5 ml each of 2,4-dinitrophenylhydrazine was added to both test tubes labelled T (sample test) and B (sample blank). Also, 0.1 ml of serum sample was added to the sample blank (B) only. The mixtures were shaken properly and allowed to stand for 20 minutes at 25 °C. Thereafter, 5.0 ml of NaOH solution was added to both test tubes and mixed thoroughly. Absorbance of the test sample was recorded against the sample blank at a wavelength of 550 nm after 5 minutes.

2.8. Assay of Alkaline Phosphatase Activity

This method is based on the principle that serum alkaline phosphatase hydrolyses a colourless substrate of phenolphthalein monophosphate giving rise to phosphoric acid and phenolphthalein, which at alkaline pH values, turns into a pink colour that can be photo metrically determined. Distilled water (1.0 ml) was pipette into 2 sets of test tubes labelled SA (sample) and ST (standard) respectively. Then one drop of chromogenic substrate was added to the distilled water in the two sets of test tubes. Their contents were mixed and incubated at 37 °C for 20 minutes in a water bath. After incubation, a standard solution of 0.1 ml was added to the standard test tube (ST) only; followed by the addition of the serum sample of 0.1 ml to the sample test tube (SA). The content was also mixed and incubated at 37 °C for 20 minutes in a water bath. A colour developer of 5.0 ml each was added to both sets of test tubes. Absorbance of the sample against the blank (water) was read at a wavelength of 550 nm. The activity of alkaline phosphatase in the serum was obtained from the formula (calculations) below:

\[
\text{ST O.D} \times 30U = \text{of Alkaline phosphatase}
\]

\[
\text{SA O.D} \times L
\]

Where:

\[\text{ST O.D} = \text{Standard Optical Density}\]
\[\text{SA O.D} = \text{Sample Optical Density}\]

2.9. In vivo Antioxidant Assay

The antioxidant assay was performed with the liver tissues of the experimental animals and evaluation of the antioxidant status was carried out by measuring the level of lipid peroxidation (Ohkawa et al, 1979) and the amount of enzymatic (Catalase: CAT) and nonenzymatic
antioxidant system (reduced glutathione: GSH) by Luck (1963) and Ellman (1959) methods respectively.

3. Results

The haemoglobin, RBC and WBC values of the positive control mice were higher than the values obtained from the negative control mice. While haemoglobin, RBC and WBC values were 11.50±0.06, 7.67±0.92 and 3.50±0.18 respectively in the negative control mice, it was 20.11±1.81, 4.36±0.12 and 9.62±0.39 respectively in the positive control mice. However, little difference in values of haemoglobin, RBC and WBC were obtained in the results of mice fed with the different extract concentrations of 400-2000 mg/kg body weight. 12.34±0.09, 5.00±0.17 and 7.00±0.48 were recorded for haemoglobin values in 400, 1000 and 2000 mg/kg body weight of mice respectively. While it was 7.43±0.12, 6.54±0.19 and 6.54±0.98 for RBC values, it was 7.43±0.08, 6.41±0.15 and 6.41±0.15 for WBC values in 400, 1000 and 2000 mg/kg body weight of mice respectively. In addition, similar results were obtained in the haemoglobin, RBC and WBC values of mice induced with the paracetamol before the administration of the various extracts (Table 1).

<table>
<thead>
<tr>
<th>Groups</th>
<th>Haemoglobin (g%)</th>
<th>RBC Million/cu. Mm</th>
<th>WBC Thousand/cu. mm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (-)</td>
<td>11.50 ± 0.06</td>
<td>7.67 ± 0.92</td>
<td>3.50 ± 0.18</td>
</tr>
<tr>
<td>Control (+)</td>
<td>20.11 ± 1.81</td>
<td>4.36 ± 0.12</td>
<td>9.62 ± 0.39</td>
</tr>
<tr>
<td>400 mg/kg b.w. Extract</td>
<td>12.34 ± 0.09</td>
<td>5.00 ± 0.17</td>
<td>7.00 ± 0.48</td>
</tr>
<tr>
<td>1000 mg/kg b.w. Extract</td>
<td>12.56 ± 0.28</td>
<td>5.64 ± 0.12</td>
<td>6.72 ± 0.98</td>
</tr>
<tr>
<td>2000 mg/kg b.w. Extract</td>
<td>12.34 ± 0.19</td>
<td>6.81 ± 0.19</td>
<td>6.54 ± 0.76</td>
</tr>
<tr>
<td>Toxicant + 400 mg/kg</td>
<td>11.46 ± 0.26</td>
<td>7.38 ± 0.17</td>
<td>4.50 ± 0.97</td>
</tr>
<tr>
<td>Toxicant + 1000 mg/kg</td>
<td>11.35 ± 0.18</td>
<td>7.45 ± 0.14</td>
<td>4.86 ± 0.78</td>
</tr>
<tr>
<td>Toxicant + 2000 mg/kg</td>
<td>11.20 ± 0.94</td>
<td>7.43 ± 0.08</td>
<td>4.09 ± 0.59</td>
</tr>
</tbody>
</table>

In the negative control mice, average values in ALT, ALT, ALP, Bilirubin and Total albumin are 29.00±3.51, 23.03±6.20, 160.65±19.54, 1.20±0.12 and 4.90±0.11 (U/L) respectively while in the positive control mice, the average values obtained in the same parameters were 67.47±4.02, 64.97±3.67, 321.12±25.81, 2.11±0.63 and 2.21±0.60 (U/L) respectively (Table 2).

Values of 40.37±3.31, 36.13±6.20 and 52.45±6.50 were observed respective as AST level of the group of mice treated with 400, 1000 and 2000 mg/kg body weight extract. The ALT level in that order was 35.81±3.75, 32.22±4.76 and 50.57±3.75. However, in the groups of mice treated with toxicant before co administration of 400 mg/kg body weight, the AST level was 50.00±3.64. ALT was 42.11±2.10 and 258.75±30.53 as the ALP level. The group co administered with 1000 mg/kg body weight was 49.04±1.75 for AST, 41.78±1.56 for ALT and 187.67±20.22 for ALP levels. The co administered with 2000 mg/kg body weight extract of mice has 48.68±2.65 as AST, 37.62±6.82 as ALT and 150.28±25.60 as ALP levels (Table 2). In the groups of mice administered the different leaf extract concentrations after exposure to Paracetamol (toxicant), close values were obtained in all the biochemical parameters. The AST values obtained in the 400 mg/kg, 1000 mg/kg and 2000 mg/kg body weight leaf extract after exposure to Paracetamol are 50.0±3.64, 49.04±1.75 and 48.68±2.65 (U/L) respectively while in ALT, values are 42.11±2.10, 41.78±1.56 and 37.62±6.82 (U/L) respectively. The ALP, Bilirubin and the total albumin as well had similar trend in values (Table 2).

The antioxidant system of control and paracetamol induced mice is reported in Table 3 where the LPO, GSH and CAT values in the negative control experiment were 98.94±6.66, 34.75±1.40 and 70.58±6.65 respectively. The positive control experiment resulted at 138.40±2.10, 22.34±2.00 and 47.36±3.76 in the LPO, GSH and CAT values respectively. Values of 105.09±3.00, 36.83±2.64 and 79.02±5.45 respectively were obtained in the mice.
Figure 1 represents the histopathology section of liver of mice treated only with normal mice diet and water (negative) where signs of damage were not noticed. Figure 2 is the representative of the liver of mice treated with toxicant and co administered with the plant extract where recovery from damages initiated by the toxicant was observed. Figure 3 represents the liver of mice which were treated only with the toxicant (positive control) and severe damage resulted.

Table 2. Effect of Ethanol Leaf Extract of C. ferruginea on Various Biochemical Parameters in Mice with Paracetamol induced Hepatotoxicity

<table>
<thead>
<tr>
<th>Group</th>
<th>AST (U/L)</th>
<th>ALT (U/L)</th>
<th>ALP (U/L)</th>
<th>Bilirubin (mg/dl)</th>
<th>Total Albumin (g/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (-)</td>
<td>29.00±3.51</td>
<td>23.03±6.20</td>
<td>160.65±19.54</td>
<td>1.20±0.12</td>
<td>4.90±0.11</td>
</tr>
<tr>
<td>Control (+)</td>
<td>67.47±4.02</td>
<td>64.92±3.69</td>
<td>321.12±25.81</td>
<td>2.11±0.63</td>
<td>2.21±0.60</td>
</tr>
<tr>
<td>400 mg/kg b.w. Extract</td>
<td>40.37±3.31</td>
<td>35.81±3.75</td>
<td>141.70±19.54</td>
<td>1.30±0.61</td>
<td>3.60±0.13</td>
</tr>
<tr>
<td>1000 mg/kg b.w. Extract</td>
<td>36.13±6.20</td>
<td>32.22±4.76</td>
<td>130.40±21.60</td>
<td>1.30±0.14</td>
<td>4.00±0.01</td>
</tr>
<tr>
<td>2000 mg/kg b.w. Extract</td>
<td>52.45±6.50</td>
<td>50.57±3.75</td>
<td>221.03±18.36</td>
<td>1.26±0.00</td>
<td>3.65±0.09</td>
</tr>
<tr>
<td>Toxicant + 400 mg/kg b.w. Extract</td>
<td>50.0±3.64</td>
<td>42.11±2.10</td>
<td>258.75±30.53</td>
<td>1.20±0.61</td>
<td>2.58±0.20</td>
</tr>
<tr>
<td>Toxicant + 1000 mg/kg b.w. Extract</td>
<td>49.04±1.75</td>
<td>41.78±1.56</td>
<td>187.67±20.22</td>
<td>1.70±0.25</td>
<td>2.80±0.13</td>
</tr>
<tr>
<td>Toxicant + 2000 mg/kg b.w. Extract</td>
<td>48.68±2.65</td>
<td>37.62±6.82</td>
<td>150.28±25.60</td>
<td>1.20±0.11</td>
<td>3.00±0.20</td>
</tr>
</tbody>
</table>

Table 3. Effect of Ethanol Leaf Extract of C. ferruginea on the Antioxidant System of Control and Paracetamol-Induced Mice

<table>
<thead>
<tr>
<th>Groups</th>
<th>LPO (nM/mg b.w.)</th>
<th>GSH (µg/mg b.w.)</th>
<th>CAT (µM of H₂O₂ Decomposed/min/mg b.w.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (-)</td>
<td>98.94±6.66</td>
<td>34.75±1.40</td>
<td>70.58±6.65</td>
</tr>
<tr>
<td>Control (+)</td>
<td>138.40±2.10</td>
<td>22.34±2.00</td>
<td>47.36±3.76</td>
</tr>
<tr>
<td>400 mg/kg b.w. Extract</td>
<td>105.09±3.00</td>
<td>36.83±2.64</td>
<td>79.02±5.45</td>
</tr>
<tr>
<td>1000 mg/kg b.w. Extract</td>
<td>109.10±3.08</td>
<td>38.86±0.52</td>
<td>87.01±2.97</td>
</tr>
<tr>
<td>2000 mg/kg b.w. Extract</td>
<td>111.46±2.11</td>
<td>41.86±8.65</td>
<td>89.35±6.20</td>
</tr>
<tr>
<td>Toxicant + 400 mg/kg b.w. Extract</td>
<td>107.10±2.89</td>
<td>40.89±1.67</td>
<td>33.98±0.67</td>
</tr>
<tr>
<td>Toxicant + 1000 mg/kg b.w. Extract</td>
<td>100.34±3.67</td>
<td>39.43±4.68</td>
<td>35.24±3.74</td>
</tr>
<tr>
<td>Toxicant + 2000 mg/kg b.w. Extract</td>
<td>95.87±6.54</td>
<td>37.65±6.89</td>
<td>42.67±1.05</td>
</tr>
</tbody>
</table>

4. Discussion

Experimental animals are used in the evaluation of the safe use of drugs and plant extracts through toxicology whose results are seen in the blood, vital organs like liver and kidney and helps in selecting a safe dose of drug in human. When rats were treated with Paracetamol in this study, it
induces hepatotoxicity by metabolic activation; therefore, it selectively causes toxicity in liver cells maintaining semi-normal metabolic function. Paracetamol hepatotoxicity has been attributed to the formation of toxic metabolites, when a part of Paracetamol is activated by hepatic cytochrome P - 450 to a highly reactive metabolite N-acetyl-p-benzoquinoneimine (NAPQI).

Treatment with C. ferruginea ethanol leaf extract recovered the injured liver to normal after 72 hr at a dose of 400 mg/kg b.w; this indicates that C. ferruginea has anti-hepatotoxic effect. In addition, the possible anti-hepatotoxic mechanism of C. ferruginea has not been reported yet. It is assumed that the effect of C. ferruginea ethanol leaf extract on liver protection is related to glutathione-mediated detoxification as well as free radical suppressing activity. From the result obtained, it could be deduced that C. ferruginea ethanol leaf extract has the functions of protecting liver from peroxidative damage in Paracetamol-intoxicated mice. The reduced necrosis of the cells in the groups of mice treated with Paracetamol before administration of the leaf extracts might be due to the presence of the phytochemical constituents of the leaf, which have hepatoprotective properties. Such liver protective properties in herbal drugs include phenols, flavonoids, organic acids, alkaloids, coumarins, lignin, essential oil, monoterpenes, glycosides and xanthenes (Gupta & Misra, 2006). In the earlier study of antibacterial and phytochemical activities of C. ferruginea by Akharaiyi et al (2012) terpenoids, glycosides, alkaloids tannins and saponins were found in appreciable quantity. The interest for oxidative stress in relation to the development of disease has gained large attention during the last decade.

5. Conclusion

Researchers have successfully used plant extracts in hepatoprotective and this is supported with the results obtained in this study with C. ferruginea ethanol leaf extract at trial concentrations of 400, 1000 and 2000 mg/kg body weight extract. Liver disease is a worldwide problem, which needs urgent attention. Despite researchers are into this study, it has been discovered that conventional drugs used in the treatment of liver diseases are sometimes inadequate and can have serious adverse effects. The present histopathological and biochemical analysis of this extract showed a good development in the Paracetamol-damaged liver cells. Therefore, the need to search for alternative drugs for the treatment of liver disease is necessary.

References


