PROTECTIVE POTENTIAL OF ETHANOL EXTRACT OF Annona squamosa AGAINST CISPLATIN INDUCED HEPATORENAL TOXICITY IN ALBINO RATS

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Abstract

The ethanol extract of Annona squamosa (As) was evaluated for its protective effect on cisplatin induced hepato and nephrotoxicity in Wistar albino rats. Their weight was around 150 – 200 g. The activity levels of liver marker enzymes such as Alanine transaminase (ALT), Aspartate transaminase (AST), Alkaline phosphatase (ALP) and Bilirubin were increased by the administration of cisplatin (5 mg/kg body weight). The increased levels were found to be decreased after treatment with ethanol leaf extract of Annona squamosa (different doses) in the experimental groups. The oral administration of ethanol extract of Annona squamosa (250, 350, 450 mg/kg of body weight) attenuated the cisplatin induced nephrotoxicity and significantly reduced the levels of kidney markers such as serum urea, uric acid and creatinine with significant normalization of histological architecture. The present results suggest that the treatment with ethanol extract of Annona squamosa might be useful to protect and restore the normal condition of hepatic and renal cells in cisplatin induced toxic liver and kidney.

Key words: Annona squamosa, Nephrotoxicity, Hepatotoxicity, Cisplatin and Wistar albino rats.

1. Introduction

The use of numerous herbs or their extracts for the treatment of different diseases including cancer has been documented in Ayurveda medical system practiced primarily in India for around 5000 years (Dahanukar et al., 2000). Nearly, 80% of the world population has been using the traditional medicinal plants to prevent and cure illnesses (Manuel et al., 2005). All over the world medicinal plants have been known to be useful in the treatment of various diseases since time immemorial. In addition, plant derived products have been used for medicinal purposes for centuries (Akinloye and Olaniyi, 2012). Medicinal plants have various effects on living systems. Some are sedatives, analgesics, antipyretics, antibacterials, antivirals, cardioprotectives antiprotozoals, hepatoprotective, nephroprotective agents (Prasenjit Manna et al., 2006). The study of medicinal plant is of great interest in the field of biotechnology. Most of the drug industries depend on plants for the production of pharmaceutical compounds (Velmurugan and Kamaraj, 2011).

Cisplatin is one of the major anti cancer drugs, frequently used in several human cancers such as different solid tumors, including gastric, testicular, ovarian, urologic, head and neck and other cancers.
(Mustafa Talip Sener et al., 2012). The clinical use of cisplatin was limited because of its undesirable side effects such as nephrotoxicity (Pabla and Dong, 2008), neurotoxicity (Barabas et al., 2008), ototoxicity (Rybak et al., 2009) and hepatotoxicity (Liao et al., 2008; El-Sayyad et al., 2009). Although, the precise mechanism of cisplatin-induced toxicity is not well understood, cisplatin is preferentially taken up and accumulated in the liver and kidney cells. While nephrotoxicity of CDDP has been recognized as the most important dose-limiting factor, little is known about CDDP induced liver injury (Zicca et al., 2012). Extensive investigations have been conducted on the hepato-renal toxicity as well as general organ toxicity of this anticancer drug (Arhoghro et al., 2012).

Annona squamosa L. (Family: Annonaceae) is a small ever green tree. It grows up to 15-25 feet tall. It is cultivated throughout India for its fruits and different parts of the plant. The different parts of Annona squamosa L. are used in folkloric medicine for treatment of various diseases. This plant is commonly called custard apple in English, sharifa in Hindi and sitaphalam in Telgu (Neha Pandey and Dushyant Barve, 2011). The plant has been documented earlier for its therapeutic effects in hepato-renal diseases. The phytochemical, pharmacological, antibacterial and antioxydual studies have already been carried out with its seed extracts (Tej Pratap Singh et al., 2014). Ayurvedic practitioners have been using an extract of the bark and leaves of Annona squamosa to cure various diseases. The leaves are thin, oblong while the flowers are greenish yellow. The fruit with purple knobby skin is very sweet. The fruit is juicy and creamy-white (Manjari Mittal et al., 2010). Annona squamosa is an indigenous uterotonic drug (Gupta, 2005). The plant is traditionally used for the treatment of epilepsy, dysentery, cardiac problems, worm infestation, constipation, hemorrhage, dysuria, fever, thirst, malignant tumors and ulcers. It also used as an abortifacient (Sobiy Raj et al., 2009). The chemical constituents found in the bark and leaves of A. squamosa such as Anonaine, roemerine, corydine, isocorydine and aporphine alkaloids are being used to cure different diseases in human beings (Baskar et al., 2007).

In the present study the folklore medicinal plant Annona squamosa (Family: Annonaceae) has been selected to evaluate its protective effect on the cisplatin induced hepato renal toxicity in male albino rats.

2. Materials and Methods

Plant Material

The leaves of Annona squamosa (Family: Annonaceae) were collected in the month of July, 2013 in and around Vellore District, Tamil Nadu, India. Its botanical identity was authenticated in the Department of Botany, C. Abdul Hakeem College, Melvisharam, Vellore district, Tamil Nadu. The voucher herbarium specimen has been deposited in the Department of Zoology, Thiruvalluvar University, Serkkadu, Vellore Districtt, Tamil Nadu. The plant materials were cleaned with distilled water and shade dried at room temperature.

Preparation of Ethanol Extract

The shade dried plant materials (leaves) were powered separately in an electrical blender and stored at 5°C until further use. An amount of 100 gms of powered leaf was mixed with 500 ml of ethanol and stirred magnetically in separate container overnight at room temperature. The residue was removed by filtration and the ethanol extract of the leaves was concentrated under vacuum to get 20% solid yield.

Animals

Healthy adult male Wistar albino rats weighing around 150 – 200 g were purchased from Tamil Nadu Veterinary and Animal Sciences University, Chennai, India. The animals were kept in polypropylene cages (three in each cage) at an ambient temperature of 25±2°C and 55 – 65% relative humidity. 12 ± 1 hr light and dark schedule was maintained in the animal house till the animals were acclimatized to the laboratory conditions, and were fed with commercially available mice chow (Hindustan Lever Ltd., Bangalore, India). They had free access to water.
Experimental Protocol

Group I: Normal rat.

Group II: Rats administered a single dose of cisplatin (5mg/kg body wt., ip).

Group III: The hepato-renal toxicity induced animals were treated with the ethanol leaf extract of *A. squamosa* (250 mg/kg body wt.,) orally by using intra gastric tubes for 30 days.

Group IV: The hepato-renal toxicity induced animals treated with the ethanol leaf extract of *A. squamosa* (350 mg/kg body wt.,) orally by using intra gastric tubes for 30 days.

Group V: The hepato-renal toxicity induced animals treated with the ethanol leaf extract of *A. squamosa* (450 mg/kg body wt.,) orally by using intra gastric tubes for 30 days.

Biochemical Assays

The biochemical parameters like serum enzymes were analyzed. They include, ALT and AST (Reitman *et al.*, 1957), ALP (King *et al.*, 1934), serum bilirubin (Malloy *et al.*, 1937), Urea (Natelson *et al.*, 1951), Uric acid (Caraway, 1963), Creatinine (Broad and Sirota 1948), Super oxide dismutase (Kakkar *et al.*, 1984), Catalase (Sinha, 1972), Glutathione peroxidase (Rotruck *et al.*, 1973), and Glutathione S-transferrorase (Habig *et al.*, 1974).

Statistical Analysis

The data of biochemical estimations were reported as mean ± SD. The statistical significance was determined by using one way analysis of variance (ANOVA) followed by Dunnet’s multiple comparison tests. P< 0.05 was used to determine statistical significance.

3. Results

Hepatoprotective activity

Administration of cisplatin led to significant liver damage in the experimental groups, as evidenced by the altered serum enzymatic levels and biochemical parameters. The concentrations of liver marker enzymes such as Alanine transaminase (ALT), Aspartate transaminase (AST), Alkaline phosphatase (ALP) and bilirubin in serum, were significantly elevated (p < 0.05) in cisplatin induced group II rats, when compared to the levels of normal animals. After the administration of ethanol leaf extract of *A. squamosa* 250, 350 and 450 mg/kg body wt., to the rats of group III, IV and V the elevated levels of the liver markers enzymes such as ALT, AST, ALP and bilirubin were significantly decreased (p < 0.05) when compared with cisplatin induced group II rats (Table - 1).

Nephroprotective activity

Administration of cisplatin led to abnormal renal functions in all experimental (Groups II, III, IV and V) rats. The levels of serum urea, uric acid and creatinine concentrations were significantly higher in the cisplatin administered animals (Group II), when compared to the normal rats (Group I). The increased levels of renal markers indicate the induction of severe nephrotoxicity (Table - 2). After the treatment with ethanol leaf extract of *A. squamosa* at a dose of 250, 350 and 450 mg/kg body wt., to the rats of Group III, IV and V respectively, the levels of renal function markers like urea, uric acid and creatinine were significantly decreased than in cisplatin induced group II rats (Table - 2).

4. Discussion

Cisplatin is commonly used to study the hepato and nephroprotective activity of medicinal plants in laboratory animals. The results of this study clearly showed the protective activity of *A. squamosa* against cisplatin induced hepato and renal toxicity in albino rats. Cisplatin is a heavy metal complex; it induces a broad range of dysfunctions and side effects including hepatotoxicity (Mansour *et al.*, 2006; Pratibha *et al.*, 2006) and nephrotoxicity (Park *et al.*, 2009). Assessment of liver function was made by estimating the activity levels of serum ALT, AST, ALP and bilirubin, which were present at higher concentration in cytoplasm. When there is hepatopathy, these molecules leak into the blood stream in compliance with the extent of liver damage (Nkosi *et al.*, 2005). Bilirubin is one of the most useful clinical clues to the severity of
necrosis and its accumulation is a measure of binding, conjugation and excretory capacity of the hepatocytes. The results suggest that the extracts protected the membrane integrity of the liver cells against cisplatin induced hepatotoxicity.

In the present study, A. squamosa was found to have protective effect over cisplatin induced toxicity. Leakage of liver marker enzymes such as ALT, AST, ALP and bilirubin were prevented by the leaf extract in dose dependent manner (Jayavelu et al., 2013). Generally, measurement of ALT, AST and ALP are commonly used as marker enzymes of hepatotoxicity (Yanpallewar et al., 2002; Asha et al., 2004; Yen et al., 2007). The significant reduction in the levels of marker enzymes like ALT, AST, ALP and bilirubin in the serum of plant extract administered animals might be due to decreased leakage of the liver marker enzymes from liver cells. This suggests that the oral administration of ethanol leaf extract of A. squamosa could prevent/repair the hepatic cell injury. Leaf extract of A. squamosa restores the cellular permeability, thus reducing the toxic effect of cisplatin induced liver toxicity. Other investigators have reported similar observations (Molina et al., 2003; Ozaras et al., 2003; Uzun et al., 2003).

The increased levels of serum urea, uric acid and creatinine are signs of renal toxicity and acute renal failure (Heidemann et al., 1989; Mckeage et al., 1993; Somani et al., 2000; Jayavelu et al., 2013) in the cisplatin administered rats. These elevated levels of kidney marker enzymes like urea, uric acid and creatinine were restored to the normal levels in the experimental groups after the administration of ethanol leaf extract of A. squamosa.

**Table - 1: Hepatoprotective activity of ethanol extract of Annona squamosa (As) on cisplatin induced liver toxicity in albino rat: Activity levels of ALT, AST, ALP and bilirubin**

<table>
<thead>
<tr>
<th>Experimental groups</th>
<th>Parameters in the serum</th>
<th>Values are mean ± S.D., n = 6. a p &lt; 0.05 compared with normal control, b p &lt; 0.05 compared with Cisplatin intoxicated.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ALT (IU/l/min/mg protein)</td>
<td>AST (IU/l/min/mg protein)</td>
</tr>
<tr>
<td>Group-I Normal control</td>
<td>49.61±2.10</td>
<td>63.56±1.93</td>
</tr>
<tr>
<td>Group-II Cisplatin Control</td>
<td>113.33±2.16 a</td>
<td>133.50±3.08 a</td>
</tr>
<tr>
<td>% of changes Normal Vs Toxic control</td>
<td>+168.75</td>
<td>+110.03</td>
</tr>
<tr>
<td>Group-III Cisplatin + As (250mg/kg body wt)</td>
<td>79.00±1.78 b</td>
<td>82.83±2.04 b</td>
</tr>
<tr>
<td>% of changes Toxic control Vs 250 mg/kg body wt</td>
<td>-25.74</td>
<td>-37.95</td>
</tr>
<tr>
<td>Group-IV Cisplatin + As (350mg/kg body wt)</td>
<td>73.80±2.01 b</td>
<td>80.20±2.48 b</td>
</tr>
<tr>
<td>% of changes Toxic control Vs 350 mg/kg body wt</td>
<td>-29.64</td>
<td>-39.92</td>
</tr>
<tr>
<td>Group-IV Cisplatin + As (450mg/kg body wt)</td>
<td>54.83±2.85 b</td>
<td>61.66±3.77 b</td>
</tr>
<tr>
<td>% of changes Toxic control Vs 450 mg/kg body wt</td>
<td>-43.87</td>
<td>-53.81</td>
</tr>
</tbody>
</table>
Table - 2: Nephroprotective activity of ethanol extract of *Annona squamosa* (As) on cisplatin induced renal toxicity in albino rats: Levels of Urea, Uric acid and Creatinine

<table>
<thead>
<tr>
<th>Experimental Groups</th>
<th>UREA</th>
<th>URIC ACID</th>
<th>CREATININE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I</td>
<td></td>
<td></td>
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<tr>
<td>Normal control</td>
<td>17.66±1.08</td>
<td>1.85±0.35</td>
<td>1.93±0.32</td>
</tr>
<tr>
<td>Group II</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Cisplatin - Control</td>
<td>54.00±2.60</td>
<td>3.71±0.44</td>
<td>4.50±0.50</td>
</tr>
<tr>
<td>% of changes</td>
<td>+205.77</td>
<td>+100.54</td>
<td>+133.16</td>
</tr>
<tr>
<td>Normal Vs Cisplatin Control</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Group-III Cisplatin + As (250mg/kg body wt)</td>
<td>35.83±3.25</td>
<td>2.74±0.41</td>
<td>2.55±0.40</td>
</tr>
<tr>
<td>% of changes</td>
<td>-33.64</td>
<td>-26.14</td>
<td>-43.33</td>
</tr>
<tr>
<td>CP control Vs As 250 mg/kg body wt</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Group-IV Cisplatin + As (350 mg/kg body wt)</td>
<td>28.81±2.61</td>
<td>2.40±0.39</td>
<td>2.45±0.50</td>
</tr>
<tr>
<td>% of changes</td>
<td>-46.64</td>
<td>-35.30</td>
<td>-45.55</td>
</tr>
<tr>
<td>CP control Vs 350 mg/kg body wt</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Group-V Cisplatin + As (450mg/kg body wt)</td>
<td>19.50±1.87</td>
<td>2.01±0.36</td>
<td>1.93±0.35</td>
</tr>
<tr>
<td>% of changes</td>
<td>-63.88</td>
<td>-45.82</td>
<td>-57.11</td>
</tr>
<tr>
<td>CP control Vs 450 mg/kg body wt</td>
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</tbody>
</table>

Values are mean ± S.D., n = 6. a p < 0.05 compared with normal control. b p < 0.05 compared with Cisplatin. ‘P’ denotes statistical significance. “a”-‘+’ and ‘-‘ indicates % of changes over the normal and “b”-‘+’ and ‘-‘indicates % of changes over the cisplatin control.

5. Conclusion

The present investigation shows the changes in the levels of liver and renal markers in serum of cisplatin induced rats. Elevated levels of serum liver marker and renal function markers were brought to nearly normal levels by the administration of the ethanol extract of *A. squamosa*. These results show the protective effect of the ethanol extract *A. squamosa*.

6. Reference


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