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**ORIGINAL RESEARCH ARTICLE**

# Evaluation Of Anti Nephrotoxic Activity of *Annona squamosa* on Cisplatin Induced Nephrotoxicity in *Albino* Rats

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## ABSTRACT

The efficacy of ethanol leaf extract of *Annona squamosa (As)* 250, 350 and 450 mg/kg body weight was evaluated on Cisplatin induced nephrotoxicity in *albino* rats. The activity levels of serum urea, uric acid and creatinine (renal markers) were increased and the activity levels of antioxidant enzymes were decreased in cisplatin induced rats when compared with the normal control group. Here Cystone was used as the reference drug. The concentration of serum urea, uric acid and creatinine and the levels of antioxidant were restored in both Cystone and plant extract treated rats when compared with cisplatin induced rats. The oral administration of the extract at a dose of 450 mg/kg body weight significantly decreased the renal marker concentrations in serum and the levels of antioxidants (SOD, CAT, GPx and GST) in kidney tissue were significantly increased. The results suggested that the extract of *Annona squamosa* effective for lowering and suppressing the nephrotoxicity in rats.

**Key words:** *Annona squamosa*, cisplatin, Cystone, ethanol extract, and Nephrotoxicity.

## 1. INTRODUCTION

Developing countries are well-known for the use of medicinal plants from ancient times to their health care system. According to the WHO, 80% of the world's population mainly depends on plant-derived medicines for their healthcare. Ancient literature also mentions herbal medicines for age-related diseases namely memory loss, osteoporosis, diabetic wounds, immune, liver and renal disorders [1] etc. for which there are no appropriate modern medicine or only palliative therapy is available. Medicinal plants consist of certain bioactive compounds which act as antioxidants and antimicrobial agents [2, 3].

Kidneys are the principal excretory organ which produces urine. Urine has nitrogenous waste products, like urea, ureic acid, excess ions and metabolites of drugs. These metabolites accumulate in the glomeruli and other parts of the kidney and cause irreversible injury to the kidney.

Cisplatin is one of the most important clinically useful anticancer chemotherapeutic agents against tumors [4]. It is used as a single agent or is combined with other cytostatic drugs, in the treatment of head and neck cancer, bladder cancer, cervical cancer, and lung cancer [5, 6]. It has tremendous antitumour effect in different malignancies, but it causes a number of harmful side effects [7, 8]. The use of this potent chemotherapeutic agent is primarily limited by a dose-related nephrotoxicity which is characterized by decreased glomerular filtration and tubular injury [9]. Previous records shows around one fourth of the patients affected with nephrotoxicity by cisplatin [10]. Many of the antitumor drugs have been known to produce hepatotoxicity, cardiotoxicity and nephrotoxicity, which are generally caused by the free radicals [11]. Cystone, is a drug with a number of indigenous ingredients with confirmed pharmacological actions. It has been reported to be a useful agent in dissolving urinary stones. It also reported its beneficial effect in various renal disorders. Cystone is very effective in supporting the urinary tract function and it reduces susceptibility to renal problems by maintaining mucosal integrity [12]. In the present study was under taken to evaluate the efficacy of *Annona squamosa* on Cisplatin induced nephrotoxicity in *albino* rats.

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## 2. MATERIALS AND METHODS

### Plant material

The leaves of *Annona squamosa* [(*As*) Family *Annonaceae*] were collected in and around Vellore district, Tamil Nadu, and authenticated at the Department of Botany, C. Abdul Hakeem College, Melvisharam, Vellore District, Tamil Nadu. The plant materials were cleaned with distilled water and shade dried at room temperature.

### Plants extract preparation

The shade dried plant materials were powdered separately in an electrical blender. The powdered material of the leaves was extracted in a Soxhlet apparatus using Ethanol (500ml for 100gms) as solvent for 3hrs. The extract was filtered and concentrated under reduced pressure on rotary evaporator to obtain (10%) the extract. The powder obtained was then subjected to phytochemical analysis to determine the chemical constituents present in the extract and the remaining was stored at 5º C for further use.

### Animals

Adult male Wister *albino* rats weighing around 175-200g 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$^{o}$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 rat chow (Hindustan Lever Ltd., Bangalore. India). Water was provided *ad libitum*. Experimental protocols and procedures with respect to the animals employed in this study were approved by the Animal Ethics Committee of Thiruvalluvar University, Serkadu, Vellore, Tamil Nadu, India.

### EXPERIMENTAL DESIGN

Cisplatin (CP) was obtained from Indian pharmaceutical company (IPCA), Mumbai. The animals were divided into six groups consisting of six animals each for different experiments.

Group I: Normal control rats. They received only feed and water.

Group II: Rats injected with Cisplatin (6mg/kg body wt., ip) for single dose.

Group III: Rats injected with Cisplatin (6mg/kg body wt., ip) and treated with Cystone (250 mg/kg body wt.,) for 30 days orally by intra gastric tube.

Group IV: The nephrotoxicity induced animals were treated with *A. squamosa* (*As*) plant extract (250 mg/kg body wt.,) for 30 days orally by intra gastric tube.

Group V: The nephrotoxicity induced animals were treated with *A .squamosa* (*As*) *plant* extract (350 mg/kg body wt.,) for 30 days orally by intra gastric tube.

Group VI: The nephrotoxicity induced animals were treated with *A. squamosa* (*As*) plant extract (450 mg/kg body wt.,) for 30 days orally by intra gastric tube.

At the end of the experiment, the animals were sacrificed. The blood samples were collected by cardiac puncture using ether anesthesia without any anticoagulant and were allowed to clot for 10 minutes at room temperature. The blood was centrifuged at 3000 rpm for 15 minutes at 30°C. The serum samples were stored at - 80°C before determination of the biochemical parameters. The liver and kidney were dissected into two parts, one portion for the preparation of homogenate and another portion for the histopathological examination. The part of the liver and kidney proposed for histopathological examination was washed in normal saline and fixed in 10% formalin for 2 days. These tissues were processed in alcohol-xylene series and stained with haemotoxylin and eosin. The 4µ thickness microtome sections were then made.

### Preparation of kidney homogenate

The kidney tissues were homogenized using glass homogenizer in 100mMpotassium phosphate buffer containing 1mM EDTA, pH 7.4 and centrifuged at 12,000×*g* for 30 min at 4º C. The supernatant was collected and used for following experiments.

### Estimation of Biochemical parameters

The biochemical parameters like serum enzymes were analyzed. They include Urea [13], Uric acid [14], Creatinine [15], superoxide dismutase [16], catalase [17], glutathione peroxidase [18], and Glutathione -S- tranferase [19].

### Histopathological Examination

Histological estimation was performed on the kidney and a portion of kidney tissue was fixed in 10% formalin and embedded in paraffin wax. Sections were cut at 4 mµ in thickness, stained with haematoxylin and eosin and mounted in neutral di-styrene-dibutyl propylene (DPX)

medium and examined under a light microscope for histological changes [20].

### 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

### Renal markers

Renal function markers like urea, uric acid and creatinine were significantly higher in Group II animals than Group I normal animals [(percentage of change Normal vs CP) 131.27%, 33.55% and 70.68%. This increase in the renal function markers shows the damage of kidney. These increased levels of functional markers were decreased significantly (CP vs *As*) by 25.96%, 8.37% and 13.63% in 250 mg/kg body weight, 48.17%, 15.64% and 22.72% in 350 mg/kg body weight and 53.50%, 22.40% and 33.33% in 450 mg/kg body weight ethanol extract of *Annona squamosa* treated animals (**Table 1**).

### Antioxidant defense enzymes

Antioxidant defense enzymes viz., superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx) and glutathione-S-transferase (GST) playing an important role in the protection of the body from toxic materials. The activity levels of kidney tissue antioxidant enzymes viz., SOD, CAT, GPx and GST were significantly declined by 58.73%, 65.30%, 52.76% and 50.00%, in the kidney tissue cisplatin induced group (Group II), when compared to normal group. The ethanol extract of *Annona squamosa* was administered to the cisplatin induced groups. Hence, the levels of antioxidants were increased significantly by 52.30%, 111.01%, 35.48% and 39.07% respectively in 250 mg/kg body weight (Group IV), 83.84%, 130.53%, 72.58% and 56.00% in 350 mg/kg body weight (Group V) and 112.88%, 152.94%, 84.07% and 75.07% respectively in 450 mg/kg body weight (Group VI) treated animals, as seen in (**Table 2**).

**Table 1: Effect of ethanol leaf extract of *Annona squamosa* on kidney marker enzyme (Urea Uric acid and Creatinine) on Cisplatin induced nephrotoxicity in rats**

| Experimental Groups | UREA mg/dl | URIC ACID mg/dl | CREATININE mg/dl |
|---|---|---|---|
| **Group I Normal** | 4.38±0.76 | 123.67±2.94 | 1.16±5.11 |
| **Group II Cisplatin - Control** | 10.13±0.77 [a] | 165.17±4.26 [a] | 1.98±2.15[a] |
| % of changes Normal vs Control | +131.27 | +33.55 | +70.68 |
| **Group III** Cisplatin + Cystone | 4.51±1.02[b] | 125.89±1.85[b] | 1.20±3.54[b] |
| % of changes Cisplatin vs Cystone 2**50 mg** | -55.47 | -23.78 | -39.39 |
| **Group IV** Cisplatin + *As*250 mg/kg body wt | 7.50±0.32[b] | 151.33±4.67[b] | 1.71± 4.76[b] |
| % of changes Cisplatin vs EtOH of *As* **2**50 mg | -25.96 | -8.37 | -13.63 |
| **Group IV** Cisplatin + *As* 350 mg/kg body wt | 5.25±0.24[b] | 139.33±3.44[b] | 1.53± 2.16[b] |
| % of changes Cisplatin + EtOH of *As* 350 mg | -48.17 | -15.64 | -22.72 |
| **Group VI** Cisplatin + EtOH of *As* 450 mg | 4.71±0.73[b] | 128.17±2.13[b] | 1.32± 3.60[b] |
| **% of changes** cisplatin vs EtOH of *As* 450 mg | -53.50 | -22.40 | -33.33 |

Values are mean of six individual observations in each group ±S.D

'P'denotes statistical significance. P<0.05. '+' and '-'indicate % of changes over the cisplatin intoxicated groups. (a) compared with normal, (b) compared with cisplatin

**Table 2: Effect of ethanol leaf extract of *Annona squamosa* on Cisplatin induced nephrotoxicity: Levels of SOD, CAT, GPX, GST in kidney tissue.**

| Experimental Groups | SOD ($U_1$/mg protein) | CAT ($U_2$/mg protein) | GPX ($U_3$/mg protein) | GST ($U_4$/mgprotein) |
|---|---|---|---|---|
| **Group I Normal** | 12.60±0.63 | 81.66±1.86 | 10.50±1.87 | 6.50±1.87 |
| **Group II Cisplatin - Control** | 5.20±0.4[a] | 28.33±1.47[a] | 4.96±1.87[a] | 3.25±0.93[a] |

| | | | | |
|---|---|---|---|---|
| **% of changes Normal vs Control** | -58.73 | -65.30 | -52.76 | -50.00 |
| **Group III Cisplatin + Cystone** | 12.31±2.48[b] | 79.53±2.54[b] | 10.03±2.03[b] | 6.03±1.58[b] |
| **% of changes cisplatin vs Cystone 2**50 mg | +136.73 | +180.72 | +102.21 | +85.53 |
| **Group IV** Cisplatin + *As* 250mg | 7.92±1.34[b] | 59.78±2.88[b] | 6.72±1.41[b] | 4.52±0.73[b] |
| **% of changes cisplatin vs EtOH of *As* 2**50 mg | +52.30 | +111.01 | +35.48 | +39.07 |
| **Group V** Cisplatin + *As* 350 mg | 9.56±0.10[b] | 65.31±1.41[b] | 8.56±1.47[b] | 5.07±1.41[b] |
| **% of changes cisplatin vs EtOH of *As* 3**50 mg | +83.84 | +130.53 | +72.58 | +56.00 |
| **Group VI** Cisplatin + *As* 450 mg | 11.07±0.15[b] | 71.66±2.16[b] | 9.13±2.16[b] | 5.69±2.36[b] |
| **% of changes cisplatin vs EtOH of *As* 4**50 mg | +112.88 | +152.94 | +84.07 | +75.07 |

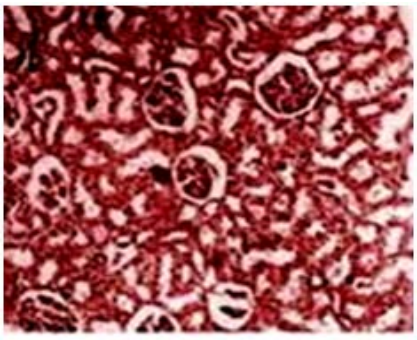
Values are mean of six individual observations in each group ± S.D.

'P'denotes statistical significance P<0.05. ; '+' and '-' indicate % of changes over the normal/CP induced groups. (a)-compared with normal, (b)-compared with cisplatin. SOD – $U_1$- One unit of activity was taken as the enzymes reaction which gives 50% inhibition of NBT reduction in one minute.CAT – $U_2$- µmoles of hydrogen peroxide consumed per minute. GPX – $U_3$- µg of glutathione consumed per minute.. GST – $U_4$- µmoles of CDNB – GSH conjugate formed per minute.

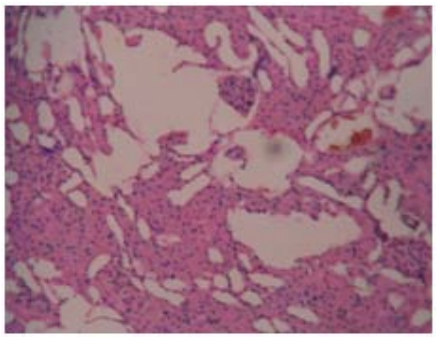
## Renal histology

Histopathological estimations of different kidney segments have been shown in Plate. 1. Sections of control group illustrated normal histology. In cisplatin administered animals the kidney sections showed extensive tubular damage by swollen and necrotic cells. Treatment with ethanol extracts of *Annona squamosa* showed nearly close to normal histology of kidney cells (dose dependent). Cystone is a well-known nephroprotective drug, has been included in the present study as a positive control, it shows close to normal kidney cells.

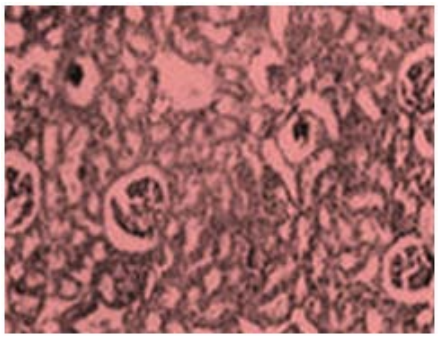
## HISTOLOGICAL SLIDES



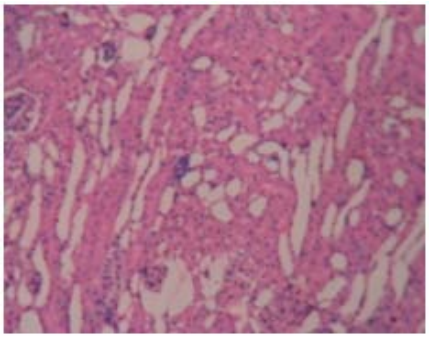
**1 (A) Normal- showing normal Glomeruli & tubules in kidney**



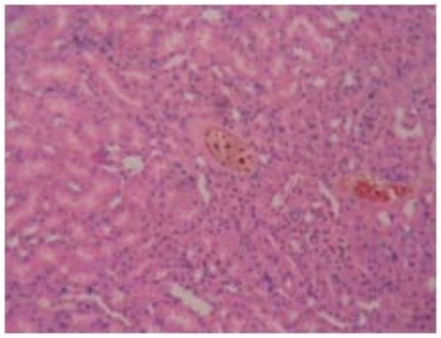
**1(B) Control- CP induced showing tubular necrosis**



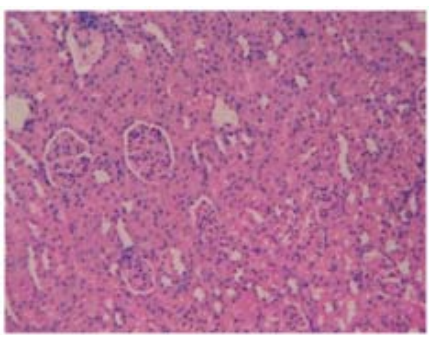
**1(C) CP+ Cytosone- Normal glomeruli & tubules**



**1(D) CP+ EtOH As 250 mg Less tubular necrosis**



**1(E) CP+ EtOH As 350 mg minimum congestion**



**1(F) CP+ EtOH As 450 mg normal glomeruli & tubules**

## 4. DISCUSSION

The results of the present study show that the nephrotoxicity by disturbing the levels of the different antioxidant was caused by the cisplatin. This impairment of renal function was managed/ cured by the treatment of ethanol extract of *Annona squamosa* (Dose dependent).

Exposure of cisplatin produces a large amount of reactive oxygen species that can overcome the antioxidant defense mechanism and oxidative damage cellular ingredients; this in turn can impair the structure and functions of the kidney cells. Glutathione antioxidant system plays the most important role against ROS and other oxidant species [21].

Intracellular antioxidants provide an important defense mechanism against ROS-induced oxidative damage. Among the first line of cellular defense, SOD and CAT commonly play a central role in the eradication of ROS. Changes in the concentration of these defense enzymes have a severe effect on the cellular resistance. In the present study a significant decrease in the activity levels of the enzymatic antioxidants, such as SOD, CAT, GST and GPx was observed in the kidney of the cisplatin induced animals. Treatment with ethanol extract of *Annona squamosa* increased the activity levels of enzymatic antioxidants, such as SOD, CAT, GST and GPx.

The hike of urea, ureic acid and creatinine has been observed in cisplatin exposed rats. The significant increase of markers of kidney was found in serum, which is linked with dysfunction of kidney cells. After the treatment with ethanol extract of *Annona squamosa* restored (Dose dependent) the levels of kidney markers such as urea, ureic acid and creatinine restored close to normal control rats. It shows the nephroprotective efficacy of ethanol extract of *Annona squamosa*.

Histopathological examination revealed that cisplatin administration caused a significant damage in structure of kidney cells with marked tubular damages. Loss of brush borders, extensive tubular casts and tubular dilatations was observed. Treatment of ethanol extract of *Annona squamosa* restored (Dose dependent) cisplatin induced alterations and kept the kidney histological almost normal.

## CONCLUSION

In conclusion, we would like to state that *A.squamosa* plays a protective/curative role against cisplatin induced renal damage. Abnormal architecture and elevated levels of renal function markers were brought to near normal levels by the extract of *Annona squamosa*. But further studies can be done to identify the bio-active found in the extract of *Annona squamosa* and the exact role of different compounds of *Annona squamosa.*

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