

# Protective Effect of *Spathodea Campanulata* Bark against Paracetamol-Induced Nephrotoxicity in Rats

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**Abstract:** The present study investigated the protective effect of 70% ethanolic extract of bark of *Spathodea campanulata* P. Beauv (EEBSC) on paracetamol-induced nephrotoxicity in rats. EEBSC was administered to *Wistar albino rats* in two different doses (250 & 500 mg/kg p.o), for 7 days. Nephro toxicity was induced by oral administration of paracetamol at the dose of 2G/kg on 5<sup>th</sup> day of treatment protocol. Paracetamol administration resulted in significant increase in the serum marker enzymes like blood urea nitrogen and serum creatinine. In addition to these, significant increase in lipid peroxidation levels and depletion of reduced glutathione levels (GSH) also occurs. Pretreatment with EEBSC p.o was found to ameliorate the effect of paracetamol on lipid peroxide formation and showed a decrease in serum marker enzymes. It also prevented the depletion of tissue GSH levels. The histopathological studies of the kidney revealed a restoration of the renal architecture. In conclusion, pretreatment with ethanolic extract of *Spathodea campanulata* bark may be useful in preventing the kidney damage induced by paracetamol.

**Key words:** *Spathodea campanulata*, Paracetamol, Nephroprotection, GSH, Lipid peroxidation.

## Introduction

Paracetamol is widely used as an analgesic and antipyretic drug belonging to the Para-aminophenol class of the non-steroidal anti-inflammatory drugs (NSAIDs). An acute paracetamol overdose can lead to potentially lethal liver and kidney failure in humans and experimental animals. Paracetamol induced renal toxicity has been increasingly reported in many literature<sup>2, 3</sup>. Paracetamol nephropathy is characterized by alterations in urine volume, glutathione status, creatinine clearance and increased products of lipid peroxidation. Several plant products are known to exhibit credible medicinal properties for the treatment of kidney ailments and need to be

explored to identify their potential application in prevention and therapy of human ailments.

*Spathodea campanulata* P. Beauv of family Bignoniaceae commonly known as African tulip tree is commonly found in gardens and avenues. In ethnic practice, the plant was used as diuretic, anti-inflammatory, kidney diseases, antidote, enemas, herpes simplex infections, stomach ache, antisecretolytic, anti parasitic, urethra inflammations, fungal skin diseases, diarrhoea, anti-HIV, anti-malarial and hypo glycaemic activity<sup>4, 5</sup>. The plant possesses steroids, cardiac glycosides, flavonoids, tannins and polyphenols as major chemical constituents. Ayurveda recommends the use of this plant in kidney disorders

and the literature also confirm the same. Hence the present study was undertaken to evaluate the *in vivo* antioxidant and nephroprotective potential of ethanolic extract of *Spathodea campanulata* bark in paracetamol-induced biochemical and histopathological changes.

## **Materials and Methods**

**Plant material and extraction:** *Spathodea campanulata* bark was collected from the surroundings of Davanagere District. The plant was identified and authenticated by Prof. K. Prabhu, Department of Pharmacognosy, S.C.S. College of Pharmacy, Harapanahalli. A voucher herbarium specimen was preserved in the college museum.

The bark was shade dried separately at room temperature and pulverized. The powder obtained was subjected to successive soxhlet extraction with solvents of increased polarity. The hydro-alcoholic extract was selected for the present study. The extract was concentrated using rotary flash evaporator and further evaporated to dryness. The percentage yield was also calculated and then the extract was properly stored.

**Chemicals:** Paracetamol is obtained from micro labs limited, Bangalore, the biochemical kits from Erba Manheim, Germany. All the used chemicals used were of analytical grade.

**Animals:** *Wistar albino rats* (weighing 150-250G) and *albino mice* (weighing 20-25G) of either sex were used in this study. They were procured from Sri Venkateshwara Enterprises, Bangalore. The animals were acclimatized for one week under standard laboratory conditions. They were housed in polypropylene cages and maintained at  $27^{\circ} \pm 2^{\circ}$  with 12 h dark / light cycles. They were fed with standard rat feed (Gold Mohur Lipton India Ltd.) and water *ad libitum* was provided. Prior Ethical clearance for handling the animals was obtained from the Institutional animal ethical committee (IAEC).

**Acute toxicity study:** Acute toxicity study was performed on albino mice (20-25G) as per OECD guideline No. 420 of CPCSEA<sup>6</sup>. The extract was found to be devoid of mortality at 2000mg/kg. Hence, 2500 mg/kg was considered as LD<sub>50</sub> cutoff value. Hence the 1/ 10<sup>th</sup> (250 mg/kg, p.o.) and 1/5<sup>th</sup> (500mg/kg, p.o.) of

the doses were selected for screening nephroprotective activity.

### **Treatment Protocol<sup>7</sup>:**

In the dose response experiment, albino rats were randomly assigned into 4 groups of 6 individuals each and the treatment was given as per the following procedure-

Group-I: Animals (negative control) were administered normal saline 1ml/kg p.o for 7 days.

Group-II: Animals (positive control) were administered normal saline 1ml/kg p.o for 7 days.

Group-III: Animals were administered 70% ethanolic extract at 250mg/kg p.o for 7 days.

Group-IV: Animals were administered 70% ethanolic extract at 500 mg/kg p.o for 7 days.

On 5<sup>th</sup> day, 30 min after the administration of normal saline to group II and 70% ethanolic extract (250 mg/kg & 500 mg/kg) to groups- III and IV, paracetamol was administered (2G/kg p.o). After 48 h of paracetamol administration, rats were sacrificed under mild ether anaesthesia.

The isolated kidney was weighed and part of the renal tissue was used for the estimation of tissue GSH levels<sup>8</sup> and lipid peroxidation<sup>9</sup>. Also, the blood samples were used to measure serum creatinine and blood urea nitrogen (BUN).

### **Histopathological Studies<sup>10</sup>**

Pieces of kidney from each group were fixed immediately in 10% neutral formalin for a period of at least 24 h, dehydrated in graded (50–100%) alcohol, embedded in paraffin, cut into 4–5  $\mu$ m thick sections and stained with hematoxylin- eosin. The sections were evaluated for the pathological signs of nephrotoxicity such as necrosis, fatty infiltration, fibrosis, lymphocyte infiltration, etc.

### **Statistical analysis**

Results were expressed as mean  $\pm$  SEM, (n=6). Statistical analysis were performed with one way analysis of variance (ANOVA) followed by Tukey's Kramer comparison test by using Graph Pad Instat Software, version 5.0. P value less than 0.05 was considered as statistically significant.

**Table No. 1- Effect of 70% ethanolic extract of *Spathodea campanulata* bark on renal damage in paracetamol-induced nephrotoxicity**

Gr. (n=6)	Treatment regimen	Blood urea (mg/dl)	Serum creatinine (mg/dl)
I	Negative Control (1ml vehicle)	36.22 ± 3.30	0.58 ± 0.02
II	Positive Control Paracetamol (2 g/kg p.o.)	86.56 ± 3.52	1.88 ± 0.03
III	Paracetamol + 70% ethanolic extract (2 g/kg p.o. +250 mg/kg p.o.)	26.49 ± 4.05***	0.71 ± 0.04***
IV	Paracetamol +70% ethanolic extract (2 g/kg p.o. +500 mg/kg p.o.)	20.25 ± 2.07***	0.63 ± 0.04***

Values are the Mean ± S.E.M. of six rats / treatment  
Significance \*\*P<0.01 and\*\*\*P<0.001(vs. Control).

**Table No.2- Effect of 70% ethanolic extract of *Spathodea campanulata* bark on tissue GSH levels in paracetamol-induced nephrotoxicity**

Treatment	Absorbance Mean ± SEM	% Increase
Negative Control (1ml vehicle)	0.810 ± 0.005	--
Positive Control Paracetamol (2 g/kg p.o.)	0.395 ± 0.003	--
Paracetamol + 70% ethanolic extract (2 g/kg p.o. + 250 mg/kg p.o.)	0.480 ± 0.026*	21.51
Paracetamol+ 70% ethanolic extract (2 g/kg p.o. + 500 mg/kg p.o.)	0.530 ± 0.069*	34.17

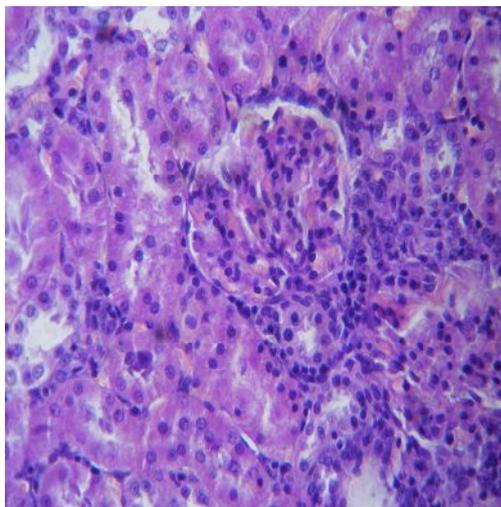
Values are the mean ± S.E.M. of six rats/ treatment.  
Significance \*P<0.005, \*\* P<0.01, compared to paracetamol treatment.

**Table No.3 - Effect of 70% ethanolic extract of *Spathodea campanulata* bark on *in vivo* lipid peroxidation in paracetamol-induced nephrotoxicity**

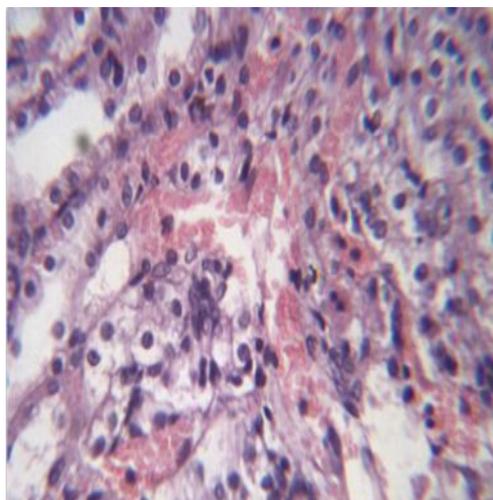
Treatment	Absorbance Mean ± SEM	% inhibition
Negative Control (1ml vehicle)	0.269 ± 0.017	--
Positive Control Paracetamol (2 g/kg p.o.)	0.317 ± 0.010	--
Paracetamol + 70% ethanolic extract (2 g/kg p.o. +250 mg/kg p.o.)	0.117 ± 0.003***	63.09
Paracetamol + 70% ethanolic extract (2 g/kg p.o. +500 mg/kg p.o.)	0.104 ± 0.003***	67.19

Values are the mean ± S.E.M. of six rats /treatment.  
Significance \*P<0.05, \*\*\*P<0.001, compared to paracetamol treatment.

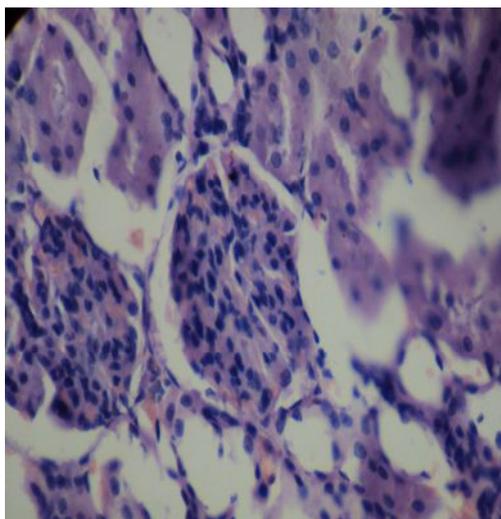
### Histopathology Figures



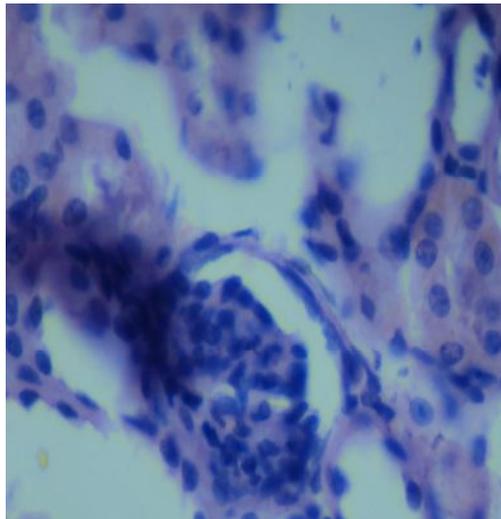
**Fig. 1 (Control)**



**Fig. 2 (Paracetamol)**



**Fig. 3 (EEBSC250 mg/kg)**



**Fig. 4 (EEBSC500 mg/kg)**

### Results

Paracetamol treated rats exhibit a significant rise in serum marker enzymes like BUN, serum creatinine. Further, there is a marked depletion of tissue GSH levels with increased lipid peroxidation when compared with control. EEBSC pretreated rats showed significant ( $P < 0.001$ ) decrease in the levels of serum markers. However, tissue GSH levels were restored to normal levels with reduction in lipid peroxidation as compared to paracetamol treated rats in a dose related manner. The results are summarized in table no.1, 2 and 3.

Histopathological study revealed the normal renal architecture in control group. But in paracetamol-

treated rats, there is presence of glomerular and interstitial congestions (Fig.2). Pretreatment with EEBSC (250 and 500mg/kg p.o) produced marked improvement in renal architecture with respect to glomeruli and no interstitial congestion (Fig. 3 and 4) when compared with paracetamol administered group.

### Discussion

Paracetamol induces acute renal damage by elevating plasma creatinine and blood urea, while depleting glutathione levels. Also tubular necrosis was observed histologically<sup>11</sup>. The fact that p-amino phenol is formed from paracetamol in the kidney by deacetylation and its excretion in urine, makes it a

candidate for its role in the pathogenesis of paracetamol induced renal damage<sup>12,13</sup>. Furthermore, hepatically derived glutathione conjugates are involved in paracetamol induced renal injury<sup>14</sup>. In one more report, it was identified that increased nitric oxide plays an important role in paracetamol induced nephropathies in rats<sup>15</sup>.

In the present study the 70% ethanolic extract was subjected to nephroprotective activity by using paracetamol-induced nephrotoxicity in rats. Biochemical markers of kidney function like blood urea nitrogen, serum creatinine levels, tissue GSH and lipid peroxidation were considered for assessing nephroprotective properties.

In the current investigation, paracetamol administration exhibited a marked depletion of tissue GSH level with increased lipid peroxidation levels. This is supported by the elevation of serum markers like blood urea and serum creatinine. Kidneys are involved in the excretion of various xenobiotics, pollutants, toxins and hence they are prone to liberate high quantities of free radicals which contribute to high oxidative stress. This is involved in the pathogenesis of kidney damage. Since the paracetamol induced nephrotoxicity was reported to be via NAPQI radical. Co-administration of test extract normalized tissue lipid peroxidation level and prevented the reduced tissue GSH level. Even the plant possess significant quantity of polyphenols like flavonoids and tannins. Infact, these principles play a major role in free radical scavenging activity and further these may

contribute to nephroprotection. However, the present study does not show the type of active polyphenols responsible for the nephroprotective property of the plant and this may be considered as one of the scope for future study on the plant. The nephro protective property of the extract is further confirmed by significant improvement of the kidney architecture by reversing the glomerular congestion, interstium with inflammatory cells, tubular necrosis, peritubular necrosis and presence of caspe suggesting massive total necrosis over paracetamol administered group.

### **Conclusion**

The results of the present investigation illustrated that ethanolic extract of the plant produced significant protection over paracetamol-induced alterations in serum marker enzymes and cellular damage. Combined effect of active principles present in the ethanolic extract of *Spathodea campanulata* might offer protection against renal damage rendered by paracetamol in rats. Thus, ethanolic extract of bark of *Spathodea campanulata* exhibited significant nephro protective activity in rats. This supported the folklore use of the title plant in renal disorders.

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