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# Nephroprotective Activity of *Benincasa hispida* (Thunb.) Cogn. Fruit Extract against Paracetamol Induced Nephrotoxicity in Rats

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**ABSTRACT**

Pharmacological research on the medicinal properties of phytochemicals has become mandatory to establish the claimed medicinal properties of herbs. Paracetamol (PCM) is a commonly used analgesic and antipyretic agent which, at high doses, causes liver and kidney necrosis in man and animals. The aim of this study was to investigate nephroprotective activity of hydro-alcoholic extract of *Benincasa hispida* (Thunb.) Cogn. (HABH) whole fruit extract of in paracetamol induced nephrotoxicity in rats. In this study, the effects of hydro alcoholic extract of *Benincasa hispida* [200 mg per kg of body weight (mg/kg) and 400 mg/kg] on PCM-induced nephrotoxicity were examined. Rats were divided into four groups containing 6 rats each. The control group received distilled water while other groups were treated with PCM alone (750 mg/kg), 750 mg/kg PCM+200 mg/kg extract (PCM+ 200-extract), and 750 mg/kg PCM+400 mg/kg extract (PCM+400-extract), respectively, for seven consecutive days. Treatment with HABH extract at doses of 200 and 400 mg/kg prevented the PCM-induced nephrotoxicity and oxidative impairments of the kidney, as evidenced by a significantly reduced in kidney weight, blood urea, blood creatinine, urinary glucose, urinary potassium level and also increased body weight, urine volume, urinary creatinine and blood total protein level. HABH significantly increased the tissue GSH levels and reduced lipid peroxidation levels. Further it was confirmed by the histopathological observation that has the degenerative changes caused by paracetamol were also restored by treatment with HABH. In conclusion, these results suggested that HABH of the whole fruit possess nephroprotective activity against paracetamol induced kidney damage.

**Keywords:** Nephrotoxicity; Benincasa hispida (Thunb.) Cogn.; Paracetamol.

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## INTRODUCTION

Acetaminophen (*N*-acetyl-*p*-aminophenol;APAP) is also known as paracetamol, is generally accepted as a safe drug for analgesic and antipyretic when administered within the therapeutic range. It is a safe drug when given in therapeutic doses but its overdose is fairly common since it has narrow therapeutic index. Its overdose can lead to hepatic and renal damage in both humans and experimental animals [1-3]. Kidney is the second target organ of acetaminophen toxicity and renal dysfunction occurs among patients with marked hepatic injury; however, acetaminophen nephrotoxicity after acute overdose may occur in the absence of hepatotoxicity [4].

The main problem with this medication remainsits misuse through intentional or unintentional ingestion of supratherapeutic dosages which usually lead to hepatic necrosis. When administered at normal doses, PCM is primarily metabolized by conjugation with sulfate and glucuronic acid. A minor pathway through CYP450 has been also reported to yield a highly reactive metabolite, *N*-acetyl-p-benzoquinonimine (NAPQI). This metabolite is generally stabilized through conjugation with glutathione (GSH) and eliminated *via* the kidney. However, when an overdose of PCM is administered, the production of NAPQI exceeds the capacity of GSH to detoxify it. The excess NAPQI then causes liver damage associated with oxidative stress [5]. PCM overdose is also known to be associated with inflammation, marked by an increase in the inflammatory cytokines; tumor necrosis-*α* (TNF-*α*), interleukins, as well as the upregulation of nitrogen oxide (NO) from serum, macrophages and hepatocytes [6]. Even in sensitive individuals, such as persons with renal insufficiency, therapeutic doses of paracetamol have also been implicated in kidney damage [7].

Oxidative stress is reported to constitute a major mechanism in the pathogenesis of PCM-induced liver and renal damage in experimental animals. Because toxic overdoses of PCM were reported to have life threatening impacts on the kidney, e.g. hepatic necrosis and renal failure in both human and experimental animals, early protection from PCM-induced nephrotoxicity has life-saving importance [8].Therefore, supplementation with antioxidants is very crucial to delay, prevent or remove oxidative damage. There are numerous reports indicating that PCM-mediated oxidative stress or hepato-renotoxicity is attenuated by use of naturally occurring antioxidants and/or free radical scavengers such as vitamins, medicinal plants and flavonoids. Recently, the flavonoids have aroused considerable interest,because of their potential beneficial effects on human health. The antioxidant capacity of these molecules seems to be responsible for many of their beneficial effects and confers a therapeutic potential in diseases such as cardiovascular diseases, gastric or duodenal ulcers, cancer and hepatic pathologies.

*Benincasa hispida* (Thunb.) Cogn. belongs to cucurbitaceae family. It is commonly known as ‘ash gourd’ or ‘Chalkumra’ or ‘Kusmanda’. It is a large climbing or trailing herb with stout hispid stems. Fruits are 30 to 45 cm long broadly, cylindric, not ribbed hairy, ultimately

covered with a waxy bloom [9]. Most of the peoples usually take its fruits as vegetable. It contains β-sitosterol, asparagines, manitol, proline, arginine, aspartic acid, glucose and vitamin B1. Moreover, the fruit of Benincasa hispida T. has been used in India for centuries in various ailments such as gastrointestinal problems, respiratory disease, heart diseases, diabetes mellitus and urinary diseases [10].

## MATERIALS AND METHODS

### Plant material

The fruits of *Benincasa hispida* were collected from Bengaluru, Karnataka. The fruit were identified, confirmed and authenticated by Dr M.D. Rajanna, Department of Botany University of Agriculture Sciences, GKVK, Bangalore, Karnataka, India.

### Extraction

The whole fruit were cut into small pieces and shade dried at room temperature. The dried material was then pulverized separately into coarse powder by a mechanical grinder. The resulting powder was then extracted directly with 70% v/v ethanol, using Soxhlet extraction apparatus [11]. The extract were asked to concentrate under reduced pressure and stored in desiccators.

### Determination of Acute Toxicity ($LD_{50}$)

The procedure was divided into two phases. Phase I (observation made on day one) and Phase II (observed the animals for next 14 days of drug administration). Two sets of healthy female rats (each set of 3 rats) were used for this experiment. First set of animals were divided into three groups, each of one in a group. Animals were fasted overnight with water *ad libitum.* Animals received a single dose of 2000 mg/kg, p.o. was selected for the test, as the test item was a source from herb. After administration of extract, food was withheld for 3-4 hrs [12].

### Phytochemical screening

Phytochemical analysis of hydro alcoholic extract of Benincasa hispida (Thunb.) Cogn. (HABH) was carried out by using standard procedures. Alkaloids, Carbohydrates, flavonoids, gum/ mucilage, phytosterols/ terpenes, proteins, saponins were qualitatively analysed [13].

### Experimental animals

Albino wistar rats weighing 150-250g rats were procured from Biogen, Bangalore. They were maintained in the animal house of Gautham College of Pharmacy. The animals were maintained under controlled conditions of temperature 23 ± 2°C and 12 h light-dark cycles. They were housed polypropylene cages containing sterile paddy husk as bedding. They had a free access to standard pellets and water was allowed *ad libitum*. All the studies conducted were approved by the Institutional Animal Ethical Committee (IAEC) of Gautham

College of Pharmacy, Bangalore (REF-IAEC/02/05/2011) according to prescribed guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals (Reg No: 491/01/c/CPCSEA), Govt. of India.

**Effect of HABH on Paracetamol Induced Nephrotoxicity in Rats [14]**

The albino rats were divided in to 4 groups and each group contains 6 rats and treatment would be as follows

**Group I:** These group animals received only normal saline through i.p route throughout the course of the experiment served as normal control.

**Group II:** The animals of this group were treated with acetaminophen suspension was given by p.o in a dose of 750 mg/kg for 7 days.

**Group III:** The animals of this group were given with 200 mg/kg, p.o HABH and acetaminophen suspension by p.o at a dose of 750 mg/kg after one hour the HABH was given to the animals for 7 days.

**Group IV:** The animals of this group were given with 400 mg/kg, p.o HABH and acetaminophen suspension by p.o at a dose of 750 mg/kg after one hour the HABH was given to the animals for 7 days.

All group of animals were kept in metabolic cages for urine collection, the urine was collected for next 24 hours and on $8^{th}$ day, animals were sacrificed with mild ether anesthesia and the kidney tissues, urine and blood samples were collected and assessed.

**Physical Parameters**

**Body Weight**

The weight of the animals before starting and at the end of the treatment was measured and percentage change in body weight was calculated in paracetamol induced nephrotoxicity.

**Kidney Weight**

The weight of the kidneys of the animals at the end of the treatment was measured in paracetamol induced nephrotoxicity.

**Urine Volume**

The urine volume of the animals was measured in paracetamol induced nephrotoxicity.

**Estimation of biochemical parameters**

The following parameters are estimated by using standard procedures of Excel, Beacon and Transasia diagnostics estimating kits: Urinary parameters: sodium, potassium, creatinine, glucose and Blood parameters: urea, creatinine, total protein.

**Estimation of antioxidant activity**

**Glutathione estimation [15]**

Tissue samples were homogenized in ice cold Trichloroacetic acid (1 gm tissue plus 10 ml 10% TCA) in a tissue homogenizer. Glutathione measurements were performed using a modification of the Ellamn procedure (Aykae, et.al.) Briefly, after centrifugation at 3000 rpm for 10 minutes, 0.5 ml supernatant was added to 2 ml of 0.3 M disodium hydrogen phosphate solution. A 0.2 ml solution of dithiobisnitrobenzoate (0.4 mg/ml in 1% sodium citrate) was added and the absorbance at 412 nm was measured immediately after mixing. % increase in OD is directly proportional to the increase in the levels of Glutathione. Hence, % increase in OD is calculated.

**Lipid peroxidation [15]**

Stock solution of TCA-TBA-HCl reagent: 15% w/v trichloroacetic acid; 0.375% w/v thiobarbituric acid; 0.25 N hydrochloric acid. This solution may be mildly heated to assist in the dissolution of the thiobarbituric acid. Combine 1.0 ml of biological sample (0.1-2.0 mg of membrane protein or 0.1-0.2 μmol of lipid phosphate) with 2.0 ml of TCA-TBA-HCl and mix thoroughly. The solution is heated for 15 min in a boiling water bath. After cooling, the flocculent precipitate was removed by centrifugation at 1000 rpm for 10 min. The absorbance of the sample is determined at 535 nm against a blank that contains all the reagents minus the lipid. % decrease in OD is directly proportional to the decrease in the levels of lipid peroxidation. Hence, % decrease in OD is calculated.

**Statistical analysis**

The values are expressed as Mean ± SEM. The data was analysed by using one way ANOVA followed by Dunnett's test using Graph pad prism software. Statistical significance was set at $P \leq 0.05$.

## RESULTS

**Effect of HABH on Change in Body Weight, Urine Volume and Kidney Weight**

There was found to be decrease of body weight and urine volume in paracetamol treated group (II). However, there was dose dependent increase of body weights and urine volume significantly ($p<0.001$) in animals treated with HABH 200 mg/kg and 400 mg/kg, p.o ($p<0.001$) (III and IV). when compared with group (II).(Table No.1)

There was found to be increase of kidney weight in paracetamol treated group (II). However, there was dose dependent decrease of kidney weight significantly in animals treated with HABH 200 mg/kg, p.o ($p<0.001$) and 400 mg/kg, p.o ($p<0.001$) (III and IV). when compared with group (II). (Table No.1)

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**Table No.1: Effect of HABH on Change in Body Weights, Urine Volume and Kidney Weight in Paracetamol Induced Nephrotoxic Rats.**

| Group | Dose | Change in body weights (g) | Urine volume (ml) | Kidney weight (g) |
|---|---|---|---|---|
| I | Vehicle | 8.820± 0.7562 | 5.533± 0.2108 | 0.6150± 0.0240 |
| II | Paracetamol 750 mg/kg p.o | -8.810± 0.2802 | 3.233± 0.3159 | 0.9467± 0.0247 |
| III | Paracetamol 750 mg/kg p.o + 200 mg/kg p.o HABH | -4.755± 0.2345*** | 5.133± 0.2275*** | 0.6733± 0.0122*** |
| IV | Paracetamol 750 mg/kg p.o + 400 mg/kg p.o HABH | -2.897± 0.1416*** | 6.300± 0.2620*** | 0.6017± 0.0144*** |

Values are Mean ± SEM (n=6) one way ANOVA followed by Dunnett's test. Where, ***P<0.001, **P<0.01, *P<0.05 and ns represents Not significant. All values are compared with Toxicant control.HABH: Hydro alcoholic extract of *Benincasa hispida (*Thunb.) Cogn.

**Effect of HABH on Urinary Sodium, Potassium, Glucose and Creatinine**

There was a decrease of sodium levels in paracetamol treated group (II) when compared to control (I). However 200 mg/kg, p.o (III) HABH and 400 mg/kg, p.o (IV) HABH have no significant effect on sodium level in urine samples were observed when compared group (II). (Tabel No.2)

Potassium levels in paracetamol treated group (II) were increased when compared to control group (I). However 200 mg/kg, p.o (III) HABH slightly decreased the levels of potassium but not significant and in the case of 400 mg/kg, p.o HABH (IV) decreased the levels of potassium significantly ($p<0.001$) in urine samples were observed when compared with group (II). (Tabel No.2)

Glucose levels in paracetamol treated group (II) were increased when compared to control group (I). However 200 mg/kg, p.o HABH (III) reduced the glucose levels significantly ($p<0.001$) and 400 mg/kg, p.o HABH (IV) reduced the glucose levels significantly ($p<0.001$) in the urine samples when compared with group (II). (Tabel No.2)

Urinary creatinine levels in paracetamol treated group (II) were decreased when compared to control group (I). However 200 mg/kg, p.o HABH (III) increased the levels of creatinine significantly ($p<0.05$) and in the case of 400 mg/kg, p.o HABH (IV) increased the levels of creatinine significantly ($p<0.001$) in urine samples were observed when compared with group (II). (Tabel No.2)

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**Table No. 2: Effect of HABH on Urinary Sodium, Potassium, Glucose and Creatinine Levels in Paracetamol Induced Nephrotoxic Rats.**

| Group | Treatment | Urinary sodium levels (mmol/l) | Urinary potassium levels (mmol/l) | Urinary glucose levels (mg/dl) | Urinary creatinine levels (g/L) |
|---|---|---|---|---|---|
| I | Vehicle | 197.1± 3.385 | 4.369± 0.2520 | 1.353± 0.1390 | 3.283± 0.2554 |
| II | Paracetamol 750 mg/kg p.o | 130.4± 2.432 | 7.278± 0.4885 | 33.72± 2.039 | 0.9625± 0.1772 |
| III | Paracetamol 750 mg/kg p.o + 200 mg/kg p.o HABH | 127.3± 1.319ns | 5.925± 0.3515ns | 13.25± 0.9117*** | 1.851± 0.1079* |
| IV | Paracetamol 750 mg/kg p.o + 400 mg/kg p.o HABH | 123.0± 1.839ns | 3.749± 0.3911*** | 7.821± 0.7541*** | 2.485± 0.3680*** |

Values are Mean ± SEM (n=6) one way ANOVA followed by Dunnett's test. Where, ***P<0.001, **P<0.01, *P<0.05 and ns represents Not significant. All values are compared with Toxicant control.
HABH: Hydro alcoholic extract of *Benincasa hispida* (Thunb.) Cong.

## Effect on Blood Urea and Blood Creatinine and Blood Total Protein

Blood urea level increased in paracetamol treated group (II) when compared with control group (I). However 200 mg/kg and 400 mg/kg, p.o HABH (III and IV) decreased urea levels significantly (p<0.001) in the blood samples when compared with group (II). (Table No.3)

Blood creatinine level increased in paracetamol treated group (II) when compared with control group (I). However 200 mg/kg p.o. HABH (III) slightly decreased blood creatinine levels but not significantly and 400 mg/kg, p.o HABH (IV) decreased creatinine levels significantly (p<0.01) in the blood samples when compared with group (II). (Table No.3)

Blood total protein level decreased in paracetamol treated group (II) when compared with control group (I). However 200 mg/kg, p.o HABH (III) increased total protein levels significantly (p<0.001) and 400 mg/kg, p.o HABH (IV) increased total protein levels significantly (p<0.001) in the blood samples when compared with group (II). (Table No.3)

**Table No. 3: Effect of HABH on Blood Urea, Blood Creatinine and Blood Total Protein Levels in Paracetamol Induced Nephrotoxic Rats.**

| Group | Treatment | Blood urea (mg/dl) | Blood creatinine (mg%) | Blood Total Protein (g/dl) |
|---|---|---|---|---|
| I | Vehicle | 50.29±2.022 | 1.587±0.2424 | 6.422±0.0748 |
| II | Paracetamol 750 mg/kg p.o | 80.73±2.192 | 2.601±0.3986 | 2.931±0.3581 |
| III | Paracetamol 750 mg/kg p.o + 200 mg/kg p.o HABH | 47.99±2.232*** | 2.029±0.3883ns | 5.145±0.3237*** |
| IV | Paracetamol 750 mg/kg p.o + 200 mg/kg p.o HABH | 33.33±1.431*** | 1.057±0.0636** | 6.049±0.3017*** |

Values are Mean ± SEM (n=6) one way ANOVA followed by Dunnett's test. Where, ***P<0.001, **P<0.01 and *P<0.05. All values are compared with Toxicant control.

HABH: Hydro alcoholic extract of *Benincasa hispida* (Thunb.) Cogn.

**Effect of HABH on Tissue Lipid Peroxidation (LP) and Glutathione (GSH)**

There was dose dependent inhibition of *in-vivo* LP by both the doses of HABH. 200 mg/kg p.o HABH showed 30.90% inhibition, whereas 400 mg/kg, p.o HABH showed 53.89% inhibition. There was a marked depletion of GSH level in paracetamol treated groups. HABH showed a dose dependent increase in the level of GSH. However at 200 mg/kg, p.o HABH showed 47.99% increased in GSH level and 400 mg/kg p.o HABH showed 20.64% increased in GSH levels.

**Table No. 4: Effect of HABH on Tissue LP and GSH Levels in Paracetamol Induced Nephrotoxicity Model.**

| Group | Dose | Absorbance (LP) | % Inhibition (LP) | Absorbance (GSH) | % Increase (GSH) |
|---|---|---|---|---|---|
| I | Vehicle | 0.2647± 0.0081 | – | 1.625± 0.1157 | – |
| II | Paracetamol 750 mg/kg, p.o | 0.8220± 0.0219 | – | 0.8487± 0.0461 | – |
| III | Paracetamol 750 mg/kg, p.o + 200 mg/kg, p.o HABH | 0.5683± 0.0109*** | 30.901 | 1.289± 0.0369* | 47.996 |
| IV | Paracetamol 750 mg/kg, p.o + 400 mg/kg, p.o HABH | 0.3797± 0.0066*** | 53.893 | 1.521± 0.0887*** | 20.64 |

Values are Mean ± SEM (n=6) one way ANOVA followed by Dunnett's test. Where, ***P<0.001, ** P<0.01, * P<0.05 and ns represents Not significant. All values are compared with Toxicant control.
HABH: Hydro alcoholic extract of *Benincasa hispida (Thunb.) Cong.*

**Histopathological Study of Kidneys in Paracetamol Induced Nephrotoxicity**

**Group I:** Negative control showed intact architecture of renal parenchyma. In glomerulus [Fig. 1, Arrow] bowman's space and mesangial cells appeared intact. Intact renal tubules [Fig. 2, Arrow] Blood vessels and Interstitium were unremarkable.

**Group II:** Positive control showed intact architecture of renal parenchyma. In glomerulus [Fig.1, Short - Arrow]: bowman's space appears decreased, extravasation of erythrocytes, mesangial cells appear increased. In renal tubules few tubules show degenerative changes [Fig.2, Arrow]. Blood vessels and Interstitium were unremarkable.

**Group III**: Treatment done with HABH 200 mg/kg, p.o showed intact architecture of renal parenchyma. Glomerulus [Fig.1, Arrow]: Bowman's space appears intact, Extravasation of erythrocytes seen, Mesangial cells appear intact. Most of the renal tubules show degenerative changes [Fig.2, Arrow]. Blood Vessels and Interstitium were Unremarkable.

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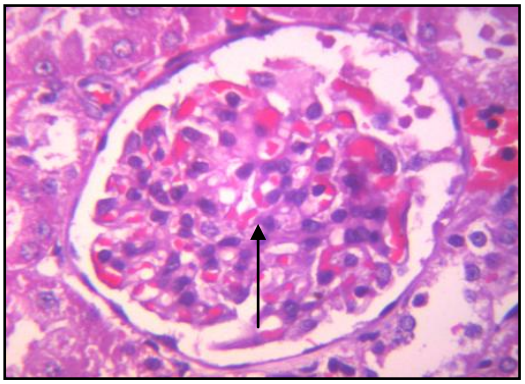
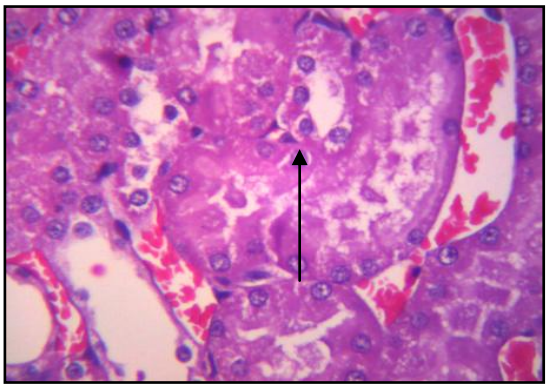
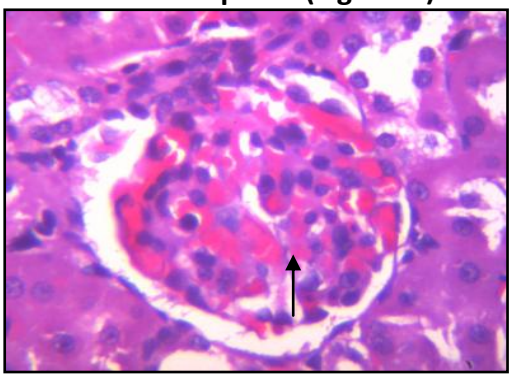
**Group IV:** Treatment done with HABH 400 mg/kg, p.o showed intact architecture of renal parenchyma. Intact glomerulus [Fig.A, Arrow]. Intact renal tubules and some of the tubules show degenerative changes [Fig.2, Arrow]. Blood vessels and Interstitium remained unremarkable.



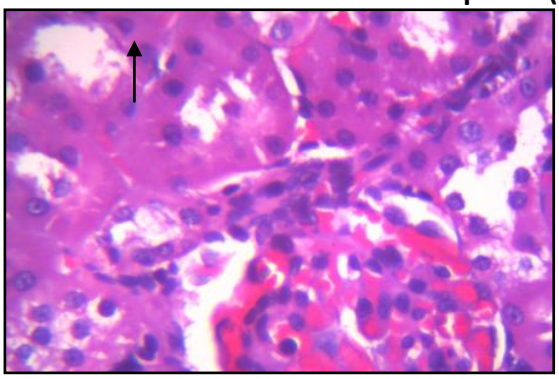
**Group-I (Fig. 1)**



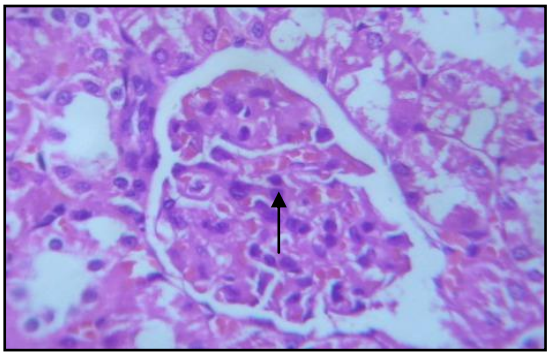
**Group-I (Fig.2)**



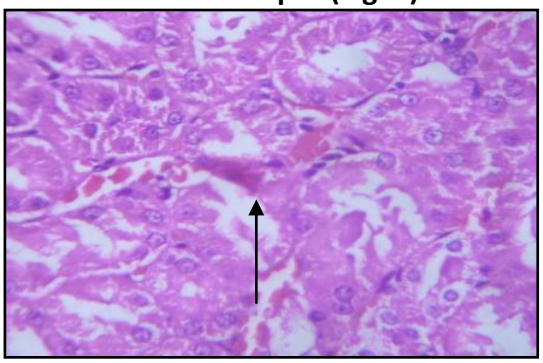
**Group-II (Fig. 1)**



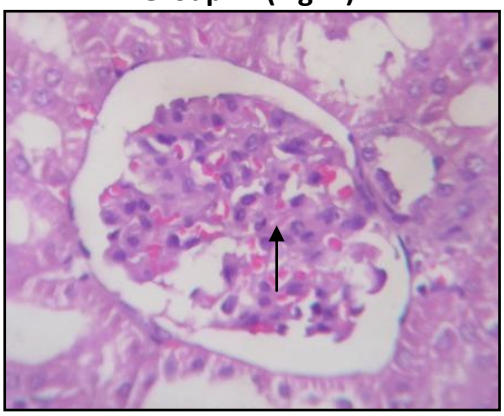
**Group-II (Fig. 2)**



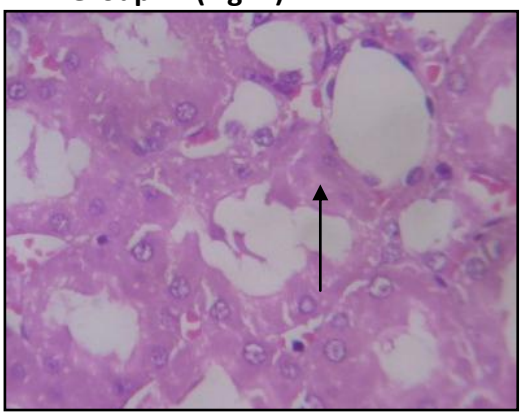
**Group-III (Fig. 1)**



**Group-III (Fig. 2)**



**Group-IV (Fig. 1)**



**Group-IV (Fig. 2)**

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**Figure No. 1: Histopathology of Kidney**

## DISCUSSIONS

Various environmental toxicants, clinically useful drugs, like acetaminophen and gentamicin and especially drugs used in the treatment of cancer and certain diseases like tuberculosis can cause severe organ toxicities . Most of these nephrotixic drugs reported to produce renal toxicity due to generation of free radicals. Thus generated free radicals over powers the inbuilt protective mechanism, resulting in the nephrotic damage and necrosis [16]. Plants may contain a wide variety of free radical scavenging molecules, such as phenolic compounds (e.g. flavonoids, quinones, coumarins, lignans, stilbenes, tannins), nitrogen compounds (alkaloids, amines, betalains), vitamins, terpenoids (including carotenoids), and some other endogenous metabolites, which are rich in antioxidant. The intake of natural antioxidants has been associated with reduced risks of organ toxicities [17].

In this study, we demonstrated that the administration of 750 mg/kg PCM for seven consecutive days was able to induce nephrotoxicity in rats. Administration of hydro-alcoholic extract of *Benincasa hispida* concurrent with PCM exposure prevented PCM-induced nephrotoxicity. Administration of 750 mg/kg PCM caused significant increase in kidney weight, urinary potassium, urinary glucose, blood urea and blood creatinine and decrease in body weight, urine volume, urinary sodium, urinary creatinine and blood total protein. It also depleted the levels of GSH and increased the levels of LP. The histological evidence showed that architecture of renal parenchyma was intact, extravasation of erythrocytes seen in glomerulus, bowman's space appears decreased, mesangial cells appear increased and most of the renal tubules show degenerative changes.

Upon treatment with 200 mg/kg and 400 mg/kg of HABH demonstrated significant dose dependant increase in depleted tissue GSH and reduction in lipid peroxidation caused by paracetamol induced nephrotoxicity and all the physical and biochemical markers brought back to the normal in a dose dependent manner. The nephroprotective activity of extract may be due to the antioxidant potential of it. Therefore, the HABH has organ protective potential against xenobiotic induced nephrotoxicity in rats.

The HABH possess alkaloids, flavonoids, saponins, terpenoids and tannins and these compounds are known to possess antioxidant activity and antioxidant activity may be involved in organ protective activity. Therefore, the antioxidant and organ protective property of HABH can be assigned to antioxidant principle of it. Our study showed that HABH was effective in protecting acetaminophen induced renal injury; the exact mechanism is not properly understood. Further investigations are required to elaborate and understanding the way HABH operates to prevent nephrotoxic effect of acetaminophen.

## CONCLUSION

The present study shows that the administration of whole fruit HABH has nephroprotective potential against paracetamol induced Nephrotoxicity, as evidenced by the physical parameters, biochemical status and histological findings. The HABH demonstrated significant dose dependant increase in depleted tissue GSH levels and

reduction in lipid peroxidation caused by paracetamol induced nephrotoxicity. The most remarkable effects were observed when the HABH extract was delivered at 400 mg/kg as compared to a lower dose of 200 mg/kg of the extract. The 70% Hydro-alcoholic extract of *Benincasa hispida* (Thub.) Cogn.fruit possess nephroprotective activity and this may be due to the presence of antioxidant principles and phytochemical consistutent in HABH.

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