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

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ABSTRACT

Cisplatin is one of the most effective chemotherapeutics against a wide range of cancers including head, neck, ovarian and lung cancers. But its usefulness is limited by its toxicity to normal tissues, including cells of the kidney and proximal tubule. The purpose of the present study is to investigate whether the hydro alcoholic whole fruit extract of *Benincasa hispida* (Thunb.) Cong.(HABH) could decrease the intensity of toxicity in cisplatin induced albino wistar rats. A single dose of cisplatin (7.5 mg/kg i.p.) induced nephrotoxicity, manifested biochemically by a significant increase of urine volume, kidney weight, urinary sodium, urinary potassium, urinary glucose, blood urea, blood creatinine and decreased in body weight, urinary creatinine and blood total protein level with multiple histological damages. Nephrotoxicity was further confirmed by a significant decrease in glutathione (GSH) and increase in lipid peroxides in kidney homogenates. Administration of HABH (200, 400 mg/kg per day p.o.) 5 days before and 5 days after cisplatin injection produced a significant protection against nephrotoxicity induced by cisplatin. The amelioration of nephrotoxicity was evidenced by significant reductions in blood urea, blood creatinine, urinary glucose, urinary sodium, urinary potassium, urine volume with a significant weight gain. In addition HABH tended to normalize decreased level of blood total protein and urinary creatinine. Moreover, HABH prevented the rise of lipid peroxidation and the reduction of GSH activities in the kidney. These results suggest that HABH has a protective effect on nephrotoxicity induced by cisplatin.

Keywords: Nephrotoxicity; Benincasa hispida (Thunb.) Cogn.; cisplatin.

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INTRODUCTION

Nephrotoxicity can be defined as renal dysfunction that arises as a direct result if exposure to external agents such as drugs and environmental chemicals. Many therapeutic agents have been shown to induce clinically significant nephrotoxicity[1]. The kidney is a central organ which maintains homeostasis, regulating water and electrolyte balance and acid-base maintenance, among other critical functions and also have an endocrine function[2]. Clinical syndromes of nephrotoxicity can be defined according to the predominant regions of the kidney affected by toxin and reversibility of the injury is likely related to the severity and nature of the injury and also to the duration of toxin exposure [3]. Since these modern medicines have certain serious side effects there is an urgent need to systematically evaluate plants for their activities. In response to this, the medicinal potential of a lot of plants have been explored [4]. There are many evidences indicates that free radicals are responsible for the birth of many disorders like inflammation, atherosclerosis, diabetes, ageing and renal toxicities and may induce renal vasoconstriction either by direct effect on vascular smooth muscle cells or via action in the juxtaglomerular apparatus that enhance the vasoconstriction induced by the tubulo-glomerular feedback response [5].

Cisplatin (Cis diamine dichloroplatinum II) is a highly effective antineoplastic DNA alkylating agent used against a wide variety of cancers. Higher doses of cisplatin in cancer treatment causes reversible and irreversible side effects including nephrotoxicity, neurotoxicity, bone marrow toxicity, gastrointestinal toxicity and ototoxicity often limit its utility and therapeutic profile. Cisplatin induces free radical production causing oxidative renal damage, possibly due to depletion of nonenzymic and enzymic antioxidant systems [6]. Various data indicate that cisplatin induces oxidative stress, lipid peroxidation and DNA damage .Therefore administration of antioxidants has been shown to ameliorate cisplatin-induced nephrotoxicity in various species of animals.The mechanism of protective effects of antioxidants against cisplatin nephrotoxicity is not fully known [7].

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 [8]. 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 [9].

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 [10]. 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 [11].

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 [12].

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 Cisplatin Induced Nephrotoxicity in Rats [13]:

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 control.

Group II: This group of animals were received a single dose of cisplatin (7.5 mg/kg, i.p) on 5^{th} day.

Group III: The animals of this group received 200 mg/kg, p.o. of HABH for 4 days, on 5th day single dose of cisplatin (7.5 mg/kg, i.p) was given, one hour after cisplatin administration, HABH was administered to the group, and the HABH treatment was continued for four days after 5th day.

Group IV: The animals of this group received 400 mg/kg, p.o. of HABH for 4 days, on 5th day single dose of cisplatin (7.5, mg/kg i.p) was given, one hour after cisplatin administration, HABH was administered to the group, and the HABH treatment was continued for four days after 5th day.

On the 9th day all group of animals were kept in metabolic cages. On the last day (10th) all animals were sacrificed under mild ether anesthesia and the kidneys, 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

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

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

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

Group	Treatment	Change in body weights (g)	Urine Volume (ml)	Kidney Weight (g)
I	Vehicle	7.737 ±0.7622	5.767 ±0.1520	0.6767 ±0.0291
II	Cisplatin 7.5 mg/kg, i.p.	-11.35 ±0.6609	14.93 ±0.9193	0.9500 ±0.0201
III	Cisplatin 7.5 mg/kg i.p + 200 mg/kg, p.o HABH	-5.742 ±0.3947***	7.967 ±0.1745***	0.7233 ±0.0217***
IV	Cisplatin 7.5 mg/kg i.p + 400 mg/kg, p.o HABH	-4.157 ±0.2487***	7.150 ± 0.0885***	0.6433 ±0.0224***

Values are Mean ± SEM (n=6) one way ANOVA followed by Dunnett's test. Where, ***P<0.001 and ** P<0.01. 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 increase of sodium levels in cisplatin treated group (II) when compared to control (I). However 200 mg/kg, p.o (III) HABH slightly increased the levels of sodium but not significant and in the case of 400 mg/kg, p.o (IV) HABH decreased the levels of sodium significantly ($p<0.05$) in urine samples were observed. When compared with group (II). (Table No.2)

Potassium levels in cisplatin 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 (IV) HABH decreased the levels of potassium significantly ($p<0.01$) in urine samples were observed. When compared with group (II). (Table No.2)

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

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

Table No. 2: Effect of HABH on Urinary Sodium, Potassium, Glucose and Creatinine Levels in Cisplatin 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	105.8 ±6.011	4.161 ±0.2494	8.265 ±0.5294	3.086 ±0.3396
II	Cisplatin 7.5 mg/kg i.p.	135.7 ±5.972	5.666 ±0.4143	238.7 ±22.10	0.665 ±0.2302
III	Cisplatin 7.5 mg/kg i.p + 200 mg/kg p.o HABH	127.0 ±9.066ns	4.503 ±0.5164 ns	139.5 ±9.645***	2.590 ±0.1316***
IV	Cisplatin 7.5 mg/kg i.p + 400 mg/kg p.o HABH	106.3 ±3.503*	3.770 ±0.2268**	98.74 ±4.284***	2.657 ±0.4243***

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 of HABH on Blood Urea and Blood Creatinine and Blood Total Protein

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

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Blood creatinine level increased in cisplatin treated group (II) when compared with control group (I). However 200 mg/kg, p.o (III) HABH slightly decrease the blood creatinine but not significant and 400 mg/kg, p.o (IV) HABH decreased creatinine levels significantly ($p<0.001$) in the blood samples. When compared with group (II). (Table No.3)

Blood total protein level decreased in cisplatin treated group (II) when compared with control group (I). However 200 mg/kg, p.o (III) HABH increased the blood total protein level significantly ($p<0.001$) and 400 mg/kg, p.o (IV) HABH 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 Cisplatin Induced Nephrotoxic Rats.

Group	Treatment	Blood urea (mg/dl)	Blood creatinine (mg %)	Blood Total Protein (g/dl)
I	Vehicle	21.3 ±2.194	1.894 ± 0.2768	7.482 ±0.4286
II	Cisplatin 7.5 mg/kg i.p.	73.89 ±5.696	4.66 ±0.4320	2.930 ± 0.4427
III	Cisplatin 7.5 mg/kg i.p + 200 mg/kg p.o HABH	42.64± 2.645***	3.590 ±0.3747ns	5.992 ±0.4397***
IV	Cisplatin 7.5 mg/kg i.p + 400 mg/kg p.o HABH	21.01 ±1.831***	2.324± 0.3110***	7.068 ±0.4453***

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 71.42% inhibition, whereas 400 mg/kg, p.o HABH showed 50.64% inhibition. There was a marked depletion of GSH level in cisplatin treated groups. HABH showed a dose dependent increase in the level of GSH. However at 200 mg/kg, p.o HABH showed 87.89% increased in GSH level and 400 mg/kg p.o HABH showed 62.94% increased in GSH levels. (TableNo.IV)

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

Group	Treatment	Absorbance (LP)	%Inhibition (LP)	Absorbance (GSH)	% Increase (GSH)
I	Vehicle	0.4590±0.0005	_	0.7607±0.0038	_
II	Cisplatin 7.5 mg/kg, i.p.	0.8197±0.0103	_	0.5377±0.0034	_
III	Cisplatin 7.5 mg/kg, i.p + 200 mg/kg, p.o HABH	0.5853±0.0014***	71.428	0.6027±0.0048***	87.896
IV	Cisplatin 7.5 mg/kg, i.p + 400 mg/kg, p.o HABH	0.3967±0.0008***	50.64	0.7363±0.0065***	62.943

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 Cisplatin 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 i.e. cisplatin treated showed focally distorted renal parenchyma architecture [mainly tubules]. In glomerulus [Fig. 1, Arrow]: intact bowman's space, extravasation of erythrocytes seen mesangial cells appear increased. Most of the renal tubules showed 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. In glomerulus [Fig.1, Arrow]: Intact Bowman's space, Extravasation of erythrocytes was seen; Mesangial cells appear decreased. Some renal tubules show degenerative changes [Fig.2, Arrow]. Blood vessels and Interstitium were unremarkable.

Group IV: Treatment done with HABH 400 mg/kg, p.o showed intact architecture of renal parenchyma. Glomerulus remained unremarkable[Fig.1, Arrow]: Renal tubules degenerative changes [Fig.2,Arrow]. Blood Vessels and Interstitium unremarkable.

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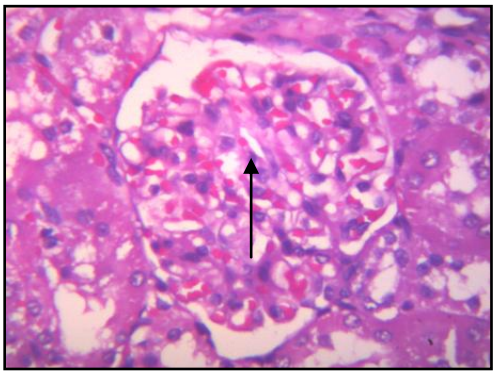
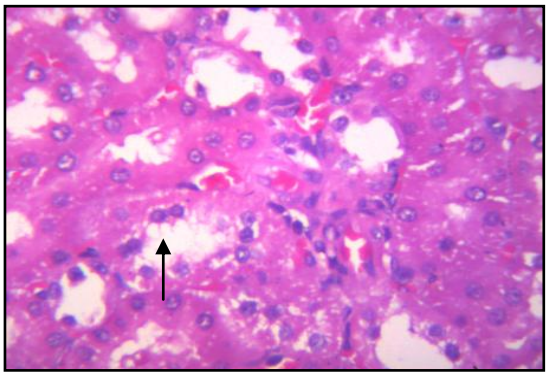
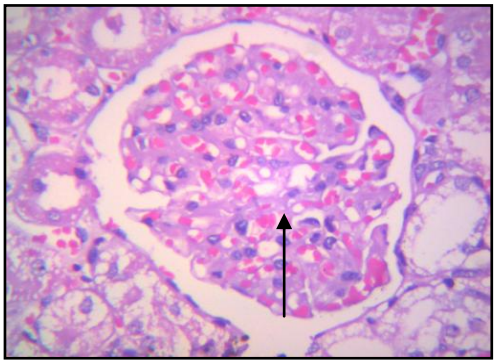


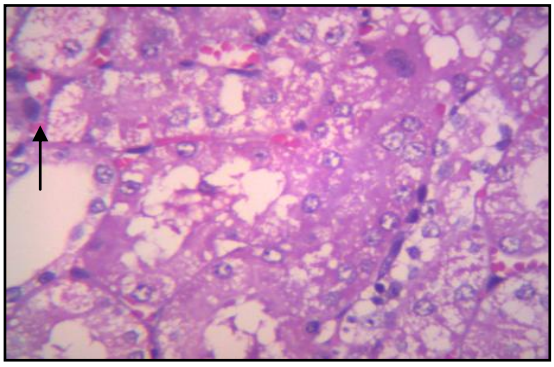
Group - I (Figure 1)



Group- I (Figure 2)



Group- II (Figure 1)



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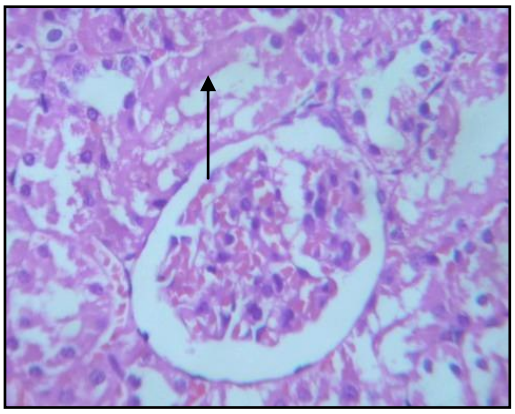
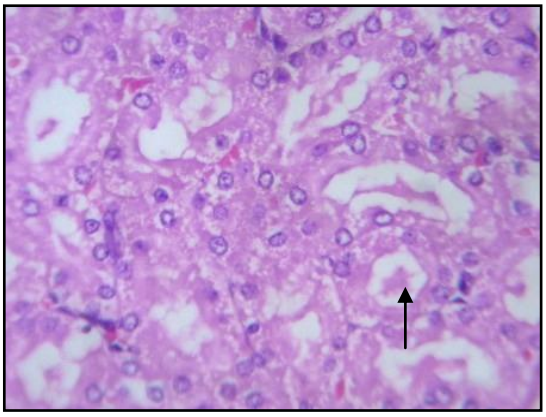
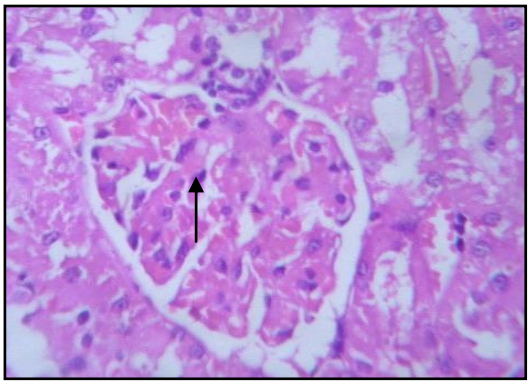


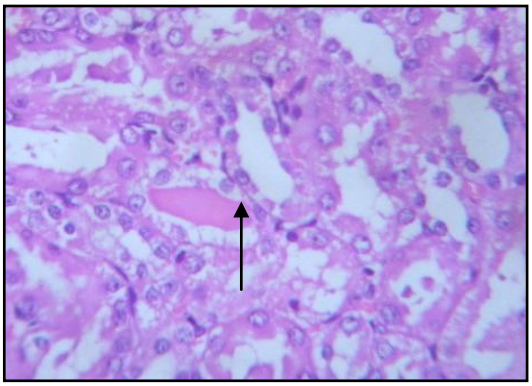
Group- III (Figure 1)



Group- III (Figure 2)



Group- IV (Figure 1)



Group- IV (Figure 2)

Figure No. 1: Histopathology of Kidney

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DISCUSSIONS

The kidney is a common target for toxic xenobiotics, due to its capacity to extract and concentrate toxic substances, and to its large blood flow share (about 20% of cardiac output)[14.] Development of nephrotoxicity can further increase load on the kidney leading to serious complications. This requires either stoppage of drug therapy or change over in the therapy. This raises a question whether any kind of nephroprotection is possible that can handle this problem. Screening of either substances from synthetic origin or herbal origin for nephroprotection can answer this question probably. There is continuing interest on the screening of medicinal plants with a view to determine new sources of natural antioxidants.

Many types of antioxidants with different functions play their role in the defense network and organ protection. It is also evident that the antioxidant supplementation helps in reducing the level of oxidative stress and in slowing or preventing the development of complications associated with diseases. Cisplatin (cis-diamminedichloroplatinum II, CP) is a major antineoplastic drug for the treatment of various forms of cancers.However, CP and its analogs accumulate in the kidney causing nephrotoxicity; thus, limiting its long-term clinical use. Several strategies and agents were utilized to prevent CP nephropathy but were not found suitable for clinical practice.

The present study aimed to evaluate the protective effect of whole fruit hydro alcoholic extract of *Benincasa hispida* (Thunb.) Cong. Plant against cisplatin induced nephropathy rats. Cisplatin administered rat (toxic control group) has encountered acute kidney dysfunction as evidenced by elevation in urine volume, kidney weight, urinary sodium, urinary potassium, urinary glucose, blood urea , blood creatinine and decreased in body weight, urinary creatinine and blood total protein level with multiple histological damages. It also depleted the levels of GSH and increased the levels of Lipid peroxidation.

Treatment with HABH at the dose level of 200,400 mg/kg b.w significantly lowered the blood urea, blood creatinine,urinary glucose,urinary sodium, urinary potassium, urine volume with a significant weight gain and increased the blood total protein, urinary creatinine level when compared with the toxic group. The histological damages in HABH treated group were minimal in contrast to the toxic rats. The HABH has shown significant and dose dependant antioxidant activity and prevented the depletion of GSH and decreased the lipid peroxidation. The nephroprotective activity of extract may be due to the antioxidant activity of benincasa hispida.

CONCLUSIONS

It can be concluded that cisplatin, when administrated at a dose of 7.5mg/kg i.p, induced renal damages as evidenced by the physical, histopathology and biochemical analysis.. On the other hand, HABH treatment was beneficial in cisplatin-induced renal dysfunction and organ damage in rats, presumably via the prevention of lipid peroxidation and preservation of antioxidant glutathione and also observed that all the physical, histopathology and biochemical analysis were brought back nearly to the normal levels with the HABH treatment.

The results of our study suggest that the HABH possesses nephroprotective potential depending on the dose levels. Extensive and multidimensional further research is needed to elucidate the exact mechanism of nephroprotective action of this plant extract on cisplatin induced nephrotoxicity.

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