WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 5.045
Volume 3, Issue 4, 1301-1313. Research Article ISSN 2277 – 7105

NEPHROPROTECTIVE ACTIVITY OF STEM EXTRACT OF *CARALLUMA UMBELLATA* HAW AGAINST CISPLATIN AND GENTAMICIN INDUCED NEPHROTOXICITY

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Article Received on
15 April 2014,

Revised on 10 May 2014,
Accepted on 03 June 2014

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ABSTRACT

Hydro-alcoholic extract (70% methanol extract) of stems of *Caralluma umbellata Haw* was subjected to preliminary phytochemical screening by qualitative tests and nephroprotective activity was assessed in gentamicin and cisplatin induced renal damage in wistar rats (150-200 *g*) by standard methods. The protective property of 70% methanol extract was assessed by measuring the levels of body weight, blood urea nitrogen, serum creatinine and total protein in administered doses. The extract significantly reduced the renal damage caused by cisplatin and gentamicin at a dose of 500 *mg/kg*.

KEYWORDS: *Caralluma umbellate Haw*, Blood urea nitrogen, Creatinine, Cisplatin, Gentamicin, renal damage.

INTRODUCTION

The term renal failure primarily denotes failure of the excretory function of kidney, leading to retention of nitrogenous waste products of metabolism in the blood. In addition, there is failure in regulation of fluid and electrolyte balance along with endocrine dysfunction. The renal failure is fundamentally categorized into acute and chronic renal failure (1, 2).

Chronic Renal Failure (CRF) is an irreversible deterioration in the renal function, which classically develops over a period of years, leading to loss of excretory metabolic and endocrine functions. Various causes of renal failure has been attributed to hypertension, diabetes mellitus, antineoplastic agents like cyclophosphamide, vincristin and cisplatin (3). Acute Renal Failure (ARF) refers to the sudden and usually reversible loss of renal function,

which develops over a period of days or weeks. There are many causes of acute renal failure, which could be prerenal (55%), renal (40%), or post renal (5%). Among the renal causes of acute renal failure, acute tubular necrosis is more common accounting for 85% of incidence. Acute tubular necrosis occurs either due to ischemia or due to toxins. The toxin can be either exogenous or endogenous. The exogenous agents are radiocontrast agents, cyclosporine, antibiotics, chemotherapeutic agents, organic solvents, acetaminophen and illegal abortifacients (1, 4).

Cisplatin (cis-diaminedichloroplatinum II) has become one of the most effective and widely used anticancer agent against various forms of solid tumours of the testes, bladder, ovary, lungs, head and neck. However, its usage has been plagued with many side effects, chiefly nephrotoxicity and testicular damage [5-7] which limits the dosage that can be administered. Several strategies have been explored to reduce the side effects of cisplatin therapy including aggressive hydration with saline, the use of less intensive treatment/or analogues and often with mannitol dilution. However, all have shown limited success. The understanding of the mechanism(s) for this side effect should allow clinicians to prevent/or treat this problem better as well as allow higher doses of cisplatin to be administered for better curative treatments. Based on these findings and others, this work investigates the effect of administration of *Caralluma umbellate Haw* extract on cisplatin-induced renal toxicity in Wistar-Albino rats.

Caralluma umbellata Haw grows wild in dry and arid regions and several Districts of Andhra Pradesh in India. It is a thick, erect, leafless, branching, and succulent a perennial herb [8]. It is medicinally important and rich in pregnane glycosides, which may possess different biological activities [9] including anti-inflammatory activity [8, 10]. A significant analgesic was exhibited by Carumbelloside-I, isolated from *C. umbellata* [11].

Previously, the tribal people of Chittoor District, Andhra Pradesh, India used *Caralluma umbellata Haw* stem juice warmed and mixed with turmeric powder for alleviation of stomach disorder and abdominal pains [12-14]. In this present context, the *in vivo* nephroprotective activity of the Hydro-alcoholic extract (70% methanol extract) of stems of *Caralluma umbellate Haw* stem was evaluated in albino male rats (Wistar strain).

MATERIALSAND METHODS

Chemicals and Standards

Urea estimation kit, Creatinine estimation kit, Protein estimation kit and Albumin estimation kit were obtained from Span Diagnostics Ltd. Cisplatin injection was procured from Celon laboratories Ltd. Gentamicin was procured from Ranbaxy Laboratories Ltd. Sodium carboxy methylcellulose was obtained from Biochem pharmaceutical laboratories Ltd. All other chemicals and reagents used were of analytical grade.

Plant samples

Fresh stems of the plant *Caralluma umbellata* were collected from Araku Valley, near Visakhapatnam (District), Andhra Pradesh (State), India in the month of November 2013. The plant was taxonomically identified and authenticated by Dr. P. Venkaiah, Professor of the Department of Botany, Andhra University, Visakhapatnam, Andhra Pradesh, India. Voucher specimens (GVSK/KLB/11/2013) have been kept in the laboratory for future reference.

Preparation of Hydro-alcoholic extract of Caralluma umbellata Haw

Freshly collected plant material was dried under shade and the dried material was milled to obtain a coarse powder. To the coarse powder (500gms) in maceration chamber, 2.5 liters of alcohol (70% v/v Methanol) was added and macerated for 5 days at room temperature. The macerated extract was obtained and concentrated under vacuum at a temperature of 45^0C by using rotary evaporator, dried completely, weighed and stored in a desiccator.

Preliminary phytochemical analysis

Screening tests were carried out for the hydro-alcoholic crude extract of *Caralluma umbellata Haw* stem using standard procedures to identify the constituents by methods described by Trease [15] and Evans and Harbone [16]. Preliminary phytochemical analysis on plant extracts was performed using the following chemicals and reagents: flavonoids (Mg metal and HCl), phenolics (FeCl3), protein and amino acid (Millon's and Ninhydrin reagent), alkaloids (Mayer and Dragendorff's reagent), saponins (Foam test), phytosterols, triterpenoids (Liebermann- Burchard Test) and carbohydrates (Fehling's solution A and B) [17 - 19].

Experimental Design

Animals

Healthy adult Wister albino rats (weighing between 130-250g, aged 60-120 days) of either sex were housed in standard polypropylene cages at a constant temperature 25±20 c in a 12 hour light and dark cycle. They were fed with standard diet i.e. regular grain chow (Rayans biotechnologies Pvt. Ltd., Hyd.) with water adlibitum throughout the experiment. All experimental protocols were approved by the institutional animal ethical committee (IAEC) Reg.no:516/01A/CPCSEA under the regulation of committee for the purpose of control and supervision of experiments on animals (CPCSEA), New Delhi.

Acute toxicity study

Acute toxicity study was conducted for hydro-alcoholic crude extract of *Caralluma umbellata Haw* stem as per OECD guidelines 420 (OECD.2001). Albino mice of single sex weighing between 20 to 25g were selected and divided into 2 groups each consisting of 6 animals. They were maintained under standard conditions (temperature at 22±3^{0}C, 12hrs light/dark) and allowed free access to water along with standard pellet diet for one week before the experiment. The animals were subjected for acute toxicity study using each extract at a dose of 2000mg/kg orally in 2 groups and observed at regular intervals of 1, 2, 4, 8, 12 and 24 hrs for skin changes, morbidity, aggressiveness, increased oral secretions, sensitivity to sound and pain as well as respiratory movements and mortality. The selected plant extract showed neither visible sign of toxicity nor mortality. The results clearly indicated non-toxicity of the extract at a dose of 2000mg/kg.

Evaluation of Nephroprotective activity against Cisplatin induced [20] **nephrotoxocity**

Four groups of animals were used for the experiment. First group (Group I served as Control) animals were treated with 1% Sodium CMC suspension orally for 10 days. Second group (Group II served as Negative Control) animals were treated with 1% Sodium CMC suspension orally for 10 days and cisplatin (5 *mg/kg, i.p*) on 11th day. The animals in third group (G III - extract treatment) were treated with hydro-alcoholic crude extract of *Caralluma umbellata Haw* at a dose of 250 *mg/kg, p.o* for 10 days and cisplatin (5 *mg/kg, i.p*) on 11th day . Animals of fourth group were (G IV - extract treatment) were treated with hydro-alcoholic crude extract of *Caralluma umbellata Haw* at a dose of 500 *mg/kg, p.o* for 10 days and cisplatin (5 *mg/kg, i.p*) on 11th day. Kidney tissues and blood samples were collected on 16th day to assess renal function, blood urea [21, 22] levels, serum creatinine

[23], serum albumin and total protein. The animals were sacrificed under mild ether anaesthesia and spinal dislocation.

Evaluation of Nephroprotective activity against Gentamicin induced [24] ***nephrotoxocity***

Four groups of animals were used for the experiment. First group (Group I served as Control) animals were treated with 1% Sodium CMC suspension orally for 8 days. Second group (Group II served as Negative Control) animals were treated with 1% Sodium CMC suspension orally for 10 days and after 2hrs Gentamicin (80 *mg/kg, i.p*) was administered intraperitoneally for 8 days. The animals in third group (G III - extract treatment) were treated with hydro-alcoholic crude extract of *Caralluma umbellata Haw* at a dose of 250 *mg/kg, p.o* for 10 days and after 2hrs Gentamicin (80 *mg/kg, i.p*) was administered intraperitoneally for 8 days. Animals of fourth group were (G IV - extract treatment) were treated with hydro-alcoholic crude extract of *Caralluma umbellata Haw* at a dose of 500 *mg/kg, p.o* for 10 days and after 2hrs Gentamicin (80 *mg/kg, i.p*) was administered intraperitoneally for 8 days. Blood samples were withdrawn on 9^{th} day to assess renal function, blood urea [21, 22] levels, serum creatinine [23], serum albumin and total protein.

Statistical analysis

Results were expressed as mean ± SEM. Statistical analysis was performed with one way analysis of variance (ANOVA). P value less than 0.05 and 0.01 was considered to be statistically significant.

RESULTS

Preliminary phytochemical analysis

Results of preliminary phytochemical analysis of hydro-alcoholic crude extract of *Caralluma umbellata Haw* stem are presented in (Table1). Phenols, flavanoids, alkaloids, steroids, terpenoids and glycosides were observed in qualitative analysis.

Effect of hydro-alcoholic extract of Caralluma umbellata in Cisplatin induced hepatotoxicity in rats

Effect on Serum creatinine and Blood urea nitrogen (BUN)

Rats treated with Cisplatin (G II) developed a significant renal damage observed as elevetaed serum levels of creatinine and blood urea nitrogen when compared to normal control (G I). Pretreatment of hydro-alcoholic extract (HAE) of *Caralluma umbellata Haw* stem along with

cisplatin at doses 250 mg/kg and 500 mg/kg (G III & G IV) produced significant ($p<0.01$) reduction in serum levels of creatinine and BUN as compared to Cisplatin treated group (Graphs 1 & 2).

The % protection against rise in serum creatinine levels by the extract at doses of 250 mg/kg and 500 mg/kg was found to be 66.42% and 73.88% respectively (Table 2).
The % protection against rise in blood urea levels by the extract at doses of 250 mg/kg and 500 mg/kg was found to be 54.43% and 68.54% respectively (Table 2).

Effect on Serum albumin and total protein

Rats treated with Cisplatin (G II) developed a significant renal damage observed as elevetaed serum levels of albumin and total protein when compared to normal control (G I). Pretreatment of hydro-alcoholic extract (HAE) of *Caralluma umbellata Haw* stem along with cisplatin at doses 250 mg/kg and 500 mg/kg (G III & G IV) produced significant ($p<0.01$) reduction in serum levels of albumin and total protein as compared to Cisplatin treated group (Graphs 3 & 4).

The % protection against rise in serum albumin levels by the extract at doses of 250 mg/kg and 500 mg/kg was found to be 34.32% and 70.88% respectively (Table 2).
The % protection against rise in total protein levels by the extract at doses of 250 mg/kg and 500 mg/kg was found to be 37.80% and 62.99% respectively (Table 2).

Effect of hydro-alcoholic extract of Caralluma umbellata in Gentamicin induced hepatotoxicity in rats

Effect on Serum creatinine and Blood urea nitrogen (BUN)

Rats treated with Gentamicin (G II) developed a significant renal damage observed as elevetaed serum levels of creatinine and blood urea nitrogen when compared to normal control (G I). Pretreatment of hydro-alcoholic extract (HAE) of *Caralluma umbellata Haw* stem along with cisplatin at doses 250 mg/kg and 500 mg/kg (G III & G IV) produced significant ($p<0.01$) reduction in serum levels of creatinine and BUN as compared to Gentamicin treated group (Graphs 5 & 6).

The % protection against rise in serum creatinine levels by the extract at doses of 250 mg/kg and 500 mg/kg was found to be 49.14% and 77.71% respectively (Table 3).

The % protection against rise in blood urea levels by the extract at doses of 250 mg/kg and 500 mg/kg was found to be 43.51% and 73.74% respectively (Table 3).

Effect on Serum albumin and total protein

Rats treated with Gentamicin (G II) developed a significant renal damage observed as elevetaed serum levels of albumin and total protein when compared to normal control (G I). Pretreatment of hydro-alcoholic extract (HAE) of *Caralluma umbellata Haw* stem along with cisplatin at doses 250 mg/kg and 500 mg/kg (G III & G IV) produced significant ($p<0.01$) reduction in serum levels of albumin and total protein as compared to Gentamicin treated group (Graphs 7 & 8).

The % protection against rise in serum albumin levels by the extract at doses of 250 mg/kg and 500 mg/kg was found to be 66.82% and 79.77% respectively (Table 3).

The % protection against rise in total protein levels by the extract at doses of 250 mg/kg and 500 mg/kg was found to be 62.69% and 78.24% respectively (Table 3).

Table no.1: Preliminary phytochemical analysis of hydro-alcoholic extract of *Caralluma umbellata haw.*

S. No	Phytoconstituents	Present/Absent
1	Carbohydrates	-
2	Glycosides	+
3	Saponins	-
4	Tannins	-
5	Phytosterols & Terpenoids	+
6	Flavonoids	+
7	Alkaloids	+
8	Quinones	-

Table no.2: % Protection of hydro-alcoholic extract of *Caralluma umbellata haw on Cisplatin induced nephrotoxicity*

Groups	Treatment	Serum Biochemical Parameters			
		Creatinine (Mg/Dl)	Bun (Mg/Dl)	Albumin (G/Dl)	Total Protein (G/Dl)
I (Normal control)	Vehicle (1%)	0.85±0.01	21.17±0.32	4.57±0.11	6.13±0.11
II (Negative control)	Cisplatin (5mg/kg i.p.)	1.52±0.04	32.15±0.61	3.45±0.10	4.86±0.12

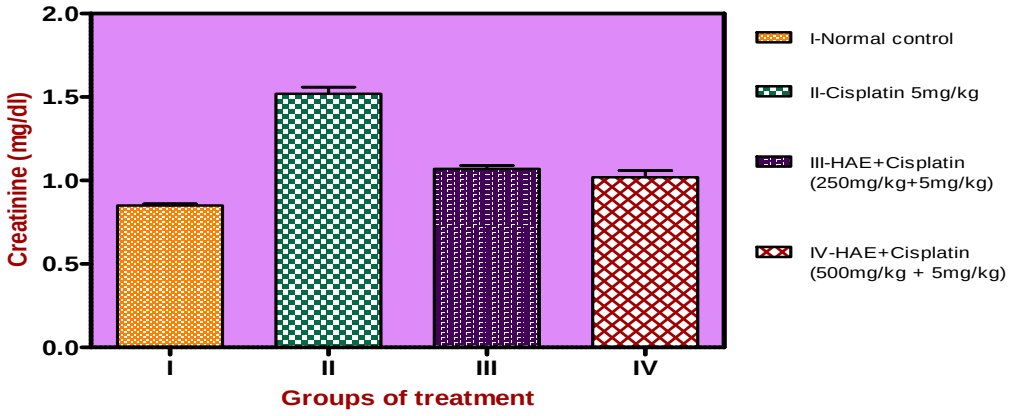
III (Prophylactic effect)	HAE + Cisplatin (250mg/kg + 5mg/kg)	1.07±0.02** (66.42%)	26.17±0.32** (54.43%)	3.83±0.06** (34.32%)	5.34±0.03** (37.80%)
IV (Prophylactic effect)	HAE + Cisplatin (500mg/kg + 5mg/kg)	1.02±0.04* (73.88%)	24.62±0.40** (68.54%)	4.24±0.03** (70.88%)	5.66±0.10** (62.99%)

Values are expressed as Mean SEM, Results were significant at *p<0.05 and **p<0.01 when compared to negative control (G II)

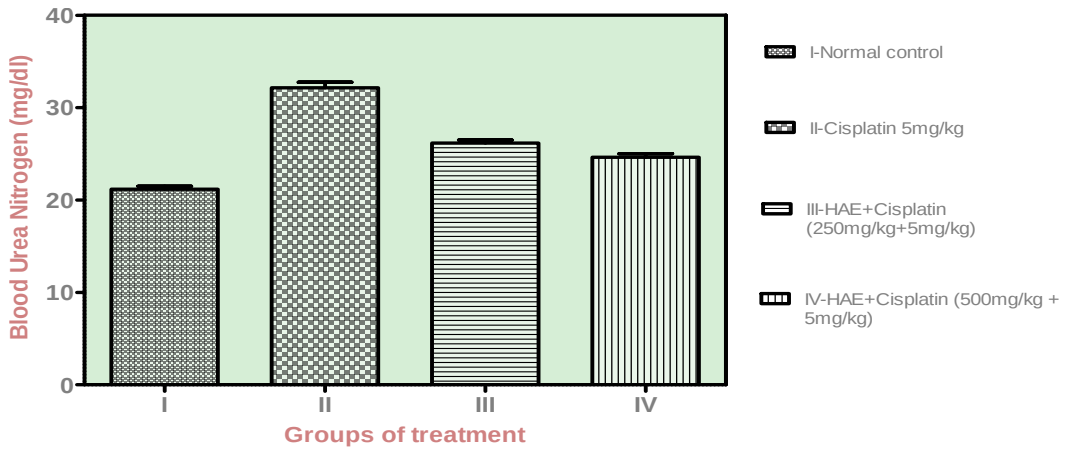
Table no.3: % Protection of hydro-alcoholic extract of *Caralluma umbellata haw on Gentamicin induced nephrotoxicity*

Groups	Treatment	Serum Biochemical Parameters			
		Creatinine (Mg/Dl)	Bun (Mg/Dl)	Albumin (G/Dl)	Total Protein (G/Dl)
I (Normal control)	Vehicle (1%)	0.85±0.01	21.17±0.32	4.57±0.11	6.13±0.11
II (Negativecontrol)	Gentamicin (80mg/kg i.p.)	1.72±0.03	36.22±0.49	3.10±0.03	4.20±0.04
III (Prophylactic effect)	HAE + Gentamicin (250mg/kg + 80mg/kg)	1.29±0.02** (49.14%)	29.67±0.37** (43.51%)	4.08±0.04** (66.82%)	5.41±0.07** (62.69%)
IV (Prophylactic effect)	HAE + Gentamicin (500mg/kg + 80mg/kg)	1.04±0.02** (77.71%)	25.12±0.29** (73.74%)	4.27±0.02** (79.77%)	5.71±0.07** (78.24%)

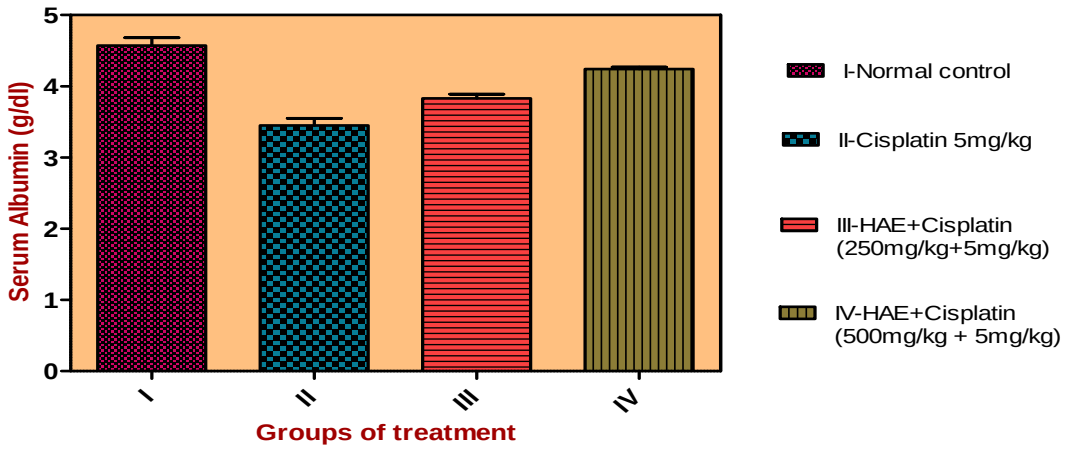
Values are expressed as Mean SEM, Results were significant at **p<0.01 when compared to negative control (G II)



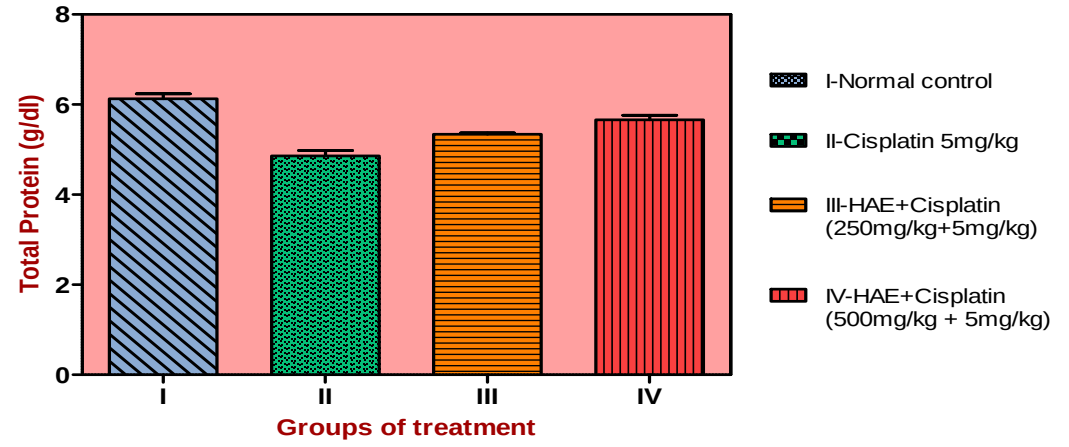
Graph 1: Effect of HAE on Serum creatinine levels in rats (Cisplatin induced Nephrotoxicity)



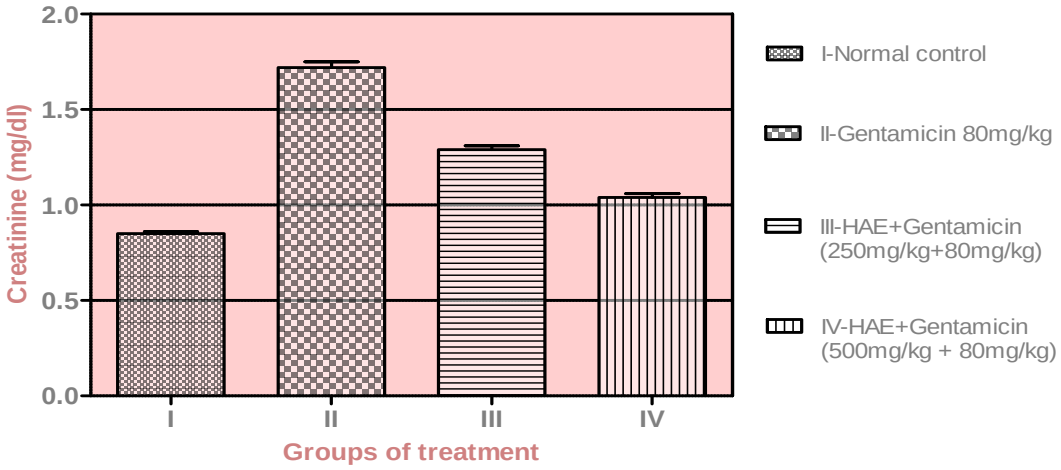
Graph 2: Effect of HAE on BUN levels in rats (Cisplatin induced Nephrotoxicity)



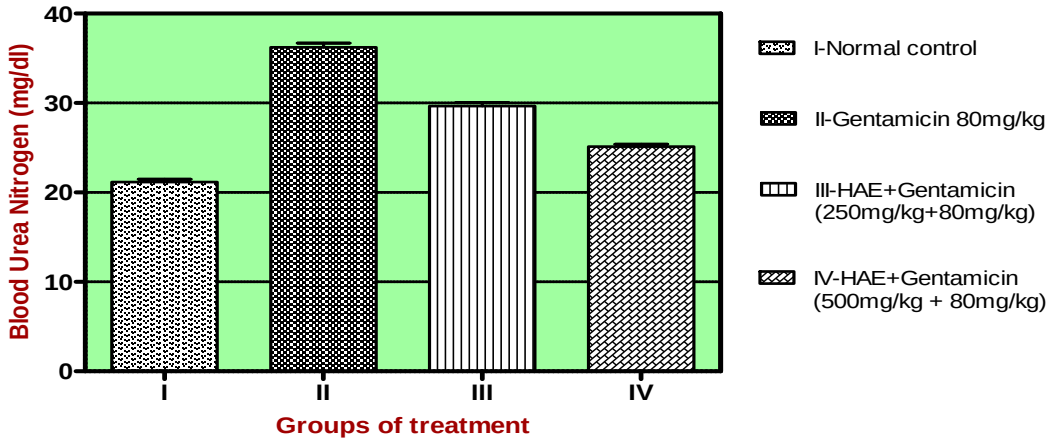
Graph 3: Effect of HAE on Serum albumin levels in rats (Cisplatin induced Nephrotoxicity)



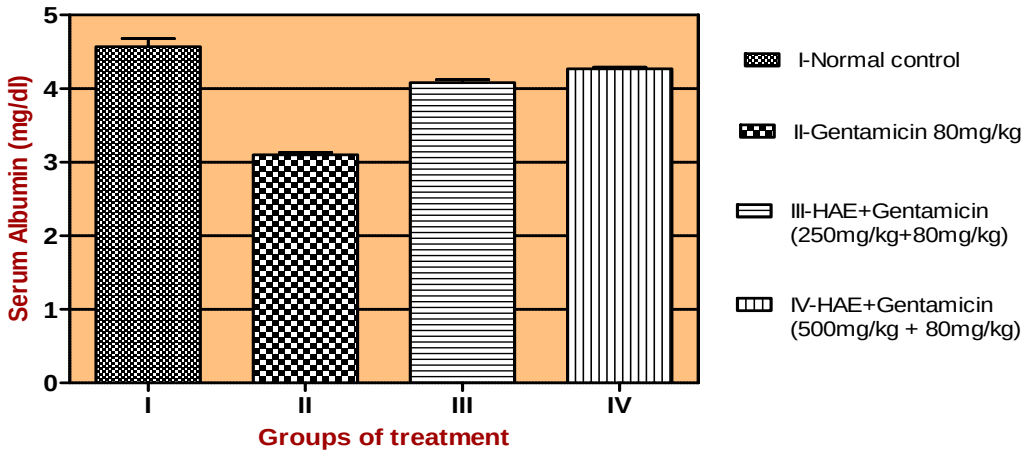
Graph 4: Effect of HAE on Total Protein levels in rats (Cisplatin induced Nephrotoxicity)



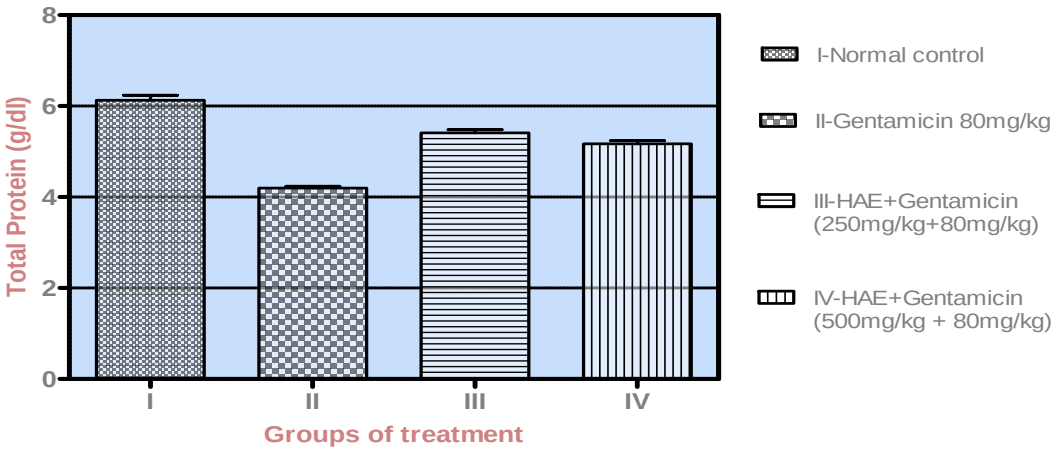
Graph 5: Effect of HAE on Serum creatinine levels in rats (Gentamicin induced Nephrotoxicity)



Graph 6: Effect of HAE on BUN levels in rats (Gentamicin induced Nephrotoxicity)



Graph 7: Effect of HAE on Serum albumin levels in rats (Gentamicin induced Nephrotoxicity)



Graph 8: Effect of HAE on Total Protein levels in rats (Gentamicin induced Nephrotoxicity)

DISCUSSION

Cisplatin nephroroxocoty was caused by decreased glomerular filtration rate that results in biotransformation of Cisplatin to mono chloro aqua di-amineplatin or diaquodi-amineplatin. These agents alkylate the purine and pyramidine bases of nuclear material and results in nephrotoxicity. Other proposed explanation of the nephrotoxicity of Cisplatin is that it induces renal damage by free radical generation. By the estimation of serum marker enzymes it was observed that 10 days administration of HAE of *Caralluma umbellate* at doses of 250 mg/kg and 500 mg/kg prior to Cisplatin (5 mg/kg, single dose) in the prophylactic regimen, effectively prevented the Cisplatin induced renal injury as evidenced by decreased BUN and serum creatinine levels and by increased albumin and total protein levels.

Gentamicin induced nephrotoxocity is multi-factorial [25], generation of free radicals may be a major factor in its nephrotoxicity [26]. Gentamicin nephrotoxicity causes increase in hydrogen peroxide production by renal cortical mitochondria [27]. By the estimation of serum marker enzymes it was observed that 10 days administration of HAE of *Caralluma umbellate* at doses of 250 mg/kg and 500 mg/kg prior to Gentamicin (80 mg/kg, single dose) in the prophylactic regimen, effectively prevented the Cisplatin induced renal injury as evidenced by decreased BUN and serum creatinine levels and by increased albumin and total protein levels.

As free radicals formation is one of the mechanisms of nephrotoxicity induced by Cisplatin and Gentamicin models of nephrotoxicity. Hence antioxidants and free radical scavengers of

natural and synthetic origin might provide nephroprotection in Cisplatin and Gentamicin induced renal injury. A relationship between oxidative stress and nephrotoxicity has been well-demonstrated in many experimental models. Thus it was assumed that nephroprotective effect might be attributed by antioxidant property of *Caralluma umbellate.* It is therefore worth study further to isolate the pure molecules responsible for nephroprotective activity and establishes the mechanism of action of *Caralluma umbellate* as nephroprotective agent.

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