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Research Article

NEPHROPROTECTIVE EFFECT OF ETHANOLIC EXTRACT OF GARLIC (*ALLIUM SATIVUM* L.) ON CISPLATIN INDUCED NEPHROTOXICITY IN MALE WISTAR RATS

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ABSTRACT

Objectives: The present study investigated the *in vivo* antioxidant and nephroprotective potential of ethanolic extract of garlic (*Allium sativum* L.) against cisplatin induced nephrotoxicity in Wistar male rats.

Methods: Nephrotoxicity was induced by a single intraperitoneal injection of cisplatin (5 mg/kg b.w.) on the first day of the experiment. The animals were treated with ethanolic extract of garlic at doses 150 mg/kg b.w. and 300 mg/kg b.w. for four consecutive days. The effect of the higher dose (300 mg/kg b.w.) of garlic extract on normal rats was also studied. The animals were sacrificed on the fifth day and enzymatic antioxidants and lipid peroxidation were assessed in kidney whereas urea, creatinine, uric acid and blood urea nitrogen (BUN) were quantified in serum samples.

Results: Cisplatin induction resulted in a decrease in the activities of kidney antioxidants with a concomitant increase in kidney weight, lipid peroxidation along with serum kidney markers like urea, creatinine, uric acid and BUN. Treatment with ethanolic extract of garlic rendered a protective effect by boosting up the antioxidant levels and reverting back the markers to near normalcy. The garlic extract at higher dose also had no significant biochemical alterations in normal rats.

Conclusion: The results from this study reveals that the ethanolic extract of garlic possess a potential nephroprotective property with no aftereffects.

Keywords: *Allium sativum*, Cisplatin, Antioxidants, Nephroprotective, Kidney markers.

INTRODUCTION

Cisplatin (cis-diamminedichloroplatinum(II)) is an antineoplastic drug used in the treatment of many solid-organ cancers which on administration results in ototoxicity, gastrotoxicity, myelosuppression, and allergic reactions [1] with the main dose-limiting side effect as nephrotoxicity [2]. Cisplatin accumulation is greater in kidney than other organs since it is the major route for its excretion and its concentration in proximal tubular epithelial cells is about five times greater than that in serum [3]. Reactive oxygen species (ROS) derived from this disproportionate accumulation of cisplatin in kidney tissue contributes to oxidative stress injury resulting in renal necrosis [4]. In order to combat the cisplatin induced oxidative stress, it is important to understand the role played by antioxidants if they can show a protective effect. Moreover, a large number of sulfur-containing compounds have been shown to reduce the nephrotoxicity of cisplatin without inhibiting its antitumor effect [5].

Garlic (*Allium sativum*) belonging to the family Amaryllidaceae is a bulbous perennial herb originated in Central Asia and has been cultivated in the Middle East for over 5000 years. In ayurvedic medicine, garlic is used to treat respiratory problems, ulcers, colic and flatulence, and garlic oil drops are used to treat ear aches [6]. Allicin, the first chemically isolated and most biologically active compound in garlic has anti-microbial effects against many viruses, bacteria, fungi and parasites [7]. The earlier reported pharmacological activities of garlic include antioxidant [8], anti-genotoxic [9], hypolipidemic [10], hepatoprotective [11], antidiabetic [12], anti-tissue atrophy [13], immunomodulatory [14] activities, reduction of allergic airway inflammation [15], prevention of cytogenetic damage [16], protection against 7, 12 Dimethyl benzanthracene induced toxicity [17] and anti-microbial activity of allicin [18]. The active components of garlic include antioxidants such as organosulfur compounds, flavonoids such as allicin, trace elements such as germanium (normalizer and immunostimulant) and selenium (for optimal function of the antioxidant enzyme glutathione peroxidase), volatile oil containing sulfur compounds, amino acids and other bio-active compounds [19].

Garlic also has protective role against cisplatin induced genotoxicity in human lymphocyte chromosome [20]. Previous reports also suggest the protective effect of garlic against renal reperfusion injury [21] and nephropathy [22]. Based on these earlier reports on garlic, the present study is centered on evaluating the protective effect of ethanolic extract of garlic against cisplatin induced nephrotoxicity.

MATERIALS AND METHODS

Chemicals

The inducer Cisplatin was purchased from Cipla and the diagnostic kits were from Piramal healthcare. All the other chemicals used in the study were of analytical grade which were bought from Himedia Laboratories Private Ltd, Merck and SD Fine Chemicals, Mumbai.

Sample preparation

Fresh garlic (*Allium sativum)* was purchased from a local market in Coimbatore, Tamil Nadu, India. Freshly purchased garlic was cleaned, skin was peeled off and was then shade dried at room temperature (25°C). The dried garlic was powdered and extracted in soxhlet apparatus using ethanol. The solvent was evaporated in a rotary vacuum-evaporator (Yamato RE300, Japan) at 50°C and the remaining was removed by lyophilization (VirTis Benchtop K, USA). The ethanol extract of garlic (EEG) thus obtained (yield 8.7%) was used for assessment of nephroprotective properties.

Experimental animals

Male Wistar albino rats weighing 150 – 200 g were obtained from the small animals breeding station, Mannuthy, Kerala, India. All the animals were housed in clean polypropylene cages and maintained under standard environmental conditions (14 h dark/10 h light cycles; Temp 25 ± 2°C; 35-60% humidity, air ventilation). The animals were fed with standard pellet diet (M/s. Hindustan Lever Ltd, Mumbai, India) and water *ad libitum*. The experimental protocol was carried out according to the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals

(CPCSEA), India and approved by the Institutional Ethical Committee (1454/po/c/11/CPCSEA).

Experimental design

The nephroprotective and the antioxidant activity of EEG were evaluated against cisplatin induced nephrotoxicity in animal model. Nephrotoxicity was induced in animals following the method of Naghizadeh et al [23]. Animals were randomly divided into five groups of six animals each. Group I and II received 10 mL/kg b.w. of distilled water. Groups II – IV received a single dose of cisplatin (5 mg/kg b.w.) intraperitoneally only on the first day of experiment. Groups III and IV were then orally administered with EEG (150 and 300 mg/kg b.w., respectively), for four consecutive days followed by the cisplatin induction. The fifth group of animals was normal which received 300 mg/ kg b.w. of EEG alone orally. The dosage was fixed based on the previous reports on active pharmacological dose of garlic. At the fifth day, all animals were anaesthetized with ether and blood samples were collected by cardiac puncture. The blood was allowed to clot for 20 min, centrifuged at 3000 rpm for 10 min and the serum obtained was used for measuring the levels of urea, uric acid, creatinine and blood urea nitrogen (BUN). The kidneys were immediately removed, washed in saline, blotted to dryness and then weighed. The kidney tissues were then homogenized in Tris Hcl buffer (pH – 7.4) to obtain a 10% homogenate which was used for measuring the antioxidant activities.

Biochemical analysis

In the 10% kidney homogenate, enzymatic antioxidants like superoxide dismutase (SOD) [24], catalase (CAT) [25], glutathione peroxidase (GPx) [26] and glutathione S-transferase (GST) [27] were assessed following standard procedures. The tissue lipid peroxidation of kidney was estimated by the method of Niehius and Samuelsson [28]. Serum urea, creatinine, uric acid and BUN were analyzed using commercially available diagnostic kits.

Statistical analysis

Data were analysed for statistical significance using one way analysis of variance (ANOVA) followed by Dunnet's multiple comparison test and results were expressed as mean ± standard deviation (SD) using SPSS version 16.0.

RESULTS

Effect of ethanolic extract of garlic on kidney weight

Table 1 shows the kidney weight of the control and experimental animals. A significant increase ($p<0.001$) in the kidney weight was observed in the cisplatin induced nephrotoxic group II rats. Administration of EEG to the nephrotoxic rats resulted in a decrease in the kidney weight when compared to their untreated counterpart. EEG extended a protective role over kidney weight in a dose dependent manner. EEG at higher dose exhibited no significant change over kidney weight of normal rats.

Table 1: Effect of EEG on kidney weight of cisplatin induced nephrotoxicity in rats

Groups	Treatment	Kidney weight (g/100g b.w.)
Group I - Control	Distilled water - 10 mL/kg	0.79 ± 0.05
Group II - Induced	CP + Distilled water - 10 mL/kg	1.09 ± 0.07$^{\#\#\#}$
Group III - EEG	CP + 150 mg/kg b.w.	0.99 ± 0.05**
Group IV - EEG	CP + 300 mg/kg b.w.	0.82 ± 0.05***
Group V - EEG	300 mg/kg b.w.	0.78 ± 0.05^{NS}

CP – Cisplatin; EEG – Ethanolic extract of garlic. $^{\#}$Change in activities at $p<0.05$ when group II compared to group I, $^{\#\#}p<0.01$, $^{\#\#\#}p<0.001$; *Change in activities at $p<0.05$ when group III - VI compared to group II, $^{**}p<0.01$, $^{***}p<0.001$; ^{NS}Not significant when group V compared to group I. Values are expressed as mean ± SD (n=6).

Effect of ethanolic extract of garlic on serum kidney markers

Nephrotoxicity of cisplatin was quite evident from the elevated levels of serum kidney markers like urea, uric acid, creatinine and BUN which was observed in the cisplatin induced untreated rats (Table 2). All these biochemical markers reverted back to their normal values on treatment with EEG. The extent of reduction in their levels was in direct proportion with the dosage of garlic extract. EEG at a higher dose (300 mg/kg b.w.) had no much effect over these kidney markers of normal rats.

Table 2: Effect of EEG on serum kidney markers of cisplatin induced nephrotoxicity in rats

Parameters	Control (10 mL/kg)	Induced (CP + 10 mL/kg)	EEG (CP + 150 mg/kg)	EEG (CP + 300 mg/kg)	EEG (300 mg/kg)
Urea (mg/dL)	9.61 ± 0.52	26.93 ± 0.90$^{\#\#\#}$	16.84 ± 0.49***	10.76 ± 0.66***	9.80 ± 0.29^{NS}
Uric acid (mg/dL)	3.65 ± 0.09	7.06 ± 0.15$^{\#\#\#}$	5.73 ± 0.94***	4.54 ± 0.10***	3.51 ± 0.06$^{\psi}$
Creatinine (mg/dL)	0.69 ± 0.07	2.73 ± 0.37$^{\#\#\#}$	1.71 ± 0.15***	0.83 ± 0.15***	0.73 ± 0.15^{NS}
Blood urea nitogen (mg/dL)	9.03 ± 0.09	20.52 ± 0.07$^{\#\#\#}$	13.30 ± 0.12***	10.81 ± 0.19***	8.88 ± 0.11$^{\psi}$

CP – Cisplatin; EEG – Ethanolic extract of garlic. $^{\#}$Change in activities at $p<0.05$ when group II compared to group I, $^{\#\#}p<0.01$, $^{\#\#\#}p<0.001$; *Change in activities at $p<0.05$ when group III - VI compared to group II, $^{**}p<0.01$, $^{***}p<0.001$; $^{\psi}$Change in activities at $p<0.05$ when group V compared to group I, $^{\psi\psi}p<0.01$, $^{\psi\psi\psi}p<0.001$; ^{NS}Not significant when group V compared to group I. Values are expressed as mean ± SD (n=6).

Effect of ethanolic extract of garlic on kidney antioxidants

The effect of EEG on enzymatic antioxidants and lipid peroxidation of kidney are depicted in the Table 3. Cisplatin induction resulted in the suppression of enzymatic antioxidant activities along with an elevation in the levels of lipid peroxides in the kidney tissue. Untreated cisplatin induced group II rats showed significantly ($p<0.001$) decreased activities of antioxidants like SOD, CAT, GPx and GST. Simultaneously, there was a significant ($p<0.001$) increase in the levels of malondialdehyde, an indicator of lipid peroxidation. On treatment with EEG for a period of four days, there was a significant ($p<0.001$) increase in the activities of enzymatic antioxidants with a concomitant decrease in the levels of lipid peroxides. EEG rendered a protective role by bringing back these antioxidant markers to near normalcy. When the normal rats were administered with 300 mg/kg b.w. of EEG, there were no remarkable changes in the activities of enzymatic antioxidants as well as in the levels of lipid peroxides on comparison with the normal control group I rats.

Table 3: Effect of EEG on antioxidants and lipid peroxidation of cisplatin induced nephrotoxicity in rats

Parameters	Control (10 mL/kg b.w.)	Induced (CP + 10 mL/kg)	EEG (CP + 150 mg/kg)	EEG (CP + 300 mg/kg)	EEG (300 mg/kg)
SOD (Units/min/mg protein)	1.03 ± 0.02	0.42 ± 0.01$^{\#\#\#}$	0.63 ± 0.01***	0.96 ± 0.03***	1.09 ± 0.02$^{\psi\psi\psi}$
CAT (µ moles of H_2O_2 consumed/min/mg protein)	44.95 ± 0.95	30.50 ± 0.94$^{\#\#\#}$	36.55 ± 0.54***	40.29 ± 0.63***	44.39 ± 0.98^{NS}
GPx (µ moles of GSH oxidized/min/mg protein)	17.82 ± 0.40	8.11 ± 0.56$^{\#\#\#}$	12.52 ± 0.59***	16.25 ± 0.65***	17.08 ± 0.64$^{\psi}$
GST (µ moles of CDNB conjugation formed/min/mg protein)	98.07 ± 1.71	40.69 ± 0.77$^{\#\#\#}$	72.23 ± 0.75***	91.89 ± 0.63***	97.97 ± 0.54 ^{NS}
LPO (µ moles/mg protein)	8.08 ± 0.54	20.06 ± 0.56$^{\#\#\#}$	13.65 ± 0.29***	9.50 ± 0.22***	8.19 ± 0.35 ^{NS}

CP – Cisplatin; EEG – Ethanolic extract of garlic. $^{\#}$Change in activities at p<0.05 when group II compared to group I, $^{\#\#}$p<0.01, $^{\#\#\#}$p<0.001; *Change in activities at p<0.05 when group III - VI compared to group II, **p<0.01, ***p<0.001; $^{\psi}$ Change in activities at p<0.05 when group V compared to group I, $^{\psi\psi}$p<0.01, $^{\psi\psi\psi}$p<0.001; ^{NS}Not significant when group V compared to group I. Values are expressed as mean±SD (n=6).

DISCUSSION

Cisplatin, being the widely used chemotherapeutic drug, its chief dose limiting side effect nephrotoxicity, has to be overcome for its better and safe use. The *in vivo* mechanisms of cisplatin nephrotoxicity are complex and involve oxidative stress, apoptosis, inflammation, and fibrogenesis. 20% of patients receiving high-dose cisplatin have severe renal dysfunction [29]. Thiol compounds have been used clinically to reduce the nephrotoxicity of cisplatin. High doses of reduced glutathione injected intravenously within 30 min of cisplatin administration renders a protective sign [30-31]. Since plant based natural products are the current field of interest for the researchers due to their cheap and high therapeutic potential without much of the side effects associated with synthetic drugs, the present study evaluated the nephroprotective property of garlic, a thiol rich natural source [32].

Cisplatin induced nephrotoxicity resulted in an increase in the kidney weight when compared to that of the normal control rats. Similar increase in kidney weight has already been reported with cisplatin toxicity [33]. This increase in kidney weight might be due to renal edema since cisplatin is known to cause inflammation [29]. Treatment with EEG resulted in a decrease in the kidney weight of nephrotoxic rats. This protective effect of garlic over kidney weight could be attributed to its anti-inflammatory properties [34] which would have abandoned the edema caused in kidney by cisplatin. The garlic extract also had no influence over the kidney weight of normal rats which shows that it does not interfere with the normal morphology and functions of the kidney.

The important parameters that should be taken into account in the evaluation of kidney damage caused by cisplatin are urea, creatinine, uric acid and BUN. These markers are the end products of various metabolic pathways that are excreted in the urine via glomerular filtration whose serum levels are an indicator of renal functions. In cisplatin induced nephrotoxicity, a significant elevation in the levels of urea, creatinine, uric acid and BUN were observed which serves as an indicator of impaired renal functions [35]. Garlic had the potency to normalize the renal functions which is quite evident from the reduced levels of urea, creatinine, uric acid and BUN in EEG treated groups.

The activities of key enzymatic antioxidants like SOD, CAT, GPx and GST were found decreased in cisplatin induced nephrotoxic rats. Reactive oxygen species like hydrogen peroxide, superoxide and hydroxyl radicals generated under normal metabolic conditions are generally detoxified by the activities of antioxidants like reduced glutathione, superoxide dismutase and catalase. But in case of cisplatin toxicity, due to excess production of highly reactive free radicals, there occur an imbalance in the oxidant-antioxidant status leading to depletion in the activities of antioxidants as well as an elevation in lipid peroxidation [36]. Similar reduction in antioxidants along with an increase in lipid peroxidation under cisplatin toxicity was observed in the earlier reports [37]. Administration of EEG revealed a protective role by enhancing the activities of antioxidants and decreasing the levels of lipid peroxides which eliminated the oxidative stress state.

Garlic oil has already been reported for its *in vitro* free radical scavenging ability [38]. Several workers reported that allicin and its metabolites are scavengers of free radicals [39-40] which might have been the reason for the increased antioxidant activities and decreased lipid peroxide levels which were observed in groups treated with EEG. Earlier reports on garlic suggests its composition of a hundred sulphur containing compounds which include allicin (70-80%), allyl-ss(o)-methyl (6-16% of total), methyl-ss(o)-allyl (3-9%), trans-1-propenyl (0.2-0.4%), trans-1-propenyl-ss(o)methyl+methyl-ss(o)-trans-1-propenyl (0.1-2.5%) and methyl-ss(o)methyl (2%) thiosulfinates [41]. Hence the nephroprotective activity of garlic could be due to its antioxidant property and thiol rich compounds.

CONCLUSION

This study concludes that garlic could serve as an economical remedy for the renal damages observed in patients undergoing cisplatin chemotherapy with no adverse effects. Further studies could be extended in analysing the active component structure and mechanism by which it renders nephroprotection.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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