

# The Journal of Phytopharmacology

(Pharmacognosy and phytomedicine Research)

## Research Article

ISSN 2230-480X

JPHYTO 2016; 5(4): 145-149

July- August

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## Nephroprotective activity of *Amomum subulatum* seeds against cypermethrin induced nephrotoxicity in rats

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### ABSTRACT

The objective of the present study was to evaluate nephroprotective activity of *Amomum subulatum* seed extract against Cypermethrin induced nephrotoxicity in albino rats. The animals were divided into five different groups consisting of six rats each. Except normal control and test drug alone group, all the rats were treated with Cypermethrin 25g/kg body weight orally for 28 consecutive days and test group IV & V (ASSE 200 & 400mg/kg body weight) were co administered with Cypermethrin orally for 28 consecutive days. On the 28th haematological, biochemical and histopathological parameters were estimated. The chronic administration of Cypermethrin caused significant elevation in the serum creatinine and urea level and increased lipid peroxidation in the kidney tissue homogenate as compared to normal control. The histological examination of kidney tissues revealed mild fatty changes especially in the sub capsular region in sections, dilatation of glomerulus along with obliteration of periglomerular space and shrinkage of glomerulus was observed. The co administration of test drug significantly attenuated the Cypermethrin induced elevated serum urea and creatinine and lipid peroxidation in tissue homogenate. Thus we can conclude the *Amomum subulatum* seed extract has significant nephroprotective effect and reversed Cypermethrin induced nephrotoxicity.

**Keywords:** *Amomum subulatum*, Nephroprotective, Cypermethrin, Large cardamom, lipid peroxidation.

### INTRODUCTION

Over the last few decades agricultural pesticides have become a common household item in rural areas of the developing countries. Due to their easy availability, pesticides have also become commonly used for intentional self-poisoning. According to World Health Organization (WHO) estimates around 3 million poisoning cases with 2, 20,000 deaths occur annually. About 99% of these deaths occur in developing countries [1]. India being the second largest producer of fruits and vegetables in the world, farmers being ignorant and use harmful pesticides like Cypermethrin, Malathion, Aldrin, Lindane and Endosulfan [2]. The wide spread use in pest control and also in public health programmes have become cause of serious environmental pollution worldwide. According to a study, pesticide poisoning causes more deaths in comparison to infectious diseases [3].

Cypermethrin is a pyrethroid insecticide first synthesized in 1974 and constitutes more than 30% of the insecticides used globally. It is known to act as both a stomach and a contact poison. Its selective toxic effects are related to the neural sodium channels. The metabolism of Cypermethrin is quite rapid, and during its metabolism reactive oxygen species (ROS) are generated. These free radicals are most active and cause oxidative stress through peroxidation of the lipid membrane. Damage may occur in certain tissues and organs, due to either the free radicals that are generated or the direct effect of the pesticide on the organs [4-6].

*Amomum subulatum* commonly known as large cardamom is a perennial herbaceous plant native of Sikkim, Darjeeling, Assam, Bhutan and Nepal [7]. The fruits are prescribed to treat indigestion, vomiting, biliousness, abdominal pains, rectal diseases, throat troubles, congestion of the lungs, inflammation of the eye lids, digestive disorder, pulmonary tuberculosis, loss of appetite, gastric troubles, liver complications and also used as diuretics [8]. The phytochemical analysis has shown the following chemicals compositions such as protocatechualdehyde, protocatechuic acid, 1,7-bis(3,4-dihydroxyphenyl)hepta-4E, 6E-dien-3-one and 2,3,7-trihydroxy-5-(3,4-dihydroxy-E-styryl)-6,7,8,9-tetra hydro-5H-benzocyc loheptene were identified [9]. It has been reported to possess different pharmacological actions such as its gastroprotective, hepatoprotective, fibrinolytic, lipid lowering, and antioxidant enhancing effects [10]. It is interesting to note that greater cardamom possess a range of organ protective effects like garstroprotective and anti-stressor against acute severe cardiac stress [11,12]. Anti-diabetic activity of *Amomum subulatum* seeds has been reported against fructose fed metabolic syndrome in rats [13]. So the present study was aimed to evaluate the cytoprotective activity of *Amomum subulatum* seed extract (ASSE) against Cypermethrin induced nephrotoxicity as a supportive treatment in pesticide toxicity.

## MATERIALS AND METHODS

### MATERIALS

#### Test drug collection and preparation

The large cardamom (*Ammomum subulatum*) was collected from SDM Pharmacy at Udipi, India and authenticated by Pharmacognosy department at SDM Centre for Research in Ayurveda & Allied Sciences, Udupi. A voucher specimen (No. 199/ 12121701) had been deposited for further future reference. The Seeds of greater cardamom were removed from the pod and the seeds were powdered. The powder was sieved using sieve no. 100 and stored in a clean and dry air tight container. The powder obtained from a single batch was used throughout the study. *Amomum subulatum* powder weighing 500g was soaked in 2L of cold distilled water for 24h was filtered and concentrated by evaporation. The concentrated *Amomum subulatum* seed extract (ASSE) was used in the present study.

#### Experimental animals

Male Wistar albino rats of weight  $200 \pm 50$  g body weight were procured from animal house attached to Pharmacology laboratory at SDM Research Centre Udupi. Animals were maintained at standards laboratory conditions such as temperature at  $25 \pm 2$  °C, humidity of 55-60% and natural day and night cycle. They were fed with Amrut brand rat pellet feed supplied by Sri Durgha feeds Bangalore and tap water given *ad libitum*. The study protocol was approved from Institutional animal ethical committee (SDMCRA/IAEC-2012-13, HSN-22) and principles of laboratory animal care guidelines were followed throughout the experimentation.

#### Acute oral toxicity test

The acute oral toxicity study was carried out as per OECD guidelines 425 using AOT software. The aqueous extract of *Ammomum subulatum* seeds was made into a suspension in 0.5% Carboxy methyl cellulose and dosed in the following order 175, 550, and 2000mg/kg body weight. After the dosing the animals were observed for 14 days for mortality. The LD 50 was determined by using AOT software.

#### Chemicals

Cypermethrin [Contropest, Dodden Kunal Industrial Area, Whitefield, Bengaluru, India Batch No.-005 MFG]

### METHODOLOGY

Wistar Albino rats were randomly divided into five different groups of six rats each. Group I served as control group- treated with 0.5% Carboxy methyl cellulose (5ml/kg body weight). Group II administered with Cypermethrin (25mg/kg body weight p.o.)-served as toxic control group. Group III considered as test drug group – treated with ASSE 200mg/kg. Group IV & V considered as test groups co administered with Cypermethrin - treated with ASSE 200mg/kg & 400mg/kg of body weight along with Cypermethrin

25mg/kg. The group specific drugs were administered once daily for 28 consecutive days [14]. On the 28<sup>th</sup> day an hour after group specific drugs, blood was withdrawn from retro-orbital puncture. The serum was examined for serum biochemical parameters. The rats were sacrificed under cervical dislocation and the kidney was rapidly isolated washed with ice cold saline and weighed. The tissue was transferred to 10% formalin for histological examination. The tissue was embedded in paraffin and the section was cut into 5µm thickness. It was stained with haematoxyline – eosin stain and mounted in diphenyl xylene. The histopathological changes of kidney tissue were observed under light microscope and microphotographs were taken for histopathological examination [15].

#### Preparation of tissue homogenate

The excised kidney tissue was cleaned with ice cold saline and stored in -20°C in deep freezer. Tissue was thawed and homogenized in phosphate buffer saline of pH 7.4, centrifuged at 4°C and supernatant was stored at -20°C. Thus obtained homogenate was subjected for determining catalase, glutathione peroxidase and lipid peroxidation using standard protocol proposed by Sinha *et. al.* 1972 [16].

#### Statistical analysis

The data obtained in the present study was expressed in Mean  $\pm$  SEM. Statistical analysis was carried out by one way analysis of variance followed by Dunnett's multiple 't' test. A level for  $p < 0.05$  was considered to be statistically significant.

### RESULT

#### Biochemical parameters

Repeated administration of Cypermethrin resulted in significant increase in the serum creatinine, uric acid and urea level as compared to the normal control. The test drug (ASSE) co-administered with Cypermethrin significantly reversed elevated serum creatinine, uric acid and urea (Table 1).

#### Anti oxidant parameters

The Cypermethrin administered group has shown significant rise in the level of catalase activity in comparison to normal control group. The test drug administered at 2 different doses has shown significant decreased the elevated catalase activity in comparison to Cypermethrin control group. There is a considerable increase in the glutathione peroxidase activity in Cypermethrin administered group where as the test drug administered at two different dose levels reduced it to nearly normal value.

There is a significant increase in the lipid peroxidation in Cypermethrin control group as compared normal control. The test drug at two different dose levels has shown remarkable decrease in lipid peroxidation and found to be statistically significant as comparison to Cypermethrin control group (Table- 2).

**Table 1:** Effect of test drug (ASSE) on biochemical parameters

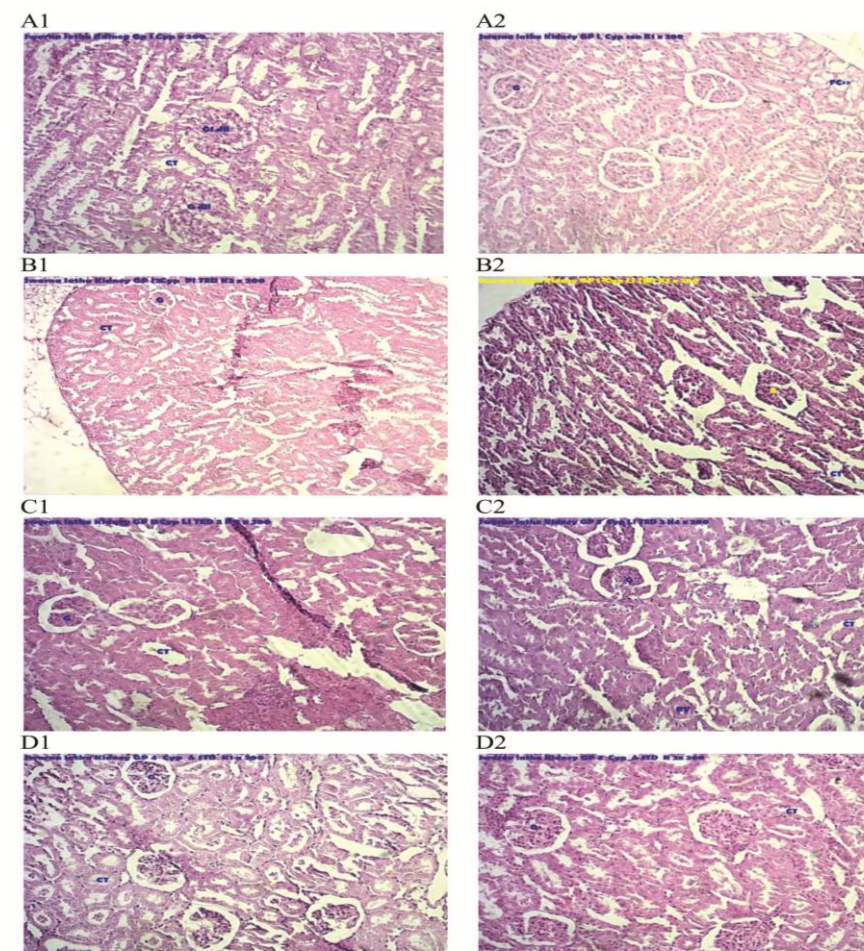
Group	Creatinine (mg/dl)	Serum Uric acid Level (mg/dl)	Serum Urea (mg/dl)	Cystatin C (mg/dl)
Control	0.7166 $\pm$ 0.04014	0.433 $\pm$ 0.0614	51.66 $\pm$ 5.8	0.0831 $\pm$ 0.01920
Cypermethrin control	1.7 $\pm$ 0.0365 <sup>@</sup>	1.1 $\pm$ 0.1095 <sup>@</sup>	81.5 $\pm$ 19.08 <sup>@</sup>	0.056 $\pm$ 0.0237
ASSE (200mg/kg) +Cypermethrin	0.465 $\pm$ 0.0310 <sup>##</sup>	1.08 $\pm$ 0.04203	42.01 $\pm$ 5.011	0.055 $\pm$ 0.0261
ASSE (200mg/kg) +Cypermethrin	1.691 $\pm$ 0.1129	0.835 $\pm$ 0.1044	29.166 $\pm$ 1.815 <sup>##</sup>	0.0751 $\pm$ 0.0182
ASSE (400mg/kg) +Cypermethrin	0.503 $\pm$ 0.03106 <sup>##</sup>	1.5166 $\pm$ 0.1138 <sup>#</sup>	25.66 $\pm$ 2.929 <sup>##</sup>	0.0991 $\pm$ 0.0227

Data expressed in Mean  $\pm$  SEM, \* $p < 0.05$ , \*\* $p < 0.01$ , @-in comparison to normal control group, # in comparison to Cypermethrin control group

**Table 2:** Effect of test drug (ASSE) on antioxidant parameters

Group	Catalase (mmole/min/mg protein)	Glutathione peroxidase (mmoles of glutathione/mg protein/min)	Lipid peroxidation (mmoles of MDA formed/g wet tissue)
Control	3.44 ± 0.48	21.20 ± 4.14	1.40 ± 0.08
Cypermethrin control	5.96 ± 1.65 <sup>@</sup>	49.52 ± 15.71	7.14 ± 1.21 <sup>@@</sup>
ASSE (200mg/kg) + Cypermethrin	5.02 ± 0.52	36.95 ± 7.5	1.33 ± 0.07 <sup>##</sup>
ASSE (200mg/kg) + Cypermethrin	2.10 ± 0.27 <sup>##</sup>	31.90 ± 10.74	2.59 ± 0.30 <sup>##</sup>
ASSE (400mg/kg) + Cypermethrin	1.43 ± 0.10 <sup>##</sup>	15.5 ± 2.54	4.35 ± 6.13 <sup>#</sup>

Data expressed in Mean ± SEM, \*p<0.05, \*\*p<0.01, @-in comparison to normal control group, # in comparison to Cypermethrin control group



**Figure- 1:** Photomicrograph representative of kidney tissue  
Cypermethrin control group (A 1& 2), Test drug only [ASSE] (B1& 2),  
ASSE 200mg/kg + Cypermethrin represented in (C1& 2). 400mg/kg +  
Cypermethrin represented in ( D1& 2).

CT: convoluted tubules, FC: fatty changes, CT: convoluted tubules, FC: fatty GC: glomerulus changes, G: glomerulus.  
Kidney exhibited mild to moderate fatty degenerative changes in cholesterol control group. In test group at 2 different doses the fatty degenerative changes were mild to moderate.

**Histopathological examination:**

**Kidney:**

Examination of kidney sections obtained from Cypermethrin administered rats showed mild fatty changes especially in the sub capsular regions, dilatation of glomerulus along with obliteration of periglomerular space and shrinkage of glomerulus was observed.

Kidney sections from test drug administered at a dose of 200mg/kg along with Cypermethrin group revealed almost normal cytoarchitecture in all the sections obtained from four rats. Kidney sections from test drug administered at a dose of 400mg/kg along with Cypermethrin group revealed almost normal cytoarchitecture in all the sections obtained from four rats and mild fatty changes were observed in sections from one rat. In only test drug administered group normal cytoarchitecture was observed in sections from all rats.



## DISCUSSION

In the present study the Cypermethrin induced nephrotoxicity was evaluated by measuring serum levels of creatinine, urea, cystatin-C and serum uric acid. Among these parameters serum creatinine, urea and uric acid levels were significantly increased in toxicant control as compared to normal control. Usually the increase in serum urea level is seen due to some renal pathology. It is also affected by hydration, hepatic metabolism of protein and reduced GFR (glomerular filtration rate). Here the elevation in the serum urea level might be the result of some muscle tissue breakdown or excess catabolism of bodily proteins. Since, serum creatinine level was also elevated significantly it may be indicative of renal function impairment. This was significantly reversed by both the dose of test drug. An increase in serum creatinine was observed in toxicant control group in comparison to the normal control group values. This is in conformity with the results of many of the previous authors. Serum creatinine elevation is mainly due to impairment in the kidney function though increased turn over in the muscle may also contribute<sup>[17, 18]</sup>.

Serum uric acid elevation was also observed. It is the product of purine catabolism. The elevation may be due to increased formation or decreased excretion. The decreased excretion may be due to impairment in the kidney function or competition for excretion between uric acid and other drugs. Ketogenic diet can impair uric acid excretion due to competition between ketones and uric acid for transport mechanism. This toxicant induced elevation was reversed in non-significant manner by the high dose of test drug indicating moderate protection.

Cystatin –C level was found to be decreased in non-significant manner in toxicant administered group in comparison to the control group. It is a polypeptide produced throughout the body, removed and broken down in the kidney. It is expected to remain in a steady level in the serum if the kidneys are working in an efficient manner and the Glomerular Filtration Rate (GFR) is normal. GFR is a marker of kidney health- there are many methods to measure it like by injecting inulin, radioisotopes etc. However, because of the complexity they are not employed in routine manner. In contrast cystatin C which is low molecular weight polypeptide is removed from the blood stream mainly by glomerular filtration in the kidneys. Decreased GFR leads to elevated level of cystatin-C. In the present study instead of elevation a moderate non-significant decrease was observed- this shows that glomerular function may not be altered by the toxicant administration. The observed decrease was reversed in non-significant manner by both the doses of the test drug.

It has been reported that the Cypermethrin is metabolized in the liver via hydrolytic ester cleavage and oxidative pathways by the cytochrome P-450 enzymes forming cyanohydrins that decompose further to cyanides and aldehydes. These substances induce free radical generation which is responsible for increasing oxidative stress in mammals. It has been shown by many that lipid peroxidation is one of the main molecular mechanism involved in the toxicity induced by Cypermethrin<sup>[19]</sup>.

Free radicals generated by various mechanisms are important for biochemical process in the body. Formation of reactive oxygen species in abnormal quantity leads to oxidative stress and it is counteracted by different anti-oxidant mechanisms. The cells can repair the damage directly or decrease the formation of free radicals by both enzymatic and non-enzymatic means. Several studies including the present one revealed that administration of Cypermethrin results in injury to kidney. It is interesting to note that earlier studies have revealed that the test drug large cardamom has good hepatoprotective activity. It has protected liver against different kinds of injurious agents like CCl<sub>4</sub> paracetamol and ethanol According to Verma et al 2012 the fruit powder of this plant has lipid lowering, fibrinolytic effect along with anti-oxidant activity<sup>[20,21]</sup>. The

protection observed in this study against Cypermethrin induced renal toxicity may be due to this anti-oxidant activity enhancing effect.

The histopathological observations have shown repeated exposure to Cypermethrin caused mild fatty changes especially in the sub-capsular region and shrinkage of glomerulus. And the dilatation of glomerulus along with obliteration of periglomerular space was observed. Kidney sections from test drug and Cypermethrin administered group revealed almost normal cytoarchitecture in all the sections. Both the above indicate protective effect of the test drug against toxicant induced degenerative changes in the above organs.

## CONCLUSION

Chronic administration of Cypermethrin resulted in considerable changes in biochemical and histopathological parameters reflecting renal injury. Most of these changes were reversed by the treatment of test drug especially at higher dose level. Thus we can conclude the *Amomum subulatum* seed extract has significant nephroprotective activity against Cypermethrin induced nephrotoxicity.

## Acknowledgement

Authors are highly grateful to revered President Dr. D. Veerendra Heggade for the constant support, and Dr. B. Yashoverma, Secretary, SDM Educational Society for the guidance.

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**HOW TO CITE THIS ARTICLE**

Puttanna GS, Nayak S, Ravi M, Ravishankar B. Nephroprotective acitivity of *Amomum subulatum* seeds against cypermethrin induced nephrotoxicity in rats. J Phytopharmacol 2016;5(4):145-149.