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Effect of *Euphorbia hirta* on nitrobenzene induced nephotoxicity with reference to renal atpases

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ABSTRACT

Euphorbia hirta is normally used to treat numerous diseases, including hypertension and edema. In this study, we evaluate the kidney damage by the induction of nitrobenzene (1000 mg/kg). The ethanolic extract (400 mg/kg) of the plant used for treatment. The level of Membrane bound enzymes, Na^+/K^+ dependent ATPase, Mg^{2+} dependent ATPase are increased in treatment group (Group III) when compared with carcinogen induced rats (Group II). The level of Ca^{2+}dependent ATPase is decreased in treatment group (Group III) when compared with carcinogen induced rats(Group II).This results shows that *E.hirta* extract prevent the kidney damage against nitrobenzene. In future, E.hirta used for therapeutic applications.

KEYWORDS

Euphorbia hirta;
Hypertension;
Kidney damage;
Nitrobenzene;
Membrane bound enzymes.

INTRODUCTION

Transmembrane (TM) proteins play important roles in signal transduction, transport of ions and small molecules, volume regulation, light harvesting, and many other physiologic processes. Important issues in membrane protein studies are how TM helices assemble and examination of the relative roles of van der Waals interactions and interhelical hydrogen bonds[1]. A Na-gradient is used in many tissues to provide the activity of different ion-exchange transport systems. The increase of intracellular Na+ concentration results in activation of Na^+/Ca^{2+} exchanger that is embedded in the plasma membrane of different cells including nephron, neurons and cardiac and smooth muscle[2].

Nitrobenzene is an important toxic compound which induces various toxicities that includes hematotoxicity, immunotoxicity, hepatotoxicity and nephrotoxicity. Nitrobenzene is primarily employed as an oxidizing agent in the synthesis of alanine and benzene compounds. It initiates the production of one ROS or may lead to the production of others through radical chain reaction. *Euphorbia hirta* Linn is one of the plants which has been widely used in several countries as an antidiarrhoeal, antidiuretic, also as a treatment of expectorant, intestinal ailments of children and various skin diseases[3]. It is a potential medication for asthma[4]. Its diuretic and purgative action has been well documented and could

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TABLE 1 : Concentration of membrane bound enzymes in kidney of control and experimental groups

Particulars	Group I	Group II	Group III	Group IV
Na^+-K^+ ATPase (μ moles of phosphorus liberated/mg protein)	4.42±1.04	2.3±0.22 [a] **	2.7±0.89 [b] **	4.0±1.45 [cNS]
Ca^+- ATPase(μ moles of phosphorus liberated/mg protein)	2.05±0.74	3.05±0.70 [a] **	2.19±0.84 [b] **	2.1±0.60 [cNS]
Mg^+- ATPase(μ moles of phosphorus liberated/mg protein)	0.13±0.02	0.05±0.05 [a] **	0.1±0.01 [b] **	0.16±0.02 [cNS]

[a]Group II compared with Group I, [b]Group III compared with Group II, [c]Group IV compared with Group I, ** Significance at p<0.01, * Significance at p<0.05, NS=Not significant

serve as an anthelmenthic agent[5].

The present study is aimed at exploring the level of membrane bound enzymes on *Euphorbia hirta* Linn against nitrobenzene induced nephrotoxicity.

MATERIALS AND METHODS

Plant collection

Fresh plants parts were collected from Pollachi, Tamil Nadu, India. The plant was authenticated by Dr. G.V.S.Moorthy, Botanical survey of India, TNAU Campus, Coimbatore. The voucher No. BSI/SC/5/23/8-9/Tech/766.Fresh plant material was washed under running tap water, air dried, and then homogenized to fine powder and stored in airtight bottles.

Ethanolic extraction

100g of dried plant powder was extracted in 500ml of ethanol for 24 hr in occasional shaker at room temperature. The supernatant was collected and evaporated to make the final volume one-fifth of the original volume. It was stored at 4°C in airtight bottles for further studies.

Animals used

The wistar strain of female albino rats weighing between 140-160g were obtained from Animal house of Karpagam Arts and Science College, Coimbatore. The animals were housed in large spacious cages and they were given food and water *ad libitum* during the course of the experiment. The animal room was well ventilated and the animals had a 10±1 hour night schedule, throughout the experimental period. The study was approved by Institutional Animal Ethical Committee (IEAC) constituted for the purpose of CPCSEA, Government of India. The animals were divided into four groups.

(1) Group I : Control rats.
(2) Group II : Nitrobenzene induced animals (1000mg/kg body weight).
(3) Group III : Nitrobenzene induced animals were treated with Ethanolic extract of *E. hirta* (400mg/kg body weight for 7days).
(4) Group IV : Ethanolic extract of *E. hirta* alone (400mg/kg body weight).

Induction of carcinogenesis

Nitrobenzene (E.Merk (India) Limited, Mumbai) was administered orally at a single dose of 1000mg/kg body wt, this dosage is known to cause renal toxicity in rats[6].

After the experimental period, the animals were sacrificed under light chloroform anesthesia the kidneys were removed and washed with ice-cold saline. A portion of kidney was homogenized in 0.1M Tris-HCl buffer pH 7.4 and used to assay the membrane bound enzymes.

Determination Membrane bound enzyme activity

Na^+/K^+ dependent ATPase was determined with the concentration of sodium by the method[7]. Ca^{2+} dependent ATPase was determined with the concentration of calcium by the method[8]. Mg^{2+} dependent ATPase was determined with the concentration of magnesium by the method[9].

Statistical analysis

The results obtained were expressed as Mean ±SD. The Statistical comparison among the groups were performed with students 't' test using a statistical package program (SPSS 10.0) at $p<0.05$ and $p<0.01$ significant level.

RESULTS AND DISCUSSION

Effect of *E.hirta* extract on Membrane bound enzymes

TABLE 1 show the Na^+-K^+ ATPase, Ca^+- ATPase and Mg^{2+}- ATPase in Kidney of Control and Experi-

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mental groups. The activity of Na^+-K^+ ATPase, Mg^{2+}-ATPase, was found to be significantly decrease in Group II rats followed by administration of alcoholic extract of *E.hira* shown reversible changes in Group III animals. The level of Ca^{2+} ATPase was found to be significantly increase in Group II rats followed by administration of alcoholic extract of *E.hira* shown reversible changes in Group III animals.

The levels of of Na^+-K^+ ATPase and Mg^{2+}- ATPase were also decreased due to renal tumor, which could also be related to the loss of membrane architecture. Ion transport across the membranes regulates a number of biochemical reactions in the cell[10].

Na^+/K^+ ATPase is an integral part of membrane and is responsible for the control of sodium and potassium transport and maintain the polarized phenotype of epithelial cells[11]. Inhibition of Ca^{2+} ATPase and Na^+/K^+ ATPases in this study reflects the blockage of ion transport, since ATPase mediate these events in cell organelles.

Mg^{2+} ATPase plays a role in endergonic processes other than ion transport. The Mg^{2+} ion functions to form Mg^{2+} ATP complex which is the substrate for the enzyme. Mg^{2+} ATPase utilizes the pool of ATP that is not directly related to change in free energy of sodium transport. Mg^{2+} activated ATPase is distributed in all renal cell compartments[12].

CONCLUSION

In this conclusion, the overall results of this study clearly shows that the ethanolic extract of *E.hirta* possess preventive role of nephroprotective against nitrobenzene. In future study, *E.hirta* could constitute a lead to discovering a novel drug which will be useful in the treatment of any type of cancer.

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