# Potential Effect of *Euphorbia Hirta* (Linn) Against Nitrobenzene Induced Nephrotoxicity

**S.Suganya[1], P.Ragavendran[2], B.Rajalakshmy Menon[2], M.A.Rathi[1], L.Thirumoorthi[1], P.Meenakshi[1], D.Guru Kumar[1] and V.K.Gopalakrishnan[1,2]**

[1]*Department of Biochemistry, Karpagam Arts and science College, Coimbatore- 641 021 (T.N.), INDIA,*

[1,2]*Department of Biochemistry, Karpagam University, Coimbatore- 641 021 (T.N.), INDIA,*

**Summary**

*Euphorbia hirta* is normally used to treat numerous diseases, including hypertension and edema. In this study, we evaluate the nephrotoxicity properties of this plant. The ethanolic extract (400 mg/kg) of the plant used for treatment. The levels of Non-protein nitrogenous compounds (Urea, Creatinine) were decreased and the level of uric acid was increased when compared with carcinogen induced rats. The level of tumor marker enzymes (ALP, ACP, LDH, γ-GT, Xanthine oxidase) and total protein were decreased when compared with carcinogen induced rats. The result from this study showed that some active fractions of *Euphorbia hirta* protect the deleterious effects on nitrobenzene induced rats. Therefore caution should be exercised in the use of *Euphorbia hirta* as medicinal plant.

**Key words**: *Euphorbia hirta,* Hypertension, Edema, Nitrobenzene, Nephrotoxicity.

**Address for Correspondence**

**Dr. V.K.Gopalakrishnan**

Professor in Biochemistry

Karpagam University

Coimbatore – 641 021

India

Ph : 091-0422-2611146
Fax: 091-0422-2611043
E-mail: gopalakrishnan_vk@yahoo.com

## Introduction

Over the past decade, herbal medicine has become a topic of global importance, making an impact on both world health and international trade. Continuous usage of herbal medicine by a large proportion of the population in the developing countries is largely due to the high cost of western pharmaceuticals in health care. Among the human diseases, mostly treated with medicinal plants is cancer which is probably the most important genetic disease.

Kidney cancer incidence and death rates are highest among American Indians/Alaskan Natives, although obesity is the only factor known to contribute to this disparity. kidney cancer is expected account for 26% of all cancer deaths in 2009 [1]. Although great advancements have been made in the treatment and control of cancer progression, a number of undesired side effects, sometimes occur. Natural therapies such as the use of plant derived products may reduce adverse side effects [2] and the compounds in plants have protective effects against environmental mutagens, carcinogens and endogenous mutagens [3] [4]. Dietary intake of such chemopreventive compounds has been suggested as an effective strategy for minimizing the deleterious effects of genotoxins or cytotoxins [5].

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 [6]. It is a potential medication for asthma [7]. Its diuretic and purgative action has been well documented and could serve as an anthelmenthic agent [8].

The present study is aimed at exploring the biochemical studies 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 $4^{o}C$ 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

Group I : Control rats.

Group II : Nitrobenzene induced animals (1000mg/kg body weight).

Group III : Nitrobenzene induced animals were treated with Ethanolic extract of *E. hirta* (400mg/kg body weight for 7days).

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 [9].

After the experimental period, the animals were sacrificed under light chloroform anesthesia. Blood was drawn from the Para-orbital venous complexes, blood, serum and kidney was separated. All the biochemical estimations were completed within 24hrs of animal sacrifice.

***Determination of Non – Protein nitrogenous compounds***

Non – Protein nitrogenous compounds such as blood urea nitrogen (BUN) was estimated by the method [10], Serum urea estimated by the method [11], uric acid estimated by the method [12]. and creatinine was estimated by the method [13].

***Determination of tumor marker enzymes***

Tumor marker enzymes are assayed by following method, Creatinine phosphokinase was determined by the method [14]. γ-Glutamyl Transpeptidase was determined by the method [15] and [16] . Xanthine oxidase was determined by the method [17].Acid and alkaline phosphatase was determined by the method [18].Lactate dehydrogenase was assayed by the method [19].

***Determination of protein***

Protein was estimated by the method [20].

***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 Non – Protein nitrogenous compounds**

Figure 1a shows that the level of BUN and Urea was significantly increased in Group II animals when compared to Group I animals. Figure 1b shows that the level of Uric acid and Creatinine was significantly decreased in group II animals when compared with Group I animals.

**Fig 1a : NON PROTEIN NITROGENOUS COMPOUNDS**

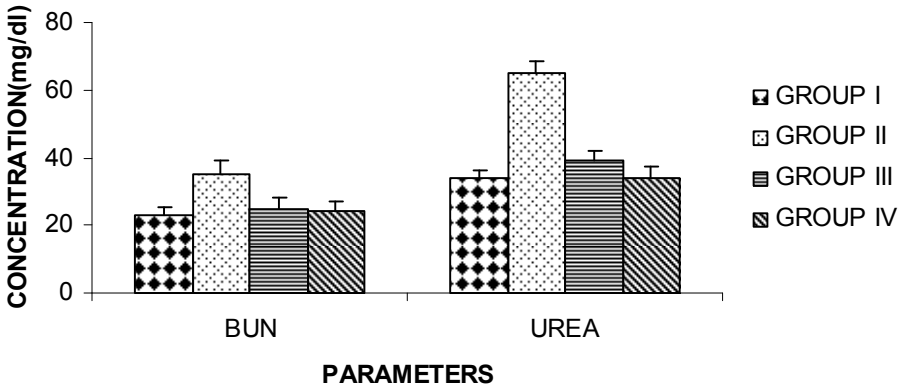
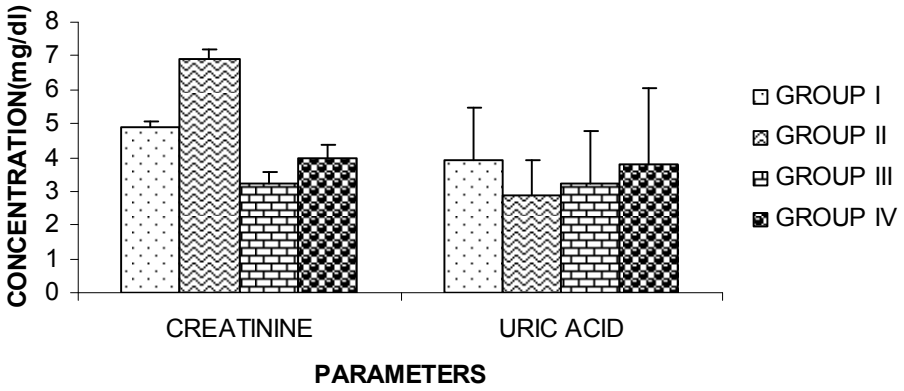



**Fig 1b : NON PROTEIN NITROGENOUS COMPOUNDS**



Increased level of Blood urea nitrogen, serum urea, uric acid and creatinine are considered as the indicators of kidney damage [21]. Non-physiological concentrations of urea have been reported to increase the levels of ROS [22].Increase in blood urea nitrogen, serum urea and creatinine indicates a marked renal injury [23].Uric acid significantly prevented the increase in plasma levels of creatinine and blood urea nitrogen (BUN) and helped to maintain a systemic nitrate/nitrite concentration and total antioxidant capacity. Uric acid attenuated the increase of renal lipid peroxidation [24].The present study demonstrates that blood urea nitrogen, urea, and creatinine were found to be significantly increased and uric acid significantly decreased in rats treated with nitrobenzene whereas treatment with the ethanolic extract of *E.hirta*(L).

***Effect of E.hirta extract on Tumor markers***

Kidney injury has been diagnosis by Tumor marker enzymes, which the level response shows the Table 1.

**Table 1: Concentration of Tumor marker enzymes in kidney of Control and Experimental Groups**

| Particulars | GROUP I | GROUP II | GROUP III | GROUP IV |
|---|---|---|---|---|
| Creatine Phosphokinase (units/mg protein) | 1.51 ±0.76 | 2.83±0.02 [a] ** | 2.06±0.04 [b] ** | 1.70 ±0.50 [cNS] |
| γ –Glutamyl transpeptidase(units/mg protein) | 2.56±1.23 | 4.01±0.71 [a] ** | 3.44±0.31 [b] ** | 2.18±0.14 [cNS] |
| Xanthine Oxidase (units/mg protein) | 1.70±0.25 | 2.70±0.26 [a] ** | 2.10±0.13 [b] ** | 1.91±0.15 [cNS] |
| Alkaline Phosphatase (µ moles of phenol liberated/mg protein) | 5.1±0.17 | 8.2±0.12 [a] ** | 7.1 ±0.15[b] ** | 5.2±0.2 [cNS] |
| Acid Phosphatase(µ moles of phenol liberated/mg protein) | 4.3±0.25 | 7.2 ±0.16[a] ** | 5.1±0.27 [b] ** | 4.0 ±0.23[cNS] |
| Lactate dehydrogenase (µ moles of pyruvate liberated/mg protein) | 1.0±0.13 | 2.2±0.15 [a] ** | 1.8±0.20 [b] ** | 1.4 ±0.15[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

The tumor marker enzymes such as ALP, ACP, LDH, γ-GT and Xanthine Oxidase level were increased in Nitrobenzene induced Group II rats. These marker enzyme levels are significantly decreased Group III when compared with Group II. No significant change shown to be in Group IV rats when compared with Group I.

Creatine Phosphokinase activity was significantly increased kidney of nephro toxicity rats. As there is an glycolytic cycle, which is an energy providing route to the system, to provide energy for the enhanced synthesis of nucleic acids and proteins, it is reasonable to except increased activity of creatine Phosphokinase in cancer [16].Increased activity of Creatine kinase has been reported in breast, colon, lung and stomach cancer [25].

γ –Glutamyl transpeptidase is an enzyme considerable importance in the renal tubule, It is concentrated in proximal tubule brush border and contains a sulphydryl group essential for enzymic activity. γ –Glutamyl transpeptidase activity has been markedly elevated during the course of tumorigenesis induced by some hepato and renal carcinogens in animals [26].

Xanthine Oxidase is an enzyme of aerobic purine degradation which catalysis the oxidation of hypoxanthine to xanthine, xanthine to uric acid. Fe-NTA (ferric nitrilotriacetate) induced oxidative stress and enhances Xanthine Oxidase [27]. Hepatocytes are the prominent cell type in liver, the major increase of alkaline phosphatase in liver tissue was due to an elevated content of the enzyme in these cells seen in both cirrhosis and in bile duct obstruction [28].

Acid Phosphatase usually consider as the classical lysosomal enzyme were found to increase in hepatotoxic animals [29] .Lactate dehydrogenase activity was significantly elevated in variety of disorders like cardiac, hepatic, skeletal muscle and renal diseases. The possible elevated level of Lactate dehydrogenase was seen in carcinoma rats may be due to enhanced glycolysis during the growth of tumor mass [30].

***Effect of E.hirta extract against tissue damage***

Tissue damage analyzed by the protein level of kidney shown in table 2. The level of protein was found to be significantly decreased in Group II rats and increased in Group III animals by the administration of alcoholic extract of *E.hira.*

**Table 2: The Concentration of Protein in Kidney of Control and Experimental Groups**

| Particulars | GROUP I | GROUP II | GROUP III | GROUP IV |
|---|---|---|---|---|
| Protein | 51.1±2.5 | 35.0±1.6 [a] ** | 45.5±1.93 [b] ** | 50.8±2.8 [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

The level of protein decreased in Group II by the production of free radicals from carcinogens affects the kidney. This result was similar to that of asphyxia induced brain damage[31]. During the treatment antioxidants produce from plant extract, scavenge the free radicals and protect the organ and protein level was increased when compared to Group II rats [32].

## Conclusion

Hence the present work was executed to evaluate the Nephro Protectivity Potential of *E.hirta* in Nitrobenzene induced carcinogenic rats. Thus plants have been a prime source of highly effective conventional drugs for the treatment of many forms of cancer. So the active compound should be isolated from this plant, which may lead to the development of potential novel drugs.

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