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NEPHROPROTECTIVE ACTIVITY OF ETHANOLIC EXTRACT OF STEM BARKS OF *CRATAEVA NURVULA* BUCH HUM

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ABSTRACT

Aim of the study: Now a days due to the hammering of movies and fashion most of the people specifically age group between 18 to 30 years, are diverted more to have the protein supplements in excess for getting good physique and well toned body shape. The people suppose to eat more calcium, vitamins and some steroids with intension that they will get done the things too fast. Without been having good trainee and medical supervisor most of the people doing exercise in the fitness club and gym and taking the diet which is one of the major cause of nephrotoxicity.

Materials and Methods: Ethanolic extract of stem barks of *Crataeva nurvula* Buch hum in the doses of 200 mg, 400 mg and 600 mg is useful as a laxative, demulcent, stomachic, and is reported to cure disorders of urinary organs. It is also very useful as anti-inflammatory drug and act as a good contraceptive for women and also used in arthritis.

Results: In the current study, effects of pretreatments with the stem barks of ethanolic extract of *Crataeva nurvula* Buch hum were investigated in Cisplatin induced nephrotoxic rats for 24 hours using renal function parameters such as serum urea (UR), uric acid (UA) and creatinine (CR). Effects of the extract pretreatments on the hematological profile in Cisplatin nephrotoxic rats were evaluated. The extract also significantly ($p<0.05$, $p<0.01$, $p<0.001$) improved packed cell volume (PCV), hemoglobin (Hb), and total leukocyte count (TLC) levels but non-significant ($p>0.05$) increase in the mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC).

Conclusions: The overall result suggests that the stem bark extract of *Crataeva nurvula* Buch hum possesses nephroprotective potential and improves hematological derangements associated with Cisplatin nephrotoxicity. Although, the active principles were not isolated and their possible mechanisms of actions were not investigated in the present study, these could constitute areas of future studies.

INTRODUCTION: Stem barks of *Crataeva nurvula* Buch hum are used in the treatment of various human diseases including drug related diseases such as fertility, diarrhoea, poisoning etc., [1, 2]. It is also useful as a laxative, demulcent, stomachic, and in blood diseases and is reported to cure disorders of urinary

organs [3]. It is also very useful as anti-inflammatory drug and acts as a good contraceptive for women. Triterpenoids, Saponins and related compounds were isolated from bark of this plant [4].

Cisplatin is a potent antitumor agent, but its clinical use is limited by its renal toxicity. Nephrotoxicity of the anticancer drug, Cisplatin involves enhanced renal generation of reactive oxygen metabolites and lipid peroxidation caused by decreased levels of antioxidants and antioxidant enzymes. Some Phytochemical studies have reported that certain Indian medicinal plants show beneficial effects on kidney and renal injury [5, 6] . Research into the etio-pathological basis of Cisplatin nephrotoxicity has recently been encouraged [7].

However, despite recognition of Cisplatin nephrotoxicity and concerted scientific efforts directed into developing therapeutic or prophylactic agents to protect against Cisplatin induced nephrotoxicity, conventional chemotherapeutic options available to either treat or prevent its development, are still limited. In the absence of reliable and effective modern nephroprotective drugs and available traditional medicines employed for the disease treatment, concerted efforts are currently channeled toward exploring complementary or alternative chemotherapy in the disease treatment and/or prevention. Its acute or chronic high doses are reported to produce hepatotoxicity, but impairment of renal function by Cisplatin as the main untoward effect is becoming increasingly reported [8].

Cisplatin nephropathy is characterized by alterations in urine volume, in glutathione status, creatinine clearance and increase products of lipid peroxidation [9]. Cisplatin nephropathy is closely associated with a significant decrease in the renal tissue concentration of glutathione and nitric oxide overproduction [10].

MATERIALS AND METHODS:

Experimental Animals: 90-120 days old, male Wistar rats were used for experiments. Nephroprotective activity was tested on adult male Wistar rats weighing between 180-200 gm which was obtained from M/S Venkateshwara enterprises (P) Ltd Bangalore. They were housed in polypropylene cages and fed with standard chow diet and water *ad libitum*. The animals were exposed to alternate cycle of 12 hrs of darkness and light each. Before each test, the animals were fasted for at least 12 hrs. The experimental protocols were subjected to the scrutinization of the Institutional Animal Ethics Committee and were cleared by the same (IAEC No: P. Col - 15/2006).

Plant Material: About 800 gm of stem barks of *C. nurvula* were collected from Shevaroyan Hills of Tamilnadu, India, in the month of November 2009 and the plant material collection, identification, authentication and specimen referencing was done under the guideline of a Botanist, ABC Botanical Garden Salem, TN, India. A voucher specimen (CCB-94) has been kept in our college museum for future reference.

Preparation of the extract and preliminary phytochemical screening: About 250 gm of the dried stem barks were ground to fine powder using Laboratory Hammer mill. The powdered material of *C. nurvula* was extracted separately by continuous hot extraction process using Soxhlet apparatus with ethanol selected as per polarity of the solvent [11]. After extraction, the extract was concentrated under reduced pressure. The powder was stored in air and moisture-tight container which was stored in a dessicator prevented from direct heat and sunlight. The dried ethanolic extract was then subjected to various chemical tests to detect the presence of different phytochemical constituents like flavonoids, alkaloids and traces of carbohydrates.

Acute Oral Toxicity Study: The animals were divided into six groups, and were treated orally with ethanol extracts of *C. nurvula* at 200, 400 and 700 mg/kg, body weight doses, separately. The animals were continuously observed for 1 hr, 4 hr and intermittently for the next 6 hrs and then again at 24 and 48 hr following drug administration. The parameters observed were grooming, hyperactivity, sedation, loss of righting reflex, respiratory rate and convulsion [12].

Experimental methods:

- **Cisplatin-induced Nephrotoxicity:** Rats were divided into 5 groups of 6 rats each such that the weight difference within and between groups does not exceed ± 20% of the average weight of the total rats. Group I rats that served as the untreated

control were administered single daily dose of 10 ml of distilled water orally and intraperitoneally, while group II rats that served as the treated or model control were administered single daily dose of 10 ml distilled water and 5 mg/kg of Cisplatin via IP route. Groups III, IV and V were pretreated with single oral dose of 200, 300 and 600 mg/kg of *Crataeva nurvula* Buch hum extract 1 hour before the IP administration of 5 mg/kg of Cisplatin.

- **Measurement of serum urea, uric acid and creatinine in nephrotoxic rats:** On termination of dose Cisplatin induced nephrotoxicity experiments 24 hrs post induction of the experiments the rats were fasted overnight. The rats were sequentially anesthetized with chloroform for about 35-45 seconds. The rats were restricted on the dissecting board and about 4 ml of whole blood for blood lipid assay were collected directly from the heart chambers by cardiac puncture with a 21 Gauge needle mounted on 5 ml syringe.

  Each blood sample obtained for each rat was collected into a well labeled 10 ml capacity plain sample bottle. The blood samples were allowed for complete clotting for about 4-5 hours before they were centrifuged. This was aimed at separating the sera from clotted blood cells. The sera were carefully separated into new, well labeled, corresponding plain sample bottles at room temperature 23 - 26 ºC. The sera were assayed for serum urea, uric acid and creatinine.

- **Measurement of hematological parameters in nephrotoxic rats:** Prior to termination of the experiments 24 hours post-induction, the rats were fasted overnight but distilled water was made available *ad libitum*. Blood samples were collected by cardiac puncture under chloroform anesthesia, using 21 gauge needles mounted on a 5 ml syringe into Ethylene Diamine Tetra-acetic Acid coated sample bottles for full blood count FBC, which included PCV, Hb, MCV, MCH, MCHC, and PLC .

- **Data Analysis:** Data were expressed as mean ± standard error of the mean (SEM) and analyzed using two ways analysis of variance on statistical software package, the data was also analyzed by one way ANOVA followed by Dunnett Multiple Comparison Test.

RESULTS:

Preliminary Phytochemical Screening: The extract showed high concentrations of glycosides, flavonoids, alkaloids, saponins, proteins, triterpenoids and tannins.

Cisplatin-induced Nephrotoxicity: Cisplatin dose has been associated with significant lipid peroxidation. As a consequence of lipid peroxidation, intracellular accumulation and covalent bonding of its highly reactive metabolite, N-acetyl-*para*-benzoquinone-imine, hepatocyte malfunction and death often result. Similar effect is often recorded for renal tissues. In addition, Cisplatin has been reported to promote hepatocyte and renal apoptosis [13, 14]. Cisplatin overdose is often associated with a wide range of metabolic disorders including serum electrolytes, urea and creatinine derangements. As such, elevations in the serum concentrations of these parameters, particularly, serum urea and creatinine are considered reliable, well documented parameters for investigating drug-induced nephrotoxicity in animals.

Blood urea nitrogen is derived in the liver from proteins/amino acids from diet or tissue sources and is normally excreted in the urine. In renal disease, the serum urea accumulates because the rate of serum urea production exceeds the rate of clearance [15]. Creatinine, on the other hand, is mostly derived from endogenous sources by tissue creatine breakdown. The plasma creatinine concentrations in normal individuals are usually affected by a number of factors such as the muscle mass, high protein diet, and catabolic state. Thus, serum urea concentration is often considered a more reliable renal function predictor than serum creatinine.

In the present study, results obtained showed that acute and repeated dose Cisplatin nephrotoxicities were reliably established with 5 mg/kg/day of intraperitoneal Cisplatin, as evidenced by significant ($p<0.5$, $p<0.01$, $p<0.001$) elevations in the serum urea, uric acid and creatinine in Cisplatin treated control (group II) rats when compared to untreated control (group I) rats (**Tables 1 and 2**). However, oral pretreatment with graded oral doses of the stem bark

extract of *Crataeva nurvula* Buch hum significantly attenuated the elevated serum concentrations of these parameters, in dose related pattern.

TABLE1. EFFECT OF GRADED ORAL DOSES OF ETHANOLIC EXTRACT OF *CRATAEVA NURVULA* BUCH HUM ON SERUM UREA (UR), URIC ACID (UA) AND CREATININE (CR) IN CISPLATIN INDUCED NEPHROTOXIC RATS

Treatment Group	UR (mmol/L)	UA (mol/L)	CR (mol/L)
Group I	5.37 ± 0.27	112.60 ± 09.88	67.21 ± 04.99
Group II	10.65 ± 01.91***	109.09 ± 26.78	99.72 ± 13.79***
Group IIII	4.98 ± 0.12**	143.01 ± 29.39	61.40 ± 04.98*
Group IV	5.23 ± 01.10**	103.01 ± 17.44	54.20 ± 03.77*
Group V	3.28 ± 0.34*	34.44 ± 11.98*	45.75 ± 05.69*

Values are expressed as Mean ± SEM of 6 rats in each group. ***P < 0.001, **P < 0.01, *P < 0.5, compared with control group. The data was also analyzed by one way ANOVA followed by Dennett Multiple Comparison Test

- Group I- 10 ml/kg of distilled water via the intraperitoneal and oral routes, respectively.
- Group II-10 ml/kg of distilled water *PO* + 5 mg/kg via the intraperitoneal route of Cisplatin.
- Group III-200 mg/kg *PO* of *Crataeva nurvula* Buch hum+ 5 mg/kg of intraperitoneal Cisplatin.
- Group IV-300 mg/kg *PO* of *Crataeva nurvula* +5 mg/kg kg of intraperitoneal Cisplatin.
- Group V-600 mg/kg *PO* of *Crataeva nurvula* Buch hum+ 5 mg/kg of intraperitoneal Cisplatin.

TABLE 2: EFFECT OF GRADED ORAL DOSES OF ETHANOLIC EXTRACT OF *CRATAEVA NURVULA* BUCH HUM ON VARIOUS BLOOD PARAMETERS IN CISPLATIN INDUCED NEPHROTOXIC RATS

Parameters	Group I	Group II	Group III	Group IV	Group V
PCV (%)	34.56 ± 0.65	33.78 ± 1.69	35.06 ± 1.68*	36.26 ± 1.46**	36.55 ± 0.52**
Hb (g/dl)	10.41 ± 0.20	10.08 ± 0.40	10.62 ± 0.38	11.03 ± 0.35*	11.28 ± 0.19*
MCV (fL)	57.80 ± 0.75	57.51 ± 0.19	57.52 ± 1.01	56.220 ± 0.31	56.16 ± 0.79
MCH (pg)	19.21 ± 1.49	17.65 ± 0.12	17.85 ± 0.39	17.43 ± 0.19	17.62 ± 0.23
MCHC (g/dL)	33.45 ± 2.11	30.73 ± 0.21	31.39 ± 0.37	31.16 ± 0.21	31.49 ± 0.30*
PLC	591.15 ± 17.72	514.23 ± 79.32	571.58 ± 69.56*	661.52 ± 85.59**	758.97 ± 51.58***

Values are expressed as Mean ± SEM of 6 rats in each group.***P < 0.001, **P < 0.01, *P < 0.5, compared with control group.The data was also analyzed by one way ANOVA followed by Dunnett Multiple Comparison Test

- Group I - 10 ml/kg of distilled water via the intraperitoneal and oral routes, respectively.
- Group II - 10 ml/kg of distilled water *PO* + 5 mg/kg via the intraperitoneal route of Cisplatin.
- Group III - 200 mg/kg *PO* of *Crataeva nurvula* Buch hum + 5 mg/kg of intraperitoneal Cisplatin.
- Group IV- 300 mg/kg *PO* of *Crataeva nurvula* Buch hum + 5 mg/kg of intraperitoneal Cisplatin.
- Group V - 600 mg/kg *PO* of *Crataeva nurvula* Buch hum + 5 mg/kg of intraperitoneal Cisplatin.

DISCUSSION: The protection offered by the extract could have been due to the presence of any of the active principles contained in the extract. Literature has shown *Crataeva nurvula* Buch hum to contain high concentrations of glycosides, flavonoids, alkaloids, saponins and tannins [16]. In addition, alkaloids have also been reported to strongly inhibit lipid peroxidation induced in isolated tissues via its antioxidant activity. Any of these or their combination could be responsible for the observed effect. In view of the above, one of the possible mechanisms of action of the extract could be via its antioxidant and/or free radical scavenging activities. However, this hypothesis requires validation. Oxidative stress occurs in cells when there is disruption of cellular redox balance [17]. Antioxidant activities by enhancing the antioxidant defense enzymes mediated

by superoxide dismutase, an important anti-lipoperoxidation enzyme in the body.

The vital function that blood cells perform, together with the susceptibility of this highly proliferative tissue to intoxication by xenobiotics, makes the hematopoeitic system unique as a target organ. Accordingly, it ranks with liver and kidney as one of the most important considerations in the risk assessment of potential environmental toxicants or xenobiotics. Certain drugs including alkylating cytotoxic agents could also affect blood formation rate and the normal range of hematological parameters. In the present acute study, treatment of rats with high dose of intraperitoneal Cisplatin did not cause significant ($p>0.5$) alterations in most of the measured hematological parameters.

Cisplatin is associated with overproduction of a highly reactive intermediate, N-acetyl-*p*-benzoquinone imine, which covalently bound to macromolecules of renal tissues [18] (Prescott, 1989) resulting in Cisplatin associated nephropathy. However, oral treatment with graded doses of *Crataeva nurvula* Buch hum reversed the significant decrease in the Hb, PCV value recorded for Cisplatin hematotoxicity and also caused a significant ($p<0.5$, $p<0.01$, $p<0.001$) dose related increase in the Hb and PLC, and PCV, respectively. Although, the extract caused non-significant ($p>0.5$) increase in lymphocyte differential, MCV, MCH and MCHC, but had no effect on other measured parameters.

Results of this study showed that the extract could contain active biological principle(s) reversing the hematotoxic effect of Cisplatin, with subsequent enhancement of hematopoiesis. The biological principle(s) could also be mediating hematopoietin-like effect or enhancing the release of hematopoietin from hematopoetic organs such as the kidneys or liver. Although, the exact hematopoietic mechanism of the extract was not investigated in the present study, this area could constitute an area for future study. Thus, the nephroprotection of *Crataeva nurvula* Buch hum extract, overall results of this study suggests that the *Crataeva nurvula* Buch hum root extract could be improving the hematological status in rats exposed to dose of Cisplatin.

CONCLUSION: The overall result suggests that the stem bark extract of *Crataeva nurvula* Buch hum possesses nephroprotective potential and improves hematological derangements associated with Cisplatin nephrotoxicity. Although, the active principles were not isolated and their possible mechanisms of actions were not investigated in the present study, these could constitute areas of future studies.

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