

Effect of the ethanolic extract of *Indigofera barberi* (L) in acute Acetaminophen - Induced Nephrotoxic Rats

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Abstract

The entire plants including the flowers of *Indigofera barberi* has been well-known in treating jaundice and renal diseases. The present study designed to evaluate the nephroprotective effect of ethanol extract of whole plant of *I. barberi* (Linn) in paracetamol induced nephrotoxicity of albino rats. The ethanol extract of *I. barberi* (250 mg and 500 mg/kg body weight) was administered orally once for 14 days. Nephrotoxicity was induced in rat by administering single dose of paracetamol (750 mg/kg). The degree of nephroprotective activity was measured by renal functional parameters such as serum urea (UR), uric acid (UA) and creatinine (CR), and hematological profile was concluded that the ethanol extract of *I-barberi* is an effective nephroprotective agent.

Keywords: *Indigofera barberi*, nephroprotective, ethanolic extract, paracetamol.

Introduction

Extensive use of plants belonging to the *Bryophyllum* species in complementary and alternative therapy has been widely reported. These plants are also used in the treatment of certain diseases like urolithiasis, hypertension and diabetes involving altered kidney function. (Sastri *et al.*, 2001 and Ojewole *et al.*, 2005). However, systematic and scientific reports on the investigation of *I-barberi* for its effects on renal function are scarce. In the present study, an effort has been made to evaluate the effects of the ethanolic extract of this plant on acetaminophen-induced nephrotoxicity in rats.

Acetaminophen is one of the most effective, over-the-counter chemotherapeutic analgesic-antipyretic agents belonging to the Para-aminophenol class of the non-steroidal anti-inflammatory drugs (NSAIDs) (Jackson-Robert II and Morrow, 2001). Its acute or chronic high doses are reported to produce hepatotoxicity, but impairment of renal function by acetaminophen as the main untoward effect is becoming increasingly reported (Perneger *et al.*, 1994; McLaughlin

et al., 1998; Fore *et al.*, 2001). Acetaminophen nephropathy is characterized by alterations in urine volume, in glutathione status, creatinine clearance and increase products of lipid peroxidation.

Acetaminophen induced nephrotoxicity is a model of acute renal failure. Therefore, the current study was designed to investigate the protective effects of 250 - 500 mg/kg/day/oral route of the ethanolic extract of *I-barberi* in acute dose (750 mg/kg/oral route) acetaminophen nephrotoxic adult male Wistar rats for 24 hours and 7 days, respectively. Doses of acetaminophen and *I-barberi* ethanolic extract used for the acute dose models were determined from results of preliminary studies earlier conducted.

Materials and methods

The plant *I-barberi* was collected from vandavasi forest area, Tiruvannamalai district, Tamilnadu, India in the month of February. The plant material was authenticated by Dr. Chelladurai, Shanthimalai Research Foundation, Tiruvannamalai, Tamil nadu, India.

Plant Extraction The whole plants was dried under shade and powdered with a mechanical grinder to obtain a coarse powder which was then subjected to successive extraction in a soxhlet apparatus using ethanol (60-80°C) solvent elimination under reduced pressure afford the ethanolic extract (18.7% w/w yield) the extract was stored in refrigerator. The resulting aqueous and ethanol extracts were then used for nephroprotective studies.

Experimental animals - Male albino rats (150 - 200g) were obtained from animal house of PG & Research Dept of Zoology C. Abdul hakeem college, melvisharam, Vellore District, Tamil Nadu, India. The animals were grouped and housed in polyacrylic cages (38x23x10 cm) with not more than 6 animals per cage and maintained under the standard laboratory conditions (temperature 25± 2°C) with dark and light cycles (12/12h). The animals were with fed balanced rodent pellet diet from poultry research station, Nandanam, Chennai, India and tap water ad libitum was provided through out the experimental periods. All the animals were acclimatized to laboratory conditions for a week before commencement of experiment. All procedure described were reviewed and approved by the university animal ethics committee.

Drugs and chemicals - the biochemical kits were procured from span diagnostics, surat, India.

Paracetamol induced nephrotoxicity in rats (Acute model) - Animals were randomized and divided into four groups (I-IV) of six animals in each groups. Group I served as untreated control and fed orally with normal saline 5ml/kg per body weight daily for seven days. Group II rats were similarly treated as group I. group III were treated with

250mg/kg body weight of the ethanol extract for seven days and group IV was treated with 500mg/kg body weight of the same ethanol extract orally daily for seven days respectively.

On the seventh day, paracetamol suspension was given by oral route, in a dose of 750 mg/kg body weight to all rats except the rats in group I. The biochemical parameters were estimated 24 hours following the last dose (Adeneye *et al.*, 2008).

Haematological and biochemical study blood samples were collected by cardiac puncture under diethyl ether anesthesia, using 21 gauge (21 G) needles mounted on a 5ml syringe (Hindustan syringes and medical devices ltd, Faridabad, India.) into ethylene diamine tetra acetic acid (EDTA) coated sample bottles for full blood count (FBC), which included DLA, Hb, PCV, MCV, MCH, MCHC, PLC and TLC. The collected blood samples were analysed using automatic haematological system (Sysmex Hematology Coagulation system, Model KX-21N, Sysmex Incorporation, Kobe, Japan).

Results

In the present study, results obtained show that acute dose of acetaminophen nephrotoxicity were reliably established with 750mg/kg/day single oral dose of acetaminophen as evidence by significant ($p < 0.05, p < 0.01, p < 0.001$) elevation in the serum urea, uric acid and creatinine in acetaminophen treated control (group II) rats when compared to untreated controls (group I) rats (Table 1). However, oral pretreatment dose of extract of *Indigofera barberi* significantly attenuated the elevated serum concentration of these parameters, in dose related pattern. The protection afforded by the extract has been due to the presence of any active principles contained in the extract. Taking into account that the flavonoids particularly quercetin, in other nephroprotective medicinal plants have been reported of inhibiting xenobiotic induced nephrotoxicity in experimental animal models (Okoli *et al.*, 2002) due to the potent antioxidant or free radical scavenging efforts (Annie S *et al.*, 2005), in addition, alkaloids have also been reported to strongly inhibit lipid peroxidation induced in isolated tissue

via its antioxidant activity (Lanher MC *et al.*, 1991, Kumaran *et al.*, 2007). Any of these are their combination be responsible for the observed effects. 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 activity. However, this hypothesis requires validation. Oxidative stress occurs in cells when there is disruption of cellular redox balance (Corcoran GB *et al.*,

also effect blood formation rate and normal range of hematological parameters. In the present acute study, treatment of rats with high oral dose of acetaminophen did not cause significant ($p > 0.05$) alteration in most of the measured parameters, except for the granulocytes differential and platelets counts which were significantly ($p < 0.05$) elevated in dose related fashion (Table 1).

Table. 1 Effect of graded oral dose of ethanolic extract of *Indigofera barberi* on serum urea (UR), uric acid (UA), and creatinine (CR) in acute acetaminophen induced nephrotoxic rats.

Treatment group	% change in body weight	UR (mmol/L)	UA (mol/L)	CR (Mol/L)
I	10.20 ± 3.10 ^b	5.55 ± 0.37	125.67 ± 10.10	65.33 ± 5.03
II	-18.27 ± 2.72	10.72 ± 2.07 ^a	120.67 ± 18.22	95.16 ± 14.67 ^a
III	8.23 ± 2.48 ^c	4.60 ± 0.23 ^b	134.83 ± 30.43	59.43 ± 5.62 ^c
IV	16.75 ± 3.82 ^b	4.62 ± 1.14 ^b	114 ± 18.63	53.23 ± 4.62 ^c

^a represents significant increase at $p < 0.001$ when compared to negative control (group-I) values while ^b and ^c represents significant decrease at $p < 0.01$ and $p < 0.001$ respectively. When compared to model control (group-II) values.

I = 10ml/kg of distilled water via the oral routes.

II = 10ml/kg of distilled water + 750mg/kg via the oral route of acetaminophen

III = 250mg/kg of ethanolic extract of *Indigofera barberi* + 750mg/kg of acetaminophen.

IV = 500mg/kg of ethanolic extract of *Indigofera barberi* + 750mg/kg of acetaminophen.

1992). Acetaminophen induced oxidative stress results in lipoperoxidation, protein thiol oxidation, mitochondrial endoplasmic reticulum injury, altered homeostasis and irreversible DNA damage characterized by protein adduct formation (Sies H 1993). Several antioxidant systems occur in the body which include superoxide dismutase, glutathione peroxidase, catalase, vitamin C and vitamin E (Sies 1993, Liu *et al.*, 1999).

The vital function that blood cells perform together with the susceptibility of this highly proliferative tissue to intoxication by xenobiotics, makes the hematopoietic 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. The various blood cells (erythrocytes, leucocytes and platelets) are produced at a turnover rate of about 1 to 3 million per second in a healthy human adult and this value could be altered in certain physiological or pathological stages including hemolytic anemia or suppressive inflammation (Guyton 1991). Certain drugs including alkylating cytotoxic reagents could

Oral dose of acetaminophen caused a significant ($p < 0.05$) decrease in the PCV level while causing non-significant ($p > 0.05$) alterations in other measured blood indices (Table 1). The recorded hematotoxicity could be secondary to the deleterious effect of acetaminophen on organs of hematopoiesis in the body which include liver and kidneys. Literature has shown acute or chronic large dose acetaminophen to be associated with overproduction of a highly reactive intermediate, N-acetyl-*p*-benzoquinone-imine (NAPQI), which covalently binds to macromolecules of renal tissues (Prescott 1989) resulting in acetaminophen-associated nephropathy (Emeigh Hart *et al.*, 1996). However, oral treatment with graded doses of *Harungana madagascariensis* reversed the significant decrease in the Hb, PCV value recorded for acetaminophen hematotoxicity and also caused a significant ($p < 0.05, p < 0.05, p < 0.001$) dose related increase in the Hb and TLC, and PCV, respectively. Although, the extract caused non-significant ($p > 0.05$) 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

Table.2 Effect of graded oral dose of indigofera barberi ethanolic extract on full blood counts in acute acetaminophen induced nephrotoxic rats.

Parameters	Group-I	Group-II	Group-III	Group-IV
PCV(%)	60.58 +0.77	59.40 + 1.75	62.70 + 1.58	63.50 + 1.65
Hb (g/dl)	8.40+0.28	8.10 + 0.52	8.65 + 0.48	9.62 + 0.40
TLC(x 103/L)	7.23 + 1.36	6.62 + 1.62	7.62 + 1.72	9.24 + 0.98
DLC				
Lymph (%)	55.70 + 7.78	65.32 + 1.18	70.13 + 4.32	52.57 + 8.15
Neut(%)	17.53 + 3.78	14.18 + 1.84	12.53 + 3.53	22.52 + 5.84
Gran(%)	14.66 + 5.70	4.50 + 1.32 ^b	4.30 + 1.64 ^b	14.56 + 3.92
MCV(fL)	56.83 + 0.73	56.53 + 0.21	58.55 + 1.02	55.22 + 0.34
MCH(pg)	19.33 + 1.59	17.68 + 0.17	17.98 + 0.42	17.45 + 0.24
MCHC(g/dL)	32.44 + 2.28	29.93 + 0.25	30.42 + 0.42	30.18 + 0.29
PLC(x103/L)	492.17 + 19.92	412.32 + 80.51	472.67 + 61.79	665.50 + 50.69 ^a

^a represent significant increase at $p < 0.05$ when compare to negative control (Group-I) values while ^b represent significant decrease at $p < 0.05$ when compare to negative control (Group-I) values.

I = 10ml/kg of distilled water via the oral routes.

II = 10ml/kg of distilled water + 750mg/kg via the oral route of acetaminophen

III = 250mg/kg of ethanolic extract of indigofera barberi + 750mg/kg of acetaminophen.

IV = 500mg/kg of ethanolic extract of indigofera barberi + 750mg/kg of acetaminophen.

active biological principle(s) reversing the hematotoxic effect of acetaminophen, with subsequent enhancement of hematopoiesis. The biological principle(s) could also be mediating hematopoietin-like effect or enhancing the release of hematopoietin from hematopoietic 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 of future study. Thus, the overall results of this study suggest that the *I-barberit* extract could be improving the hematological status in rats repeatedly exposed to high dose of acetaminophen.

Discussion

In recent time, the safety of acute use of acetaminophen at therapeutic dose as generated a lot of hot debate (Watkins *et al.*, 2006). Acetaminophen over dose as been associated with significant glutathione depletion and consequent lipid peroxidation, as a consequence of lipid peroxidation intra cellular accumulation and covalent bonding of its highly reactive metabolite, N-acetyl-para-benzoquinone-imine (NAPQI), Hepatocyte mal function and necrosis often

result. Similar effect is often recorded for renal tissues. The selective renal accumulation of non steroidal anti inflammatory nephrotoxins including acetaminophen in animal and human is thought to result in a chain of biochemical reactions which culminate in acute or chronic nephropathies (Schnellman 2001) in addition, acetaminophen as been reported to promote hepatocyte and renal apoptosis (Ray SD *et al.*, 2000).

Acetaminophen over dose (acute or chronic) is often associated with a wide range of metabolic disorders including serum electrolyte, urea and creatinine and derangements. As such elevations in the serum concentrations of this parameters, particularly serum urea and creatinine are considered reliable, well documented parameters for investigating drug induced nephrotoxicity in animals and man (Adelman *et al.*, 1981).

Blood urea nitrogen is derived in the liver protein / amino acid 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 (Mayne PD

1994). Creatinine, on the other hand, is mostly derived from endogenous sources by tissue creatinine 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 (Mayne PD 1994), thus serum urea concentration is often considered the more reliable renal function predictor than serum creatinine.

In conclusion, the overall result suggests that the ethanol extract of *I-barberit* possesses nephroprotective potential and improves hematological derangements associated with single dose acetaminophen 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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