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THE RENOPROTECTIVE EFFECTS OF *URTICA DIOICA* L. AGAINST CARBON TETRA CHLORIDE INDUCED TOXICITY IN RATS

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ABSTRACT

Urtica dioica (UD) has been used as herbal medicine since prehistoric times for the treatment of ailments including liver cirrhosis, benign prostatic hyperplasia (BPH), rheumatoid arthritis, Type 1 Diabetes Mellitus, hypertension and colitis. The renal protective effects of UD are limited therefore, this study was intended to investigate the protective effects of UD against renal toxicity induced by carbon tetrachloride in male wistar rats. The study included male albino wistar rats divided in to four groups (n = 6). Group I remained healthy control rats, group II, received CCl_4 (0.8 mL/Kg body weight, subcutaneous, for 8 weeks, twice a week), group III received CCl_4 (0.8 mL/Kg body weight, subcutaneous, for 8 weeks, twice a week) together with UD (2 mL/kg UD extract i.p daily for 8 weeks), group IV received UD (2 mL/kg UD extract i.p daily for 8 weeks) . Biochemical analysis included; plasma urea, creatinine and BUN. Renal antioxidant enzymes catalase, SOD & GSH & lipid peroxidation product (MDA).The sixty day treatment of rats with CCl_4 resulted in renal impairments exhibited via increased plasma urea, creatinine, BUN and renal MDA levels whereas decrease antioxidant enzymes catalase, SOD & GSH levels in CCl_4 treated group compared with the control group. The toxic effects of CCl_4 were reverted by *Urtica Dioica* treatment and indicated by reduced urea, creatinine, BUN & MDA and enhanced catalase, SOD & GSH levels. The histologic findings indicated tubulointerstitial fibrosis with no mesengial proliferation in CCl_4 treated rats. UD treatment along CCl_4 has been shown to revert tubulointerstitial fibrosis in rats.

Key word: Urtica dioica, renotoxicity, CCl_4, antioxidant, tubulointerstitial fibrosis

INTRODUCTION

Kidneys accomplish an extensive range of dynamic functions in the healthy body. Kidney disorders have become a major health problem worldwide with increased indisposition and mortality. Although a number of treatment strategies are available for kidney diseases but researchers are now motivated towards the use of herbal medicines due to the side effects of chemicals and drugs. *Urtica dioica* L. a member of family Urticaceae grows in many regions of the world. *Urtica dioica* has a long history of domestic use as a herbal remedy since ancient times. This medicinal plant is a rich source of many natural products and different parts of the plants have been used in various medical practitioners for the relief of a variety of illnesses. It has been used in Russian folk, Lithuanian, Eclectics, African, British & French folk medicine (Seliya and Kothiyal, 2014). Research studies have documented that it has been widely used for the treatment of benign prostatic hyperplasia (BPH), rheumatoid arthritis (Yang *et al.*, 2013), Type 1 Diabetes Mellitus (Ozkol *et al*., 2013), hypertension, colitis (Genc *et al*., 2011), chronic knee pain (Randall *et al*., 2008), gout, hair loss, and mild bleeding (Grieve , 1971). According to some in vitro and in vivo studies it is an acute diuretic, natriuretic and hypotensive agent (El-Haouari *et al*., 2006). It was found to be effective against renal and hepatic lesions induced by ischemia, biliary obstruction, peptic ulcer and brain lesions in various animal models (Oguz *et al*., 2013; Burkova *et al*., 2011). It has also been used as a suitable herbal therapy for the symptomatic relief of micturition disorders like nocturia, pollakisuria, dysuria and urine retention in the early stages of benign prostatic hyperplasia (BHP (British Herbal Pharmacopoeia) 1996; ESCOP 1996 and 1997).

Oxidative stress is the basis of many pathological processes with worst outcomes. Excessive production of reactive oxygen species (ROS) may result in inflammation, aging, genotoxicity & cancer (Kourounakis *et al*., 1999).Several studies have indicated that *Urtica dioica* provides protection against hepatic injury induced by ROS via enhancing activity of antioxidant enzymes catalase, paraoxonase & arylesterase (Oguz *et al*., 2013; Kanter *et al*., 2005).

CCl_4 is a volatile organic compound well known to induce toxicity in experimental models. Various research studies have indicated that besides liver it can also cause toxicity in kidneys, lungs & testis principally via generation of ROS (Ahmad *et al*., 1987; Ozturk *et al*., 2003) which instigate a cascade of reactions that results in lipid peroxidation with subsequent membrane damage that further leads to cellular destruction (Aleynik *et al*.,

1997). Kidney and liver are particularly susceptible to damage induced by CCl_4 in rats (Bruckner *et al*., 1984) and its role in the pathogenesis of renal diseases in humans has been observed by many researchers (Ruprah *et al*., 1985; Gosselin *et al*., 1984). According to some in vivo and in vitro studies CCl_4 causes reduction of renal microsomal NADPH cytochrome P450, ratio of reduced/oxidised glutathione (GSH/GSSG) in kidney cortex as well as renal microsomes and mitochondria thereby enhancing lipid peroxidation (Rungby and Ernst, 1992). The clinical use of nettle seeds as a herbal therapy for the treatment of renal dysfunctions was first described by North American Herbalist David Winston (Winston *et al*., 2001) another study indicated the protective effects of UD against proximal tubular damage after ischemia/reperfusion injury in rat kidney. Data relevant to the effects of *Urtica dioica* on renal functions to date is limited. The present study therefore was aimed to investigate the protective effects of *Urtica dioica* against renal toxicity induced by CCl_4 in rats.

MATERIALS AND METHODS

Twenty four male Albino wistar rats weighing 190-250g were purchased from the animal house of ICCBS (International center for chemical and biological sciences, Karachi, Pakistan) for the study. Prior to experimental phase, animals were adapted to the lab animal house settings for one week. Animals were kept in cages separately in a proper ventilated and moderately temperature controlled room (24±4°C). Rats had free access to typical prepared diet and water.

UD seeds were purchased from a local herb store of Karachi, Pakistan. The seeds were powdered in a mixer and fixed oil of UD was extracted with the help of a rotary evaporator using diethyl ether as solvent.

ETHICAL GUIDELINES

Animals were handled according to globally acknowledged ethics for lab use and caution in animal examination (health research extension act of 1985 & Ethical guidelines of Institutional ERB.

STUDY DESIGN

The rats were randomly divided in to four experimental groups, each of six rats. The experimental phase lasted for 60 days. CCl_4 and other chemicals used in the present study were purchased from BDH laboratory supplies, Fisher Scientific UK limited and Fluka AG.

Group I: Untreated control
Group II: CCl_4 treated
Group III: CCl_4 + UD treated
Group IV: UD treated

Group I served as control and received only 2 mL/kg normal saline solution for 60 days. Group II (CCl4 treated) & III CCl_4+UD treated) received CCl_4, 0.8 mL/kg of body weight, subcutaneously, twice a week for 60 days. Group III (CCl_4 + UD treated) & IV (UD treated) received daily intra-peritoneal injections of 2 mL/kg UD extract for 60 days. At 60^{th} day rats of all groups were decapitated. The blood was collected through decapitation in the lithium heparin coated tubes and centrifuged to collect plasma. Liver was excised, trimmed of connective tissues, rinsed with saline to eliminate blood contamination, dried by blotting with filter paper and weighed. The tissues then kept in freezer at -70 ºC until analysis.

Assessment of Plasma Urea, Creatinine & BUN

Plasma Urea (Fawcett and Scott, 1960; Patton and Couch, 1977), Creatinine (Hare, 1950; Kostir and Sonica, 1952) & BUN were analyzed using commercially prepared reagent kits from Randox.

Preparation of Post Mitochondrial Supernatant

Liver were homogenized after perfusing with saline in cool KCl (1.17%) through an electric homogenizer and the homogenates thus obtained were centrifuged at 8000g for 5 minutes at 4°C to isolate the nuclear fragments. The supernatant so achieved was again subjected to centrifugation at 10,500 g for 20 minutes at 4°C to acquire supernatant (post mitochondrial) which then utilized to test hepatic enzymes & MDA.

Estimation of Thiobarbituric Acid Substances:

The TBARS was measured by the method of Ohkawa *et a*l, (1979). The concentration of TBARS is proportional to the amount of MDA in the tissue that in turn is reflective of lipid peroxidation.

Estimation of Catalase:

Catalase activity was assayed by the method of Sinha (Sinha, 1972).

Estimation of Superoxide Dismutase:

Superoxide Dismutase Levels in the cell free supernatant were measured by the method of Kono (1978).

Estimation of Glutathione Reductase:

GSH activity was determined by continuous spectrophotometric rate determination (Carlberg and Mannervik, 1985).

Statistical analysis:

Results are presented as mean± standard deviation. Statistical analysis was done by one-way analysis of variance (ANOVA) followed by the Least Significance Difference Post hoc multiple comparison test (LSD) test. Statistical significance was tested at least at $p<0.05$

RESULTS

Effects of CCl_4 and UD treatment on Renal Function test (Urea, Creatinine & BUN) in control and treated rats.

Plasma concentration of urea ($P<0.001$) & BUN ($P<0.001$) were increased significantly whereas creatinine non-significantly in CCl_4 treated rats as compared with control. Treatment with *Urtica dioica* (UD) has been shown to reduce significantly the concentration of urea ($P<0.05$) and BUN ($P<0.05$) in CCl_4+UD treatment group as compared with CCl_4 treated group. Although creatinine level was reduced in CCl_4+UD treatment group as compared with CCl_4 treated group but results are statistically non-significant. Plasma levels of Urea, Creatinine and BUN were almost similar in UD treated groups and controls (Table 1).

Table 1. Comparison of Plasma Urea, Creatinine and BUN concentration in Control, CCl_4 treated, CCl_4 + UD treated &UD treated rats.

Previous:	CONTROL	CCl4[1]	CCL4 + UD treated 1,2	UD treated 1,2,3
Urea (mg/dl)	16.23 ± 2.98	61.96 ± 6.18 c	43.65 ± 13.47 b, a	17.51 ± 2.28 n, c, b
Creatinine (mg/dl)	0.24 ± 0.05	0.72 ± 0.19 n	0.49 ± 0.26 n, n	0.57 ± 0.54 n, n, n
BUN (mg/dl)	7.57± 1.39	28.9 ± 2.88 c	20.37 ± 6.28 b, a	8.17 ± 1.06 n, c, b

The data is expressed as mean ± standard deviation.

1= As compared with control; 2= As compared with CCl_4

3= As compared with CCl_4+*Urtica dioica*; a= $P<0.05$, b= $P<0.01$, c= $P<0.001$, n= $P>0.05$(Non-significant)

Table 2. Comparison of Renal antioxidant enzymes and MDA activity in Control, CCl_4 treated, CCl_4 + UD treated &UD treated rats.

	CONTROL	CCl4[1]	CCL4 + UD treated 1, 2	UD treated 1,2,3
Catalase (µmol/g tissue)	40.93±8.49	23.81±4.32 c	31.82±6.5 a, a	31.59±4.26 a, n, n
SOD (Unit/g tissue)	25.65±5.55	1.32±0.56 c	10.71±4.09 b, a	10.8±8.16 b, a, n
GSH (Unit/g tissue)	11.47±5.07	0.47±0.06 c	0.86±0.58 c, n	0.97±0.36 c, n, n
MDA (µmol/g tissue)	0.46±0.09	2.48±2.46 a	0.66±0.14 n, a	0.56±0.13 n, a, n

The data is expressed as mean ± standard deviation.

1= As compared with control; 2= As compared with CCl_4

3= As compared with CCl_4+ *Urtica dioica*; a= $P<0.05$, b= $P<0.01$, c= $P<0.001$, n= $P>0.05$(Non-significant)

Effects of CCl_4 and UD treatment on renal Concentration of Catalase in Control and Treated Rats

Administration of CCl_4 in rats resulted in significant ($P<0.001$)reduction of renal catalase activity in CCl_4 treated group as compared with control, UD treatment along with CCl_4 resulted in elevation of catalase level significantly($P<0.05$) in CCl_4+UD treated group. There was no statistically significant change observed in catalase level among control group & UD treated groups (Table 2).

Effects of CCl4 and UD treatment on renal Concentration of SOD in Control and Treated Rats

Activity of SOD was significantly reduced in CCl_4 treated rats as compared with control ($P<0.001$). UD treatment in CCl_4+UD group has been shown to increase significantly SOD activity ($P<0.05$) as compared with CCl_4 treated group. Activity of SOD was more or less identical in UD treated group; CCl_4+UD group and control group (Table 2).

Effects of CCl_4 and UD treatment on renal Concentration of GSH in Control and Treated Rats

Activity of renal glutathione reductase was reduced significantly in CCl_4 treated rats as compared with control ($P<0.001$). UD administration in CCl_4+UD group has been shown to increase non-significantly the reduced renal GSH activity as compared to CCl_4 treated group. No statistically significant elevation in GSH activity was observed in UD treated group when compared with control (Table 2).

Effects of CCl_4 and UD treatment on renal Concentration of MDA in Control and Treated Rats

Levels of MDA were raised non-significantly in CCl_4 treated group as compared with control. UD treatment along with CCl_4 in CCl_4+UD treated group has been shown to decrease MDA level significantly ($P<0.05$) as compared with CCl_4 treated group. MDA activity was almost similar in UD treated group & control (Table 2).

Histopathological findings in Control, CCl_4 treated, CCl_4 + UD treated &UD treated rats

Histological examination revealed absence of mesengial proliferation and tublointerstitial fibrosis in control & UD treated rats. CCl_4 treatment has been shown to cause tubulointerstitial fibrosis with no mesengial proliferation in rats. UD treatment along with CCl_4 has been shown to revert tubulointerstitial fibrosis in rats (Table 3).

Table 3. Histopathological features in Control, CCl_4 treated, CCl_4 + UD treated &UD treated rats.

	CONTROL	CCl4	CCL4+UD treated	UD treated
Mesengial proliferation	0	0	0	0
Tublointerstitial fibrosis	0	01	0	0

0= none, 01= mild, 02= moderate, 3= severe

DISCUSSION

CCl_4 is a well-known environmental toxicant used experimentally to induce liver and kidney damage. It causes cellular damage after being metabolized by cytochrome P450 2E1 to a highly reactive trichloromethyl (CCl_3-) radical that arbitrate lipid peroxidation with subsequent deposit of lipid peroxidation products thereby causing hepatic and renal injuries (Aleynik *et al*., 1997). During the present study rats treated with CCl_4 showed renal impairments exhibited via marked elevation in urea, creatinine and BUN (Table 1). The mechanism involved is principally through generation of ROS during the course of CCl_4 metabolism through activation of endoplasmic reticulum enzymes (Slater and Sawyer 1971). *Urtica dioica* acquire analgesic, antibacterial, antioxidant & anti-inflammatory properties (Terzi *et al*., 2010; Gulcin *et al*., 2004; Mittman *et al*., 1990). In this study treatment of rats with Urtica dioica resulted in reduction of urea, creatinine and BUN thereby endorsing the beneficial effects of UD against nephrotoxicity induced by CCl_4 in those rats (Table 1).

Oxidative stress contributes to the progression of structural & functional renal impairments. Generation of ROS can lead to the alteration in antioxidants status, release of vasoconstrictors, inactivation of nitric oxide, direct cellular damage, microvascular endothelial dysfunction (Bonetti *et al*., 2003). Oxidative stress can lead to a broad range of renal abnormalities among which glomerular damage & acute or chronic failure are important. Several studies have indicated that substances exhibiting antioxidant activities in various experimental disease models have attenuated renal injuries and glomeruloscelerosis generated by CCl_4. In the present study the activity of renal antioxidant enzymes Catalase, SOD, & GSH was reduced after CCl_4 treatment in rats (Table 2). The alteration in antioxidant enzyme status indicates a state of nephrotoxicity being produced via generation of ROS. Treatment of rats with UD caused increase in catalase, SOD & GSH activities suggesting a free radical scavenging effects of UD against nephrotoxic effects of CCl_4 in those rats (Table 2).

The concentration of MDA was increased in CCl_4 treated rats (Table 2) which is a lipid peroxidation product and strong indicator of lipid peroxidation. Lipid peroxidation is the basis of many pathological processes and result in membrane damage via oxidation of lipids and proteins that further lead to tissue damage (Dogukan *et al*., 2003). The rise in MDA by CCl_4 was attenuated by *Urtica dioica* in UD treated rats (Table 2) suggesting its significance against lipid peroxidation either via scavenging ROS or increasing the activities of antioxidant enzymes.

ROS may cause tubulointerstitial and glomerular fibrosis (Chade *et al*., 2002) through activation of the TGF-β pathway thereby smoothing the progress of extracellular matrix (ECM) accumulation (Iglesias *et al*., 2002). In addition free oxygen radicals can directly reduce the activity of MMP-2 and oxidation of ECM that may further leads to glomerulosclerosis (Mattana *et al*., 1998). Histological examination revealed mild tubulointerstititial fibrosis without mesengial proliferation in CCl_4 treated rats suggesting acute toxicity created by CCl_4 in those rats (Table 3). *Urtica dioica* ameliorate the changes induced by CCl_4 in CCl_4+UD treated rats as proved by no sign of tubulointerstititial fibrosis and mesengial proliferation in those rats (Table 3). The histological findings suggest that *Urtica dioica* has successively attenuated the renal pathophysiological changes induced by CCl_4 thus may be effective against nehrotoxicity induced by CCl_4 and our results are consistent with other studies in which renal ischemic-perfusion injuries were attenuated by *Urtica dioica* in rats (Sayhan *et al*., 2012).

Conclusion

The efficiency of *Urtica dioica* against CCl_4 induced nephrotoxicity in rats and suggested the free radical scavenging effect of *Urtica dioica* that may be useful against various renal diseases together with hepatic diseases and would improve renal functions and prevent renal damage without any side effects.

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