

A Review on Evaluation of Nephroprotective activity

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ABSTRACT

The study was performed to evaluate the nephroprotective activity on Carbon Tetrachloride induced nephrotoxicity in Wister rats. Rats were divided into group of five. Group I received normal saline solution. Group II received only CCl₄ treatment. Group III received silymarin as standard drug. Group IV and V were treated p.o with ethanolic plant extract at low and high dose respectively. The kidney function test (estimation of serum creatinine, total protein and protein urea), physical analysis of urine, determination of biochemical enzymatic parameters and histological studies were conducted. CCl₄ treatment caused nephrotoxicity as it showed marked changes in physical, urinary and blood parameters. Co-administration of plant extract with CCl₄ has markedly improved all the parameters with a significant reduction in lipid peroxidation and rise in GSH levels. These results suggest that the ethanolic plant extract may be useful in reducing the CCl₄ induced Nephrotoxicity.

Keywords: Nephrotoxicity, Carbon tetrachloride, Kidney function test.

I. Introduction

Kidneys play an important part in the maintenance of our endocrine and acid-base balance, blood pressure, erythropoiesis etc. The main functions of kidney can be categorised as formation of urine, water and electrolyte balance and production of hormones and enzymes [1]. Nephrotoxicity is the most common kidney problem, occurs when the body is exposed to a drug or toxin. Nephrotoxicity is manifested functionally by decreased urine concentrating capacity, tubular proteinuria and decreased ammonium excretion, lowering of glomerular filtration rate, creatinine clearance and increase in serum blood urea nitrogen, and serum creatinine level with kidney tissue morphological alteration [2].

Drug-induced nephrotoxicity tends to be very common among certain patients or in specific clinical situations. Thus, successful prevention requires knowledge of pathogenic mechanisms of renal injury, drug-related risk factors, and preemptive measures, coupled with early intervention. General preventive measures include using alternative non-nephrotoxic drugs whenever possible; assessing baseline renal function before initiation of therapy, followed by adjusting the dosage; monitoring renal function and vital signs during therapy; and avoiding nephrotoxic drug combinations [3].

Exposure to various compounds including a number of environmental pollutants and drugs can cause cellular damages through metabolic activation of those compounds to a highly reactive oxygen species (ROS) [4]. Carbon tetrachloride (CCl₄) is a toxic chemical, widely used in the dry cleaning industry, in filling fire extinguishers, in the fumigation of grains, and as an insecticide [5].

Studies have demonstrated that CCl₄ can cause generation of reactive oxygen species (ROS) in many tissues other than the liver including the kidney, heart, lung, testis, brain, and blood [6]. Kidney tissue has great affinity for CCl₄ due to the presence of the CYP450 in the renal cortex. CCl₄ toxicity is caused by its bioactivation to trichloromethyl free radical by CYP450. The trichloromethyl radical reacts with oxygen to form trichloromethyl peroxy radical that is one reactive oxygen species (ROS) [7].

According to WHO, about 75 – 80% of the world populations still rely mainly on herbal remedies; because it is safe and without any side effects etc [8]. The ethanolic plant extract is used for generations to energize, vitalize, and improve sexual function and physical performance in men [9]. It is a taproot and perennial herb that best grow in dry, loose and sandy soil. It is found mostly along with tracks and around habitation, especially, in inland districts [10].

Its various parts contain a variety of chemical constituents which are medicinally important, such as flavonoids, flavonol glycosides, steroidal saponins, and alkaloids [11]. The whole plant is of medicinal value as the leaves are considered to possess stomachic properties and paste prepared from them is used in the treatment of bladder stone. Root possesses aperient and tonic properties while the fruit is credited with diuretic, tonic, cooling, demulcent, and aphrodisiac properties. This indigenous medicinal plant is also used in the Indian and Chinese system of medicine for the treatment of various male reproductive disorders [12]. Several pharmacological studies have revealed its cardioprotective, hepatoprotective, antitumor, and anti-proliferative activity on mouse carcinoma and breast cancer [13].

The present study aimed at evaluates nephroprotective against CCl₄ induced nephrotoxicity in rats with special reference to biochemical, antioxidants parameters and histopathological studies.

II. MATERIALS AND METHODS

- Source of data:** Experiment was performed as described in the standard bibliography, literatures and text books. The reputed journals and publications were obtained from college library and through web search.
- Preparation of extract:** 100 grams powdered leaves were subjected to successive extraction in a soxhlet extractor with ethanol. The extract obtained, was concentrated in a rotary shaker, where it was evaporated to dryness to get a constant weight [14].
- Acute toxicity studies:** The acute toxicity study was performed by using up and down procedure (OECD/OCDE GUIDELINE) 423 Adopted on 17th December 2001. [15]
- Experimental animals:** Wistar rat were used for the experiment. It is characterized by its wide head, long ears, and tail length that is always less than its body length. Adult male Wistar rats (150-200gm) were used to evaluate the nephroprotective and anti-oxidant activity. The animals were maintained under standard laboratory conditions in polypropylene cages under 12 hr light/dark cycle, controlled temperature(24±2°C), fed with commercial pellet diet and water in an animal house approved by the Committee for the Purpose of Control and Supervision on Experiments on Animals.

III. EXPERIMENTAL METHODS:

Animals were divided into 5 groups, with 6 animals in each group:

Group I: Served as normal control group, which received normal saline solution (1 mL, p.o.).

Group II: Served as diseased control group, which received CCl₄ at the dose of 1.25 mL/kg, p.o. [16].

Group III: Served as standard group, which received silymarin (10 mg/kg p.o.).

Group IV: Test group, received the ethanolic plant extract at the low dose.

Group V: Test group, received the ethanolic plant extract at the high dose.

Method: All the groups received the treatment for five days. On the 6th day, group II – V received a single dose p.o. of CCl₄ (1.25 mL/kg). 24 hours after the administration of CCl₄, rats were sacrificed and blood samples were collected by heart puncture and the serum was separated for evaluation of various biochemical parameters [16].

A) Physical analysis of urine: Urine samples were assayed for red blood cells (RBCs) count, white blood cells (WBCs) count, urobilinogene, pH, and specific gravity by using standard diagnostic kits (MediScreen Urine Strips, Organics, France)[17].

B) Biochemical analysis of urine and serum: Estimation of total protein, albumin, urea, creatinine, and creatinine clearance was done by using standard diagnostic kits (MediScreen kit France) [17].

C) Determination of biochemical enzymatic parameters: The biochemical parameters such as aspartate aminotransferase (AST), alanine aminotransferase (ALT), gamma-glutamyltranspeptidase (GGT), alka-line phosphatase (ALP) and total bilirubin were estimated by reported methods [18]. The enzyme activities were measured using diagnostic strips (Roche, Basel, Switzerland). Serum creatinine and blood urea were assayed using Randox Diagnostic kits (Randox Laboratories Ltd., Crumlin, U.K.) by the reported method [19].

D) Determination of total protein (TP): The kidney samples were cooled in a beaker immersed in an ice bath. The tissues were homogenized in 0.02 M EDTA in a Pot-ter-Elvehjem type C homogenizer (Sigma-Aldrich, St. Louis, MO, USA). Parts of the homogenate were treated with 0.7 mL of Lowry's solution, mixed and incubated for 20 min in dark at room temperature. Diluted Folin's reagent (0.1 mL) was then added, and samples were incubated at room temperature in the dark for 30 min. The absorbance of the resulted solutions was then measured at 750 nm [20].

E) Histopathological studies: Kidney tissues were excised from sacrificed animals, individually weighed, and thin kidney slices were cut, fixed in 4% paraformaldehyde and were sequentially embedded in paraffin wax blocks. Thin sections (3 μm) were made using microtome and stained with Mayer's hematoxylin solution and counterstained in eosin-phloxine solution for conventional morphological evaluation, then examined under light microscope (BX50; Olympus, Tokyo). The images were obtained by a digital camera system (Pixcera Co., Osaka, Japan) attached to the microscope. A minimum of 10 fields for each kidney slide were examined and scored. The scoring was done as none (-), mild (+), moderate (++) and severe (+++). [4]

F) Statistical analysis: The results were expressed as mean \pm SD of different groups. The differences between the mean values were evaluated by ANOVA, further comparisons among groups were made using Dunnett's multiple comparisons test. The F-value was found statistically significant ($p < 0.05$).^[16]

IV. RESULTS:

After the extract was subjected to biochemical enzymatic analysis, it was reported that the group with silymarin as standard drug resulted in a significant decrease in the elevated AST, ALT, GGT, ALP, and bilirubin level in rats. The group that used the total ethanolic extract of *T. terrestris* showed a weak protective effect at the two used doses^[16].

Kidney is responsible for regulation of plasma ionic composition like magnesium, chloride, sodium, potassium, calcium. It also plays a major role in the removal of nitrogenous metabolic waste products such as urea, creatinine and uric acid^[21]. The toxicity due to CCl₄ is reflected by the elevation of the serum levels of LDH, creatinine-kinase, urea, uric acid, creatinine, sodium, potassium and calcium. Administration of silymarin reversed these effects and thereby showed protective effect with different degrees. The extract showed a dose-dependent effect and highly significant reduction in the urea and creatinine levels. Improvement in the levels of sodium, potassium and calcium was also observed^[16].

The results support the ability of the ethanolic plant extract \pm SD to protect the kidney against CCl₄ induced toxicity.

V. DISCUSSION:

Chemical and drug-induced acute kidney injury is a major cause of death worldwide. Previous published research studies have reported that CCl₄ exposure causes damage to the kidney due to enhanced production of reactive oxygen species^[22]. Carbon tetrachloride induced nephrotoxicity employed in the present study is one of the most widely accepted models to evaluate nephroprotective activity in laboratory animals.

The present investigation showed that administration of CCl₄ increased the levels of creatinine and blood urea nitrogen (BUN). Creatinine and BUN are the nitrogenous end product of metabolism in the blood, distributed throughout the total body water and are normally removed from blood by the kidney. After the kidney is exposed to CCl₄, its function slows down and increases the levels of creatinine and BUN in the blood. The treatment with extract significantly reduced the increased levels of creatinine and BUN.

Ozturk *et al.* (2003) recorded similar histopathological alterations in the kidney of rats that were treated with CCl₄ exhibited tubular epithelial cell alterations including vacuolization, atrophy, detachment of epithelial cells and tubular necrosis. With these histopathological changes, the capacity of tubular absorption may have been altered and functional overloading of nephrons with subsequent renal dysfunction was observed (Khan *et al.*, 2010)^[23].

It was reported that CCl₄ metabolized by cytochrome p- 450 generates a highly reactive free radical, and initiates lipid peroxidation of the cell membrane of the endoplasmic reticulum and causes a chain reaction. These reactive oxygen species are responsible for causing oxidative damage in DNA, proteins and lipids (Melin *et al.*, 2000). Various studies have demonstrated that CCl₄ causes free radical generation in many tissues including kidney^[23].

The plant extract contains various phytoconstituents which are rich in flavonoids, anthraquinone and phenolic compounds. It is because of these constituents that it is able to show its protective activity. They are able to donate electrons to reactive radicals, converting them to more stable species, thereby preventing them from reaching the various biomolecules like lipoproteins, polyunsaturated fatty acids, DNA amino acids, proteins and sugar in biological systems.

VI. CONCLUSION:

From this study, it can be clearly seen that the medicinal plants play a vital role against various kinds of diseases. The extract of the plant have significant nephroprotective activity in animal models. The nephroprotective activity is probably due to the presence of flavonoids in the plant. The result of this study indicates that the extract have good potential for use in renal disease. The present study also gave evidential explore mechanism of action of the plant against CCl₄ induced nephrotoxicity.

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