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Evaluation Of Trichosanthes Dioica Roxb. Extract For Nephroprotective Potential

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Abstract:

Trichosanthes dioica Roxb. is known by a common name of *parwal* (Pointed gourd) and is cultivated mainly as a vegetable. It is an easily available common plant, the fruit of which is integral part of an average Indian diet, being consumed as a vegetable. The aims of this study were to validate the medicinal uses of plant extract and to evaluate its nephroprotective potential on scientific grounds. Acetaminophen is frequently used for analgesia and is considered safer than nonsteroidal anti-inflammatory drugs for the kidneys. However, there is little epidemiological evidence of the association between drug and acute kidney injury. Acetaminophen was used to induce nephrotoxicity in the animal model. Two doses of the methanolic extract of methanolic extract of plant (METD; 200 and 400 mg/kg) were utilized in addition to silymarin (50 mg/kg/ d). Treatments were administered once daily for 14 days. The levels of renal function markers such as serum creatinine, blood urea nitrogen, and serum uric acid levels also evaluated, and a significant retrieval was found in a dose-dependent fashion. Statistical analysis was done using analysis of variance to determine the significance of differences among the data.

Key-words: *Trichosanthes dioica Roxb.*, Plantextract, Nephroprotective activity

Introduction

The renal system, characterized by a significant amount of blood supply from the cardiac output, carries out several vital tasks, including eliminating exogenous medications and toxins from the blood, controlling water fluid levels, and adjusting the body's acid–base balance [1]. This vital organ's high metabolic activities and transporter capacity make it more

vulnerable to nephrotoxicity and increase its sensitivity to several types of failure [2]. Renal impairment caused by medication treatments is a regular occurrence in clinical care. The use of nephrotoxic drugs is responsible for about 20% of renal insufficiencies among hospitalized patients [3]. An acute acetaminophen (paracetamol, N-acetylp-aminophenol; APAP) overdose may results to potentially fatal hepatic and renal necrosis in humans and experimental animals [4]. The initial step of its toxicity is formation of the reactive intermediate N-acetyl-p-benzoquinone imine (NAPQI) by cytochorom P450 which at therapeutic doses is removed by conjugation with gluthation solfidryle (GSH). High doses of acetaminophen result in the depletion of cellular GSH which allows NAPQI to bind to cellular proteins and initiate lipid peroxidation, leading to renal injury [5]. Acetaminophen -induced renal injury could also be due to hepatic-derived acetaminophen metabolites, particularly GSH conjugates [6].Their main function is to maintain the volume, composition, and acid−base balance of the total fluid. Many environmental xenobiotics and drugs influence these functions. Aminoglycosides produce nonoliguric acute renal failure in 10−25% of therapeutic courses. This type of renal failure manifests as a decreased urine concentrating capacity, tubular proteinuria, lysosomal enzymuria, mild glycosuria, and alterations in the electrolyte balance. Moreover, it also reduces ammonium excretion, depresses the glomerular filtration rate, and increases the amount of serum creatinine and blood urea nitrogen. The cellular mechanisms of gentamicin (G.M)-induced nephrotoxicity are still poorly understood. Reactive oxygen species (ROS) have an important role in the pathogenesis of this toxicity. The production and accumulation of ROS results in the induction of apoptosis and tubular necrosis as well as the increased infiltration of leukocytes [7]. Gentamicin has been showed to increase the generation of reactive oxygen species such as superoxide anions,4−6 hydroxyl radicals, and hydrogen peroxide in kidneys, and all of these may lead to renal issues. Moreover, gentamicin reduces the efficiency of kidney antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPX), and glutathione (GSH). Free radicals have also been implicated in both glomerular diseases and neutrophil-mediated glomerular diseases [8]. The drug is nephrotoxic because a small but sizable proportion (about 5%) of the administered dose is retained in the epithelial cell linings of proximal renal tubules. Drugs that can mitigate the toxicity of aminoglycoside always remain a fertile area of research. In the scientific literature, we found that various drugs, such as deferoxamine, methimazole, vitamin E, vitamin C, diethyldithiocarbamate, histidinol, and thymoquinone, have been used to prevent G.Minduced nephrotoxicity. Howeverall, all these drugs are far from ideal in practice. Phytotherapy research is an area that in the recent times has been proven to successfully manage several morbidities. *Trichosanthes dioica Roxb.* is known by a common name of *parwal* (Pointed gourd) and is cultivated mainly as a vegetable. It is an easily available common plant, the fruit of which is integral part of an average Indian diet, being consumed as a vegetable. The plant belongs to family Cucurbitaceae, which has given us many important medicinal plants. Samhita mentioned the protective role of *Trichosanthes dioica Roxb.* on important body organs such as liver, spleen, heart, etc., many of which are now scientifically proven. Clinical investigation on peptic ulcer with polyherbal formulation, where *Trichosanthes dioica Roxb.* was an integral part, has shown promising results.*Trichosanthes dioica Roxb*, a genus of family Cucurbitaceae, is an annual or perennial herb distributed in tropical Asia.

Material And Methods

Extraction Method: The fresh leaves of the plant of *Trichosanthes dioica Roxb.* were collected from local area of indore and identified by taxonomist. The fresh leaves were dried in the shade at room temperature. The dried plant was converted into a powder and soaked in methanol for 14 days. After soaking, the mixture was filtered with a muslin cloth, followed by whatman filter paper. The filtrate was evaporated using a rotary evaporator and stored at

room temperature in a container. Moreover, the extract was mixed with normal saline and stored for experimental use.

Experimental Animals: Wistar male mice of either sex weighing 25-30g were selected, and were obtained from the environmental conditions of temperature, a 12 h light dark cycle, and humidity were provided. All the in vivo experiments were conducted according to guidelines of animal use for experimental purposes recommended by the CPCSEA. Over the course of the experiment, the rats were provided with a proper diet. Water was given for all 24 h of the day [9].

Experimental Design: Mice were randomly divided into 5 groups, each consisting of 6 animals. All Animals were fasted over night before the experiment. Group1 received normal saline as control negative group. Group2, the standardgroup, received silymarin, and group3 the acetaminophen group, received a single dose of acetaminophen (500 mg/kg). Groups 4 and 5 as test groups were treated with *Trichosanthes dioica Roxb.* extract (at doses of 200 and 400 mg/kg) and a single dose of acetaminophen (500 mg/kg.) at the same time. Acetaminophen and *Trichosanthes dioica Roxb.* methanol extract were given to the animals by gavage method at the same time. *Trichosanthes dioica Roxb.* extract was diluted with distilled water and acetaminophen suspension was prepared by gum tragacant (0.5%) in normal saline (13). Twentyfour hour after administration of acetaminophen, the mice of each group were anesthesized and the kidneys were removed and kept in %10 formalin solution for histopathology tests [10-11].

Biochemical tests

Blood samples collected from the jugular arteries of the mice ´s necks. Blood samples were centrifuged at 2500 rpm for 10 min, and serum was taken to perform serum tests, e.g., Blood urea nitrogen, Creatinine and Uric acid concentration was assessed as markers of nephrotoxicity. Blood urea nitrogen, Creatinine and Uric acidwere determined spectrophotometrically from serum samples using commercially available kits (Sigma).

Histopathological determination: The kidney was isolated and cut into small pieces, preserved, and fixed into 10% formalin for two days for the proposed study. The kidney pieces were the washed, then keep in solution containing 70% isopropyl alcohol for 12 h, then, keepfor 12 h into absolute alcohol and alcohol was removed using xylene for the study. Subsequently, the kidney pieces were subjected to paraffin infiltration in an automatic tissue processing unit. Hard paraffin was liquefied and transferred into square-shaped blocks. The kidney pieces were then placed in the block containing paraffin and allowed to cool. The blocks were then cut using microtome to get sections with a thickness of 5 μM. The sections were taken on a microscopic slide onto which a sticky substance, egg albumin, had been applied. The sections were placed in an oven at 60 °C for 1 h. Subsequently, the paraffin melts and egg albumin denatured, thereby fixing the tissue slide. Staining involved the use of eosin, an acid stain that stained all the basic cell constituents pink, and hematoxylin, a basic stain that stained the entire acidic cell components blue. The slide was immersed in the hematoxylin stain for 1−2 min and then in eosin dye for 30 s. The tissue was dehydrated with the successive use of 80%, 90%, and 100% isopropyl alcohol and finally with xylene for 20−30 min. The coverslip was placed on the slides using one drop of desterene dibutyl phthalate xylene (DPX). Care was taken not to leave air bubbles, and samples were then left to dry overnight to make the permanent slide. All slides were observed for changes in histopathological characteristics, and photographs were taken by using photo microscope at 100 × resolution. The purpose was to determine how much tubular necrosis, epithelial cell damage, and inflammation was present in cells due to reactive oxidative stress [12].

Statistical Analysis: All data are presented as the mean ± SEM. One-way analysis of variance (ANOVA) was used to analyze the data using Graph Pad Instat software package ver. 7.2. The difference was considered significant if the if P value was <0.05.

RESULTS

Effect of the Methanolic Extract of *Trichosanthes dioica Roxb.* (METD) on Kidney Function Parameters: Acetaminophen intoxication significantly ($P < 0.005$) increased the levels of serum creatinine, uric acid, and blood urea nitrogen as compared to those of the normal control. However, silymarin and both doses of METD (200 and 400 mg/kg) significantly ($P < 0.005$) improved all of these parameters in a dose-dependent fashion. The only exception is blood urea nitrogen, where the effects of the low dose are better than those of the higher dose. Biochemical tests results are shown in table 1. The serum markers were significantly decreased in treatment group compared to the acetaminophen-treated mice

Table1. Effect of *Trichosanthes dioica Roxb.* methanol extract on Blood urea nitrogen, Creatinine and Uric acid in mice

Treatment	Blood urea nitrogen	Creatinine	Uric acid
Control	133±16	4.17 ±0.04	9.4 ±0.05
Standard	130 ±15	3.98 ±0.04	9.3 ±0.05
acetaminophen	186 ±17	5.47 ±0.04	15.53 ±0.11
Trichosanthes dioica Roxb. methanol extract (200 mg/kg)	164 ±12	4.95 ±0.04	14.42 ±0.12
Trichosanthes dioica Roxb methanol extract (400 mg/kg)	138 ±7	4.39 ±0.04	10.14 ±0.08

(Mean ± S.E.M.)

Histopathological Assay: To evaluate the effect of *Trichosanthes dioica Roxb.* extract on the histological changes in the kidney, H&E staining was performed. Histopathological sections from kidney tissues of acetaminophentreated rats showed degeneration, desquamation, and necrosis in tubules in addition to swelling in glomerulus. Treatment with acetaminophen caused acute renal damages in glomerulus and proximal tubules. There was also intertubular haemorrhage and acute leukocyte infiltrations in the inter tubular region. All parts of kidney showed normal appearance in control group.

The kidney of *Trichosanthes dioica Roxb.* group showed normal architecture. Glomerolus damages were evident by glomerular bleeding and partial endothelial rupture in capsule. Proximal tubules were dilated with loss of cellular boundary. Intraluminal cell debris, karyorrhexis and glassy pink cytoplasm were observed as indicators of the cell death wree observed. The proximal tubule also showed loss of brush border. Debris and granules from epithelial cells leaked into the tubular lumen

A significant change in morphologic appearance, recovered tubular epithelial cell damage, and a normal morphological view of medulla were observed with 400 mg/kg *Trichosanthes dioica Roxb.* extract.

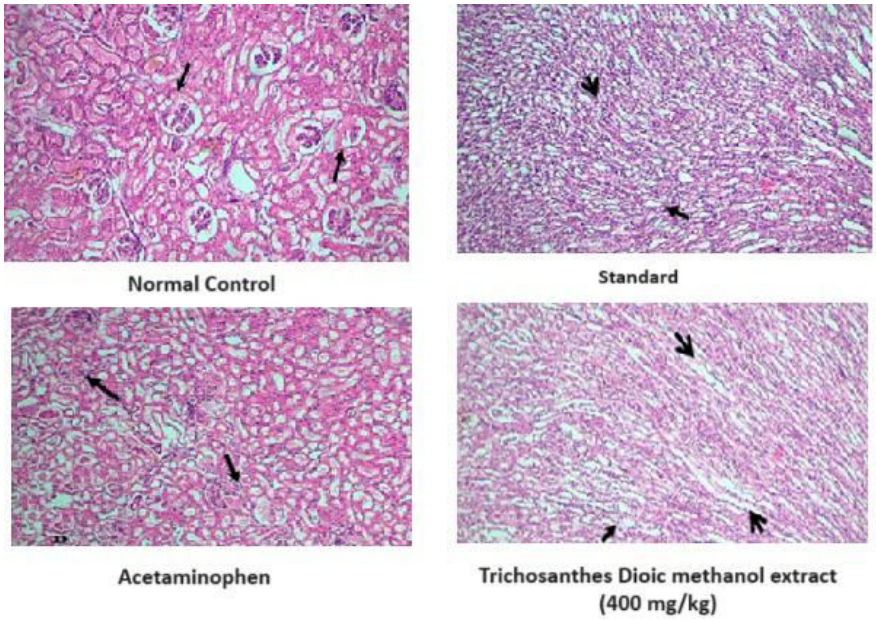

Figure 1: Effect of the methanolic extract of *Trichosanthes dioica Roxb.* on acetaminophen induced nephrotoxicity

Discussion

The major mechanism behind the acetaminophen-induced nephrotoxicity is the production of ROS and inflammatory mediators. In the present study, *Trichosanthes dioica Roxb.* was tested to validate its medicinal use as a nephroprotective agent in kidney diseases. Acetaminopheninduced nephrotoxicity is distinguished by increased levels of urea, uric acid, creatinine, and blood urea nitrogen in plasma as well as urine.

The plants extract doses (200 and 400 mg/kg) are critically observed and compared with the acetaminophen group. The plant extracts decreased the levels of serum toxicity biomarkers such as serum creatinine, serum urea, and uric acid.

The histopathological view of renal sections in the acetaminophentreated group, which compared to the control group showed degeneration, desquamation, and necrosis in tubules as well as swelling in the glomerulus. Similarly, in histopathological examination in the present work, we observed damage in the structures of the kidneys of acetaminophentreated mice. Glomerular and tubular epithelial changes were considerably mild in the groups treated with the methanolic extract of plant extract dose (200 and 400 mg/kg), and the restoration of normal histopathology was also observed.

Two weeks of treatment with *Trichosanthes dioica Roxb.* methanolic extract at a dose of 200 mg/kg showed reductions in tubular necrosis and tubular degeneration, as was observed from histopathology; however, slight leukocyte infiltrations in the intratubular area were present. In case of animals treated with *Trichosanthes dioica Roxb.* methanolic extract at a dose of 400 mg/kg, the regeneration of tubular epithelial cells was observed, and there was no sign of necrosis, degeneration, or mild inflammation. It can be concluded that morphological changes in kidneys were caused by the acetaminophen injection, but these changes were considerably mild in the acetaminophen plus *Trichosanthes dioica Roxb.* methanolic extract (400 mg/kg)treated animals. The extract dose of 200 mg/kg seems to normalize kidney parameters less effectively as compared to 400 mg/kg.

Conclusion

Methanolic extract of *Trichosanthes dioica Roxb.* significantly improved the acetaminopheninduced nephrotoxicity in animal models, which might provide the rationale for the medicinal uses of methanolic extract of proposed plant. Thus, this study validates the

traditional uses of this plant and opens a new era for carrying out further molecular level research after the isolation and purification of various known and unknown compounds.

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