



Hepatoprotective activity of *Michelia champaca* L. against carbontetrachloride induced hepatic injury in rats

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ABSTRACT

Michelia champaca (Magnoliaceae), commonly known as *Svarna champa* is a glorious traditional Indian medicinal plant. The aim of this study was to evaluate the hepatoprotective activity against CCl_4 induced liver injury in rats. Methanolic flower extract of *Michelia champaca* was investigated against CCl_4 induced hepatotoxicity and compared with standard drug silymarin. Liver marker enzymes (AST, ALT, ALP and GGT) and Renal markers (Urea, Creatinine and Total Bilirubin) were evaluated in control and experimental rats. CCl_4 treated rats elevated the liver marker enzymes and renal markers. However treatment with *M. champaca* significantly reversed the above changes compared to the control group as observed in the CCl_4 treated rats. The results clearly indicate that flower extract of *Michelia champaca* possess promising hepatoprotective effect.

Key words: *Michelia champaca*, Liver markers, Renar Markers, Silymarin

INTRODUCTION

Liver is the key organ for detoxification and disposition of endogenous substances. It is continuously and widely exposed to xenobiotics, hepatotoxins, and chemotherapeutic agents that lead to impairment of its functions [1]. Liver diseases are mainly caused by toxic chemicals, excess consumption of alcohol, infections and autoimmune disorders. Most of the hepatotoxic chemicals damage liver cells mainly by inducing lipid peroxidation and other oxidative damages [2]. Hepatotoxicity is one of very common ailments resulting into serious debilities ranging from severe metabolic disorders to even mortality. Hepatotoxicity in most cases is due to free radicals. Free radicals are fundamental to many biochemical processes and represent an essential part of aerobic life and metabolism [3].

Reactive oxygen species mediated oxidative damage to macromolecules such as lipids, proteins and DNA has been implicated in the pathogenesis of major diseases like cancer, rheumatoid arthritis, degeneration process of aging and cardiovascular disease etc. Antioxidants have been reported to prevent oxidative damage caused by free radicals by interfering with the oxidation process through radical scavenging and chelating metal ions [4].

Liver disease is still a worldwide health problem. Unfortunately, conventional or synthetic drugs used in the treatment of liver diseases are inadequate and sometimes can have serious side effects. In the absence of a reliable liver protective drug in modern medicine there are a number of medicinal preparations in Ayurveda recommended for the treatment of liver disorders. In view of severe undesirable side effects of synthetic agents, there is growing

focus to follow systematic research methodology and to evaluate scientific basis for the traditional herbal medicines that are claimed to possess hepatoprotective activity [5].

Michelia champaca L. (Magnoliaceae) commonly known as Svarna champa, a tall handsome tree with yellow fragrant blossoms, is commonly used by many traditional herbal preparations and it is also reported to have significant wound healing [6], antimicrobial [7], antidiabetic [8], antitumor [9], anti-inflammatory [10], antioxidant [11] and anti infective [12] properties.

EXPERIMENTAL SECTION

Collection of plant material

The *Michelia champaca* flowers were procured from the local areas of Udumalaipettai, Coimbatore District, Tamilnadu. The collected plant material was botanically identified and confirmed by Dr. S. John Britto, The Director, Rapinat Herbarium, St. Joseph's College, Tiruchirappalli, Tamilnadu.

Preparation of Extract

Flowers were shade dried and were finely powdered. The 150g of powdered material was dissolved with 250ml of 70% methanol and extract was prepared using soxhlet apparatus for 30-40 hours. The extract was filtered and concentrated on a water bath at temperature below 50° C to syrup consistency (yield: 12%). Then it was stored in refrigerated condition for further use.

Source of chemicals

All the chemicals and solvents used were of analytical grade and were procured from Ranbaxy Fine Chemicals Ltd., Mumbai, India

Experimental Animals

Healthy Wistar albino rats of male, weighing about 150-200g were obtained from Tamil Nadu Veterinary and Animal Science University, Chennai, India. Animals were maintained under standard conditions (12 h light / dark cycle; 25 ± 2° C with 65 ± 5% humidity) and were fed with standard rat feed (Sai Durga feeds and Foods, Bangalore, India) and water *ad libitum*. All the animals were acclimatized to laboratory conditions for a week before commencement of the experiment. The study was conducted at Srimad Andavan Arts and Science College, Trichy. All the experimental protocols were reviewed and approved by the Institutional Animal Ethical Committee (IAEC) prior to the initiation of the experiment and the care of the laboratory animals was taken as per the CPCSEA regulations (Registration Number: 790/03/ac/CPCSEA).

Experimental design

The animals were divided into 5 groups consisting of 6 animals in each group. Group I rats received saline (0.5 ml/kg b.wt) orally for 21 days. Group II rats administered with CCl₄ (0.5ml/kg b.wt) dissolved in olive oil (1:1 ratio) injected intraperitoneally for 21 days alternatively. Group III administered with CCl₄ treated with methanolic extract of *M.champaca* (300 mg/kg b.w) orally for 21 days. Group VI, the CCl₄ induced rats were treated with silymarin (25 mg/kg b.w) orally for 21 days. Group V rats were treated with methanolic extract of *M.champaca* alone (300 mg/kg/b.w) orally for 21 days. The animals were sacrificed at the end of the experimental period by cervical decapitation under mild anesthesia. Blood sample was collected in centrifuging tubes and allowed to clot for 45 min at room temperature. Serum was separated by centrifugation at 2500 rpm for 15 minutes.

Biochemical Estimation

The separated serum was used for the estimation of some biochemical parameters like AST and ALT were measured according to the method of Rietman and Frankel [13]. ALP was measured according to the method of Kind and King [14], and The Rosalki and Rau method is used for estimation of gamma glutamyl transferase (γ -GT) [15]. Also, measurement of Urea was done according to the method of Natelson [16]. The Brod and Sinota method [17] was used to evaluate the Creatinine levels. Bilirubin was measured according to the method of Malloy and Evelyn [18].

Statistical Analysis

Statistical analysis of the results was performed using one-way ANOVA followed by Duncan's Multiple Range Test (DMRT) using SPSS (Version 13, SPSS Inc., and Chicago, IL, USA). A value of $P < 0.05$ was considered

statistically significant between the measurements of the two compared groups. All values were reported as mean \pm SD.

RESULTS

The effect of methanolic extract of flowers of *Michelia champaca* on liver markers such as AST, ALT, ALP, and GGT is summarized in Table 1. There was a significant ($P < 0.05$) increase in AST, ALT, ALP and GGT levels in serum was increased in CCl_4 treated rats when compared to control. The methanolic extract of flowers of *M. champaca* treatments reversed the level of AST, ALT, ALP and GGT when compared to CCl_4 alone treated rats. Silymarin treated animals also showed significant ($P < 0.05$) decrease in AST, ALT, ALP and GGT levels when compared to CCl_4 treated rats. No significant statistical changes were distinguished in rats treated with *M. champaca* flower extract alone compared to that of control.

Table 1: Effect of methanolic extract of flowers of *Michelia champaca* on serum liver marker enzymes (AST, ALT, ALP and GGT) in CCl_4 treated rats

Treatment	AST (IU/L)	ALT (IU/L)	ALP IU/L)	GGT (IU/L)
Group I	12.73 \pm 2.97 ^a	29.20 \pm 6.89 ^{ab}	66.59 \pm 8.99 ^a	43.09 \pm 3.11 ^c
Group II	115.47 \pm 12.21 ^d	141.37 \pm 16.59 ^d	200.63 \pm 9.70 ^b	142.66 \pm 5.58 ^d
Group III	24.41 \pm 2.26 ^c	37.36 \pm 5.26 ^c	74.74 \pm 4.74 ^a	37.19 \pm 3.34 ^a
Group IV	20.41 \pm 2.12 ^{b,c}	34.18 \pm 2.85 ^{ab}	72.99 \pm 4.28 ^a	41.62 \pm 5.54 ^{ab}
Group V	14.15 \pm 3.51 ^{ab}	25.16 \pm 2.34 ^a	68.23 \pm 8.00 ^a	42.16 \pm 3.86 ^{ab}

Values are given as mean \pm S.D (n=6). Values not sharing a common superscript letter significantly at ($p < 0.05$) (DMRT)

Table 2 shows the effect of methanolic extract of flower parts of *Michelia champaca* on renal markers in CCl_4 induced toxicity. Administration of CCl_4 significantly ($P < 0.05$) increased the levels of urea, creatinine and bilirubin when compared to control group of rats. The serum Urea, creatinine and bilirubin were significantly ($P < 0.05$) decreased in CCl_4 with methanolic extract of flowers of *M. champaca* treated rats (Group III) and as well as standard drug (Group IV) compared with CCl_4 treated rats (Group II). There was no significant deviation changes observed in rats treated with *M. champaca* alone compared to that of control group of rats (Group V).

Table 2: Effects of methanolic flower extract of *Michelia champaca* on renal markers (Creatinine and Urea and Total Bilirubin) in CCl_4 treated rats

Treatment	Urea (mg/dl)	Creatinine (mg/dl)	Total bilirubin (mg/dl)
Group I	33.05 \pm 3.92 ^a	0.79 \pm 0.02 ^a	0.83 \pm 0.14 ^a
Group II	55.12 \pm 7.52 ^c	1.87 \pm 0.02 ^c	1.12 \pm 0.19 ^d
Group III	40.50 \pm 3.54 ^b	1.25 \pm 0.02 ^b	0.90 \pm 0.33 ^{ab}
Group IV	39.54 \pm 3.09 ^b	1.33 \pm 0.09 ^b	0.80 \pm 0.09 ^a
Group V	38.21 \pm 3.14 ^b	0.80 \pm 0.02 ^a	0.75 \pm 0.04 ^a

Values are given as mean \pm S.D (n=6). Values not sharing a common superscript letter significantly at ($p < 0.05$) (DMRT)

DISCUSSION

Carbon tetrachloride (CCl_4), an industrial solvent, a well-established hepatotoxin, it was demonstrated that liver is not the only target organ of CCl_4 but it causes free radical generation in other tissues also such as kidneys, heart, lung, testis, brain and blood in various studies by researchers [19-22]. CCl_4 gets converted to halogen free radical by hepatic cytochrome P_{450} [23]. It has also been reported that exposure to CCl_4 induces acute and chronic renal injuries. Such case control studies and various documented case reports increasingly establish that hydrocarbon solvents produce renal diseases in humans.

Of all the macromolecules that leak from the damaged tissues, enzymes, because of their tissue specificity and catalytic activity are the best markers of tissue damage [24]. Determination of the activity of hepatic enzymes released into the blood by the damaged liver is one of the most useful tools in the study of hepatotoxicity [25]. The specific and non specific biochemical parameters which were known to be altered by hepatotoxins were measured as markers for evaluating the hepatoprotective activity of many drugs [26].

Normally, AST and ALP are present in high concentrations in liver. Due to hepatocyte necrosis or abnormal membrane permeability, these enzymes are released from the cells and their levels in the blood increase. ALT is a sensitive indicator of acute liver damage, and elevation of this enzyme in non-hepatic diseases is unusual. ALT is more selectively a liver parenchymal enzyme than AST [27]. Assessment of liver function can be made by estimating the activities of serum ALT, AST, ALP and bilirubin, which are enzymes originally present in higher concentrations in cytoplasm. When there is hepatopathy, these enzymes leak into the bloodstream in conformity with the extent of liver damage [28].

Total bilirubin, a byproduct of the breakdown of red blood cells in the liver, bilirubin is a good indicator of liver function. High levels will cause icterus and are indicative of damage to the liver and bile duct [29].

Elevation of urea and creatinine levels may indicate diminished ability of the kidneys to filter these waste products from the blood and excrete them in urine. The effective control of total bilirubin levels by flower extract indicating its protective effect over liver and improvement in its functional efficiency [30]. Based on the findings, the flower extract of *Michelia champaca* may enhanced the ability of the kidneys to remove these waste products from the blood as indicated by reduction in serum urea and creatinine levels and confer protective effect on the kidney [31].

On the basis of the present investigation was observed as the flower extract of *Michelia champaca* shows a significant hepatoprotective activity against CCl₄ induced liver injury in rats. Further studies are needed to isolate active principles and also to evaluate the exact mechanism of action for liver diseases.

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