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BIO EFFICACY OF *MICHELIA CHAMPACA* LINN ON MEMBRANE BOUND ENZYMES IN CCl₄ INDUCED HEPATOTOXICITY IN RATS

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ABSTRACT: The aim of the present study is to evaluate the protective effect of flower extract of *Michelia champaca* on membrane bound enzyme (serum ATPase) levels on male albino rats. The membrane bound enzymes (Ca²⁺ ATPase, Mg²⁺ ATPase, Na+K+ ATPase) levels of methanolic flower extract of *Michelia champaca* at a dose of 300 mg/kg was evaluated during exposure to CCl₄. There was a significant decrease in membrane bound enzymes such as Ca²⁺ ATPase, Mg²⁺ ATPase, Na+K+ ATPase was observed in CCl₄ treated rats. Therapeutic treatment with plant extract has significantly ameliorated to near normalcy in the curative group. These results of the study concluded that *Michelia champaca* was found to be effective in preventing the biochemical abnormalities caused by toxins.

INTRODUCTION: *Michelia champaca* L. (Magnoliaceae) commonly known as Yellow champaca. Medium sized evergreen tree with yellow fragrant blossoms. Leaves ovate-lanceolate, coriaceous, glabrescent. Flowers solitary, axillary, pale yellow. Fruit an aggregate of follicles¹. *M. champaca* is used in the treatment of fever, colic, leprosy, eye disorder, inflammation, cough rheumatism, gonorrhoea, cephalgia and gout^{2,3}. It has been used in India for the treatment of abdominal tumour⁴. The plant is also reported to have significant wound healing⁵, antimicrobial⁶, antidiabetic⁷, antitumour⁸ anti-inflammatory⁹, antioxidant¹⁰, and anti infective¹¹ properties.

The aim of the study was designed to evaluate the curative effect of the methanolic extract of *Michelia champaca* flowers on the membrane bound enzymes (Na+K+ ATPase, Ca²⁺ ATPase and Mg²⁺ ATPase) in CCl₄ induced hepatotoxicity in rats.

MATERIALS AND METHODS:

Collection and extraction of Plant Material:

The *Michelia champaca* flowers were collected and botanically identified by the botanist in Rapinet Herbarium of St. Joseph's College, Tiruchirapalli. The herbarium specimens was preserved for further reference (Voucher No.001). The flowers were chopped into small pieces, shade-dried and coarsely powdered. 150g of the powdered material was dissolved with 250ml of 70% methanol and the extract was obtained using soxhlet apparatus. The extract was filtered and concentrated at temperature below 50° C to syrup consistency (yield: 12%).

Experimental Animals: Healthy adult wistar strain of albino rats of both sexes, weighing 150-

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200g were obtained from Tamil Nadu Veterinary and Animal Sciences University, Chennai, India. The animals were allowed to acclimatize to laboratory conditions for a period of 5 days to the experiment. Animals were housed in standard polypropylene cages and maintained under standard condition of 12-hours light/dark cycle and at $23 \pm 2^\circ \text{C}$ with $65 \pm 5\%$ humidity. Animals were fed with standard rat chow pellet obtained from Sai Durga feeds and Foods, Bangalore, India and water *ad libitum*. The study was approved by Institutional Animal Ethical Committee (IEAC) constituted for the purpose of CPCSEA, Government of India (Approval No: 790/03/ac/CPCSEA).

Experimental Design:

The rats were randomized into five groups comprising 6 animals each. Group I served as normal control, which received 0.5ml/kg b.wt of normal saline orally for 21 days. Rats of Group II received CCl₄/olive oil (1:1 v/v, 0.5ml/kg,ip) for 21 days. Group III rats were administered a single daily dose of methanolic extract of the flowers of *Michelia champaca* (300mg/kg b.wt p.o) for 21 days. Group IV, standard group was administered a single daily dose of Silymarin (25mg/kg,po) for 21 days. Rats were administered CCl₄ with Silymarin at a dose of 25mg/kg b.wt p.o. Group V rats were administered a single daily dose of methanolic extract of the flowers of *Michelia champaca* (300mg/kg b.wt p.o) alone for 21 days.

After the experimental period, the animals were sacrificed by cervical dislocation. Blood was collected and used for biochemical estimations. Liver tissues were homogenized, in 0.1M phosphate buffer at pH 7.4. The liver tissue was used to estimate Na⁺/K⁺ ATPase¹², Ca²⁺ ATPase¹³ and Mg²⁺ ATPase¹⁴, in which the liberated phosphate was estimated spectrophotometrically.

Statistical Analysis:

The data were expressed as mean \pm SD, (n=6). They were further analyzed using One way analysis of Variance (ANOVA) with the help of SPSS and the group of means compared by Duncan's Multiple Range test (DMRT). Values of $P \leq 0.05$ were considered statistically significant.

RESULTS:

The membrane bound enzymes levels in all the group of animals are shown in **Table 1**. The activity of membrane bound enzyme in liver homogenates of CCl₄ -induced animals (Group II) was significantly ($P < 0.05$) lower than that of group I. Treatment of the animals with the methanolic flower extract of *Michelia champaca* (Group III) and Silymarin (Group IV) significantly ($P < 0.05$) increased the membrane bound enzymes level compared to the CCl₄ -induced animals (Group II). The enzymes level in methanol extract alone treated animals (Group V) was almost close to control animals (Group I).

TABLE 1: EFFECT OF METHANOLIC EXTRACT OF MICHELIA CHAMPACA ON MEMBRANE BOUND ENZYMES IN CONTROL AND EXPERIMENTAL ANIMALS

Groups	Na ⁺ / K ⁺ ATPase	Ca ²⁺ ATPase	Mg ²⁺ ATPase
Control	43.15 \pm 1.67 d	30.92 \pm 1.24 c	42.12 \pm 0.59 c
CCl ₄	12.96 \pm 1.06 a	7.59 \pm 0.91 a	8.75 \pm 0.52 a
CCl ₄ + Plant extract (300mg/Kg)	33.77 \pm 2.14 b	26.33 \pm 1.81 b	36.13 \pm 1.01 b
Silymarin (25mg/Kg)	40.12 \pm 1.68 c	27.20 \pm 1.80 b	37.68 \pm 0.88 c
Plant extract alone (300mg/Kg)	44.03 \pm 2.78 d	29.76 \pm 1.40 c	39.38 \pm 1.88 d

Values are expressed as mean \pm SD for six animals, values not sharing common superscript letters differ significantly at $P < 0.05$. Units – Na⁺/K⁺ ATPase, Ca²⁺ ATPase and Mg²⁺ ATPase - μg of Phosphorus liberated/min/ g tissue

DISCUSSION: Membrane Na⁺ K⁺ ATPase play an important role in active transport of Na⁺ and K⁺ ions across the plasma membrane¹⁵. The enzyme Na⁺ K⁺ ATPase utilizes the energy derived from ATP hydrolysis to pump out Na⁺ from inside the cell to transfer K⁺ from outside to cytosol. Its activity has been frequently used as a marker for

plasma membranes and has been followed as a probe for monitoring membrane integrity alteration in the physical state of various biological membranes¹⁶. Na⁺ K⁺ ATPase, Mg²⁺ ATPase and Ca²⁺ ATPase in the plasma membrane keep the intracellular sodium low but intracellular

magnesium and potassium high when compared with the levels in extracellular fluids¹⁷.

Increased oxidative stress in high-fat fed rats might have contributed to the decreased Ca²⁺, Mg²⁺-ATPase activity¹⁸. Reduction in the activity of these enzymes might be due to enhanced lipid peroxidation by free radicals. Arsenic enhances the production of free radicals in the liver of rat and interferes with the antioxidant defence system leads to the alteration of structural integrity of membrane lipids and secondarily affects the membrane bound enzymes. In the present study, reduced activity of Mg²⁺ ATPase and Na⁺/K⁺ ATPase are responsible for ionic imbalance caused by As which damages the membranous lipids. Moreover, Arsenic decreased the activity of Ca²⁺ ATPase observed in the result due to high affinity for SH groups. This could be due to the ability of THC to protect the sulfhydryl group from oxidative damage through the inhibition of lipid peroxidation by scavenging of free radicals¹⁹. Interestingly, the restoration of membrane bound enzymes supported by the restoration of serum marker enzymes

CONCLUSION: In conclusion, the present study suggests that *M.champaca* prevented the CCl₄ induced oxidative stress and cellular damage to that liver tissue. Further studies are needed to elucidate the exact mechanism of action of *Michelia champaca* and its pharmacological application.

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