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Effect of *Portulaca quadrifida* Linn on Mercury-Induced Hepatotoxicity In Swiss Albino Mice.

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Research Article

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ABSTRACT

Portulaca quadrifida Linn. has been evaluated for its hepatoprotective activity in Swiss albino mice using the mercury induced hepatotoxicity. The ethanolic extract showed remarkable activity against mercury-induced hepatotoxicity as judged from biochemical parameters such as serum aspartate amino transferase (AST), alanine amino transferase (ALT), alkaline phosphatase (ALP) and levels of lipid peroxides (LPO) in liver. Animals treated with *P. quadrifida* extract before and after mercury intoxication showed a significant decrease in LPO level, AST and ALT activities and increase in ALP activity and glutathione (GSH) content. The extract treatment alone did not alter the biochemical parameters. The results suggest that oral administration of *P. quadrifida* provide protection against mercury induced toxicity in Swiss albino mice, but it was dose dependent, which was comparable to the control.

INTRODUCTION

Mercury is a transition metal, it promotes the formation of reactive oxygen species (ROS) such as hydrogen peroxides. These ROS enhances the subsequent iron and copper-induced production of lipid peroxides and the highly reactive hydroxyl radical [1,2,3]. These lipid peroxides and hydroxyl radical may cause the cell membrane damage and thus destroy the cell. Mercury also inhibits the activities of the free radical quenching enzymes catalase, superoxide dismutase and glutathione peroxidase [4]. Exposure to mercury cannot be avoided since it is being widely used in the industrial, medical, agriculture and other fields. Thus it is important to develop an effective drug to provide protection against mercury-induced toxicity. Several naturally occurring dietary and non-dietary constituents and parts of several species of edible plants having pharmacological activity, influence the antioxidant enzymes and provide protection against free radical induced damage.

Portulaca quadrifida Linn. (portulacaceae)a prostrate fleshy annual or stoloniferous perennial herb with somewhat base but sometimes with simple main stems, 5-40 cm tall and generally widespread in warm countries. It is a small diffused, succulent, annual herb found throughout the tropical parts of India. It is used as a vegetable and also used for various curative purposes. It is said to be useful in asthma, cough, urinary discharges, inflammations and ulcers. A poultice of the plant is applied in abdominal complaints, erysipelas and haemorrhoids [5]. *Portulaca quadrifida* Linn. has been reported to possess antifungal activity against *Aspergillus fumigates* and *Candida albicans* [6] and the neuropharmacological activities were reported by Syed *et al* [7]. A review of literature afforded no information on the hepatoprotective aspects of this plant. So the present study is therefore an attempt to assess the efficacy of this indigenous herb for its hepatoprotective activity against mercury induced toxicity model in mice.

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MATERIALS AND METHODS

Plant material

The aerial parts of the plant were collected from the foothill of Yercaud, Salem, in the month of June 2013 and cleaned to remove the debris. The collected plant was identified and authenticated by a botanist Dr. A. Marimuthu, Department of Botany, Government Arts College, Attur. A voucher specimen (PQM-1) has been kept in our museum for future reference. The plant parts were dried at room temperature for 10 d and coarsely powdered with the help of a hand-grinding mill and the powder was passed through sieve No. 60.

Preparation of the extract

The powder of aerial parts of *P. quadrifida* was extracted separately by continuous hot extraction process using soxhlet apparatus with different solvents in increasing order of polarity from petroleum ether, chloroform, acetone, alcohol, to finally chloroform:water. After extraction, the extracts were concentrated under reduced pressure in tared vessel. The marc of crude drug powder was then once again subjected to successive extraction with other solvents and the extractive values were calculated with reference to the air-dried drug. The dry extracts were subjected to various chemical tests to detect the presence of different phytoconstituents.

Test animals

Swiss albino mice of either sex and of approximately the same age, weighing about 20-25 g were used for the study. They were housed in polypropylene cages and fed with standard chow diet and water *ad libitum*. The animals were exposed to alternate cycle of 12 h of darkness and light each. Before each test, the animals were fasted for atleast 12 h. The experimental protocols were subjected to the scrutinization of the Institutional Animal Ethics Committee and were cleared by the same.

Hepatoprotective study

For determining the hepatoprotective activity animals were divided into four groups containing 6 animals each. Group I served as control and received orally 1 ml of propylene glycol daily for 7 consecutive days. Group II was treated with ethanolic extract of *P.quadrifida* (200 mg/kg, p.o.) for 7 days. Group III was served as positive control and received Mercuric chloride (5 mg/kg) in normal saline through i.p. for 7days [8]. Group IV was treated with ethanolic extract of *P.quadrifida* (200 mg/kg, p.o.), before mercuric chloride administration and until 7 days of mercuric chloride administration. The animals were autopsied at 7th day after mercuric chloride administration. The liver was excised and processed for GSH [9] and LPO [10] estimation. Blood from autopsied animals were collected by cardiac puncture and the biochemical parameters such as ALT [11], AST [12] and ALP [13] were estimated as reported earlier. A small portion of liver was cut from the animals from each group and preserved in neutral buffered formalin and was processed for paraffin embedding, following the standard microtechnique [14]. 5 μ section of the livers stained with alum haemotoxylin and eosin and studied for degenerative and necrotic changes.

Statistical analysis

All values were expressed as mean±SEM. The data were statistically analyzed using one way ANOVA followed by Newman Keul's multiple range test and differences below $P<0.05$ are considered as significant.

RESULTS

The plant *P.quadrifida* was collected from the foothill of Yercaud, Salem, air-dried and extracted by continuous hot extraction process using soxhlet apparatus. The average percentage yield of ethanolic extract of *P.quadrifida* was found to be 3.8 % w/w. The LD_{50} was found to be 2000 mg/kg for ethanolic extract of *P.quadrifida*.

The ethanolic extract did not exhibit and toxic effects up to 1000 mg/kg when administered to mice as a single i.p. dose. The results of biochemical parameters revealed to the elevation of enzyme level in mercury chloride treated group indicating that mercury induces damage to the liver. Mercuric chloride induces pathological changes in the liver such as cytoplasmic vacuolization, karyohexsis, karyolysis, pycnosis and centrilobular necrosis. However, in group IV reduced cytoplasmic vacuolization and centrilobular necrosis were observed. Animals treated with ethanolic extract of *P.quadrifida* before and after mercury intoxication showed a significant decrease in LPO level, AST and ALT activities and increase in ALP activity and GSH content (Table 1). The enzyme levels were almost restored to the normal. So the animals treated with ethanolic extract of *P.quadrifida* exhibited statistically significant ($P<0.05$) protection against mercury-induced hepatotoxicity in mice, which is comparable to the control. The histopathological studies support the biochemical findings. Hepatotoxicity induced by mercury manifested itself

by the 7th d with the liver showing massive degeneration enveloping the not so visible necrotic areas as compared to the normal.

Table 1: Effect of ethanolic extract of *P.quadrifida* on Mercury induced hepatotoxicity in mice

Treatment	Dose mg/kg, p.o.	AST U/ml	ALT U/ml	ALP KAU	GSH μ mole/g	LPO content n mole of MDA/mg
Control	1 ml	21.50±1.55	14.33±0.82	6.10±0.05	95.33±1.79	4.62±0.03
P.quadrifida	200	21.65±1.22	13.12±0.70	6.77±0.12	99.83±3.33	3.33±0.17
Mercuric chloride	5	52.67±2.63	45.67±2.65	2.62±0.10	42.67±1.40	16.93±0.60
P.quadrifida + Mercuric chloride	200	24.50±1.22*	22.83±1.57*	4.76±0.17*	69.83±0.90*	7.33±0.26*

**P*<0.05 when compared with control. Number of individuals used=6 in each group. Days of drug treatment=7. Values are expressed as mean±S.E.M

DISCUSSION

Mercury intoxication showed a significant increase in ALT and AST levels. The increase in ALT and AST in serum may be due to hepatocellular necrosis, which causes increase in permeability of the cell membrane resulting in the release of transaminases in the blood stream [15, 16]. Further, there was a decrease in the serum alkaline phosphatase activity after mercuric chloride intoxication. In the liver it is closely connected with lipid membrane in the canalicular zone, so that any interference with the bile flow, whether extra hepatic or intra hepatic leads to decrease in serum alkaline phosphatase activity [16]. Mercury causes cell membrane damage (lipid peroxidation), which leads to the imbalance between synthesis and degradation of enzyme protein [17], thus lowering the enzyme activity. Present findings are in agreement with the findings of El-Demerdash [18], who showed that mercuric chloride (0.5 μ mol/ml) intoxication significantly decreases the alkaline phosphatase activity in rats.

GSH is a major thimol, which binds electrophilic molecular species and free radical intermediates. It plays a central role in the antioxidant defence system, metabolism and detoxification of exogenous and endogenous substances [19, 20]. Mercury has high affinity for GSH and causes the irreversible excretion of up to two GSH tripeptides [21]. The metal-GSH conjugation process is desirable in that it results in the excretion of the toxic metal in to the bile. However, mercury can deplete the GSH from the cell and decrease the antioxidant potential. In the present investigation it was observed that mercuric chloride treatment significantly reduced the GSH content thus reducing the antioxidant potential and accelerating the lipid peroxidation, resulting in cellular damage.

The efficacy of any hepatoprotective drug is dependent on its capacity of either reducing the harmful effect or maintaining the normal hepatic physiology, which has been disturbed by a hepatotoxin. The extracts decreased mercury induced elevated levels of the enzymes in group IV, indicates the production of structural integrity of hepatocytic cell membrane or regeneration of damaged liver cells by the extracts.

So, the result of present investigation indicates that the ethanolic extract of *P.quadrifida* possess good hepatoprotective activity. Further investigations are required to characterize the active hepatoprotective principle and its mechanism of action.

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