PREVENTIVE AND CURATIVE EFFECTS OF CALENDULA-OFFICINALIS LEAVES EXTRACT ON ACETAMINOPHEN-INDUCED HEPATOTOXICITY

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ABSTRACT

Objective: *To asses the hepatoprotective potential of leaves of Calendula officinalis against experimentally produced liver damage in animals using acetaminophen as model hepatotoxin.*

Material and Methods: *This study was conducted at Department of Pharmacy, University of Peshawar. Preliminary experiments were performed in mice to estimate the protective effect of plant material against lethal dose of acetaminophen (1gm/kg). Acute toxicity of plant material up to a dose of 3 gm was assessed in mice to note any behavioural changes and mortality. Hepatic damage in rats was induced by oral acetaminophen (640 mg/kg). The effect of methanolic extract of leaves of Calendula officinalis was investigated against acetaminophen-induced hepatic damage in 30 male, albino rats.*

Results: *Acetaminophen produced 100% mortality at a dose of 1 gm/kg in mice, while pretreatment of mice with Calendula officinalis (1.0 gm/kg) reduced the death to 30%. Pretreatment of rats with leaves extract (500 mg/kg orally, four doses at 12 hours interval) prevented ($p< 0.05$) the acetaminophen (640 mg/kg) induced rise in serum transaminase (GOT, GPT), serum bilirubin and serum alkaline phosphatase. Post treatment with three successive doses of leaves extract (500 mg/kg, 6 hourly) restricted the hepatic damage induced by acetaminophen ($P<0.05$).*

Conclusion: *These results indicate that the crude extract of Calendula officinalis leaves exhibits hepato-protective action.*

Key Words: *Calendula-Officinalis, Acetaminophen, Hepatotoxicity.*

INTRODUCTION

Calendula officinalis (locally known as Gul-e-Ashrafi) belongs to Composite family. It is an annual herb widely cultivated in Pakistan and India in the months of February and March as an ornamental plant. The plant material (leaves and flower) is considered to be an indigenous source of medicine. The flowers are used as stimulant, antiseptic and emmenagogue, while the leaves are used as resolvent and diaphoretic.[1] The plant can relieve constipation and colic.[2] The tincture made from the leaves and flowers is useful in the treatment of jaundice due to liver damage.[3]

It contains salicylic acid, a bitter substance, Calendulin and traces of an essential oil.[4] The present work was undertaken in an attempt to asses its hepatoprotective potential against experimentally produced liver damage in animals using acetaminophen as model hepatotoxin.

MATERIAL AND METHODS

i. PLANT MATERIAL

Calendula officinalis was grown in the winter season in the gardens in front of the Department of Pharmacy, University of Peshawar and at PCSIR Laboratory, Peshawar. The plant was authenticated with the help of a taxonomist. The leaves were shade dried, powdered, and macerated in 80% methanol-(BDH Ltd. Pole, England) for one week with occasional shaking. The extract was filtered and concentrated to a dark green residue under reduced pressure on a rotary evaporator, with an approximate yield of 12%.

ii. PHARMACOLOGICAL MATERIALS

a) Acetaminophen (Welcome Pakistan Ltd.)

b) Normal saline (0.9 % NaCl)

c) Ketamine hydrochloride (Medimpox Budapest, Hungary)

d) Methylcellulose (Sigma chemicals company, St. Louis. Mo. USA)

e) Acetaminophen and plant materials were suspended in 1% melthylcellulose.

iii. ANIMALS

a) Swiss male mice (20-25 gm)

b) Male Albino rats (200-250 gm)

The animals were housed in cages and they had free access to tap water and food.

EXPERIMENTAL PROCEDURES

1. LETHALITY STUDY IN MICE

Preliminary experiments were performed in mice to estimate the protective effect of plant material against lethal dose of acetaminophen (1gm/kg). Animals were divided into 2 groups having 10 animals in each group. One group was given leaves extract orally (1.0 gm/kg) followed by oral administration of acetaminophen one hour after plant material. The second group served as control and received the same treatment except that normal saline (0.9% NaCl) was given instead of plant extract. The mortality was observed for 24 hours post administration of acetaminophen.

2. ACUTE TOXICITY OF PLANT MATERIAL

Thirty mice divided into six groups (A, B, C, D, E, F) each having 5 animals were given the leaves extract orally at a dose of 0.5 gm, 1.0 gm, 1.5gm, 2.0 gm, 2.5 gm and 3.0 gm/kg respectively and were kept under constant observation for 6 hours to note any behavioural changes and mortality was recorded after 24 hours of plant administration.

3. LIVER FUNCTION STUDY IN RATS:

a. INDUCTION OF HEPATIC INJURY

Hepatic damage in rats was induced by acetaminophen, (640 mg/kg) administered orally, whereas control animals received an equal volume of vehicle (1% methylcellulose).

b. MULTIPLE-DOSE PRE-TREATMENT IN RATS

Animals were divided into three groups (AB & C) each having 10 male, albino rats. Group A, served as vehicle control and received normal saline (0.9% NaCl, 10ml/kg) and vehicle (1% methylcellulose 13 ml/kg body weight). Group B received four doses of normal saline at 12 hour interval and acetaminophen (640 mg/kg) was administered orally 1 hour post treatment of the last dose. Group C was treated as group B, except that leaves extract of Calendula officinalis (500mg/kg) was administered orally, instead of saline.

Animals were anaesthetized by intramuscular administration of Ketamine HCl (100 mg/kg) 24 hours after the last treatment. Blood (3 ml) was collected by cardiac puncture using sterile disposable syringes, Serum was separated by centrifugation (3000 rpm for 15 minutes) and S. Bilirubin SGOT, SGPT and Alkaline Phosphatase (ALP) were estimated on the same day spectrophotometrically using diagnostic kits.

c. MULTIPLE-DOSE POST TREATMENT IN RATS

In this series of experiments, the curative effect of the plant extract was evaluated. Rats were divided into groups E. F. and G having 10 animals each. Group E served as vehicle control received vehicle 1% methylcellulose followed by three successive doses of normal saline (10ml/kg). Groups F was given acetaminophen (640 mg/kg) orally at O hour and then after every 6 hours three successive doses of normal saline (10 ml/kg) were administered orally, Group G was treated similar to that of group F, except that leaves extract of Calendula officinalis (500 mg/kg) was substituted for saline. All other procedures i.e. blood collection, serum separation and Bilirubin, SGOT, SGPT and Alk, phosphatase estimation were performed at 24 hours as mentioned above.

Statistical analysis

The results were expressed as means ± SEM and all statistical comparisons were made by means of student "t" test and $P< 0.05$ was regarded as significant.

RESULTS

1. EFFECT ON ACETAMINOPHEN INDUCED LETHALITY

Acetaminophen at a dose of 1 gm/kg induced 100% lethality in mice, but in animals treated with the leaves extract of Calendula officinalis before acetaminophen administration, the number of deaths was 3 out of 10, resulting in 70% protection against lethal effect of acetaminophen.

2. ACUTE TOXICITY OF PLANT

No behavioural changes were observed in the animals and all of them were alive after 24 hours, meaning thereby that the plant material was found safe upto an oral dose of 3.0 gm/kg.

3. PREVENTIVE EFFECT ON HEPATOTOXICITY

In a study pertaining to assess the

protective effect of Calendula officinalis leaves extract on acetaminophen-induced hepatotoxicity, the control (saline + vehicle) values of S. Bilirubin, SGOT, SGPT and serum Alk, Phosphatase (ALP) in rats were found to be 0.52 ± 0.0467 mg/dl, 54.5 ± 1.45, 34.0 ± 1.55 and 207.2 ± 4.33 (I.U/L (n=10) respectively, while a toxic dose of acetaminophen (640 mg/kg) significantly raised ($p< 0.05$) the respective serum values to 2.34 ± 0.0733 mg/dl, 246.3 ± 6.46, 247 ± 4.61 and 393 ± 7.54 IU/L in group B. In a group of animals treated with leaves extract, the values of S. Bilirubin, SGOT, SGPT and serum ALP were found to be 1.08 ± 0.01 mg/dl, 87 ± 1.70, 80±3.16 and 211.8 ± 4.87 IU/L respectively, which are lower ($p< 0.05$) than the values of toxic group.

4. CURATIVE EFFECT ON HEPATOTOXICITY

The post treatment effect of leaves extract on acetaminophen-induced hepatic damage in rats was also determined by the same parameters and the estimated control values of serum bilirubin SGOT, SGPT and serum ALP were 0.52 ± 0.0467 mg/dl, 54.5 ± 1.45, 34.0 ± 1.55 and 207.2 ± 4.33 IU/L respectively (n=10), while the acetaminophen intoxication raised significantly ($p<0.05$) the respective serum values to 2.55 ± 0.099 mg/dl, 152±6.84, 126±3.64, 318±4.78 IU/L. Group G animals were treated with leaves extract and the respective value of S. Bilirubin, SGOT, SGPT and serum ALP were found to be 1.1±0.051 mg/dl, 88.6±3.97, 66.5±2.13 and 221.3±3.5 IU/L, which were significantly lower ($p<0.05$) than the serum values of the toxic group and comparable to the normal values.

DISCUSSION

Liver injury by acetaminophen and carbon tetrachloride are commonly used models for the screening of hepatoprotective drugs.[5,6] The rise in serum levels of transaminases (GOT and GPT) and ALP has been attributed to the damaged structural integrity of the liver,[7] because these are cytoplasmic in location and released into circulation after cellular damage.[8] Acetaminophen is converted to its reactive metabolite N-Acetyl-p-benzoquinoneimine (NAPQI) by specific isoenzymes of cytochrome p-450.[9] Physiologically important protective mechanisms involving both vitamin E (á-tocopherol) and glutathione are available to curtail progression of cellular damage.[10] However, the massive production of reactive metabolites may lead to depletion of protective moieties ensuring widespread propagation of alkylation as well as peroxidation causing damage to both lipids and proteins present in bio-membranes of microsome and mitochondria.[11,12]

The methanolic leaves extract of Calendula officinalis, when administered prophylactically exhibited protection against acetaminophen-induced liver injury, as manifested by the reduction in toxin mediated rise in serum bilirubin, SGOT, SGPT and serum ALP in rats as well as protection against the lethal dose of acetaminophen in mice. One of the possible mechanisms of hepatoprotection is through microsomal drug metabolizing enzyme (MDME) inhibition. The inhibition of MDME can impair the bioactivation of acetaminophen into its reactive metabolites and hence provide protection against the prevailing hepatocellular damage.[13,14] It is also known that MDME inhibitory activity is common in medicinal plants.[15] The inhibitors of MDME can provide protection against the hepatotoxicity only when they are given before the metabolic activation of hepatotoxin (acetaminophen) and are unable to provide any protection after generation of the reactive metabolites. The calcium content in the hepatocytes are increased during the process of experimental hepatic damage[16,17] and calcium ion channel blocking drugs i.e. nifedipine, diltiazim and verapamil were found to inhibit the development of hepatic damage induced by different hepatotoxins including acetaminophen.[18,19] This is another possible mechanism of hepatoprotection. The hepatoprotection may also be due to the presence of anti-oxidants in medicinal plants as these antioxidants inhibit the covalent binding of NAPQI, to vital macromolecules[20] and consequently can minimize toxic damage. The acetaminophen toxicity following NAPQI generation is chiefly due to oxidative stress and can effectively be ameliorated by antioxidant.[21] The exact mode of hepatoprotective action of the plant Calendula officinalis may be speculative at this stage and whether the hepatoprotective action is mediated through inhibition of MDME, presence of certain antioxidant and/or calcium ion channel blocking activity, needs further investigation. The plant material is safe as is obvious by the lack of any symptoms of acute toxicity at and oral dose of as high as 3.0 mg/kg. This study, thus, provides scientific basis for the traditional use of Calendula officinalis leaves in hepatobiliary diseases.

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