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Studies on the Protective Effect of Calendula-officinalis Leaves Extract on Paracetamol-induced Hepatotoxicity.

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SUMMARY

The hepatoprotective activity of methanolic extract of calendula officinalis leaves was investigated against paracetamol-induced hepatic damage.

Paracetamol, produced 100% mortality at a dose of 1 gm/kg in mice, while pre-treatment of mice with plant material (1.0gm/kg) reduced the death ate to 30%. Paracetamol at a dose of 640 mg/kg produced liver damage in albino rats, which was manifested by the rise in serum levels of Bilirubin, glutamic oxaloacetic transaminase (GOT), glutamic pyruvic transaminase (GPT), and alkaline phosphatase (ALP) to 2.34±0.0733 mg/dl, 246.3±6.46, 274±4.61 and 393±7.54 IU/l (n=10) respectively, compared to respective control values of 0.52±0.0467 mg/dl, 54.5±1.45, 34±1.55 and 207.2±4.33 IU/l. Rats were treated with calendula officinalis leaves extract (500mg/kg) orally (four doses at 12 hours interval) and then paracetamol was given (640 mg/kg) one hour after the last dose of plant extract. This pretreatment significantly lowered (P< 0.05) the respective serum Bilirubin, GOT, GPT and ALP levels to 1.08±0.011 mg/dl, 87±1.70, 80±3.16 and 211.8±4.87 IU/l. These results indicate that the calendula officinalis leaves possesses hepatoprotective activity and can be used in hepatic disorders.

INTRODUCTION

Calendula officinalis (Gul-e-Ashrafi) belongs to composite family. It is an annual herb with fibrous roots. Natives of France and southern European countries generally cultivated the plant. It is now widely cultivated in Pakistan and India[1].

The flowers have a folkloric reputation as stimulant, antiseptic and emmenagogue, while the leaves are used as resolvent and diaphoretic[2]. The plant can relieve constipation and colic[3], while flowers can relieve pain and swelling when rubbed over the affected part[4]. A tincture made from the leaves and flowers is useful in the treatment of jaundice due to liver damage[5]. Calendox cream and soap (5% extract of calendula officinalis by Phar-Man laboratories, Karachi), is useful for cuts, abrasions, burns, sunburn and Ichthyosis[6].

Scientific studies on its usefulness in liver damage are lacking. In the present study, the plant extract (leaves) was tested against paracetamol - induced liver damage to validate the folkloric use of calendula officinalis in hepatic damage.

MATERIAL AND METHODS

Plant material

Calendula officinalis was grown in the months of Feb & March in the gardens in front of pharmacy department and at PCSIR laboratories Peshawar and authenticated with the help of a taxonomist at PCSIR laboratories Peshawar. The leaves were powdered and macerated in 80% methanol (BDH Ltd,. Poole, 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%.

Pharmacological materials

1. Paracetamol (Wellcome Pakistan Ltd)
2. Ketamine hydrochloride (Medimpox, Budapest, Hungary).
3. Methylcellulose (Sigma chemicals Company, St. Louis, Mo, USA).
4. Paracetamol and plant material were suspended in 1% methylcellulose.

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 on mice to estimate the protective effect of plant material against lethal dose of paracetamol (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 paracetamol one hour after the 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 paracetamol.

2. **Acute toxicity of plant**
   Thirty mice, divided into six groups (A, B, C, D, E and F) having 5 animals per group were used in this study. Groups A, B, C, D, E and F animals, were given the plant extract orally at a dose of 0.5gm, 1.0gm, 1.5gm, 2.0gm, 2.5gm, and 3.0gm/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 functions study**
   Hepatic injury in albino rats was induced by paracetamol (640mg/kg). Rats were divided into 3 groups having 10 animals each. Group A, served as a control, received normal saline (10 ml/kg) and vehicle (1% methylcellulose; 13ml/kg, orally). Group B was given 4 doses of normal saline orally at 12 hours intervals and paracetamol was given orally 1 hour post-treatment of the last dose of saline. Group C was given 4 doses of the plant extract (500mg/kg) orally and paracetamol was given orally 1 hour post-treatment of the last dose of plant material.
   Animals were anesthetized with ketamine Hcl (100mg/kg IM) 24 hours after the last treatment and blood (3ml) was collected by cardiac puncture using sterile disposable syringes. Serum was separated by centrifugation (3000 r.p.m for 15 minutes) and serum Bilirubin, GOT, GPT and ALP were estimated on the same day spectrophotometrically using diagnostic kits.
   Statistical analysis: The results are expressed as mean$\pm$S.E.M. All statistical comparisons were made by means of student's "t" test and $P < 0.05$ was regarded as significant.

RESULTS

Lethality study in mice

Paracetamol in a dose of 1gm/kg induced 100% lethality in mice, but in animals treated with the leaves extract of calendula officinalis before paracetamol the number of deaths were 3 out of 10, resulting in 70% protection against lethal effect of paracetamol (Table 1).

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 3gm/kg.

Liver functions study

Control (saline$\pm$vehicle) serum value of Bilirubin, GOT, GPT and ALP in rats were found to be 0.52 ± 0.0467 mg/dl, 54.5 ± 1.45, 34 ± 1.55 and 207.2 ± 4.33 IU/L ($n=10$) respectively, while a toxic dose of paracetamol (640 mg/kg) significantly raised ($P<0.05$) the respective serum levels to 2.34 ± 0.0733 mg/dl, 246.3 ± 6.46, 247 ± 4.61 and 393 ± 7.54 IU/L in group B. Group C animals were pre-treated with plant extract (500gm/kg orally twice daily for 2 days)

to determine its effect on paracetamol-induced rise in serum values of Bilirubin, GOT, GPT and ALP. The serum values in the pre-treated group were found to be 1.08±0.011 mg/dl, 87±1.70, 80±3.16 and 211.8±4.87 IU/l respectively which are lower ($P<0.05$) than the value of toxic group (Table 2).

Table 1: Effect of Calendula officinalis leaves extract on Paracetamol-induced lethality in mice.

Group	Treatment	No. of Deaths	% Lethality
1.	Plant 1.0mg/kg + Paracetamol 1 gm/kg	3/10	30%
2.	Saline 10 ml/kg + Paracetamol 1 gm/kg	10/10	100%

Table 2: Effect of Calendula officinalis leaves extract on Paracetamol-induced elevation of serum Bilirubin (mg/dl), GOT, GPT, and ALP (IU/L) in rats (means ± SEM of 10 determinations).

G.	Treatment	Bilirubin	GOT	GPT	ALP
1.	Saline + Vehicle	0.52 ± 0.0467	54.5 ± 21.45	3.4 ± 1.55	207.2 ± 4.33
2.	Saline + Paracetamol	2.34 ± 0.0733	246.3 ± 6.46	247 ± 4.61	393 ± 7.54
3.	Plant + Paracetamol	1.08 ± 0.011	87 ± 1.70	80 ± 3.16	211.8 ± 4.87

DISCUSSION

Liver injury induced by paracetamol and carbon tetrachloride are commonly used models for the screening of hepatoprotective drugs[7,8]. The rise in serum levels of ALP, GOT, and GPT has been attributed to the damaged structural integrity of the liver[9], because these enzymes are cytoplasmic in location and are released into circulation after cellular damage[10]. Paracetamol is converted to its reactive metabolite (N-acetyl-p-benzoquinoneimine (NAPQI) by the hepatic cytochrome P-450[11]. The massive production of reactive metabolite leads to depletion of protective physiological moieties (glutathione).

The methanolic extract of calendula officinalis leaves used in this study seems to preserve the structural integrity of the hepatocellular membrane. This was evident from the protection provided to mice against the lethal dose of paracetamol as well as a significant reduction in the paracetamol-induced rise in serum Bilirubin, GOT, GPT and ALP level in rats.

The paracetamol toxicity following NAPQI generation is chiefly due to oxidative stress and can effectively be ameliorated by antioxidants[12].

Moreover, it has been proved that calcium contents in the liver cells are increased during the process of experimental hepatic damage[13,14] and calcium channel blocking drugs i.e, nifedipine, diltiazem and verapamil were found to inhibit the development of hepatic damage, induced by different hepatotoxins including paracetamol and CCl_4[15,16]. The inhibitors of microsomal drug metabolizing enzymes (MDME) can impair the bioactivation of paracetamol into its reactive metabolite (NAPQI), and thus provide protection against the prevailing hepatocellular damage[17]. It is reported that mdme inhibitory activity is common in medicinal plants[18]. The inhibitors of MDME can provide protection against the hepatotoxicity only when they are given before the metabolic activation of hepatoxin and fail to provide any protection after generation of reactive metabolites. The exact mode of hepatoprotective action of the plant extract may be speculative at this stage, and whether the hepatoprotective action is mediated through inhibition of MDME, presence of certain antioxidants and/or calcium channel blockers need further investigations.

The plant material is safe as is obvious by the lack of any symptom of acute toxicity at an oral dose of as high as 3.0 gm/kg. This study, thus, provides scientific basis for the traditional use of calendula officinalis leaves in hepatobiliary diseases.

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