# EVALUATION OF THE LIVER PROTECTIVE POTENTIAL OF CALENDULA OFFICINALIS GLOWER EXTRACT ON PARACETAMOL INDUCE DAMAGE

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## SUMMARY

**The hepatoprotective activity of methanolic extract of calendula officinalis flowers was investigated against Paracetamol induced hepatic damage. Paracetamol at a dose of 640 mg/kg body weight produced liver damage in rats, as mainfested by the rise in serum levels of Glutamate Oxaloacetate Transaminase (GOT), Glutamate Pyruvate Transaminase (GPT) and Alkaline Phosphatase (ALP) to 246 ± 6.46, 247 ±4.61 and 393.0 ± 7.54 IU/L (n=10) respectively, compated to respective control values of 54.5 ± 1.45, 34 ± 1.55 and 207.2 ± 4.33 IU/L. pre-treatment of animals with plant extract (500 mg/kg body wt) significantly lowered ($p<0.05$) the respective serum GOT, GPT and ALP to 76.7 ± 1.74, 68.3 ± 3.9 and 208,8 ± 4.48 IU/L. these results indicate that the crude extract of Calendula officinalis flowers exhibits hepatoprotective action.**

## INTRODUCTION

Calendula officinalis (pot marigold) belongs to composite family. It is locally known as Gul-e-Ashrafi. It is small to medium sized annual herb with fibrous roots. Flowers are yellow or orange in colour and have a disagreeableodour.[1] It is grown in all parts of Pakistan in the winter as an ornamental plant. It contain salicylic acid, a bitter substance Calendulin and traces of an essential oil.[2] The plant is considered as an indigenous source of medicine. It has got anti-septic, diaphoretic and resolvent properties.[3] Flowers can be used as analgesic and anti-inflammatory agent when rubbed over the affected part.[4] The flowers and leaves are also useful in the treatment of jaundice due to liver damage.[5] The plant can relieve colic and constipation6 and is also used in cuts, burn and abrasions.[7] The present study was undertaken in an attempt to validate the traditional use of Calendula officinalis flowers in hepatic damage.

## MATERIAL AND METHODS

### 1. PREPARATION OF CRUDE EXTRACT

Calendula officinalis was grown in the months of Feb and march in the gardens in front of pharmacy department and at FCSIR laboratories Peshawar and authenticated with the help of a taxonomist at PCSIR laboratories Peshawar. The flowers were powdered and maceratedin 80% methanol (BDH Ltd., Poole, Eengland) for one week with occasionalshaking. The extract was filtered and concentrated to a dark yellow residue under reduced pressure on a rotary evaporator, with an approximate yield of 16%.

### 2. PHARMACOLOGICAL MATERIALS

Paracetamol (Wellcome Pakistan Ltd)
Ketamine Hydrochloride (Medimpox, Budapest, Hungary)

Methylcellulose (sigma chemicals company. St. Louis, Mo, USA). Paracetamol and plant material were suspended in 1% methylecullose.

## 3. ANIMALS

a. Swiss male mice (20-25 gm)

b. Male Albino rats (200-250 gm)

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

### 1. ACUTE TOXICITY OF PLANT

Thirty mice, divided into six groups (ABCDEF) having 5 animals per group were used in this study. Group ABCDEF animals, were given flower extract oraly at a dose of 0.5 gm, 1.0 gm, 2.0 gm, and 3.0 gm/kg body wt. 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.

### 2. LIVER FUNCTIONS STUDY

Hepatic injury in albino rats was induced by Paracetamol (640 mg/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 [3] (1% methylecllulose; 13 ml/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 3 doses of calendula officinalis flower extract (500 mg/kg) orally and Paracetamol was given orally 1 hour post-treatment of the last dose of plant material.

Animals were anaesthetized with ketamine Hcl (100 mg/kg i.m) 24 hours after the last treatment and blood (3 ml) was collected by cardiac puncture using sterile disposable syringes. Serum was separated by centrifugation (3000 r.p.m. for 15 minutes) and serum GOT, GPT and ALP were estimated on the same day spectrophotometrically using diagnostic kits.

Statistical analysis: The results are expressed as mean + S.E.M. All statistical comparisons were made by means of student's "t" test and $p<0.05$ was regarded as significant.

## RESULTS

### 1. ACUTE TOXICITY OF PLANT

No behavioural changes were observed in all 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 gm/kg.

### 2. LIVER FUNCTIONS STUDY

Control (saline + vehicle) serum value of GOT, GPT and ALP in rats were found to be 54.5 $\pm$ 1.45, 34 $\pm$ 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 level to 246.3 $\pm$ 6.64, 247 $\pm$ 4.61 and 393 $\pm$ 7.54 IU/l in group B.

Group C animals were pre-treated with flower extract (500 mg/kg) orally twice daily for 2 days) to determine its effect on paracetamol induced rise in serum values of GOT, GPT and ALP. The serum values in the pre treated group were found to be 76.7 $\pm$ 1.74. 68.3 $\pm$ 3.9 and 208.8 $\pm$ 4.48 IU/l respectively which are significantly lower ($p<0.05$) than the values of toxic group.

## DISCUSSION

The methanolic extract of flowers of calendula officinalis has shoen hepatoprotection against paracetamol- induced liver damage by preventing rise in serum levels of GOT, GPT and ALP.

Liver injury indueced by paracetamol is a commonly used model for the screening of hepatoprotective drugs.[8,9] The rise in serum levels of transaminases have been attributed to the damaged structural integrity

of the liver,[10] because these enzymes are cytoplasmic in location and are released into circulation after cellular damage.[11]

Paracetamol is converted to its reactive metabolic (N-acetyl-p-benzoquinoneimine, NAPQI) by the hepatic cytochrome P 450.[12] The massive production of reactive metabolic leads to depletion of protective physiological moieties (glutathione), causing damage to the macro-molecules in vital biomembranes.[13]

The exact mode of hepatoprotective action of the plant extract may be speculative at this stage, through there are many hypothesis to explain drug induce liver injury.

The toxicity produced by Paracetamol following NAPQI generation is chiefly due to oxidative stress and can effectively be ameliorated by anti-oxidants.[14] Calcium contents in the liver cells are increased during experimental hepatic damage[15] and Ca++ channel blocking agents, i.e. nifedipine, diltiazem and verapamil were found to inhibit the development of hepatic damage.[16,17] Some hepatoprotective drugs have proved to have Ca++ channel blocking constituents.[18]

The other possible mechanism is through microsomal drug metabolizing enzyme (MDME) inhibition. The inhibitors of MDME can impair the bioacivation of Paracetamol into its reactive metabolite and hence provide protection against the prevailing hepatocellular damage,[19,20] and since MDME inhibitory activity is reported to be common in medicinal plants,[21] the plant, Calendul officinalis flowers must be investigated for possessing any such activity or not.

The plant material in safe as is obvious by the lack of any symptoms of acute toxicity at an oral dose of as high as 3.0 gm/kg. This study lends some support for the traditional use of Calendulla officinalis flowers in hepatobiliary diseases.

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