



## INTRODUCTION

*Ocimum sanctum* (Tulsi), an erect hairy annual herb, has been claimed to possess numerous properties.

It has been investigated for its antioxidant activity and found to offer substantial protection against free radical induced damage in rat liver microsomes (1, 2). More studies revealed that *ocimum sanctum* decreased lipid peroxidation and increased the activity of superoxide dismutase (3).

The antiulcer activity of *ocimum sanctum* is well documented. A hydroalcoholic extract (4), a hydrodistilled leaf extract (5) and the fixed oil (6) of *ocimum sanctum* have been reported to exhibit antiulcer activity. Some recent studies (7,8) have reestablished the antiulcer activity of *ocimum sanctum*.

In the present study, we decided to further explore the antioxidant activity of *ocimum sanctum* in some animal models of gastric and duodenal ulcer, with the aim of ascertaining any correlation between the antiulcer and the antioxidant activity of *ocimum sanctum* which has not been done earlier.

## MATERIAL AND METHODS

### Animals

Animals were procured from National Institute of Nutrition, Hyderabad after taking permission from institutional ethical committee and were caged in the animal room of Department of Pharmacology,

M.G.I.M.S., Sevagram under standard conditions.

(i) *Albino rats*: Animals weighing between 120–180 gm and belonging to either sex were used to estimate plasma malondialdehyde (MDA) and serum superoxide dismutase (SOD) in ethanol treated and pyloric ligated groups respectively.

(ii) *Guinea pigs*: Animals of either sex, weighing between 400–600 gm were used for estimating plasma MDA and serum SOD in response to histamine treatment.

### Drugs and Chemicals

*Test drug*: *Ocimum sanctum* was identified by a botanist. Fresh leaves of the plant were shade dried and powdered. A hydroalcoholic extract (70% v/v) was prepared from 50 grams of the powder. The menstrum collected was again shade dried, yielding 12 grams of dried extract. Thus, the extraction yield was 24%. This dried extract was used to prepare aqueous solutions in the desired concentrations.

**Ranitidine** (Cadila pharma): 10 mg/ml in distilled water.

**Ethanol** (Analar, BDH): Absolute solution.

**Histamine acid phosphate** (BDH, England): 1 mg/ml in distilled water.

### For estimation of MDA

(a) Malondialdehyde bis (Dimethyl acetal) [Sigma, USA].

(b) Trichloroacetic acid (20% in distilled water) [Loba chemie, India]

- (c) Thiobarbituric acid (0.2 gm/dl of sodium sulphate solution) [Loba chemie, India]
- (d) Sulphuric acid 0.05 M (Pluto chemicals, India)
- (e) n-butyl alcohol (Mallincrodt Inc., USA)
- (f) Sodium sulphate solution (2 M) [Global chem., India]

#### For estimation of SOD

- (a) Superoxide dismutase (From bovine erythrocytes) [Sigma, USA]
- (b) Pyrogallol, (E. Merck, India)
- (c) Ethylene diaminetetraacetic acid (EDTA) [Chemo fine Industries, India]
- (d) Diethylene triaminepentaacetic acid (Loba chemie, India)

### METHODS

Before experimentation, the animals were kept fasting for 24 hours but allowed free access to water. Oxidative stress was induced by ethanol treatment, histamine treatment and pyloric ligation which are experimental peptic ulcer producing methods. For each of these three methods, the animals were divided into six groups of six animals each. Group I served as diseased control in which distilled water (10 ml/kg) was administered orally as placebo for seven days. Group II, III and IV received the test drug (*ocimum sanctum* leaf extract) in doses of 50 mg/kg, 100 mg/kg and 200 mg/kg respectively orally, once daily for seven days. Group V was administered ranitidine (10 mg/kg orally) once daily for 7 days and served

as standard for comparison. Group VI animals received only distilled water (10 ml/kg) and consisted of healthy control for baseline MDA and SOD levels.

#### 1. Ethanol induced oxidative stress in albino rats :

Absolute ethanol was administered to induce oxidative stress, 30 minutes after drug treatment on the seventh day to animals in group I to group V (9). After one hour of ethanol administration, blood samples were collected from animals in all the six groups for estimation of plasma MDA.

#### 2. Histamine induced oxidative stress in guinea pigs :

After 30 minutes of drug treatment on the seventh day, the animals in group I to group V were administered histamine acid phosphate (10). Blood samples were collected from animals in all the six groups for estimation of plasma MDA and serum SOD after 4 hours of histamine administration.

#### 3. Pyloric ligation induced oxidative stress in albino rats :

Pyloric ligation was done on the seventh day, after 30 minutes of drug treatment, in animals of group I to group V (11). Blood samples were collected from animals in all the six groups, after 5 hours of pyloric ligation, for estimation of serum SOD.

#### Collection, processing and storage of blood

In rats, blood samples were collected from the orbital plexus and in guinea pigs from the marginal ear vein. From each

animal 2–3 ml of blood was collected for MDA and 1–2 ml for SOD estimation.

**(a) For MDA estimation :**

Blood was collected in EDTA containing vials from each animal of the ethanol treated rat group and histamine treated guinea pig group. Plasma was separated by centrifugation at 3000 rpm for 10 minutes and stored at  $-20^{\circ}\text{C}$  for estimation of MDA (12, 13).

**(b) For SOD estimation :**

Blood samples were collected in plain vials from each animal in the pyloric ligated rat group and histamine treated guinea pig group. Serum was separated and stored at  $-20^{\circ}\text{C}$  to estimate SOD (14).

**Examination of Ulcers**

After collection of blood samples, the animals were sacrificed with an overdose of ether anaesthesia and confirmation of ulcers was done by gross as well as histopathological examination.

**Statistical analysis :**

Data were analyzed by Student's 't' test. The animals in group II, III, IV, V and VI were compared to group I animals.

All data are expressed as mean  $\pm$  SEM of six animals in each group. P values of less than 0.05 are considered significant.

**RESULTS**

All results are expressed in comparison to the diseased control (Group I).

**a) Plasma MDA estimation in :**

**(i) Ethanol induced oxidative stress in albino rats :**

Ocimum sanctum leaf extract (100 mg/kg and 200 mg/kg) significantly decreased the plasma MDA levels, whereas the effect of ranitidine on this parameter was insignificant.

Plasma MDA was significantly low in the healthy control (Table I).

**(ii) Histamine induced oxidative stress in guinea pigs :**

Both ocimum sanctum leaf extract (100 mg/kg and 200 mg/kg), as well as ranitidine decreased plasma MDA levels, though the effect of ranitidine was less significant.

The plasma MDA was significantly low in the healthy control (Table I).

**(b) Serum SOD estimation in :**

**(i) Pyloric ligation induced oxidative stress in albino rats :**

An elevation in the serum SOD levels was caused by ocimum sanctum leaf extract (100 mg/kg and 200 mg/kg) as well as by ranitidine, the effect of ranitidine being more significant.

In the healthy control also the serum SOD levels were significantly high (Table I).

**(ii) Histamine induced oxidative stress in guinea pigs :**

A significant increase in the serum SOD levels was caused by ocimum sanctum leaf

TABLE I: Effect of ocimum sanctum leaf extract on oxidative stress in peptic ulcer models.

Group/drug (n=6)	Dose (mg, ml/kg) (P.O.)	Plasma MDA levels (nmole/ml)		Serum SOD levels (units/ml)	
		Ethanol treated rat group (Mean±SEM)	Histamine treated guinea pig group (Mean±SEM)	Pyloric ligated rat group (Mean±SEM)	Histamine treated guinea pig group (Mean±SEM)
(I) Diseased control (D.W.)	10*	4.87±0.11	4.66±0.11	1.29±0.06	1.32±0.07
(II) OSE	50	4.53±0.13	4.55±0.19	1.32±0.08	1.45±0.09
(III) OSE	100	2.45±0.29**	2.45±0.12**	1.78±0.12**	2.10±0.11**
(IV) OSE	200	2.40±0.14**	2.37±0.16**	1.89±0.08**	2.20±0.14**
(V) Ranitidine	10	4.22±0.15	3.54±0.19*	2.08±0.10***	1.81±0.12*
(VI) Healthy control (D.W.)	10*	2.06±0.07***	1.78±0.10***	2.41±0.15***	2.64±0.15***

The animals in group II, III, IV, V and VI are compared to group I animals.

n=Number of animals

\*P<0.05, \*\*P<0.01, \*\*\*P<0.001

P.O.=Per orally

SEM=Standard error of mean

D.W.=Distilled water

OSE=Ocimum sanctum leaf extract

extract (100 mg/kg and 200 mg/kg). Ranitidine also produced a similar though less significant effect.

The serum SOD levels were significantly high in the healthy control. (Table I).

## DISCUSSION

In the present experimental work, ocimum sanctum leaf extract showed significant antioxidant activity by decreasing the levels of MDA and by elevating the levels of SOD in response to oxidative stress due to induction of peptic ulcers in various animal models.

MDA (15) is a stable secondary aldehyde degeneration product of lipid peroxidation and is used as a biological marker for the assessment of lipid peroxidation. SOD (16) is an enzymatic antioxidant, which protects organisms from reactive oxygen species mediated damage to cell components (17).

The lowering of plasma MDA by ocimum sanctum leaf extract in response to oxidative stress induced by ethanol in albino rats could be due to its antioxidant activity where direct damage to the mucous membranes by ethanol is prevented. Ranitidine did not cause any such effect, possibly because its main role is blockade of H<sub>2</sub> receptors.

In the histamine treated guinea pig model, plasma MDA was reduced by ocimum sanctum leaf extract as well as by ranitidine, though the effect of ranitidine was less significant. It goes to suggest that while ranitidine antagonizes the action of histamine only at the H<sub>2</sub> receptors, ocimum sanctum might be exerting an additional antioxidant activity by preventing histamine induced damage through other receptors.

Ocimum sanctum leaf extract increased serum SOD though less significantly than ranitidine in the pyloric ligated rat model. Since, ranitidine showed a better antisecretory

activity (unpublished observation) and hence a better antiulcer activity than *ocimum sanctum* in the pyloric ligated rat model, a consequent better antioxidant activity and more increase in serum SOD resulted in comparison to *ocimum sanctum* leaf extract.

In response to histamine induced oxidative stress in guinea pigs, *ocimum sanctum* leaf extract caused a more significant increase in serum SOD than ranitidine. A possible explanation could be

that ranitidine antagonizes the activity of histamine only at the H<sub>2</sub> receptors whereas *ocimum sanctum* may be preventing histamine induced tissue damage by acting on other receptors.

To sum, the decrease in plasma MDA and the increase in serum SOD produced by *ocimum sanctum* leaf extract in various animal models of peptic ulcer indicates its antioxidant property which could be a mechanism of action for its antiulcer activity.

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