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EFFECT OF *ACACIA FARNESIANA ON* SOD AND CAT ENZYMES IN ETHANOL INDUCED GASTRIC ULCER RATS

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ABSTRACT

Experimentation was conducted to test the hypothesis that Acacia farnesiana leaves has antioxidant activity. For this purpose, a study was designed using alcoholic extracts of Acacia farnesiana leaves produce significant antioxidant activity. 125 to 150g weighing rats (Wistar albino) aging around one or two months of age are selected for this experimentation. The activity was tested in wistar albino rats at dose levels of 200mg/kg, orally and compared with ranitidine (20mg/kg) as standard. During the ulcer condition, there was a decline in the levels of Super oxide dismutase [SOD] and catalase [CAT]. This indicated that the generation of relative oxygen species during stress might be the causative factor for the inactivation of these enzymes. The methanol leaf extract of Acacia farnesiana has shown ulcer protective effect as dose dependently against ethanol induced gastric ulcer in rats. The said extract of Acacia farnesiana was found to decrease ulcer and acid pepsin secretion. A change was also seen in SOD, CAT, levels in rat gastric mucosa due to antioxidant property of alcoholic leaves extract of Acacia farnesiana. Antioxidant defense mechanism of the extract was probably due to metabolizing and scavenging H_2O_2.

KEY WORDS

Acacia farnesiana, SOD, CAT, Ranitidine.

INTRODUCTION

Now a day's hyperacidity and gastric ulcers became very common health problem in humans. Imbalance between damaging factors and defensive mechanisms within the gastrointestinal tract, anxiety, emotional stress, hemorrhagic surgical shock, burns and trauma are known to cause severe gastric irritation **[**Rao *et al.,* 2000**].** Free radicals such as reactive oxygen species are implicated in the pathogenesis and variety of clinical disorders including gastric damage, caused by physical, chemical and psychological factors that lead to gastric ulceration in human and experimental animals.

Although a number of antiulcer drugs such as H_2 receptor antagonists, proton pump inhibitors and cytoprotectants are available for treating ulceration, but all these drugs have side effects and limitations **[**Ariyoshi *et al.,* 1986**].** Herbal medicine is considered as safer because of the natural ingredients with no side effects **[**Clouatre & Rosenbaum 1994**].** *Acacia farnesiana* is indigenous plant of several states of India, popularly known as Kasturi Jali or Kasturi Gibbali in rural parts of Karnataka; moreover, it is generally called as Aroma as well as sweet acacia. The gummy roots of the plant are chewed for sore throat and it is often planted in gardens also. The surface root extracts of the plant are likewise utilized for suppressing muscle spasms [spasmolytic], sexually stimulation[erotic], cellular contraction [astringent], mucus protection [demulcent], reducing fever[febrifuge], stopping loose bowels [Anti-diarrheal] and suppressing rheumatism [antirheumatic] **[**Duke, 1981**].** The flower infusion of this plant used as a stomachic **[**Morton, 1981**].** It is also used for dyspepsia and neuroses. The root decoction of *Acacia farnesiana* has been suggested as a folk remedy for tuberculosis. The decoction of the root, used in hot baths, is said to

help stop stomach cancer. A plaster, made from the pulp, is said to alleviate tumors **[**Hartwell, 1971**].** Preliminary phytochemical investigations revealed that leaves contain lipids, carotenoids, alkaloids, flavonoids and reducing and non-reducing sugars and seven polyphenols. Quercetin, another phytochemical of this plant was evaluated for its antioxidant activity **[**Stanford, 1969**].** Recent reports have revealed that many flavonoids possess antiulcerogenic activity. Ether extracted fractions of flavonoids has exhibited a decent level of gastric security when given orally. Flavonoids exhibit several biological effects such as anti-inflammatory, anti-hepatotoxic and antiulcer actions **[**Bors *et al.,* 1990**].**

The present study was undertaken to explore the effect of methanol extract of *Acacia farnesiana* on the antioxidant enzymes SOD and CAT in ulcer induced animal models.

MATERIALS AND METHODS

Plant

The whole plant of *Acacia farnesiana* (Aroma) was collected from the out fields of Tumkur district; Karnataka. It is identified and authenticated. Leaves of the plant were used for experimentation.

Methanol extract

The leaves of *Acacia farnesiana* (Aroma) were shade dried and reduced to coarse powder in a mortar and pestle. The powdered material is subjected to extraction in a soxhelet apparatus by hot percolation method using methanol as solvent. The extract was evaporated at 45^0 C to get a semisolid extract. The percentage yield of methanolic extract was found to be 55.5% w/w and the extract was taken for phytochemical screening through qualitative tests for the presence of plant secondary metabolites such as carbohydrates, alkaloids, tannins, flavonoids, proteins, saponins and glycosides were carried out on extracts using standard procedure studies.

Experimental Design

Animals

Male rats [albino Wister] between 1 to 2 months of age and weighing 125-150g were maintained as per the guidelines of Institutional animal ethical committee [IAEC] Animal User's Manual. Post Graduate Department of Pharmacology Laboratory, Sree Siddaganga College of Pharmacy, Tumkur, with Regd. no: 123/PO/C/99/CPCSEA.

Rats were acclimatized for 7 days to animal house, maintained at temperature of 20-24^0c. The light source in the animal room was regulated with 12 h light period followed by 12 h dark schedule. Two to three animals were housed per cage sized (41× 28 × 14 cm). Rats were maintained in a good hygienic environment by regularly changing the bedding with husk and cleaning the floor of the cages with mild disinfectants. The rats were fed on a standard rodent pellet diet, and water ad libitem.

Design of experiment

The animals were divided into four groups, each consisting of six rats. Group I represented the Normal control group, which received distilled water orally. Groups II represented the Control group, which received Ethanol 1ml/100g.b.w. Rats from group III has depicted the treatment with methanol extract of *Acacia farnesiana* (MEAF) 200 mg/kg body weight and group IV rats has been treated with 20 mg/kg body weight of Ranitidine, a reference drug for ulcers. The gastric ulcers were induced in rats by administrating absolute ethanol (95%) (1 ml/100 g b.w.) Orally. To avoid consumption of faeces [coprophagia] by the rats that has undergone treatment with MEAF and ranitidine, they were stored in specified cages immediately after 45minutes of incubation

Animal sacrifice and Stomach collection

After the experimental period, the animals from all four groups were sacrificed by cervical dislocation and immediately Stomach was dissected out and washed thoroughly with ice-cold 0.9% NaCl (saline), and suspended in 0.15 M potassium chloride in polypropylene containers, sealed with parafilm, labeled carefully and stored at -80° C until assays were carried out.

ASSAY OF ANTIOXIDANT ENZYMES

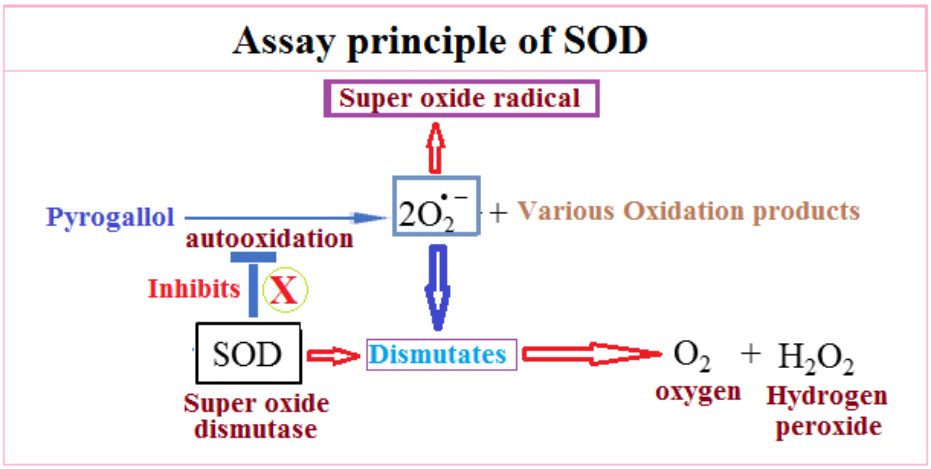
For assaying antioxidant enzymes like superoxide dismutase (SOD) and Catalase (CAT) gastric mucosa was excised from the glandular region of the stomach portion and placed in 5.0 ml of ice-cold potassium phosphate buffer solvated in 0.1% Triton X -100 with pH 7.4. Later the mixture is centrifuged at 1000 rpm for 10 min. Then the tissue homogenate was used for assay of superoxide dismutase (SOD) and Catalase (CAT).

SUPEROXIDE DISMUTASE

SOD activity was measured based on the ability of the enzyme to inhibit the oxidation of Pyrogallol. A procedure described by Soon and Tan, 2002 [9] was followed. The assay system contained 2.1ml of

phosphate buffer (50 mM, pH 7.8 containing 1 mM EDTA buffer), 0.02 ml of enzyme source (35 pg protein) and 0.86 ml of distilled water. Addition of 0.02 ml of 10mM Pyrogallol (0.01 N HCl) initiates the reaction. After 5 minutes change in optical density was read at a wavelength of 420nm. The percent inhibition was calculated on the basis of comparison with a blank assay system. One unit of SOD was defined as the amount of enzyme required to inhibit the oxidation of Pyrogallol by 50 % in standard assay system of 3 ml. The activity was expressed as units/ minutes/mg protein.

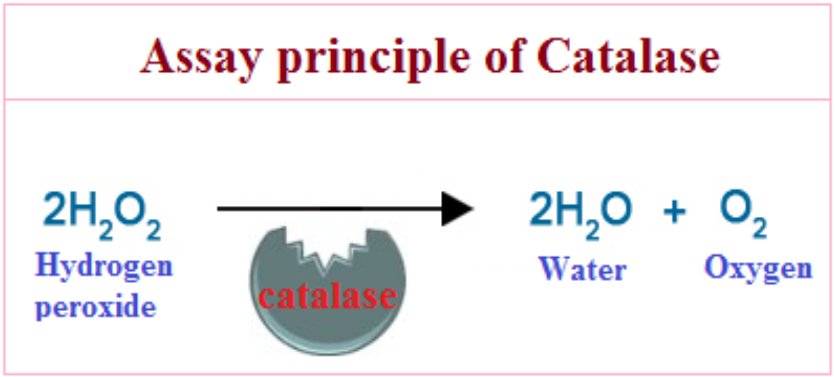
Figure 1. Principle of Superoxide dismutase assay.



CATALASE

Catalase catalyses the breakdown of H_20_2 to H_20 and 0_2 and the rate of decomposition of H_20_2 was measured spectrophotometrically at 240 nm following the method of Beers and Seizer **[**Beers & Sizer, 1952**].** The assay system contained 1.9 ml of sodium phosphate buffer (0.05 M, pH 7.0) and 1.0 ml of H_20_2 (0.059 M, in buffer) and the reaction was initiated by the addition of 0.1 ml enzyme source (45 pg). The decrease in absorbance was monitored at 1 min interval for 5 min at 240 nm and the activity was calculated by using a molar absorbance coefficient of H_20_2 as 43.6. The activity was expressed as mmoles of H_20_2 decomposed/min/mg protein.

Figure 2. Principle of Catalase assay.



Statistical Analysis

The values are represented as Mean ± S.E.M. and Statistical significance between treated and controlled group was analyzed using of one-way ANOVA, followed by Dunnett's test where P< 0.05 was considered statistically significant.

Results and discussion

Methanol extracts of *Acacia farnesiana* leaves showed a dose dependent antioxidant activity (p<0.001) in all anti-ulcer models. Ethanol produced depletion of enzymatic antioxidant enzymes CAT and SOD. This indicated that the generation of relative oxygen species

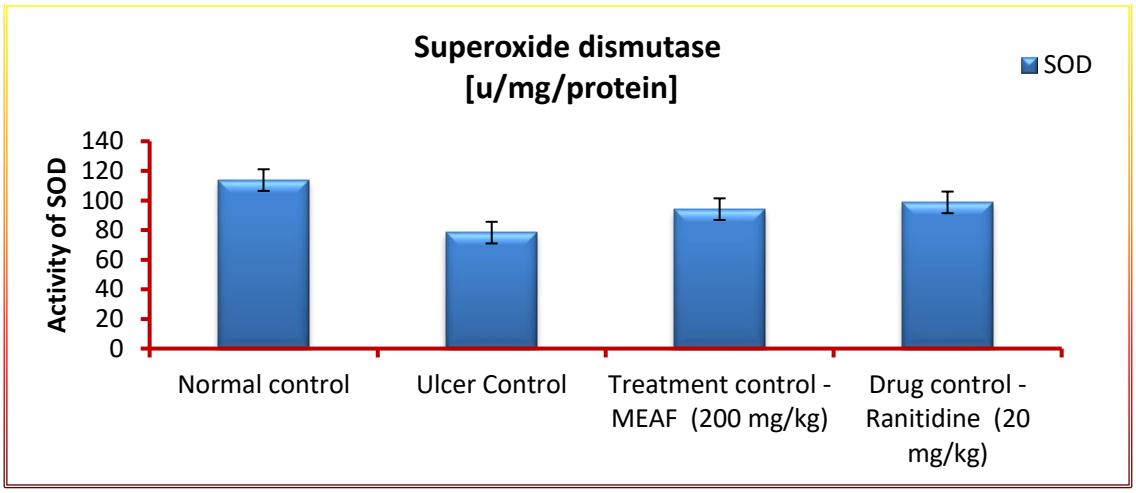
during stress might be the causative factor for the inactivation of gastric peroxidase. Treatment with MEAF elevated the levels of CAT, SOD enzymes to near normal in ulcer models.

Super oxide dismutase [SOD]

Table 1. Effect of MEAF on SOD activity.

Groups	Superoxide dismutase [u/mg/protein]
Normal control	113.79 ± 1.325
Ulcer Control	78.312 ± 1.126
Treatment control - MEAF (200 mg/kg)	94.144 ± 1.521
Drug control - Ranitidine (20 mg/kg)	98.717 ± 1.523

Figure 3. Effect of *MEAF* on SOD activity.

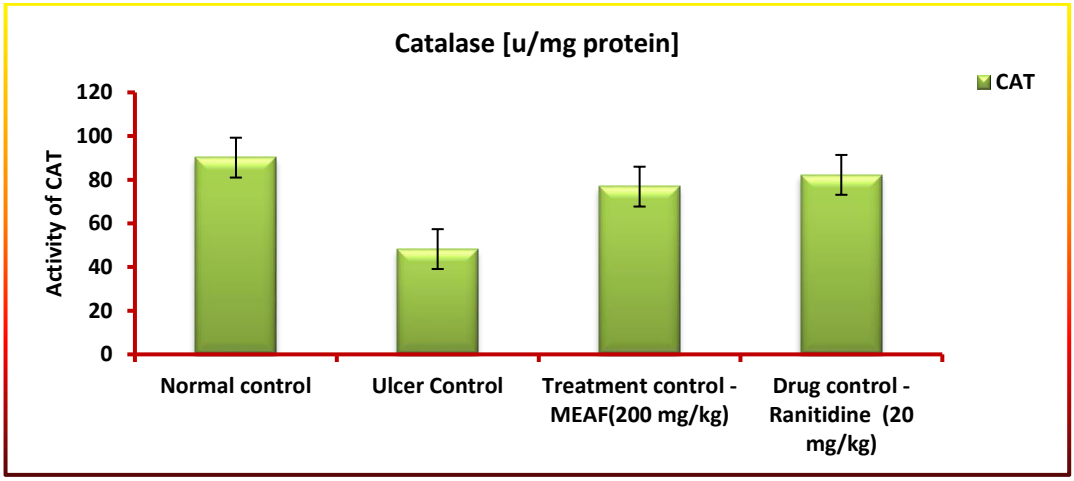


Catalase [CAT]

Table 2. Effect of MEAF on catalase activity.

Groups	Catalase (u/mg protein)
Normal control	90.124 ± 1.28
Ulcer Control	48.235 ± 0.65
Treatment control - MEAF (200 mg/kg)	76.843 ± 1.21
Drug control - Ranitidine (20 mg/kg)	82.23 ± 1.11

Figure 1.Effect of MEAF on catalase activity.



CONCLUSION

In this present study we have evaluated the anti-oxidant activity of methanol extract of *Acacia farnesiana* [MEAF] leaf through regaining of SOD and CAT enzyme levels, in ulcer induced experimental rats and It is further investigated and confirmed by using bio analytical studies of different another antioxidant enzymes also by taking the consideration of phytochemical compounds of *Acacia farnesiana*.

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