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GASTROPROTECTIVE AND ANTIOXIDANT EFFECTS OF SIDDHA DRUG (*Musa paradisiaca* Bhasma) ON EXPERIMENTALLY INDUCED GASTRIC ULCERS IN RATS

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Abstract : Antiulcer activity of a siddha drug-ripe fruit *Musa paradisiaca* bhasma was studied in rats in which gastric ulcers were induced by oral administration of ethanol (80%) in acute model and by 0.5ml of 100% acetic acid on anterior serosa surface in chronic model. The bhasma was administered in the dose of 10 and 20 mg/kg orally 1 hour prior to ulcer induction in acute model and administered daily for period of 10 days in chronic model. The antiulcer activity was assessed by determining and comparing the ulcer index, mucin content, non-protein sulfhydryl group in the test group with that of vehicle as control group. Antioxidant studies like catalase, superoxidase dismutase, lipid peroxidation were also estimated. Sucralfate was used as a reference drug. The ulcer index of the *Musa paradisiaca* bhasma treated animals was found to be significantly less in both the models compared to vehicle control animals. The antiulcer property was more prominent in animals in which ulcers were induced by ethanol and acetic acid. The antiulcer activity of *Musa paradisiaca* bhasma was however, less than that of sucralfate. Our results suggest that *Musa paradisiaca* bhasma possess significant antiulcer property which could either due to cytoprotective action of the drug or by strengthening of gastric and duodenal mucosa and thus enhancing mucosal defence.

Keywords: *Musa paradisiaca* bhasma, Ethanol, Acetic acid, Gastro protective effect, Antioxidants, Siddha system of medicine.

Introduction

Siddha system of medicine is one of the ancient systems of medicine in India. According to Siddhars, peptic ulcer is known as *Valigunmam* with its signs and symptoms as detailed in Siddha literature matching modern terminology of peptic ulcer **(Formulary of Siddha Medicines, 1993)**. In Ayurveda and Siddha Bhasma refers to calcinated metals and minerals **(Suresh B. 1994)**.

Musa paradisiaca Linn (Family – Musaceae) is a tall herb with aerial pseudo stem dying after flowering **(The Wealth of India, 1970; Indian Medicinal Plants, 1997)**. The plant is well known for its various medicinal properties

1. Roots are anthelmintic, antiscorbutic, depurative and tonic and are useful in veneral diseases, helminthiasis, scabies, inflammation, opthalmopathy, blisters and burns.
2. Fruits are sweet, demulcent, astringent, emollient, cooling, anthelmintic, aphrodisiac, antiscorbutic and tonic. They are used in vitiated conditions of *pitta*,

strangury and general debility.

3. Flowers are good for dysentery, diabetes, ascites and dropsy.
4. The inflorescence axis (stem) is specific for renal and vesical calculi. 5. Ash obtained by burning plant is antiscorbutic, anthelmintic and are used in hyperacidity, heartburn, colic and verminosis **(Yoganarasimha, 2000)**.

2. Materials and Methods

2.1 Plant Material

The plant used in this study was collected from Mettupalayam, Tamilnadu, India. It was authenticated by Prof. T.Subburaju, Head, Pharmacognosy Department; J.S.S. College of Pharmacy, Ooty. A specimen was submitted to the herbarium of the same institute.

2.2 Preparation of *Musa paradisiaca* bhasma

The ripe fruits of *Musa paradisiaca* Linn. were cut longitudinally and covered with paste of *Cissus quadrangularis* Linn. which is then covered with muslin cloth smears with mud, dried at room temperature and then incinerated at 700^0C for several hours till whole mass becomes red hot, then cooled. Adhering soil and other materials were removed carefully. White colour ash was separated and stored in an air tight container and used for experimental animal studies.

2.3 Animals

Adult male albino rats of Wistar strain weighing 150-180 gms. were procured from the animal house, JSS College of Pharmacy, Ooty. They were maintained under standard laboratory conditions with standard pelleted diet (M/S Hindustan Lever Foods, Bangalore, India) and water *ad libitum*. All animal experiments were carried according to the guidelines of the Institutional Animal Ethics Committee.

2.4 Ethanol induced acute gastric mucosal lesions in rats (Reshma *et al.* 2000)

24 hours fasted albino rats weighing about 180-250 gms were selected and divided into four groups of 6 animals each. 1ml of 80% ethanol was used orally to produce gastric ulcers. Rats were pretreated with following groups, 1-hour before ethanol treatment.

* Group I received solvent control 0.3% carboxy methyl cellulose with water orally.
* Group II received sucralfate 270mg/kg orally.
* Group III and group IV received *Musa paradisiaca* bhasma 10mg/kg and 20mg/kg orally.

One hour after ethanol administration animals were sacrificed by cervical dislocation under Pentothal sodium 40mg/kg, stomach was removed and cut along greater curvature. Ulcer index was calculated **(Asuzu *et al.* 1990)**.

Lesions were counted with aid of hand lens (X10) and given severity rating as follows:

Less than 1mm (Pin Point)= 1.
1-2 mm = 2.
Greater than 2mm & above= 3.

Overall total in each group was divided by factor of 10, to get ulcer index. % Ulcer Index was calculated **(Njar *et al.* 1994)**.

$$\frac{\text{Ulcer Index in Control - Ulcer Index in Test}}{\text{Ulcer Index in Control}} \times 100$$

2.5 Acetic Acid induced chronic gastric mucosal lesions in rats (Wong. M. 1990; Surendar Singh, 1999)

24 hours fasted albino wistar rats were used for experiment. Under pentothal sodium, a midline epigastric incision exposed stomach and cylindrical plastic mould (6.5 mm in diameter) was firmly placed upon anterior serosa surface of gastric wall. 0.05 ml of 100% acetic acid was pipetted into mould and allowed to remain for 60 seconds. Acid solution was then removed using syringe. The mould was rinsed 3 times with 0.9%w/v sodium chloride to prevent possible damage to surrounding tissues close to point of acid application. Abdomen was closed and once recovered; animals were fed on normal diet. From 2nd day after operation, group I received solvent control 0.3% carboxy methyl cellulose with water orally. Group II received sucralfate 270mg/kg orally. Group III and IV received *Musa paradisiaca* bhasma 10 mg/kg and 20 mg/kg orally. Rats were sacrificed at the end of 10th day after operation by a sharp blow and open along greater curvature. Ulcer index was calculated **(Asuzu *et al.* 1990)**.

3. Gastric Mucosal Defensive Factors

3.1 Estimation of Mucous barrier (Kulkarni et al. 1996)

Glandular portions of stomach of 24 hrs fasted rats were everted and soaked for 24 hrs in 10 ml of 0.1% alcian blue 8GX dissolved in 0.16 M sucrose buffered with 0.05 M sodium acetate adjusted to pH 5.8 with Hcl. Uncomplexed dye was removed by two successive washes of 15 and 45 minutes in 0.25 N sucrose. Dye complexed with mucous was dilated by immersion in 10 ml aliquots of 0.5 M magnesium chloride for 2 hrs. The resulting blue solutions were shaken with equal volume of diethyl ether and optical density of aqueous phase was measured at 605 nm. The barrier mucous was expressed in terms of microgram of alcian blue dye/g of wet stomach glandular tissues.

Mucous barrier [(microgram of alcian blue dye/g of wet stomach glandular tissues (g)]

$$= \frac{\text{Absorbance} \times 10^5}{\text{E1\% 1cm} \times \text{wt. of glandular tissues}}$$

E1% for alcian blue = 189

3.2 Estimation of Non–Protein Sulfhydryl (NP-SH) Group (Sedlak et al. 1968)

The glandular part of the stomach was homogenized in ice-cold 0.02m EDTA. Aliquots (5ml) of the homogenates were mixed in 15ml test tubes with 4 ml of distilled water and 1ml of 50% tricholoroacetic acid. The tubes were shaken intermittently for 10 to 15 minutes and centrifuged at 3000 r.p.m. Two ml of supernatant was mixed with 4ml of Tris buffer pH 8.9; 0.1ml of 5, 5 -dithio-bis 2(nitro-benzoic acid) was added and the sample was shaken. The absorbance was read within 5 min of addition of 5, 5 – dithio-bis 2(nitro-benzonic acid) at 412 nm against a reagent blank with no homogenate.

4. Biochemical Estimation

Lipid peroxidation products were estimated by assaying malondialdehyde formation **(Das *et al.* 1994)**. The determination of total tissue sulphydryl (thiol) group (reduced glutathione level) was carried **(Ellman, G.L. 1959)**. The auto-oxidation of epinephrine was noted by determining the activity of Superoxide

Dismutase (SOD) **(Misra *et al.* 1972)**. Catalase (CAT) activity was measured by following decomposition of H_2O_2 **(Beers *et al.* 1952).**

5. Statistical Analysis

Statistical significance was determined by one way analysis of variance (one way ANOVA) followed by Dunnet's *"t"* test.

6. Results

Analytical report of the bhasma by atomic absorption spectroscopy showed the presence of various metals like zinc (0.0002 ppm), copper (0.001 ppm) and iron (0.007 ppm).

The results of the experiment (acute model) are given in **Table 1 and Table 2.** Percentage healing promotion of ulcer for 10 mg and 20 mg/kg of the bhasma were 45.63and 64.80 respectively which reflects that there is significant decrease in ulcer index [F (3, 23) – 115.1]. There is a significant increase in mucin content [F (3, 23) –30.03] in 20 mg/kg of the bhasma treated groups which was comparable and equipotent with that of the sucralfate ($p<0.01$), but 10 mg/kg showed no significant

Table 1. Effect of *Musa paradisiaca* bhasma on gastric lesions induced by ethanol in rats.

Treatment and Dose (mg/kg. b wt)	No. of animals	Ulcer index	Percentage of Inhibition
Ethanol induced ulcer	6	11.68± 0.50	-
Ethanol + pretreated with Sucralfate (270mg/kg)	6	2.066± 0.18**	82.31
Ethanol + pretreated with (MPB) (10mg/kg)	6	6.35 ± 0.48**	45.653
Ethanol + pretreated with (MPB) (20mg/kg)	6	3.06 ± 0.21**	77.79

Values are Mean ± SEM P value: ** P<0.01

Table 2. Effect of *Musa paradisiaca* bhasma on lipid peroxidation and antioxidant enzymes on ethanol induced gastric lesions in rats.

Parameters	Group I	Group II	Group III	Group IV
Lipid peroxidation (units/mg of tissue)	0.446 ± 0.0159	0.268 ± 0.0098 **	0.310 ± 0.006 **	0.281 ± 0.008 **
Superoxide Dismutase (units/mg of tissue)	0.068 ± 0.022	0.140 ± 0.015 *	0.091 ± 0.009 ns	0.122± 0.0032 *
Catalase activity (units/mg of tissue)	0.032± 0.0014	0.071 ± 0.0014 **	0.055 ± 0.0012 **	0.068 ± 0.0032 **
Reduced sulphydryl group (ìmol/g tissue)	0.151 ± 0.006	0.531 ± 0.070 **	0.305 ± 0.004 *	0.475 ± 0.012 **

Values are Mean ± SEM P value: ** P<0.01 * P<0.05 ns = Not significant

increase in mucin content. There is a significant increase in the non-protein sulfhydryl group [F (3, 23) –22.81] in 20 mg/kg of the bhasma treated group which was comparable and equipotent with that of sucralfate ($p<0.01$) but 10 mg/kg showed no significant increase in NP-SH. There is significant increase in catalase enzyme level groups [F (3, 23) – 77.25] and superoxide dismutase enzyme level groups [F (3, 23) – 4.97] and there is significant decrease in lipid peroxidation groups [F (3, 23) – 49.42] which were comparable and equipotent with that of sucralfate. It was evident that 20 mg/kg of bhasma showed promising cytoprotective effect.

The results of the experiment (chronic model) are given in **Table 3 and Table 4.** Percentage healing promotion of ulcer for 10 mg and 20 mg/kg of the bhasma were 43.65 and 64.80 respectively which reflects that there is significant decrease in ulcer index [F (3, 23) – 64.69]. There is a significant increase in mucin content [F (3, 23) – 11.36] in 20 mg/kg of the bhasma treated groups which was comparable and equipotent with that of the sucralfate ($p<0.01$), but 10 mg/kg showed no significant increase in mucin content. There is a significant increase in the non-protein sulfhydryl group [F (3, 23) – 83.95] in 20 mg/kg of the bhasma treated group which was comparable and

Table 3. Effect of *Musa paradisiaca* bhasma on acetic acid induced gastric lesions in rats.

Treatment and Dose (mg/kg. b wt)	Number of animals	Ulcer index	Percentage of Inhibition
Acetic acid induced ulcer **(GROUP I)**	6	7.33 ± 0.36	-
Acetic acid induced ulcer + pretreatment with Sucralfate (270mg/kg) **(GROUP II)**	6	2.5 ± 0.22**	65.89
Acetic acid induced ulcer + pretreatment with (MPB) (10mg/kg) **(GROUP III)**	6	4.13 ± 0.27**	43.65
Acetic acid induced ulcer + pretreatment with (MPB) (20 mg/kg) **(GROUP IV)**	6	2.58 ± 0.24**	64.80

Values are Mean ± SEM P value: ** P<0.01

Table 4. Effect of *Musa paradisiaca* bhasma on lipid peroxidation and antioxidant enzymes on acetic acid induced gastric lesions in rats.

Parameters	Group I	Group II	Group III	Group IV
Lipid peroxidation (units/mg of tissue)	0.356 ± 0.005	0.277± 0.009**	0.255± 0.011 **	0.206 ± 0.034 **
Superoxide Dismutase (units/mg of tissue)	0.094± 0.003	0.146± 0.003 **	0.112± 0.001 **	0.134± 0.0029 **
Catalase activity (units/mg of tissue)	0.048± 0.0011	0.079± 0.0026 **	0.066± 0.0003 **	0.084± 0.0028 **
Reduced sulphydryl group (ìmol/g tissue)	0.417± 0.009	0.676± 0.022**	0.445± 0.004 **ns**	0.619± 0.012 **

Values are Mean ± SEM P value: ** P<0.01 * P<0.05 ns = Not significant

equipotent with that of sucralfate ($p<0.01$) but 10 mg/kg showed no significant increase in NP-SH. There is significant increase in catalase enzyme level groups [F (3, 23) – 62.60] and superoxide dismutase enzyme level groups [F (3, 23) – 70.92] and there is significant decrease in lipid peroxidation groups [F (3, 23) – 32.14] which were comparable and equipotent with that of sucralfate. It was evident that 20 mg/kg of bhasma showed promising cytoprotective effect.

Discussion

Under normal physiological conditions, the antioxidant enzyme, superoxide dismutase (SOD), catalase (CAT), glutathione peroxide (GSH-PX) and glutathione reductase (GR) provide cytoprotective defense against oxy radical toxicity **(Somani** *et al.* **1996).**

Impaired cytoprotective defenses against oxidative challenge are related with the decreased antioxidant enzyme activity in various tissues **(Remacle** *et al.* **1992)**. Sulfhydryl containing agents increase the concentration of non-protein sulphydryls in rat gastric mucosa where they bind electrophilic radicals that mediate tissue damage **(Lamont** *et al.* **1983)**, they could protect this mucosa against ischaemic injury. Substances containing sulfhydryl groups are capable of chemically binding various free radicals and so they influence the physical and chemical properties of gastric mucus **(Szabo** *et al.* **1981)**. Ethanol induced depletion of gastric glutathione is counteracted by maintenance of normal glutathione tissue concentration which inhibit gastric mucosal injury possibly through scavenging ethanol generated metabolites **(Olsen, 1988)**.

Our result of the analytical report showing the presence of zinc, copper and iron is supported by the previous literature **(Wealth of India, 1970)** that copper and iron were the major element and zinc is considered as minor element. The antioxidant enzymes contain metal (Cu, Zn, Mn, Fe and Se) at the catalytic site. These cofactors are essential for enzymatic activity and have the potential to limit the expression of enzyme activity **(Somani** *et al.* **1996)**. The results of antioxidant studies with various enzymes after treatment with *Musa paradisiaca* bhasma at both the dose levels (10 mg and 20 mg/kg) show that CAT, SOD and LPO were significantly altered. Except in ethanol induced acute gastric model at 10 mg/kg the SOD was not altered significantly. This particularly shows that in the acetic acid (model) chronic ulcer repeated dose administration is necessary in order to protect the animal from ulceration. In acute model, the higher dose is necessary to protect the animal from ulceration in order to alter the anti oxidant enzymes and for scavenging the free radicals.

Hence with the antioxidant studies (CAT, SOD, LPO) gastric mucosal defensive factors (NP-SH, mucin content, ulcer index), it was evident that at 20 mg/kg the bhasma showed promising cytoprotective effect.

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