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Toxicological and Pharmacological Assessment of Godanti Bhasma

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Godanti bhasma is an ayurvedic herbal mineral formulation used as antiulcer and antipyretic drug in Indian traditional systems of medicine. Godanti bhasma was evaluated for its toxicity, antiulcer and antipyretic activity in experimental animals. The experimental paradigms used for antiulcer activity were cold restraint stress induce ulcer model and diclofenac induced ulcer model in rat. Yeast induce pyrexia model in rat was used for antipyretic activity. Acute oral administration of Godanti bhasma showed no mortality in mice in the period of 14 days. The animals were found to be safe up to a maximum dose of 2000 µg/kg body weight in acute toxicity studies. Godanti bhasma demonstrated significant ($p < 0.001$) protection of 44.72 % in cold restraint stress induced gastric ulcer and 45.35 % protection in diclofenac induced ulcer ($p < 0.001$). TBARS of stomach in ulcer induced rat was also reduced ($p < 0.001$) by Godanti bhasma but serum calcium level was not altered. A significant ($p < 0.001$) reduction in hyperpyrexia in rat was also produced by the bhasma. The results suggest that this ayurvedic preparation possess significant gastro protective and antipyretic activity in lower doses of therapeutic range and the effect is not dose dependent ($p > 0.05$).

Key Words: Ayurvedic formulation, Calcination, Gypsum, Antipyretic, Antiulcer.

INTRODUCTION

Bhasma are unique oral inorganic preparation synthesized by calcination of metal and mineral in Ayurvedic systems of Indian Traditional Medicine. These preparations have been used since long and are claimed to be very effective. Bhasma are potent medicament given orally, in very small doses (250-500 µg). Bhasma are administered as such or as a paste with honey, butter or ghee[1-3]. However, the mechanism of action of these unique preparations is not clearly understood and very few scientific data on efficacy of the bhasma is available. The literature reveals that at present attempts have been made to obtain scientific data on use of some bhasma.

Evaluation of chemical constituents and free-radical scavenging activity of swarna bhasma (gold ash) was reported[4]. Toxicological and free radical scavenging activity of tamra bhasma revealed that some Ayurvedic herbal medical products including some bhasma contain potentially harmful levels of lead, mercury and arsenic[5]. Further, they concluded that the users of Ayurvedic medicine may be at risk of heavy metal toxicity and testing of Ayurvedic products for toxic heavy metals should be mandatory. The literature reveals the need of scientific methods for assessing and maintaining quality of Ayurvedic preparations. Traditional preparation and physico-chemical evaluation of godanti bhasma (gypsum ash) revealed that Godanti bhasma is calcium containing bhasma prepared using gypsum as source of calcium[6,7]. The medicinal properties of the preparation have been discussed in ancient Ayurvedic and Unani systems of medicine. It is used traditionally as antipyretic, antimalarial and antiulcer drug at a dose of 250-500 µg daily. Godanti bhasma is not only a widely used marketed ayurvedic preparation but it is also used as one of the key ingredients of many marketed ayurvedic preparations. Since no data is available on its toxicological and pharmacological activity, it was thought worth while to investigate its acute toxicity, antipyretic and antiulcer activity.

EXPERIMENTAL

Raw materials were procured by Bhardwaj Pharmaceuticals Ltd. Indore, (M.P.) and authenticated by experts of Govt. Astang Ayurvedic medical college Indore, (M.P.). Transparent plate like crystals of Godanti [gypsum] was selected for synthesis of bhasma. *Nimbu swarus* is fresh filtered lemon juice obtained from fruits of *Citrus lemon*. Aloe vera gel was obtained by peeling off outer layer of leaf of *Aloe vera* (L.)

Burm. f. diclofenac sodium, paracetamol and ranitidine were procured from Schon Pharmaceuticals Ltd., Dhar, Madhya Pradesh, India as gratis sample. Commercially available dried Baker Yeast (*Saccharomyces Cerevisiae*, Saf do Brasil Produtos Alimentycios Ltd, Brazil) was suspended in pyrogen-free 0.9 % w/v sodium chloride solution in a water bath at 37 ºC for 5 min. Other chemicals were of analytical grade and procured commercially.

Wistar albino rats were housed in standard cages at room temperature 22 ± 2 ºC and 50 ± 5 % relative humidity, under a light/dark cycle of 10/12 h, for 1 week before the experiments. Animals were provided with standard rodent pellet diet (Amrut, India) and water *ad libitum*. The animals were deprived of food for 24 h before experimentation, but had free access to drinking water. All experiments were performed in the morning. The study was approved by the institutional ethical committee, (465/01/96/CPSCSEA) which follows the guidelines of CPSCEA (Committee for the Purpose of Control and Supervision of Experimental on Animals), which complies with international norms of INSA.

Traditional preparation of Godanti bhasma: Godanti bhasma was prepared under guidance of an authentic traditional medical practitioner, in whose family these bhasmas have been synthesized for a few generations. Godanti bhasma was prepared by following the method described in Ayurvedic texts. Small pieces of Godanti (gypsum) were first cleaned with hot water and then boiled for 1.5 h in *nimbu swarus* in dola yantra (swedana). The shodhit Godanti (cleaned Godanti) was then treated with aloe vera gel (bhavana) and triturated for 8 h. The mixture was pressed in form of cakes and dried in shade to obtain chakrikas (cakes). Chakrikas were placed in sarava samputta (sealed earthen pot) and ignited (Marana) at 650 ± 10 ºC in gajaputa (traditional furnace). The procedure of bhavana and marana was repeated two more times till the sample showed all the traditional test for bhasma positive, to obtain the final product[1].

Three batches of Godanti bhasma were prepared and approved by two experts of Ayurveda unaware of the procedure. Godanti bhasma used for biological evaluation is a mixture of Godanti bhasma of all the three batches of Godanti bhasma prepared.

Acute toxicity studies and dose determination: Nulliparouse and non-pregnant, 10 to 12 week old female Albino rats of Wistar strain weighing between 150-200 g were kept fasting for 24 h prior to drug administration. Godanti bhasma suspension in normal saline was administered as single oral dose equivalent to 2000 µg/kg body weight to single animal, followed by dosing of further four animals at this level. Food was withheld for further 4 h. A total of five animals were used. Animals were observed individually at least once during first 0.5 h after dosing, periodically during the first 24 h (with special attention during first 4 h) and daily thereafter for a period of 14 days. Once daily cage side observation included changes in skin and fur, eyes and mucous membrane (nasal) and also respiratory tract, circulatory (heart and blood pressure), autonomic and cervical system changes. Mortality if any was determined over a period of 2 weeks[8].

Antiulcer studies: Albino rats of Wistar strain of either sex weighing between 150-200 g were used. Godanti bhasma was prepared and administered orally in 2 % v/v sodium carboxymethyl cellulose solution as vehicle at a dose of 250 and 500 µg/kg body weight.

Cold restraint stress (CRS)-induced ulcers: Rats were divided in four groups consisting of eight each. Group I and group II received Godanti bhasma orally at a dose of 250 and 500 µg/kg body weight, respectively. Group III received ranitidine orally at a dose of 20 µg/kg body weight. Control group IV received vehicle only. Animals were immobilized after 1 h of dosing by strapping the fore and hind limbs in restraint cage and placed in a cold chamber (4 ± 1 ºC). 3 h later, the animals were sacrificed under the influence of anesthetic ether; the stomach was removed, washed carefully with 5.0 mL of 0.9 % w/v sodium chloride and fixed on a cork plate.

Diclofenac induced ulcer[9]: Rats were divided in four groups consisting of eight each. All four groups received diclofenac (DCL) suspension (80 µg/kg body weight) in saline orally. After 1 h group I and group II received Godanti bhasma orally at a dose of 250 and 500 µg/kg body weight, respectively. Group III received ranitidine orally at a dose of 20 µg/kg body weight. Control group IV received saline only. After 3 h, the animals were sacrificed under the influence of anesthetic ether. The stomach was removed, washed carefully with 5.0 mL of 0.9 % w/v sodium chloride and fixed on a cork plate. The number and severity of ulcer per stomach, in the glandular portion of the stomach was registered with clinical microscope by a person unaware of the experimental protocol.

The following arbitrary scoring system was used to grade the incidence and severity of lesions (0 = No ulcer, 1 = superficial ulcer, 2 = deep ulcers, 3 = perforations).

The mean Ulcer Index U_I was calculated using following formula:

$$U_I = U_N + U_S + U_P \times 10^{-1}$$

where U_I = Average of number of ulcer per animal, U_N = average of severity score. U_P = Percentage of animals with ulcers.

Biochemical estimations: Serum calcium[10] and gastric tissue lipid peroxidation were estimated in rats that developed ulcers. The stomach homogenates were prepared in chilled 0.15 M KCl and lipid per oxidation was determined by estimating TBARS (thiobarbituric acid reacting substances)[11]. Protein estimations of tissue homogenates were made according to method given by Lowry *et al*[12].

Antipyretic studies (induction of bakers yeast induced pyrexia)[13]: Godanti bhasma was prepared and administered orally in 0.9 % w/v sodium chloride solution as vehicle at a dose of 250 and 500 µg/kg and paracetamol (150 µg/kg) as standard antipyretic drug was administered subcutaneously.

All experiments were performed in the morning. The animals were transferred to the experimental room before 2 h and the experiments for acclimation to the environment. Animals were housed in group of eight and initial basal rectal temperature was measured. All animals were injected with baker yeast (0.135 µg/kg, i.p.). Temperature changes were recorded every hour up to 12 h using digital thermometer (SK-1250MC, Sato Keiryoki Mfg. Co. Ltd., Japan) and expressed as the difference from the basal value. Since it has been previously reported

that handling and temperature measuring-related stress alter rectal temperature, these animals were habituated to the injection and measuring procedure for 2 days before experiments were carried out. Animals received treatment at 4 h after yeast administration. Group II received paracetamol injection i.p. 120 µg/kg. Group III and IV were treated with Godanti bhasma 250 and 500 µg/kg body weight.

The statistical analysis was carried out using GraphPad Prism, version 5.0. All *in vivo* experimental results were expressed as mean ± SEM. Data were analyzed using two way analysis of variance (ANOVA) with Bonferroni post test.

RESULTS AND DISCUSSION

Acute toxicity studies: The LD_{50} of bhasma as per OECD guideline falls under class IV with no signs of acute toxicity up to a maximum dose of 2000 µg/kg. There were no changes in normal behavioral pattern and no signs and symptoms of toxicity and mortality were observed. The dose selected for antipyretic studies is 250 and 500 µg/kg body weight.

Antiulcer studies: Godanti bhasma at the doses of 250 and 500 µg/kg produced significant ($p < 0.001$) reduction in ulcer index as compared to control (Fig. 1). It is interesting to note that Godanti bhasma at 500 µg/kg dose shows comparable activity to 250 µg/kg dose as difference was not significant at $p > 0.05$. The activity was less than that of ranitidine. The biochemical estimation showed reduction in thiobarbituric acid reactive substances (TBARS) content of stomach tissue in Godanti bhasma treated group. No significant difference was noted in serum calcium activity between the groups (Tables 1 and 2).

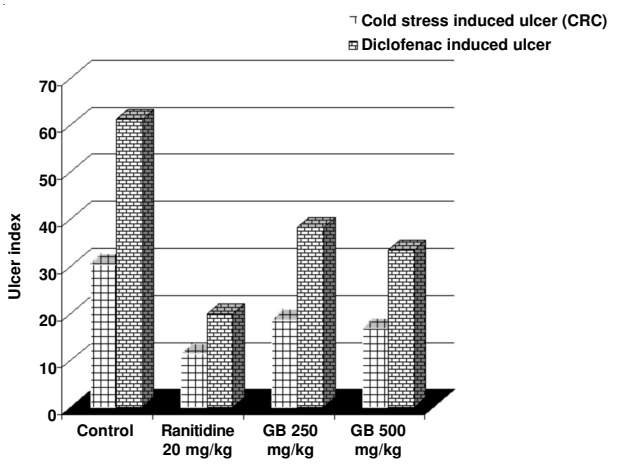

Fig. 1. Antiulcer effect of Godanti bhasma

Antipyretic studies: The subcutaneous injection of yeast suspension markedly elevated the rectal temperature. Treatment with bhasma at the dose of 250 and 500 µg/kg body weight decreased the rectal temperature of the rats but not in a dose dependent manner. The antipyretic effect started from first hour was maintained for 4 h after administration of the bhasma (Table-3).

The result obtained from both the standard and bhasma treated rats were compared with the control group and a significant reduction ($p > 0.001$) in the yeast induced hyper pyrexia was observed (Fig. 2). It is interesting to note that Godanti bhasma at 500 µg/kg dose shows comparable activity to 250 µg/kg dose as difference was not significant at $p > 0.05$.

TABLE-1
EFFECT OF GODANTI BHASMA ON COLD STRESS INDUCED ULCER, SERUM CALCIUM AND TISSUE TBARS IN RATS

Groups	Cold restraint stress induced Ulcer			
	Total no of Ulcers†	Protection (%)	Serum calcium (mg/dl)	TBARS† (nmol/mg protein)
Control vehicle	8.9 ± 1.24	–	9.95 ± 0.22	14.78 ± 0.39
Ranitidine (20 mg/kg)	0.62 ± 0.72	62.49	9.86 ± 0.47	9.54 ± 0.48
Godanti bhasma (250 mg/kg)	3.4 ± 0.26	38.80	10.38 ± 0.28	10.93 ± 0.30
Godanti bhasma (500 mg/kg)	2.75 ± 0.25	44.72	10.98 ± 0.39	9.33 ± 0.27

†Mean ± SEM; number of animal used (n = 8).

TABLE-2
EFFECT OF GODANTI BHASMA ON DICLOFENAC INDUCED ULCER, SERUM CALCIUM AND TISSUE TBARS IN RATS

Groups	Diclofenac induced ulcer			
	Total no of Ulcers†	Protection (%)	Serum calcium (mg/dl)	TBARS† (nmol/mg protein)
Control (DCL) (80 mg/kg)	20.37 ± 0.98	–	10.23 ± 0.31	17.88 ± 0.46
Ranitidine (20 mg/kg)	4 ± 0.46	67.43	9.73 ± 0.23	10.35 ± 0.33
Godanti bhasma (250 mg/kg)	11.12 ± 0.91	37.67	10.48 ± 0.14	11.53 ± 0.21
Godanti bhasma (500 mg/kg)	9.75 ± 0.72	45.35	10.52 ± 0.25	9.73 ± 0.21

†Mean ± SEM, number of animal used (n = 8).

TABLE-3
ANTIPYRETIC ACTIVITY OF GODANTI BHASMA

Drug (mg/kg)	Basal rectal temperature	Difference in rectal temperature in ºC at time (h)†				
		Before drug administration	After drug administration			
		4th Hour	5	6	7	8
Control (yeast)	37.80 ± 0.12	0.94 ± 0.34	1.30 ± 0.02	1.13 ± 0.03	1.13 ± 0.03	1.22 ± 0.03
PCM (150 mg/kg	37.77 ± 0.16	0.79 ± 0.04	0.37 ± 0.02[a]	0.59 ± 0.01[a]	0.62 ± 0.02[a]	0.74 ± 0.01[a]
GB (250 mg/kg	37.68 ± 0.28	0.82 ± 0.03	0.59 ± 0.01[a]	0.65 ± 0.01[a]	0.72 ± 0.01[a]	0.87 ± 0.01
GB (500 mg/kg)	37.74 ± 0.19	0.86 ± 0.03	0.53 ± 0.01[a]	0.63 ± 0.01[a]	0.68 ± 0.01[a]	0.83 ± 0.01

†The results given are mean ± SEM; number of animal used (n = 6) [a]$p < 0.001$ experimental groups were compared with control.

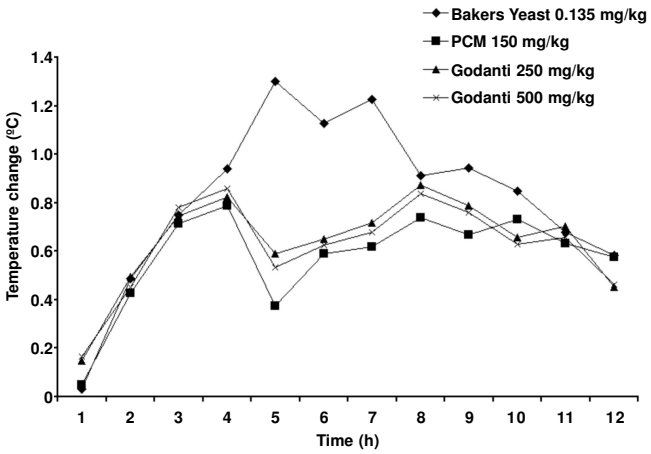

Fig. 2. Antipyretic effect of Godanti bhasma

Cold restraint stress induced ulcer represents an unique ulcer model in examining the cause, course, consequence and treatment of peptic ulcer. Pharmacological effects of diclofenac sodium are related to the inhibition of the conversion of arachidonic acid to prostaglandins, which are the mediators of the inflammatory processes. NSAID induced ulceration causes accumulation of oxygen free radicals, which play a crucial role in the pathophysiology of gastric ulceration. Oxygen derived free radicals cause lipid peroxidation, which leads to membrane fluidity, resulting in reduced membrane integrity of surface epithelial cells, thereby causing gastric ulcers[14]. It is found that the ulcer severity and lipid peroxidation are aggravated during cold resistance stress, which is also indicated by thiobarbituric acid reacting substances (TBRAS) content under stress as compared to unstressed rat, whereas inhibition of lipid peroxidation on Godanti bhasma administration indicates the antilipid peroxidative effect of Godanti bhasma which could have prevented lipid peroxidation mediated ulcerative damage to gastric mucosa. In this study, we have also observed protection offered by Godanti bhasma in cold restraint stress induced gastric ulcers. There is extensive experimental evidence that indicates certain substances, through scavenging of free radicals, protect the gastric mucosa[15]. The thiobarbituric acid reactive substance (TBARS) is used as an indicator of lipid peroxidation and free radical scavenging activity in biological sample[11]. In the present study, Godanti bhasma exhibits a potent antiperoxidative effect without altering serum calcium level. Hence, it can be suggested from our study that Godanti bhasma provides antiulcer activity in rats. It may act as gastric cytoprotective agent by modulating scavenging of free radicals. Further studies like, acids and mucopolysaccharides estimations by pyloric ligated models are required to establish the role of Godanti bhasma in protection against gastroduodenal ulcer.

Yeast-induced fever is called pathogenic fever. Its etiology includes production of prostaglandins, which set the thermoregulatory center at a lower temperature. The present results show that bhasma possesses a significant antipyretic effect in yeast-provoked elevation of body temperature in rats and its effect is comparable to that of paracetamol (standard drug). So inhibition of prostaglandin synthesis could be the possible mechanism of antipyretic action as that of paracetamol[16]. Also, there are several mediators or multi-processes underlining the pathogenesis of fever. Inhibition of any of these mediators may bring about antipyresis, which requires further investigation. Further, the antipyretic activity of Godanti bhasma is not dose-dependent.

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