Explor. Anim. Med. Res.,
Vol.3, Issue - 1, 2013, p. 48-56

ISSN 2277- 470X (Print), ISSN 2319-247X (Online)
Website: www.animalmedicalresearch.org

ANTI-ULCER ACTIVITY OF *SHANKHA BHASMA* (CALCINED CONCH-SHELL)

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ABSTRACT: The anti-ulcer activities along with chemical identification of purified *Shankha Bhasma* (SBM) or calcined conch-shell was undertaken. *Shankha Bhasma* (SBM) was prepared by traditional process used in India. The chemical composition of SBM was studied by atomic absorption spectrometry and infra-red spectral analysis. Acute oral toxicity of SBM was done in Swiss mice. SBM (25 mg/kg and 50 mg/kg) was studied for anti-ulcer effect on ethanol induced and pylorus ligature induced model in Wistar rats. SBM showed significant (P<0.001) positive results in both the models in comparison with respective control and standard control (omeprazole 20 mg/kg and ranitidine 50 mg/kg).

Key words: Calcined conch-shell, Anti-ulcer activity.

INTRODUCTION

Peptic ulcer results when the gastro-duodenal mucosal defenses are unable to protect the epithelium from the corrosive effects of acid and pepsin. Multiple chemicals, neural and hormonal factors participate in the regulation of gastric acid secretion. The proteolytic effect of pepsin in concert with the corrosive properties of secreted gastric acid contributes to the tissue injury that produces peptic ulcer (Loren 1998). In recent years, consorted effort has been directed towards identifying new anti-ulcer drugs from natural resources. In Indian traditional system of medicine *Shankha Bhasma* (SBM) is a powder prepared from the conch shell (*Gastropoda*, Class: *Mollusca*), is well known for its antacid and digestive properties (Sen and Sen 1947, Nadkarni 1982, Sastri 1995). *Bhasma* means preparation from inorganic or organic substances that burnt into its ash and the process of burning is known as *putapaka* or calcinations (Pandit *et al*. 1999). *Bhasma* is prepared to improve the quality of conch-shell to use it medicinally (Mishra 2004).

SBM showed protection against duodenal ulcer in rats (Nadar and Pillai 1889). Earlier in our laboratory, it was reported that SBM has

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potential role in restoration of indomethacin-induced and restraint stress-induced gastric ulcers in rats (Pandit *et al*. 2000, Sur *et al*. 2003). But its role in the regulation of acid-pepsin secretion is still obscure. The current study was aimed to establish the anti-ulcer activity of SBM in rats particularly its role in acid pepsin secretion. Moreover, to develop any drug from natural resources, the first step is chemical standardization. There is no report of chemical analysis of SBM till date, though it has been used from 1300 AD in Indian sub-continent (Mishra 2004). In the present research program, a detailed chemical analysis of SBM and anti-ulcer activity were evaluated.

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METERIALS AND METHODS

Shankha Bhasma preparation

SBM was prepared by maintaining the traditional process as described in the ancient Indian text (Sharma 1978). The outer shell of conch (500 g) was made into finer particles by cutting and crushing, washed with distilled water and subjected to boiling with fresh juice of *Citrus limonum* (1.5 L) for 3 h at 70-80^{o}C. After cooling these particles were washed with distilled water and dried in air. Thereafter, the particles were placed in an earthen covered crucible, sealed properly and heated at low flame for 12 h (Pandit *et al*. 2000). Thus the powder of SBM was prepared and collected in sterile glass container.

Chemical analysis of *Shankha Bhasma*

SBM treated with few drops of conc. entreated HCl and its aliquot was taken for analysis of different metals. Calcium was determined titrimetrically with EDTA using Patton and Reed indicator (Gowenlock *et al*. 1988). Other metals present in trace amounts were determined by atomic absorption spectrometry using Perkin-Elmer spectrometer. Infra-red spectra were recorded on Perkin-Elmer FT IR spectrophotometer in the region 500-4000 cm^{-1}.

Animals

Male albino Swiss mice (20-25 g) and male Wistar rats (175-180 g) were taken for the study. The animals were housed in polypropylene cages with dust free rice husk as bedding material. 10 hour light : 14 hour dark cycles was maintained throughout the experimental period (CPCSEA guidelines 2003). They were supplied pellet diet and water *ad libitum*. Institutional animal ethics committee approved the study protocols.

Acute oral toxicity study

The doses of SBM were selected on the basis of previous work (Pandit *et al*. 2000) and administered at 0.001, 0.01, 0.1 and 1.0 g per kg of Swiss mice in oral route. The animals were observed continuously for 72 h (Raymond *et al*. 1996).

ANTI-ULCER EVALUATION OF *SHANKHA BHASMA*

Activity of *Shankha Bhasma* against ethanol-induced ulcer

The ethanol induced lesions assay was carried out according to the method of Morimoto *et al*. 1991. A total of 32 rats were divided into four groups. All rats were pretreated with vehicle, test drug and standard for 10 days by oral route. The dosage of SBM was selected on the basis of our previous findings (Pandit *et al*. 2000, Sur *et al*. 2003). The dosages of different drugs for different groups were as follows: group I, 2% gum acacia;

Ulcer Index = (number of lesion I) + (number of lesion II) X 2 + (number of lesion III) X 3

Lesions description on ulcer production	Score
presence of edema, hyperemia and single submucosal punctiform hemorrhages	1
presence of submucosal punctiform hemorrhagic lesions with small erosions	2
presence of deep ulcer with erosions and invasive lesions	3

group II, SBM 25 mg/kg; group III, SBM 50 mg/kg and group IV, omeprazole 20 mg/kg. On day 10, ethanol (50% v/v, 5 mL/kg) was orally administered to the 24 h fasted animals that had only 1 h previously been treated with the last dose. Two hours after ethanol ingestion, the animals were sacrificed under anesthesia. The stomach was incised along the greater curvature and examined for ulcers. The gastric lesions were counted and the ulcer index was calculated (Pandit *et al*. 2000, Sur *et al*. 2003).

Activity of *Shankha Bhasma* against pylorus ligature-induced ulcer

A total of 32 rats were divided into four groups and pretreated for 10 days as describe earlier. Only ranitidine at 50 mg/kg was used as standard drug (group IV) instead of omeprazole, as ranitidine is a potent proton pump inhibitor and suitable for this secretory study. On day 10, pylorus ligature was performed as described by Shay *et al.*, 1945. The animals were sacrificed after 4 h under anesthesia, the abdomen was opened and another ligature was placed around the esophagus close to the diaphragm. The stomachs were dissected out and its contents drained into a graduated centrifuge tube and centrifuged at 2000 rpm. The supernatant volume of the gastric content, pH, free and total acid concentration (Dhuley 1999), mucin (Edward 1979) and pepsin activity (Debnath *et al.* 1975) were assessed. The stomach was opened along the greater curvature and examined for ulcer as mentioned before.

Table 1: Composition of purified *Shankha Bhasma* (calcined conch shell)

Element	Concentration
Ca	48.6%
Na	0.31%
Al	272.0 ppm
Mg	260.0 ppm
Fe	340.0 ppm
Cr	40.0 ppm
Co	12.4 ppm
K	13.0 ppm
Mn	23 ppm
Zn	20 ppm
Ni	4.9 ppm
Co	2.72 ppm

Calcium was analyzed tritimetrically, all other metals were analyzed by atomic absorption spectral study (Perkin-Elmer spectrometer), ppm = particles per million.

Table 2: Effect of purified *Shankha Bhasma* (calcined conch shell) on gastric ulcer index in rats.

Medicine / Drug	Ulcer Index Ethanol (50% v/v, p.o.)	% inhibition	Ulcer Index Pylorus ligation (4h)	% inhibition
Control	35.50 ±1.71	_	26.50±2.01	_
SBM-25	26.12±2.44*	26.4	16.12±2.68*	39
SBM-50	19.50±2.10*	45	11.75±3.15*	55.6
Omeprazole	14.69±1.86*	58.6	_	_
Ranitidine	_	_	10.47±2.40*	60.5

Values (ulcer index) are expressed as mean±S.D. (n=8), SBM= *Shankha Bhasma*, *p<0.01

Table 3: Effect of purified *Shankha Bhasma* (calcined conch shell) on gastric juice content in pylorus ligated rats.

Parameter	Control	SBM-25	% Change	SBM-50	% Change	Ranitidine	% Change
Gastric volume (ml/100g)	3.45 ±0.14	2.72±0.15*	-21	1.34±0.23*	-61	1.52±0.46*	-56
pH	1.51±0.12	2.33±0.14 *	+32.6	3.26±0.13*	+69.7	4.32±0.58*	+92
Total acid (mEq/100g/4h)	459.97±12.86	347.76±13.70*	-24.3	252.3111.82*	-45	232.58±10.92*	-53.7
Free acid (mEq/100g/4h)	47.45±3.89	33.53±2.72*	-29	21.96±1.67*	-53.7	22.46±2.85*	-52.6
Mucin (mg/L)	4.34±0.26	5.50±0.20*	+26.7	5.97±0.33*	+37.5	4.52±0.18	+4
Pepsin (U activity)	6.86±0.11	3.57±0.15*	-47.9	2.76±0.13*	-59.7	2.08±0.60*	-69.6

[a] Values (ulcer index) are expressed as mean±S.D. (n=8), SBM= *Shankha Bhasma*, *p<0.0

Statistical analysis

Results are presented as mean±S.D. Statistical significant of non-parametric data was determined by one-way analysis of variance followed by Duncan's test, with the level of significance set at $P<0.05$. Statistical significant of parametric data was determined by one-way analysis of variance followed by Student's Newmann-Keul test.

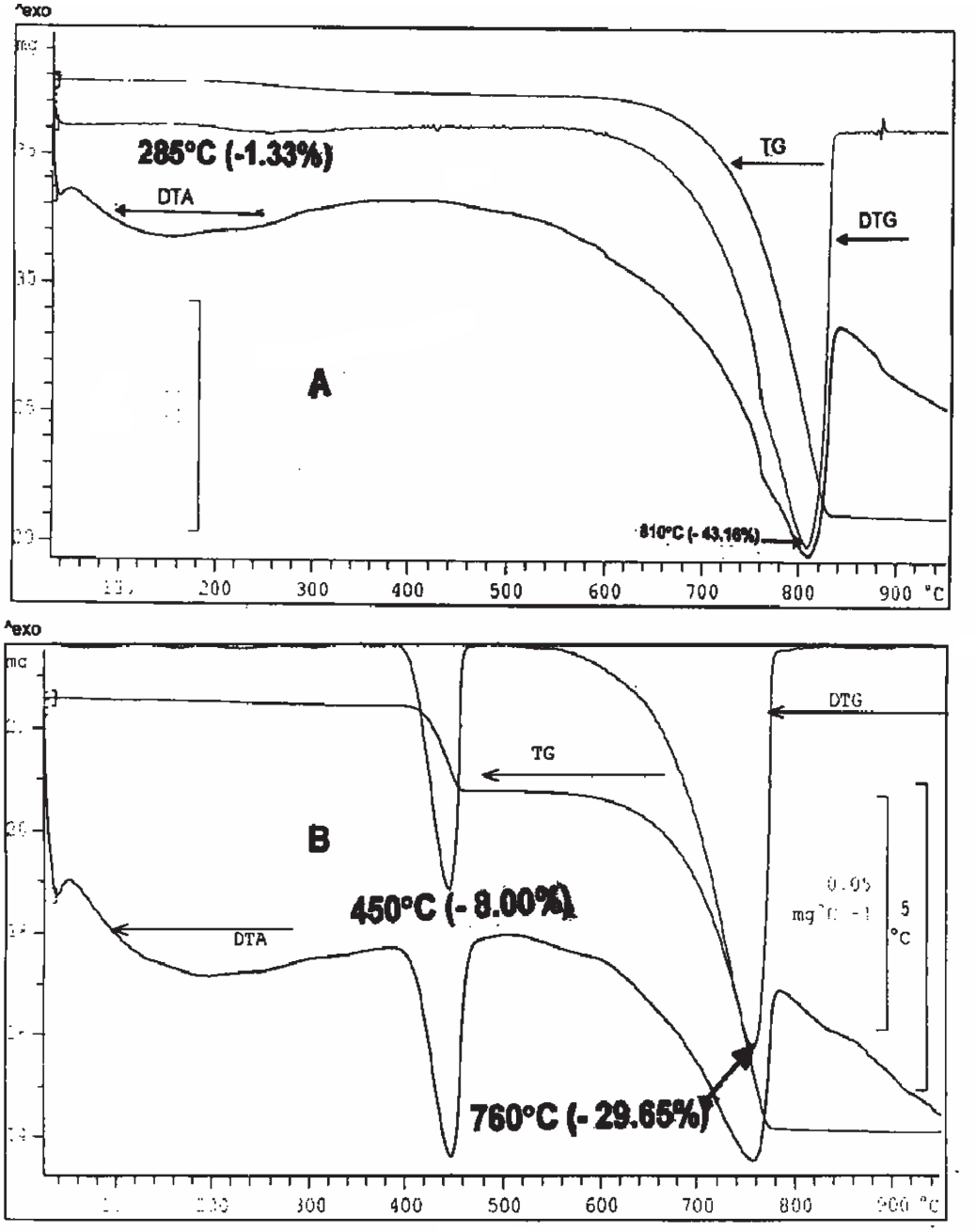
RESULTS AND DISCUSSION

In ancient India, marine products (including Conch) were used since the period of *Charaka Samhita* for the treatment of different diseases. *Shankha* or conch shells (Fig. 2) are built on a matrix of proteins filled with calcium carbonate that can crystallize into calcite and aragonite (Ghosh 1987). The infra-red spectrum of SBM was quite interesting. The bands attributable to carbonate as in calcite was present in the spectrum (Fig. 1). The calcite was decomposed to calcium oxide on temperature during the preparation of SBM. The product thus formed absorbs water and carbon dioxide to form a mixture of calcium hydroxide and calcium carbonate (Oomori *et al*. 1987). Further, atomic absorption spectral analysis showed calcium was the main constituent of SBM. Beyond calcium, other major trace elements on SBM were Na, K, Fe, Al, Mg, Cr, Co, Mn, Cu, and Zn (Table 1). Most of the elements present in SBM have been reported to scavenging action in free radical generation (Garribba *et al*. 2001), while, oxidative stress induced free radical generation is one of the prime causes of gastric ulcer formation (Das et *al.* 1997). It has also been reported, calcium and magnesium has anti-oxidant properties and has mediatory scavenging action in free radical generation and thereby they can be protecting gastric ulcer (Smith *et al.* 1988). Further, calcium has acid neutralizing action. It may be postulated that components present in SBM may be responsible for its anti-ulcer action.

Earlier, we reported that SBM have effective role in the prevention of gastric ulcers either induced by indomethacin or restraint stress in rats (Pandit *et al*. 2000, Sur *et al*. 2003). In this study, SBM further showed inhibition in the genesis of ulcers induced by both ethanol and pylorus ligature in rats. It was observed that there was significant inhibition of ulcer formation in rats pretreated with SBM (26.4 % and 45 %) on ethanol-induced gastric lesions. These results were almost same as in the case of omeprazole (58.6 %) standard drug treated group (Tab 2). Ethanol (50%) is used in rats by direct damage to the gastric tissue but independent of gastric acid secretion (Morimoto *et al.* 1991). The mechanism of ethanol induced gastric damage involves either by the disruption of the defensive factors such as the gastric mucosal barrier, gastric mucus and mucosal circulation or by enhancing the toxic oxygen radicals. This explains why ethanol-induced ulcers are not inhibited by anti-secretary agents such as ranitidine, but are greatly inhibited by agents that enhance mucosal defense factors, such as omeprazole (Morimoto *et al.* 1991, Rodriguez *et al.* 2001).

The effect of accumulated gastric juice in the induction of gastric ulcers is well documented in the pylorus-ligation model (Dhuley 1999, Debnath *et al.* 1974). By applying pylorus ligation it was noted that the test drug, SBM has anti-secretary action. It showed dose dependent diminution in the secretion of gastric juice as also free and total acid concentrations. Pretreatment of SBM at doses of 25 and 50 mg/kg significantly inhibited (39% and 55.6%) the

Fig. 1: Infrared (IR) spectrum of *Shankha* (A) and its *Bhasma* (B) in KBr matrix.



Thermal Analysis of *Shankha* (A) and its *Bhasma* (B)

Fig.2 : Photograph of Sankha (Conch) and different conch shaped creatures.

appearance of gastric lesions induced by pylorus ligature (Table 2). The pepsin secretion was also significantly inhibited by SBM. Further, SBM was able to reduce the gastric volume (21% and 61%), free acid (29% and 53.7%), total acid (24.3% and 45%), and pepsin (47.9% and 59.7%) concentrations significantly when compared to control in pylorus-legated rats (Table 3). The results were almost same as in the case of standard drug, ranitidine at 50 mg/kg. Ranitidine inhibited gastric lesions (60.5 %) and also reduced the gastric volume (56%), free acid (52.6%), total acid (49.4%) and pepsin (69.6%). Further, SBM pretreatment showed increment in pH level (32.6% and 69.7%) and mucin concentration (26.7% and 37.5%) in the gastric juice of pylorus-ligated rats than vehicle treated control (Table 3). However, reference drug ranitidine, only significantly enhanced the pH level (92%) level but did not alter mucin concentration. Recently in another study, *Shankha bhasma* has also been shown effective in the treatment of gastro-esophageal reflux disease patients (Ranade and Chary 2013). Moreover, the doses up to 1.0 g/kg body weight after oral administration of SBM did not cause toxicity or mortality in mice and therefore, purified SBM is practically non-toxic. From the above discussion it may, therefore, establish a cytoprotective action of purified SBM as it was found effective against both the models *viz.* indomethacin (Pandit *et al*. 2000, Sur *et al*. 2003) and ethanol used for producing cyto-destructive damage in the gastric mucosa of rats (Rodriguez *et al.* 2001).

CONCLUSION

Purified *Shankha bhasma* has promising cytoprotective and anti-secretary action and that may be due to its oxidative stress negating action in gastric tissue.

ACKNOWLEDGEMENT

The authors are thankful to Director of GSI, Kolkata for providing necessary facilities to conduct AAS study.

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