ISSN 0975-6299 Vol 3/Issue 2/April – June2012

International Journal of Pharma and Bio Sciences

RESEARCH ARTICLE PHARMACOLOGY

GASTRIC ANTISECRETORY, ANTIULCER AND CYTOPROTECTIVE PROPERTIES OF TAMIRA PARPAM (BIO-COPPER) FROM EARTHWORM

M.KRISHNAVENI*[1], J.ANBU[2], S.NITHYA[2], ASHWINI ANJANA[2] AND S.PREMA[3]

[1]Department of Pharmacology, Govt. Siddha medical College, Arumbakkam, Chennai
[2]Department of Pharmacology, School of Pharmaceutical Sciences, (VISTAS) Vels University, Pallavaram, Chennai-600117
[3] Department of Siddha, Tamil University, Tanjore-613010

M.KRISHNAVENI
Department of Pharmacology, Govt. Siddha medical College, Arumbakkam, Chennai

*Corresponding author

ABSTRACT

In the present study we investigated the anti-ulcer properties of *Tamira Parpam* (bio-copper) from earthworm against Aspirin plus pylorus ligation induced gastric ulcer in rats, HCl-Ethanol induced ulcer in mice and water immersion stress induced ulcer in rats. The number of lesions in HCl-Ethanol induced peptic ulcer model and mean score value of ulcer inhibition in water immersion stress induced ulcer model. A significant antiulcer activity of *Tamira Parpam* was observed in all the models. Pylorus ligation model showed significant reduction in gastric volume, free acidity and ulcer index as compared to control. Results revealed that the *Tamira Parpam* showed significant ($P<0.01$) ulcer inhibition in HCl-Ethanol induced ulcer and ulcer protection index ($P<0.01$) in stress induced ulcer. This study indicates that *Tamira Parpam* have potential anti-ulcer activity in the three models tested.

KEYWORDS

Tamira Parpam, Aspirin, Pylorus ligation, Ulceration, Stress ulcer.

INTRODUCTION

Ulcers occur due to persistent erosions causing damage of the stomach and duodenum wall that might become perforated and developed into peritonitis and massive hemorrhage as a result of inhibition in the synthesis of mucus, bicarbonate and prostaglandins.[1] Various factors can contribute to the formation of ulcer such as the infection of stomach by *Helicobacter Pylori* [2], the frequent use of non-steroidal anti-inflammatory drugs (NSAIDs) [3] and consumption of alcohol.[4]

Bio-copper element from earthworm has been known for many centuries as a therapeutic drug source for various diseases in India and China. However practical pharmacological studies have not been performed and reviewed.[5] Earthworm copper can be given orally, which has a potent inhibitory effect on gastric secretion which is effective against peptic ulcer especially gastric ulcer. Moreover, its tonic properties also make it beneficial support for the liver and other organ systems. In Korea, Vietnam and most Southeast Asia, the earthworm copper has been used for their therapeutic benefits, [6] particularly for the promotion of general health and prevention of wide variety of diseases. Furthermore, the earthworm is a primary ingredient in the traditional Vietnamese remedy known as, miracle medicine. Several Vietnamese based studies have demonstrated the safety and effectiveness of the earthworm for supporting immunity and cardiovascular health.[7] Because it originates from the soil, the earthworm has dense nutritional content as well as anti-oxidant properties. In Guyana, there have been experiments carried out isolating enzymes from the earthworm powder and converting it into dietary supplement. Glycoprotein (G-90), a mixture obtains from tissue homogenate of *Eisenia foetida* exhibits anti-bacterial properties.

The commercially available metallic copper preparations and antiulcer drugs in the treatment of gastric ulcer are usually over-shadowed by various side effects and adverse drug reactions. For examples, H_2-receptor antagonists (e.g. cimetidine) may cause gynecomasia in men and galactorrhea in women,[8] while proton-pump inhibitors (e.g. omeprazole and lansoprazol) can cause nausea, abdominal pain, constipation, and diarrhoea.[9] Moreover the metallic preparation of copper may increase the tissue concentration of copper which leads to untoward reactivity. [10, 11] Due to these side effects of allopathic metallic copper preparations, there is an urgent need to discover and isolate new anti-ulcerogenic bio-copper with potentially less or no side effects from medicinal plants and worms like megadriles (earthworm). Earthworms are rich sources of bio-copper and used for the treatment of gastric ulcer.[12] Moreover, the structure of copper molecule from earthworm is naturally complex and gets in-to a modified molecule against toxicity when correlated with structure of metallic copper. Till now there are no reports pertaining to bio-copper from earthworm have been published particularly with reference to antiulcer properties. Against this backdrop this study is aimed to determine the antiulcer activity of *Tamira parpam* from earthworm.

MATERIALS AND METHODS

(i) Experimental animals

The study was conducted on Albino rats (Wistar) of 150-200 g and maintained under standard conditions (room temperature 24-27^{o}C and humidity 60-65%) with 12 h light and dark cycle. The food in the form of dry pellets (Sai meera foods Pvt Ltd., Bangalore) and water were *ad libitum* were provided to

the experimental animals. Rats of either sex were randomly allocated to groups of six animals each. Animals used in the study were housed and cared in accordance with the CPSCEA guidelines, India, and Institutional Animal Ethics Committee (IAEC/SPS/VU/48/11/2009) of School of Pharmaceutical Sciences, Vels University, Chennai, India has approved the study.

(ii) Extraction of Tamira parpam:

The collected earthworms are let inside the milk to shed their muddy particle to purify them. Then they were mixed with cow dung and rolled in small spheres, dried under the sun. It is then taken in a pot containing spheres sealed by mud cloth. The sealed pot is heated for 12 h and cooled, dissolved in vinegar. The ash was filtered to get the sediments of copper at the bottom of the pot. Later the copper was mixed with mercury, which forms bolus. Borax is added to the bolus and placed in a crucible melted by heating. Mercury gets separated and the extracts of earthworm (poonaga sathu) settle at the bottom.

The extracts of earthworm were grounded well with *Clinus lotoides* juice and dried well. The dried spheres were placed in a small earthen vessel. The mouth of the earthen vessel is covered by another earthen vessel and the joint is closely sealed with a mud-smeared cloth to prevent passage of air. A crucible pit, one yard in length, breadth and height was dug and half the pit was filled with cow dung cakes. The earthen vessel containing the bundle of medicine is placed upon the heap of cow dung cakes. The rest of the pit is filled with cow dung cakes. Then fire is set to this oven made up of cow dung cakes. When it gets cooled down, the earthen vessel are taken out and broken to get the bundles. Then the bundles are opened and *Tamira Parpam* extract was collected safely.

(iii) Drugs:

The standard drugs such as Ranitidine, Omeprazole and the test extract of *Tamira parpam,* were suspended in 1% sodium carboxy methyl cellulose (SCMC) and used for anti-ulcer studies. Each drug suspension was prepared freshly just before the administration. Drugs and vehicles were administered orally. The present study followed three approaches of antiulcerogenic mechanism of the test extract: Aspirin plus pylorus ligation induced gastric ulcer in rats (antisecretory mechanism), HCl-Ethanol induced ulcer in mice (cytoprotective mechanism), Water immersion stress induced ulcer in rats (proton pump inhibition mechanism).

***(iv) Aspirin plus pylorus ligation induced gastric ulcer*:** [13, 14, 15]

The wistar albino rats were divided into 4 groups (n=6). All the animals received (200 mg/kg, P.O) aspirin. The different groups of animals are assigned as follows. Group I (control): received (2ml/kg of 0.5%, p.o.) Carboxy methyl cellulose. Group II (standard): treated with (8 mg/kg. p.o.) Omeprazole. Group III (T1) & Group IV (T2) received graded doses of *Tamira parpam* (15 & 30mg/kg, p.o.). Under light ether anesthesia, the abdomen was opened and the pylorus was ligated. The abdomen was then sutured. At the end of 4 h after ligation, the animals were sacrificed with excess of anesthetic ether, and the stomach was dissected out. The length of each lesion was measured under the dissecting microscope (X 10). The sum of the length (mm) of all lesions for each rat was used in lesion index. [16] The gastric content was analyzed for volume, acidity, pH and peptic activity. For determination of acidity, 0.2 ml of gastric juice was taken and diluted to 2 ml with distilled water in small conical flask; 1 drop of 1% phenolphthalein was then added to it. The total acidity was determined by titrating with 0.01 N NaOH and was expressed in meq/L/G. Peptic activity was determined. [17]

Table-1.
Ulcer Scores

S.No	Stomach colour	Ulcer score
1	Normal colour	0
2	Red colour	0.5
3	Red spots	1.0
4	Haemorrhagic streaks	1.5
5	3>5 Ulcers	2
6	< 5 Ulcers	3

Mean ulcer score for each animal is expressed as Ulcer Index.
Percentage ulcer protection = Ut / Uc X 100
Where, Ut = Ulcer index of treated group and Uc = Ulcer index of the control group
Total acidity was calculated using the formula
Volume of NaOH×Normality of NaOH ×100/0.1 meq /lt/ 100g

(v) Ulcer lesion Index method (HCl - Ethanol induced ulcer):

Wistar albino rats were divided into 4 groups (n=6). The different groups of animals are assigned as follows. Group I (control) received (2ml/kg of 0.5%, p.o) Carboxy methyl cellulose. Group II (reference standard) received ranitidine (50mg/kg, p.o) further Group III (T1) & Group IV (T2) received graded doses of *Tamira parpam* (15 & 30mg/kg, p.o.) After 30 minutes of drug administration to their respective groups, all the animals were treated with 0.2 ml of HCl - Ethanol mixture p.o (0.3 M Hydrochloric acid and 60% ethanol) to induce gastric ulcer. After 1 hour, animals were sacrificed with excess of anesthetic ether. The stomach was excised and lesion index was determined by measuring each lesion in mm along its greater length.[18] Cleared of residual matter with saline, the inner surface was examined for ulceration. Ulcer index and percentage of ulcer protection were calculated.

***(vi) Water immersion stress induced ulcer*:**[19, 20]

Stress ulcers were induced by forced swimming in the glass cylinder (height 45 cm, diameter 25 cm) containing water to the height of 35 cm maintained at 25°C for 3h 10h. Rats were fasted for 24h prior to the experiment and divided in–to 4 groups (n=6). The different groups of animals are assigned as follows. Group I (control), received (2 ml/kg 0.5%) of CMC. Group II (standard)–treated with (8 mg/kg, p.o) Omeprazole. Group III (T1) & Group IV (T2) received graded doses of *Tamira parpam* (15 & 30mg/kg, p.o.) similar to the previous. After the drug treatment animals were allowed to swim in water for 3 hour. The stomach of each animal was removed and the extent of gastric damage was assessed.

(vii) Statistical analysis:

The statistical analysis was carried out using one-way ANOVA followed by Dunnett's multiple comparison tests. All the results obtained in the study were compared with the vehicle control group. INSTAT V3 computer software tool was used for statistical analysis. P values <0.05 were considered statistically significant.

RESULTS

In aspirin and pylorus ligation induced gastric ulcer model, the *Tamira parpam* group recorded low volume, free acidity, total acidity, and ulcer index thus showing the anti-secretary mechanism involved in the test drug for their anti-ulcerogenic activity.

Table-2.
Effect of Tamira parpam against Aspirin and pyloric ligation induced ulcers in rats

Group and dose	Gastric volume (ml/100 g)	Total acid output *T. parpam* (mEq/L/100 g)	Free acidity (mEq/mL)	pH	Ulcer index	%Ulcer inhibition
Control	2.85±0.07	213.85 ± 5.70	522.45± 6.634	2.8	3.2±0.37	-
***T. parpam*(15 mg/kg)**	1.98 ± 0.62	204.63 ± 6.54	481.31± .062*	4.1	0.98±0.18*	69.37
***T. parpam*(30 mg/kg)**	1.25 ±0.75**	164.52±4.39**	478.93±.035**	4.9	0.54±0.57**	83.12
Omeprazole(8 g/kg)	1.25 ±0.99**	107.37±5.25**	312.67±3.021**	5.4	0.28±0.21**	91.25

The values are *P<0.05, **P <0.01.

In case of vehicle control, aspirin plus pylorus ligation increased the acid secretion, which in turn caused increase in gastric volume, low pH, increased free and total acidity resulting in increase in ulcer index. Pretreatment of rats with *Tamira parpam* (15-30 mg/kg) produced a dose dependent protection from the Aspirin and Pyloric ligation induced ulceration, as compared to control animals. The protection was statistically significant at 15 and 30 mg/kg. Omeprazole (8 mg/kg) produced significant protection as compared to control group.

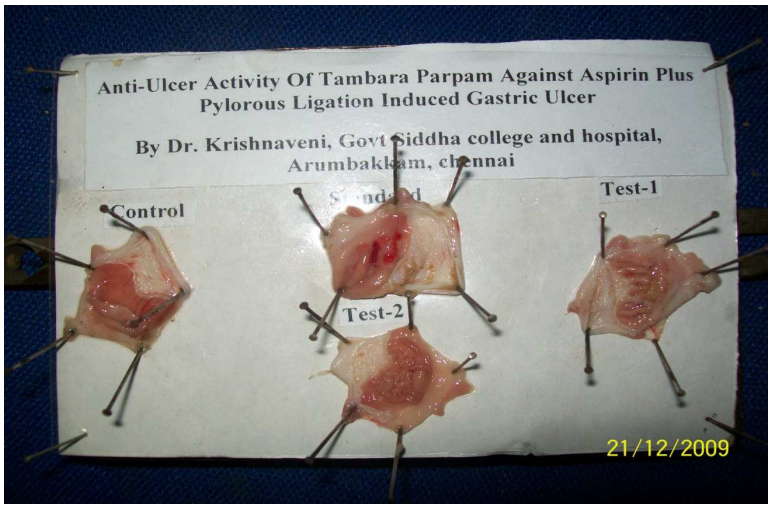

Figure 1.
Anti-ulcer activity of Tamira Parpam against Aspirin and pyloric ligation induced ulcers in rats.

Table 3.
Gastric cytoprotective effect of Tamira parpam on HCL and ethanol induced gastric ulceration

Group and dose	Gastric juice volume (ml/4 h)	Ulcer index	Ulcer inhibition (%)
Control	2.84±0.34	8.54±1.91	-
***T. parpam* (15 mg/kg)**	1.29±0.18**	5.63±0.98**	34.07
***T. parpam* (30 mg/kg)**	2.15±0.26*	4.82±0.74**	43.55
Ranitidine (50 mg/kg)	1.14±0.13**	1.96±0.36**	77.04

The values are *P<0.05, **P<0.01.

Pretreatment of rats with *Tamira parpam* from earthworm (15-30 mg /kg) produced a dose dependent protection from ethanol induced ulceration, as compared to control animals. However, the protection was not statistically significant at 15 mg/kg dose. Ranitidine
(50 mg/kg) produced significant gastric ulcer protection as compared to control group. *T. parpam* from earthworm in the doses of 15-30 mg/kg produced a significant reduction in the ulcer index. However, it failed to produce any significant effect on gastric volume, total acid output and pepsin activity. Ranitidine produced significant reduction in gastric ulcer and total acid output as compared to control group.

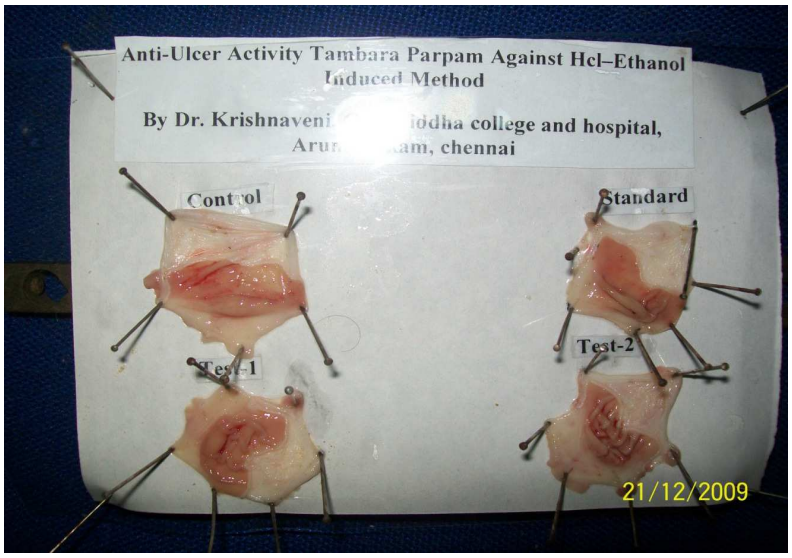

Figure 2.
Anti-ulcer activity of Tamira parpam against HCL and Ethanol induced method

Water immersion stress is one of the best models of stress in rats to induce ulcer. The model provides both emotional stress as well as physiological stress to the animal. In case of water immersion induced stress in rats, the *Tamira parpam* from earthworm showed significant (P<0.05) ulcer inhibition.

Table 4.
Effect of Tamira parpam on water immersion stress induced ulcer in rats

Group and dosage	Gastric acid content (μEq H^+)	pH	Ulcer index	Ulcer inhibition (%)
Control	123.31±15.16	4.19±0.14	4.29±0.95	-
***T. parpam* (15 mg/kg)**	79.57±5.78**	6.28±0.10**	3.86±0.86*	10.02
***T. parpam* (30 mg/kg)**	66.11±5.22**	6.99±0.19**	4.11±0.70	9.7
Omeprazole (8 mg/kg)	58.39±4.95**	6.57±0.15**	3.34±0.73**	22.144

The values are *P<0.05, **P value <0.01.

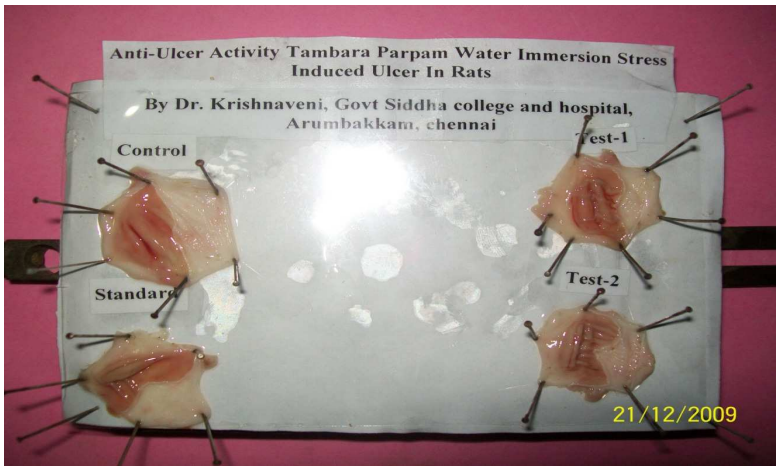

Figure 3.
Anti-ulcer activity of Tamira parpam water immersion stress induced method

DISCUSSION

The anti-ulcergenic activity was evaluated by employing aspirin/alcohol/stress induced ulcerations in rats. As the defence potential of mucus perimeter of gastric mucosal defence depends upon a delicate balance between the processes affecting the synthesis and secretion of its mucin constituents, the effect of *Tamira Parpam* from earthworm on gastric volume, free acid, and total acid was evaluated in pyloric ligated rats. [21] In addition, the effect of *Tamira Parpam* from earthworm on mucus secretion was also studied. *Tamira Parpam* from earthworm prevented the mucosal lesions induced by aspirin/alcohol/stress. Ranitidine (H_2–receptor antagonist) and omerprazole (an inhibitor of the proton pump) show gastro protective effect which suggests that mucosal injury by ethanol is not acid dependent. [22] The primary therapeutic approach of anti-ulcer agent involves maintenance of a delicate balance of factors controlling the synthesis, secretion, and break-down of its proteins, glycoproteins and lipid components, so as to strengthen the mucosal integrity. In the present study, *Tamira Parpam* from earthworm showed prevention of gastric lesions in the experimental models. Amongst the mechanisms by which *Tamira Parpam* provides gastro-protection in these models, involvement of a process of mucosa adaptation by means of an increase in the activity of defense mechanisms such as mucous secretion is probable.

Tamira Parpam from earthworm was found to increase the mucous and decrease the acid volume, free and total acid contents in rats. Intense staining of gastric mucosa by alcian blue at the apical region and in the deeper

mucosal layer as compared to control suggest that *Tamira Parpam* from earthworm, treatment promotes mucus secretion by mucosal cells. These effects of *Tamira Parpam* treatment on the parameters that influence the initiation and induction of ulceration may be considered as highly desirable property of anti-ulcerogenic agent. Thus, *Tamira Parpam* treatment affects the parameters that influence the initiation and perpetuation of ulceration. On the other hand, it is not possible to discount the possibility of the involvement or regulation of leukotrienes release in aspirin induced gastric lesions. Leukotrienes exert various biological actions, beside the vasoconstrictor effect, that could contribute to their role as mediators of ischemic and tissue damage. On the basis of the data presented here and the available reports, it can be concluded that the gastro protective effect elucidated by *Tamira Parpam* from earthworm could be mainly due to the modulation of defensive factors through an improvement of gastric cytoprotection and partly due to acid inhibition property. This study revealed a significant anti-ulcer effect of *Tamira Parpam* from earthworm in experimental models of gastric lesion induced by ethanol-acid and by a non-steroidal, anti-inflammatory drug, Aspirin.

Under the experimental conditions, different doses did not alter the gastric mucosal lesions in the stress model compared to the control. Anti-inflammatory drugs like Aspirin administered in toxic doses (200 mg/kg), produce visible gastric ulcers in animals. Aspirin is a potent inhibitor of prostaglandin biosynthesis. Prostaglandins are known to play an important role in maintaining mucosal integrity. An increase in certain endogenous prostaglandins can enhance gastric mucosal resistance to ulcerogenic agents. The mechanisms involved in prostaglandin action are multiple, including stimulation of mucus and bicarbonate output, gastric mucosal blood flow, decreasing gastric motility, increasing the release of endogenous mediators of gastric injury vasoactive amines and leucotrienes and stimulation of cellular growth and repair. [23]

On the day of ulcer induction, hemorrhagic lesions with disruption and edema covering the total glandular area of the stomach was evident in the untreated ulcerated mice. At peak ulceration, the severity of the gastric mucosal damage reached maximum as revealed from the exfoliation of gastric epithelial cells and disruption of mucosal layer. Loss of foveolar as well as cryptic architecture was also evident. The ulcerative process led to the generation of a large number of inflammatory cells with altered chief and parietal cells. Treatment with two doses of *Tamira Parpam* from earthworm did not significantly restore the gastric architecture as compared to ulcer-untreated rats in Asp+PL model. However, administration of *Tamira Parpam* from earthworm low dose in HCl-ethanol model prevented the inflammatory and necrotic debris, but the damage to chief and parietal cells were not completely healed. In contrast, the TP mid and high doses showed high tendency of inhibition on inflammatory cells and death cell debris, with more number of healthy cells. In addition, it also helped in the reduction of inflammatory cells thereby minimizing the inflammatory burden. The effects of *Tamira Parpam* from earthworm were comparable and are better than that of Omeprazole. Thus, the *Tamira Parpam* from earthworm helped in reversing the damage of the gastric surface epithelial cells, as well as mucosal layer of gastric lumen caused by HCl-ethanol, which led to a faster ulcer healing. Water immersion stress is one of the best models for stress induced ulcer in animals. The model provides both emotional stress as well as physiological stress to the animal. The extract showed significant ($P<0.05$) ulcer inhibition in both the dosages (15 and 30 mg/kg).

CONCLUSION

The anti-ulcer effect observed in the present study might be due to a possible relationship between protection of mucosal injury, inhibition of acid secretion and the

antioxidant nature of *Tamira parpam*. Tamira parpam possess antisecretory, cytoprotective and proton pump inhibition mechanism. This study indicates that *Tamira parpam* has a potential anti-ulcer activity.

REFERENCES

1. Wallace J L., Prostaglandins, NSAIDs and gastric mucosal protection: why doesn't the stomach digest itself. Physiol Rev, 88, (4): 1547-1565, (2008)
2. Phillipson M, Atuma C, Henriksnas J and Holm L., The importance of mucus layers and bicarbonate transport in preservation of gastric juxtamucosal pH. Am J Physiol Gastrointest Liver Physiol, 282, (2): 211-219, (2002)
3. Bighetti A E, Antonio M A, Kohn L K, Rehder V L G, Fogli M A, Possenti A, Vilela L and Carvalho J E., Antiulcerogenic activity of a crude hydroalcoholic extract and coumarin isolated from *Mikania laevigata* Schultz Bip. Phytomedicine, 12, 72–77, (2005)
4. Bandyopadhyay U, Biswas K, Chatterjee R, Bandyopadhyay D, Chattopadhyay I, Ganguly C K, Chakraborty T, Bhattacharyya K and Banerjee R K., Gastroprotective effect of Neem (*Azadirachta indica*) bark extract: Possible involvement of H+-K+-ATPase inhibition and scavenging of hydroxyl radical. Life Sci, 71, 2845–2865, (2002)
5. Zhao Dan, Wang Ying, Wu Ying and Zhang Jing-jing., Analysis of Trace Elements in Earthworm. Journal of Shenyang Normal University (Natural Science Edition), (2009)
6. Ansari A and Kaminie Sitaram., An investigation on the Anti-microbial and Anti-fungal properties of earthworm powder obtained from Eisenia fetida. Intern J micro res, 1,(2): 62-66, (2010)
7. Hartenstein R, Leaf A L, Neuhauser E F and Bickelhaupt D H., Composition of the earthworm Eisena foetidu (Savigny) and assimilation of 15 elements from sludge during growth. Comp Biochem Physi, 66, 187-192, (1980)
8. Feldman M and Burton M E., Histamine 2-receptor antagonists. Standard therapy for acid-peptic diseases. N Engl J Med, 323, (24): 1672-1680, (1990)
9. Reilly J P., Safety profile of the proton-pump inhibitors. Am J Health Syst Pharm, 56, (23) :4,S11-17, (1999)
10. Van der Voet, G B, Sarafanov A, Todorov T I, Centeno J A, Jonas W B, Ives J A and Mullick F G., Clinical and analytical toxicology of dietary supplements: a case study and a review of the literature. Biol Trace Elem Res, 125, (1): 1-12 (2008)
11. Paganini C L and Bianchini A., Copper accumulation and toxicity in isolated cells from gills and hepatopancreas of the blue crab (Callinectes sapidus). Environ Toxicol Chem, 28,(6): 1200-1205, (2008)
12. Rates S M., Plants as source of drugs. Toxicon, 39, (5): 603-613, (2001)
13. Brodie D A., Experimental peptic ulcer. Gastroenterol, 55, (1):125-133,(1968)
14. Bhatnagar M, Jain C P and Sisodia S S., Anti-ulcer activity of *Withania somnifera* in stress and pyloric ligation induced gastric ulcer in rats. J Cell Tis Res, 5, (1): 287-292, (2005)
15. Bhave A L, Bhatt J D and Hemavathi K G., Antiulcer effect of amlodipine and its interaction with H2- blocker and proton pump inhibitor in pylorus ligated rats. Ind J Pharmacol, 38, (6): 403-407, (2006)
16. Okabe S, Takata Y, Takeuchi K, Naganuma T and Takagi K., Effects of carbenoxolone Na on acute and chronic gastric lesion reduced by NSAIDs drugs in mice Agents Action, 21, 316-319, (1976)
17. Debnath P K, Gode K D, Govind Das D and Sanyal A K., Effect of propanolol on

gastric secretion in albino rats. Br J Pharmacol, 51, 213-16, (1974)
18. Yesilada E, Gurbutz I and Ergun E., Effect of *Cistus laurifolius* L flowers on gastric and duodenal lesions. J Ethnopharmacol, 55, 201-11, (1997)
19. Alder R., In Breakdown in human adaptation to stress. Martinus Ninjihoff Boston, 653-658, (1994)
20. Alphine R S., Word JW: Antihistaminic activity and ulceration. European J Pharmacol, 6, 61-6, (1999)
21. Sanyal A K, Mitra P K and Goel R K., A modified method to estimate dissolved mucosubstances in gastric juice. Ind J Exp Biology, 21, 78-80,(1983)
22. Shay H, Komarov S A, Fels S S, Meranze D, Gruenstein M and Siplet H., A simple method of the uniform production of gastric ulceration. Gastroenterology, 5, 43-61, (1945)
23. Derelanko M J and Long J F., Carbenoxolone sodium protects rat gastric mucosa against ethanol-induced necrosis. Proc Soc Exp Biol Med, 166, (3): 394-397, (1981)