**Abstract.** – **Background:** For thousands of years, earthworm and its products have been used for its therapeutic benefits. The traditional medical knowledge of indigenous people throughout the world more particularly in Asia, including India, Myanmar, China, Korea and Vietnam has played vital role in identifying, extracting and using biologically active compounds from earthworms.

**Materials and Methods:** Effect of various doses (20, 40, 80, 160 and 320 mg/kg, po) of earthworm paste (*Lampito mauritii*, Kinberg) was studied on aspirin-induced gastric ulcer and compared to ranitidine.

**Results:** The earthworm paste showed significant ulcer protective effects. The cytoprotective effect of the earthworm paste seems to be not only due to the anti-secretory action but also to the effects on mucosal glycoprotein. The decrease in total acidity, pepsin and protein content of the gastric juice proved the antiulcer activity of earthworm paste. Histopathological studies revealed that the earthworm paste was more effective in gastric cytoprotection than ranitidine in preventing lesion formation.

**Conclusions:** These observations show that the earthworm paste possess antiulcer potential.

**Key Words:** Earthworm, *Lampito mauritii*, Aspirin, Anti-ulcer.

**Introduction**

The organic and functional dyspepsia are common clinical syndromes characterized by pain associated with the stomach. Organic dyspepsia can be caused by peptic ulcer disease, a common gastro-intestinal disorders in clinical practice. The increase in acid-pepsin secretion and decrease in mucosal resistance appears to be the basic causes for peptic ulceration. The aetiology of gastric ulcer is mainly due to defective mucosal resistance. Although the cause of ulceration is not known, gastric acid and pepsin are responsible for maintaining the lesion, once it is produced. The mucosal resistance is lowered by the *Helicobacter pylori*-gram negative bacillus that colonizes the stomach and duodenum. Aspirin a non-steroidal anti-inflammatory drug which induces the peptic ulcer causes increase in gastric secretion, reduces the pH leads to acidity which damages the epithelial cells and sub mucosa layer. Davenport suggested first that the normal resistance of the gastric mucosa to back diffusion of luminal acid can be disrupted by topical administration of lipid soluble damaging agents such as acetylsalicylic acid or aspirin. Oral administration of aspirin has the ability to transform the gastric mucosa rapidly from a hydrophobic to a more hydrophilic state.

Over the last 25 years, a remarkable revolution in the pathophysiology and treatment of gastric and duodenal ulcers have occurred. Several therapies that have been successful in healing peptic ulcers include neutralizing gastric acid, inhibiting acid secretion and protecting the gastro duodenal mucosa. Efforts are being made to discover a effective anti-ulcerogenic drug which will not only heal the ulcer but also prevent its recurrence. Search for a suitable drug for the treatment of ulcer from natural products is an ongoing process. Considering the several side effects of modern medicine, indigenous drugs possessing fewer side effects should be looked for as a better alternative for the treatment of ulcer.

Earthworms, a soil macro-invertebrate possess many therapeutic role is recorded in many ancient literature and they are used still in countryside for numerous ailments. For a long period of time, earthworms have been a valuable source of natural products for maintaining human health, especially in India, China and most of the Asian countries. Earthworm derivatives have been used as...
drugs against various diseases in China and the Far East for a few thousand years. Earthworms have been used in medicine for various remedies since 1340 A.D. and their medicinal properties have been described by Bristow. According to the description given by Vohora and Khan earthworms have largely been used internally and externally as powerful aphrodisiacs. Mihara et al. and Ismail et al. reported that earthworm has the potentiality in the treatment of thrombosis and actually, orally administrated earthworm powder was capable of digesting intravascular fibrin clots. Bhatnagar and Palta reported that earthworms ingested into our body system increase body heat and are of value in neurologic disorders, bronchitis, tuberculosis and holding a substance effective in curing rheumatism. Recently earthworm protein and its coelomic fluid are known to have cytolitic, agglutination, proteolytic, haemolytic, mitogenic, anti-pyritic, tumorstatic, anti-inflammatory and antibacterial activities. Earthworm tonic is also used for balancing the sympathetic and parasympathetic functions of the central nervous system. In Korea taking “earthworm soup” before going to bed is a traditional habit, which enriches general health and prevents various ailments. Earthworm is formulated as “earth dragon” by allergy research group and used as a suitable drug for the gastro-intestinal disorders caused in gastro-intestinal tract. Hence in the present study efforts have been made to find the antiulcerant property of earthworm paste compared with a reference drug ranitidine in rats.

Materials and Methods

Preparation of Earthworm Paste
Earthworms Lamptio mauriti (Kinberg) were obtained from the stock culture, Division of Vermibiototechnology, Department of Zoology, Anna Malai University, Tamil Nadu, India. 500 sexually mature, clitellate worms (900 mg/worm) were washed with running tap water and then fed with wet blotting paper for 18-20 hours for gut clearance. The gut cleared worms were again washed with distilled water. The worms were kept in plastic trough covered tightly with polythene cover and exposed to sunlight for three days to kill the earthworms. Mucus and coelomic fluid that oozed out from the worms digested the dead worms forming a brown coloured paste called “earthworm paste”.

Animals Used
Healthy and pure strain male albino rats (Rattus norvegicus), ranging from the body weight of 150-200 gm were procured from the Department of Experimental Medicine, Central Animal House, Rajah Muthiah Medical College, Anna Malai University, Annamalainagar and used for the experimental study. The animals were housed in polypropylene cages at 24°C ± 2°C and were fed with proper food and water ad libitum throughout the experiment. The experiment got clearance from the Institutional Animal Ethical Committee (IAEC).

Drugs
Aspirin, “earthworm paste” and standard drug ranitidine were suspended in 1% carboxymethyl cellulose before experiment and given orally for 10 days during the experiment.

Aspirin Plus Pyloric Ligation Induced Ulcer Model
The methods of Goel et al., Shay et al. and Parmar were followed for the evaluation of anti-ulcer activity. The animals were divided into 8 groups of 6 animals each. Out of 8 groups, group I served as normal control and received water. Group II served as aspirin control receiving aspirin (200 mg/kg). The remaining 6 groups served as treated groups receiving ranitidine (50 mg/kg) – a standard drug and “earthworm paste” administered in different doses (20, 40, 80, 160 and 320 mg/kg). All the doses were administered orally once daily for 10 days. From the 6th day onwards, animals in groups III to VIII received aspirin (200 mg/kg) orally, one hour after the administration of the earthworm paste was done. On the 11th day pylorus ligature was carried out on the 18 hours fasted rats.

Collection and Analysis of Gastric Juice
Gastric juice was collected by a 4 h pyloric ligation. Normal rats and those from experimental groups were anaesthetized with ether and cut open through a midline incision in the abdomen. The pylorus was secured and ligated with silk sutures after which the wound was closed and the animals allowed to recover. Drinking water was withheld for 4 h. The rats were then killed by an overdose of chloroform vapours. The stomach was removed after clamping the oesophagus and the gastric contents were collected. The gastric juice was centrifuged at 3500 rpm for 15 min. Aliquots from this was used for the assay of
Gastroprotective effect of earthworm paste (*Lampito mauritii*, Kinberg)

pepsin$^{22}$ and total protein$^{23}$. Total acidity was determined by titrating with 0.01N sodium hydroxide using phenolphthalein as indicator.

**Histopathological Studies**

The stomach was washed with normal saline and fixed in Bouin’s fluid. After 24 hours the tissues were processed following standard techniques$^{22}$. Sections of 6 thickness were stained in Heidenhain’s haematoxylin and counterstained with eosin for microscopic observation.

**Statistical Analysis**

Results are expressed as means ± SD. Statistical analysis was done using Student’s t-test. A value of $P<0.05$ was considered significant.

**Results**

**Gastric Secretion**

The total acidity, pepsin activity and protein content of the gastric juice of normal and experimental animals are presented in Table I. The aspirin-ulcerated rats showed significantly higher pepsin activity, protein content and total acidity as compared to normal rats. Rats pretreated with earthworm paste and ranitidine showed a significantly lower activity of pepsin, protein content and total acidity of gastric juice compared to the ulcerated group. Particularly 160 mg/kg of earthworm paste shows better reduction in pepsin, protein content and total acidity of gastric juice when compared to the ranitidine treated animal.

**Histopathological Observations**

The histopathology of stomach of aspirin treated animals showed ulceration with haemorrhage and discontinuity in the mucosal epithelial lining. The aspirin induced ulcer model shows various changes in the histopathological architecture of the stomach. The aspirin induced gastric lesion is multifactorial with the depletion of gastric wall mucous content. The gastric mucosa or the mucous membrane is damaged thereby producing ulcer, the mucosal layer was severely affected by the administration of aspirin. The blood vessels in the mucosal layer were damaged. Venular constriction were found to be observed in the epithelial cells of mucous membrane. Pretreatment with 50 mg/kg ranitidine was found to reduce the occurrence of gastric lesion (Figure 3). There was lesser ulcer in the gastric mucosa. The intensity of haemorrhage was significantly reduced upon the pretreatment with ranitidine. The mucosal necrosis of the gastric epithelium was found to be reduced. Sub-mucosal venular constrictions in the epithelial cells were reduced. Similarly no gastric lesions were observed in the animals treated with earthworm paste. Treatment with 160 mg of earthworm paste/kg was found to show better results than the ranitidine treated animals (Figure 7). This was followed by 80 mg/kg (Figure 6), 320 mg/kg (Figure 8) and 40 mg/kg (Figure 5) treatments. But 20 mg of earthworm paste/kg (Figure 4) had the least effect.

**Discussion**

In general ulcers were caused by the back diffusion of H$^+$ ions into the mucosal cells$^{22}$. The histopathological observations on the ulcer in the stomach induced by aspirin revealed gastric lesions, damage to mucosal epithelium, blood ves-

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Table I. Effect of pretreatment with different doses of earthworm paste and ranitidine on the total acidity, and protein content in aspirin-treated rats (*Rattus norvegicus*) ($P<0.05$).

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Total acidity*</th>
<th>Pepsin activity*</th>
<th>Protein*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal control</td>
<td>15.29 ± 0.27</td>
<td>1.36 ± 0.4</td>
<td>0.995 ± 0.1</td>
</tr>
<tr>
<td>Aspirin induced ulcer control</td>
<td>53.15 ± 0.17*</td>
<td>3.01 ± 0.2*</td>
<td>4.25 ± 0.2*</td>
</tr>
<tr>
<td>Standard drug (Ranitidine 50 mg/kg)</td>
<td>24.68 ± 0.16**</td>
<td>1.95 ± 0.1**</td>
<td>1.27 ± 0.3</td>
</tr>
<tr>
<td>Earthworm paste (mg/kg) 20</td>
<td>33.17 ± 0.40**</td>
<td>2.99 ± 0.5**</td>
<td>3.51 ± 0.1**</td>
</tr>
<tr>
<td>40</td>
<td>30.60 ± 0.54**</td>
<td>2.42 ± 0.2**</td>
<td>2.72 ± 0.4**</td>
</tr>
<tr>
<td>80</td>
<td>22.39 ± 0.21**</td>
<td>2.01 ± 0.3**</td>
<td>2.15 ± 0.2**</td>
</tr>
<tr>
<td>160</td>
<td>20.09 ± 0.41**</td>
<td>1.75 ± 0.6**</td>
<td>1.25 ± 0.3**</td>
</tr>
<tr>
<td>320</td>
<td>29.30 ± 0.29**</td>
<td>2.07 ± 0.4**</td>
<td>2.20 ± 0.4**</td>
</tr>
</tbody>
</table>

Values are means ± SD from six rats in each group. *Compared to normal $P<0.05$; **Compared with aspirin treated $P<0.05$

*Expressed as mM Eq/l; **Expressed as µg tyrosine liberated/kg protein/h; *Expressed as mg/ml.
sels and constriction in the veins. These damages were found to be prevented by the pretreatment with the standard drug ranitidine and treatment with different doses of earthworm paste.

Ulcerogens like ACTH, cortisone, ethanol, aspirin and phenylbutazone reduce the rate of secretion of mucus and reduce the concentration of protein bound carbohydrates in the secretion. These agents injure the gastric mucosa by reducing its ability to form a protective layer of mucus. In addition, the ACTH and cortisone decrease the rate of renewal of surface epithelial cells while aspirin and phenylbutazone increases the rate of exfoliation of surface epithelial cells. Aspirin induces gastric lesion (ulcer) by causing back diffusion of H+ ions into the mucosal cells. The ethanol or aspirin induced gastric lesions are multifactorial with the depletion of gastric wall mucus content. Mucosal blood flow has also been attributed to be an important factor in the damage caused by non-steroidal anti-inflammatory drugs (NSAIDs) and is modulated by prostaglandin. Submucosal venular constriction by NSAIDs drugs and eventual injury is caused to perturbations of superficial mucosal cells. Aspirin causes leakage of plasma protein into gastric juice. This reflects the increased protein concentration in the gastric juice of aspirin control rats. In the present study pretreatment with earthworm paste decreases the protein concentration in the gastric juice, thereby preventing the plasma membrane from the damage. The administration of 200 mg/kg of aspirin had induced gastric ulcer by producing mucosal damage, gastric lesion, constriction in epithelial cells and decrease in number of cells. Sanmugapriya et al. suggested that the presence of polyphenolic compounds prevents damages to the gastric mucosa. Some flavonoids have been shown to increase the mucosal content of prostaglandin and mucus in gastric mucosa, showing their cytoprotective effects. Some flavonoids have been shown to increase the mucosal content of prostaglandin and mucus in gastric mucosa, showing their cytoprotective effects.
Gastroprotective effect of earthworm paste (Lampito mauritii, Kinberg)

damages reduced the gastric lesion and haemorrhage. Falling in line with the above observations in the present study, the pretreatment with earthworm paste had reduced the gastric lesions, increased in number of epithelial cells and reduced the mucosal damage. This may be due to the presence of polyphenolic compounds in earthworm tissue and SH group containing amino acids, which prevents the gastric damage by increasing the mucin activity. The effect of earthworm paste against gastric ulcer is much better than the ranitidine treatment. Particularly treatment with 160 mg earthworm paste/kg showed better cure than ranitidine. The antiulcer effect of the earthworm paste may be due to the formation of mucin like gel layer on the surface of the mucosa or via the formation of protecting complexes between gel and mucus as a barrier against the agents introduced into the stomach or against endogenously formed acid and pepsin in the stomach. The histopathological observations showed that upon earthworm paste treatment, the mucosal epithelium had retained normal histarchitecture, less haemorrhage and more significant on reduction in ulcer which proves the anti-ulcer activity.

References

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Dietary supplementation with the earthworm paste to Wistar rats significantly increased the percentage of growth rate and number of litters delivered when compared to the rat fed on standard rodent diet and given water ad libitum (normal control). The percentage of growth rate of experimental male and female rat fed on the earthworm paste showed a significant increase (p<0.01) when compared to the respective normal control groups. The results clearly indicate that earthworm paste is a good growth and reproductive stimulator.