Heteromorpha trifoliata (Dombwe) accelerates acetic acid-induced peptic ulcers : a preliminary study in the rat

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Objectives: To investigate the effect of *H. trifoliata* on: (a) acetic acid-induced ulcers, (b) food intake, (c) water intake, (d) weight gain, (e) gastric acid secretion in rats.

Design: Comparative study.

Setting: Laboratory.

Subjects: 20 female Sprague Dawley rats (220 to 250g) with acetic acid-induced peptic ulcers randomly assigned to test and control groups (n=10). The test rats were allowed water and normal rat diet comprising 20% *H. trifoliata* (*'Dombwe)* and *'Imfe nkulu'* in Shona and Ndebele respectively) for 17 days after ulceration while control rats were allowed water and normal rat diet for 17 days after ulceration. Thirty six other rats were prepared to study the effect of *H. trifoliata* on gastric and acid secretion stimulated by histamine, gastrin and carbachol.

Main Outcome Measures: Photographs of the gross anatomy and hisotology of test and control rat stomachs were taken. Daily food and water intake, weekly weight gain and gastric acid secretion were measured in the test and control rats.

Results: 17 days following the consumption of the *H. trifoliata* containing diet, macroscopically, no ulcers were found on the outer surface of the stomach walls of test rats. However, histological examination revealed traces of ulcer at the sites where ulcers were induced previously. In contrast, 70% of the control rats still had ulcers on the surface of their stomach walls. Histological examination showed massive denuded mucosa and submucosa at the ulcer sites which are signs of severe ulceration. Food intake in both groups was not significantly different except during the first three days when test rats consumed significantly less food ($p< 0.01$) than control rats. Daily water intake and weekly weight gain were also not significantly different in the test and control groups. *H. trifoliata* had no significant effect on gastric acid secretion stimulated by histamine, gastrin and carbachol.

Conclusions: *H. trifoliata* does not affect daily food and water intake and weekly weight gain in rats. It also does not affect histamine, gastrin and carbachol-stimulated acid secretion in rats. However, *H.trifoliata* accelerates the healing of acetic acid-induced peptic ulcer in rats. This may validate the use of *H. trifoliata* in the treatment of peptic ulcer in humans.

Introduction

Peptic ulceration is common in Zimbabwe. In 1992 alone, there was a prevalence rate of ulceration of 456 per 100 0000 new hospital cases and the incidence appears to be increasing more in Zimbabwe than in Western countries.[1] The majority of Zimbabweans live in the rural areas and most of them visit traditional healers for medical treatment owing to low cost and easy accessibility. Some traditional healers in Zimbabwe use a local herb known as *Heteromorpha trifoliata* (L) to treat peptic ulcers, and claim a considerable success in their cure of the disease (Duri, personal communication).

H. trifoliata , a member of the *Umbelliferae* (carrot) family is a deciduous shrub which is widely distributed in Zimbabwe. The Shona refer to it as *'Dombwe'* and the Ndebele as *'imfe nkulu'*. The roots of *H. trifoliata* have been extensively used in traditional medicine in southern Africa to treat a variety of disorders. In Zimbabwe, the root bark of *H. trifoliata* has a variety of other medicinal uses including the treatment of headache, abdominal pains, chest pains and cough.[2] However, many people are skeptical regarding the use of traditional medicines owing to the lack of scientific validation of their efficacy and safety. It is, therefore, necessary to provide scientific validation of the efficacy and safety of traditional (herbal) therapies.[3] There is a move by the Ministry of Health to recognise the traditional healers and register herbal therapies used by the Zimbabwe Traditional Healers Association

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(ZINATHA). The move is being hampered by the lack of scientific validation of the efficacy and safety of the therapies.

To our knowledge, the effect of *H. trifoliata* on the healing of peptic ulcer has not been scientifically validated. In view of the high incidence of peptic ulceration in Zimbabwe, it was necessary to revisit the herbal therapy, *H. trifoliata* used in the treatment of peptic ulcers. Therefore, the major aim of the study was to ascertain whether *H. trifoliata* accelerates the healing of an experimental peptic ulcer using the rat as experimental animal. Its effects on food, and water intake, weight gain and stimulated gastric acid secretion were also investigated.

Materials and Methods

Induction of Acetic Acid Peptic Ulcers in Rats.

The method of Wang *et al*,[4] was used in the induction of peptic ulcers.Twenty female Sprague Dawley rats were used. The dissecting instruments used for this study were sterilized by autoclaving them for 30 minutes. Under Pentobarbitone anaesthesia (35mg/Kg,ip), the skin overlying the abdomen was shaven and cleaned with 70% alcohol. The stomach was exposed and 0.02ml of 90% acetic acid was injected into the submucosal layer of the antral oxyntic border on the anterior wall of stomach, to induce an ulcer. The abdomen was closed with catgut (3/0) and its skin with silk thread (4/0). The rats were allowed free access to water and normal rat diet for five days.

On the fifth day, well defined ulcers that penetrated the muscularis mucosal and mucosa had developed. The fifth day was therefore selected as the day of ulceration. Indomethacin (1mg/kg) suspended in traces of Tween 80 and saline was given subcutaneously to all the rats for 17 days following the ulceration. The indomethacin was to maintain the ulceration throughout the duration of the experiment. Rats with acetic acid-induced ulcers were randomly assigned to test and control groups of 10 rats each. Rats in the test group were fed a powdered rat diet comprising 20% dry powdered *H. trifoliata* and water while those in the control group were allowed a powdered normal rat diet and water .

Preparation of Ground Powder and Aqueous Extract of *H. trifoliata*.

The preparation of the extract of *H.trifoliata* was similar to the method used by Parry *et al*.[5] The roots of *H. trifoliata* were collected from Marirangwe area of Zimbabwe. The plant was authenticated at the National Herbarium in Harare where voucher specimens were deposited. The root bark was scrapped off and sun dried. It was then ground into fine powder using a pestle and mortar. The fine powder was used for two sets of experiments. The first set was the feeding experiments where the fine powder of *H. trifoliata* was added to powdered normal rat feed (National foods, Harare, Zimbabwe). The test diet comprised the ground normal rat diet comprising 20% of the ground *H. trifoliata* while the control diet comprised the ground normal rat diet only. The test and control diets were used in feeding experiments to determine the effect of *H. trifoliata* on the healing of acetic acid-induced ulcers. The daily food intake and water intake as well weekly weight gain in rats were also recorded.

The second set of experiments was on the effect of intraperitoneal administration of aqueous extract of *H. trifoliata* on gastric acid secretion stimulated by histamine, gastrin and carbachol. For these experiments, the powder was soaked in water for five days. The resulting solution was filtered and the filtrate was freeze dried. The solid material was subsequently reconstituted in a known volume of distilled water and then serially diluted. The extract solutions of *H.trifoliata* thus obtained, were stored at -4°C until use.

Gross Anatomy and Histology of Rat Stomachs.

Seventeen days following the consumption of control and test diets, the rats were deprived of food for 18 hours but allowed free access to water to empty their stomachs. The animals were killed by an overdose of sodium pentobarbitone (80mg/kg ip). The abdominal wall was incised and their stomachs exposed. The stomach ends were tied and inflated with 5ml of 10% formal saline. The gross anatomical features were noted and the photographs of the stomach outer surface were taken to compare the healing of ulcers in the control and test rats.

For histological studies, the stomach wall of the ulcerated area and the area around the ulcer-induced site in non-ulcerated rats were incised and fixed in 10% formal saline for a minimum of 24 hours. Five micron sections were cut and stained with haematoxylin and eosin after routine histological preparation. The sections were observed under light microscope and the appropriate areas were photographed.

Effect on Stimulated Gastric Acid Secretion.

High acidity is a common factor in the aetiology of peptic ulcers. Therefore, to find the probable mechanism by which *H. trifoliata* could accelerate the healing of acetic acid-induced ulcers, it was necessary to investigate its effect on cholinergic, histamine and gastrin-stimulated gastric acid secretion which are the major pathways for gastric acid secretion. Carbachol was used as the cholinergic drug.

Gastric acid secretion was measured by the method of Gosh and Schild[6] and modified by Amure and Ginsburg[7] and Osim *et al*.[8] Briefly, the rats were starved for 18 hours and each animal was anaesthetized with 25% urethane at a dose of 0,6ml/100g body weight ip. By incision, a tracheal cannula was inserted to ensure free breathing throughout the period of experiment. The pylorus was semi-transected at its junction with the duodenum and a pyloric cannula was inserted and tied in place. An oesophageal cannula for infusion of normal saline (pH:7,0) was passed through the mouth into the stomach. The normal saline was kept at a temperature of 37°C throughout the experiment. The stomach was perfused with the normal saline at a rate of 1ml per minute. Gastric acid was collected via the pylorus cannula at 10 minute intervals. The volume of the gastric perfusate was about 10ml. Determination of acidity was done by titration of the 10ml perfusate sample against 0.01 NaOH solution using phenolphthalein as indicator. The titratable acidity was expressed in μEq/10mins.

Eighteen rats were used to study the effect of the secretagogues (histamine, carbachol and gastrin were obtained from Sigma,UK) on acid secretion in either control and test animals. In the control and test animals, histamine (100mg/Kg), carbachol (10μg/Kg) or gastrin (200μg/Kg) was administered subcutaneously into subgroups of six rats each, thus making a total of three control and three test subgroups.

In the control animals, after a steady basal acid output was obtained for an hour, histamine, carbachol or gastrin was administered subcutaneously to stimulate acid secretion, and the perfusate collection was continued for the following two hours. In the test groups, *H. trifoliata* (100mg/Kg) was administered intraperitoneally after one hour of basal acid output collection. Ten minutes following *H. trifoliata* administration, histamine, carbachol or gastrin was administered to determine if *H. trifoliata* could block the acid secretion induced by the secretagogues.

Statistical Analysis.

Differences between the percentages of healed ulcers in both control and test groups were assessed by Fisher's exact test. Other differences between control and test rats were assessed using an unpaired student's test. Data were presented as means and standard error of the mean (SEM). A p value less than 0,05 was considered significant.

Results

Gross Anatomy and Histology of Test Control Rat Stomachs.

Comparison of the stomach walls of 10 test rats and 10 control rats showed that *H. trifoliata* accelerated the healing of acetic acid-induced ulcers in rats. Seventeen days after the consumption of *H. trifoliata* containing diet by test rats, macroscopic examination showed that the outer wall of their stomachs had healed. However, histologically, the mucosal lining was not healed completely. The regenerated mucosa at the ulcer induced site showed irregular arrangement and slight dilatation of the gastric glands in the *lamina propia*. A slight thickening of the stomach wall at the induced site was also noted. The mucosal lining was transformed into normal columnar epithelium with some luminal carnification indicating the metaplastic transformation of epithelium at the induction site (Plates I and II).

In contrast, 70% of control animals' gross anatomy exhibited a well defined ulcer at the induction site. On macroscopic examination, the ulcerated region of the stomach wall was adhered to the adjoining liver tissue and black patches of

*Plate I: A typical photograph of the gross anatomy of a test rat which received a normal rat diet comprising 20% **H. trifoliata**. It shows that the ulcers on the outer wall of the stomach had healed before autopsy.*

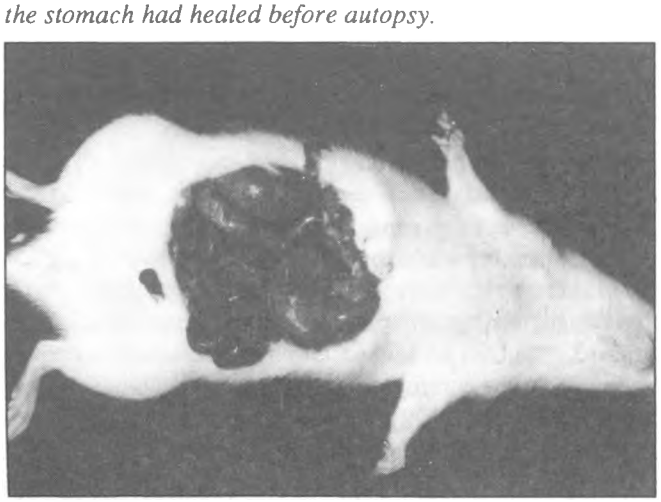


*Plate II: A typical photograph of the histology of test rat which received a normal rat diet comprising 20% **H. trifoliata**. It shows a nearly regenerated mucosa and near normal histology of the stomach at the site of ulcer induction.*

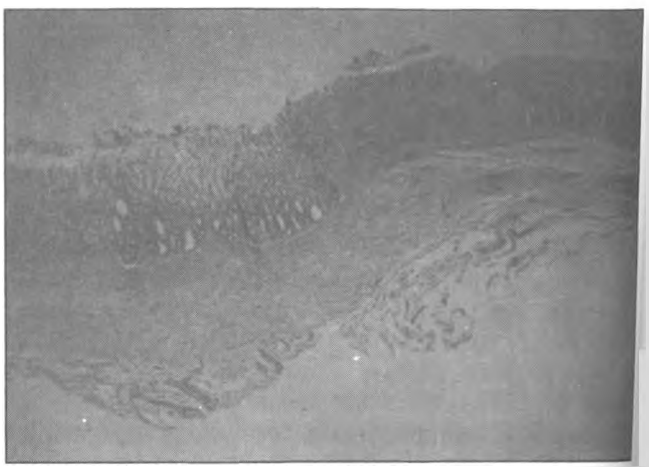


Plate III: A typical photograph of a control rat that was fed on a normal rat diet showing a well defined ulcer on the outer surface of the stomach wall.

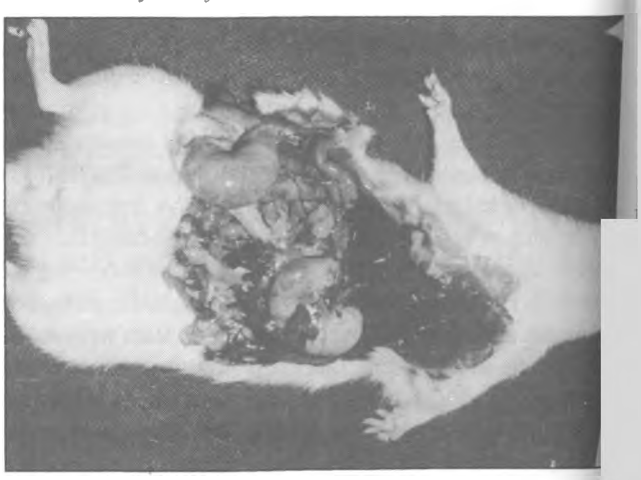
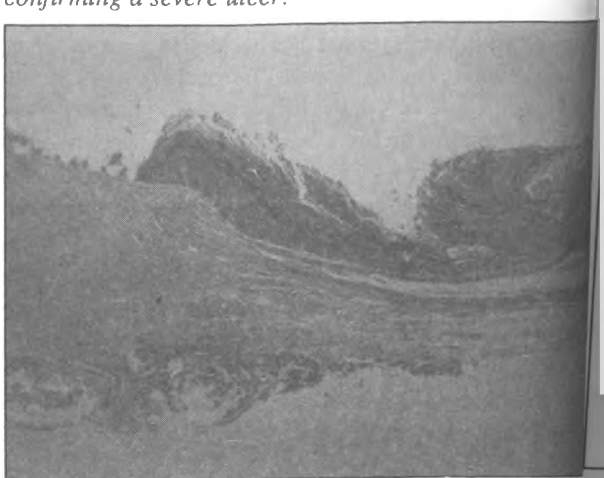


Plate IV: A typical presentation of the histology of all control rats fed on normal rat diet only. It shows an abnormal histology structure with a denuded mucosa t confirming a severe ulcer.



stomach wall were observed in the interior of the stomach at the induction site. Histologically, the stomach wall exhibited denuded mucosa and submucosa at the induction site confirming the presence of severe ulceration. The area of stomach wall adjoining the denuded mucosa and submucosa showed that the mucosa was attempting to regenerate. The stomach wall at the ulcer site was very thick and consisted of connective tissue, confirming that the ulcer site was attempting to heal but at a slow pace (Plates III and IV). The difference between the percentages of healed ulcers on the outer wall of the stomachs of test and control rats was significant (p=0.003).

Daily Food Intake, Water Intake and Weekly Weight Gain in Test and Control Rats.

Test and control rats (n=10) were placed in separate cages. The weight of the food contents and volume of water in a water bottle were measured before and after every 24 hour period. The weight of the rats was measured weekly. *H. trifoliata* did not affect daily food and water intake and weekly weight gain. The only significant difference was in the food intake during the first three days when test rats consumed significantly less food ($p<0,01$) than control rats. The results showed that the average food consumed per day by each control rat was 17.1 ± 0.3g and each test rat consumed 17.3 ± 0.3g per day after the third day up to the 17th day (Figure I).

The average daily water intake in each control and test rat was 27.6 ± 0.5 and 27.7 ± 0.4ml respectively (Figure II). The average weekly weight gain in control rats was 2.3 ± 0.3g. Although the average weekly weight change in test rats was not significantly different from control rats, the body weight in the test rats showed a slight decrease in body weight in the first week following consumption of the test diet. The decrease in body weight was, however, not significant from its original weight (Figure III).

Gastric Acid Secretion in Control Rats.

Basal gastric acid secretion in the first subgroup of six control rats was 3,42 ± 1,28µEq/10 minutes. Following histamine administration, (100mg/kg body wt, s.c.), the gastric acid secretion increased to a peak of 20,3 ± 3,5µEq/10 minutes after a period of 60 minutes. The difference between the basal acid output and the peak gastric acid secretion following histamine administration was statistically significant ($p<0,001$).

In the second subgroup of six control rats, the basal gastric acid secretion was 3.48±1.30µEq/10mins. Following carbachol administration (10µg/Kg body wt, s.c.), the gastric acid secretion increased to a peak of 15,25 ± 2,91µEq/10 minutes after a period of 50 minutes. The difference between the basal acid output and the peak acid secretion following carbachol administration was statistically significant ($p<0,001$).

In the third subgroup of six control rats, the basal gastric acid secretion was 3.37±1.5µg Eq/10 minutes. Following

*Figure II: Daily water intake in rats given test diet comprising of 20% **H. trifoliata** and control diet comprising normal rat diet only.*

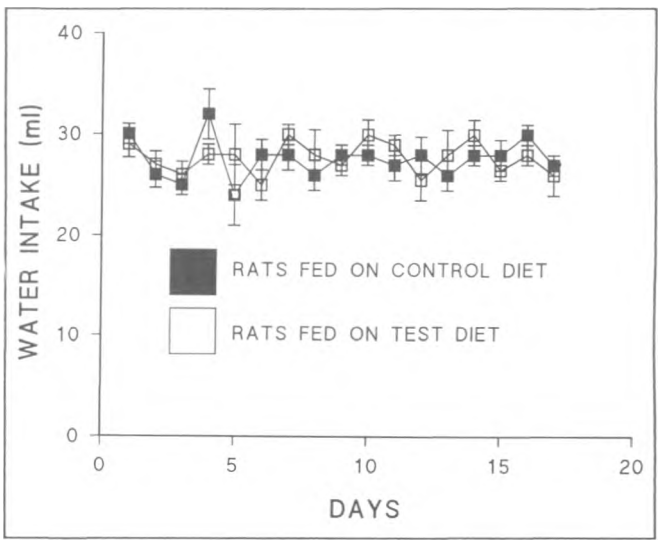


*Figure I: Daily food intake in rats given test diet comprising 20% H. **trifoliata** and control diet comprising normal rat diet only.*

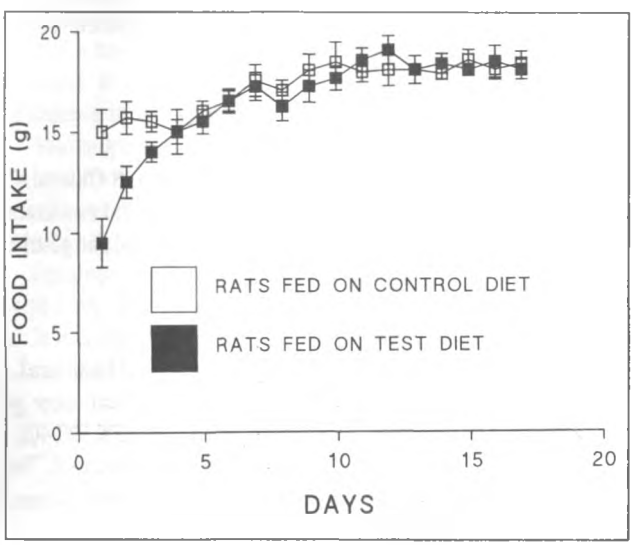
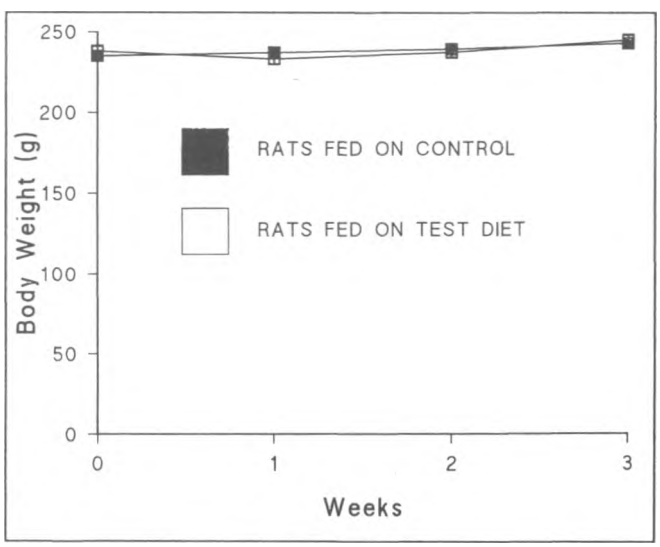


*Figure III: Body weight of rats allowed test diet comprising 20% **H. trifoliata** and control diet comprising normal diet only.*



gastrin administration (200µg/Kg body wt, s.c), the gastric acid secretion increased to a peak of 23,40 ± 3,94 ± µEq/10 minutes after a period of 70 minutes. The difference between the basal acid output and the peak gastric acid secretion following gastrin was statistically significant ($p<0,001$).

Gastric Acid Secretion in Test Rats.

Basal gastric acid secretion in a group of six test rats was 3.95 ± 1.10µEq/10 minutes. Following the administration of aqueous extract of *H. trifoliata* (100 mg/kg body wt, i.p.) and histamine (100 mg/Kg, s.c.) 10 minutes after the *H. trifoliata*, the gastric acid secretion increased to a peak of 20.90 ± 2.37µEq/10 minutes after 70 minutes. The peak acid output following histamine administration was not significantly different from that obtained previously in control rats.

Basal gastric acid output in a second group of six test rats was 3.65 ± 1.05µEq/10min. Gastric acid secretion following aqueous extract of *H. trifoliata* (100mg/kg, i.p.) and carbachol (10µg/kg body wt, s.c.) 10 minutes after the *H. trifoliata* increased to a peak of 18.67 ± 2.48µEq/10 mins after 60 minutes. The peak acid output following carbachol administration was not significantly different from that obtained previously in control rats.

Basal gastric acid output in a third group of six test rats was 3.47 ± 1.20µEq/10min. Gastric acid secretion following aqueous extract of *H. trifoliata* (100mg/kg, i.p.) and gastrin (200µg/kg body wt, s.c.) 10 minutes after the *H. trifoliata* increased to a peak of 20,50 ± 4,15µEq/10 minutes after 60 minutes. The peak acid output following gastrin administration was not significantly different from that obtained previously in controls.

Discussion

H. trifoliata did not significantly affect food and water intake, and weight gain in rats. Food intake in the control and test groups was the same except in the first three days when test rats ate significantly less food than the control rats. Weekly, instead of daily measurement of body weight, probably obscured the fall in body weight that might have occurred in the test rats during the three days that the test animals ate less food than the control animals. However, the results showed that rats became used to the palatability of the test diet after three days which is also a measure of compliance of the rats to the medication. The method of drug administration used in the experiments is similar to that used by humans where the ground dried powder of roots of *H. trifoliata* is usually added to the porridge of peptic ulcer patients. The lack of effect on the weight of the animals probably shows that *H. tr* no untoward side effects such as tissue wasting loss that could result from anorexia or toxic ef tissues.

H. trifoliata accelerated the healing of acetic acid-induced peptic ulcers in rats. The method of Wang *et al*[4] used involved high acidification of the stomach. High acidity is a common factor in the aetiology of peptic ulcer. In preliminary experiments, ulceration in the stomach was confirmed, both macroscopically and microscopically five days following the administration of acetic acid into the submucosal layer of the antral oxyntic border of the stomach in rats. Seventeen days following the consumption of *H. trifoliata* containing diet, no ulcer was observed macroscopically on the outside wall of the rat stomachs. In contrast, 70% of the control rats had ulcers on the outside of their stomach wall. However, histological examination showed that test rats still had traces of ulcer whereas the control rats showed massive denuded mucosa and thickening of the stomach wall at the ulcer sites which are signs of severe ulceration.

The mechanism whereby *H. trifoliata* accelerated the healing of peptic ulcers in rats is not very clear. As mentioned earlier high acidity is a common factor in the aetiology of peptic ulcer. The mechanism of gastric acid secretion involves cholinergic, histaminic, gastrin and proton pump pathways. Aqueous extract of the roots of *H. trifoliata* did not inhibit acid secretion stimulated by histamine, gastrin and carbachol which is a longer acting cholinergic drug than acetylcholine. It is also likely that *H. trifoliata* did not inhibit the proton pump, since the proton pump is always the final pathway in acid secretion. If *H. trifoliata* was a proton pump inhibitor it would have inhibited acid secretion stimulated by both histamine, gastrin and carbachol.[9] It is unlikely, therefore, that *H. trifoliata* accelerated the healing of peptic ulcer in rats via an acid inhibiting mechanism.

H. trifoliata may posses an intrinsic wound healing property. This proposal is based on the fact that rats receiving *H. trifoliata* had an accelerated healing of both the ulcer and the suturing on the abdominal wall when compared to control rats. So, *H. trifoliata* might exert its action as a mucosal protective agent, a mechanim employed by some clinically used anti ulcer drugs eg, sucralfate and bismuth colloidal products. This proposed mechanism is similar to the mechanism in wound healing situations of plants of the same family (*Umbilliferae*) documented by Hostetmann *et al*.[10] It is likely therefore, that like other members of this family, *H. trifoliata* promoted wound healing by acting as a protective agent and that its cellulose fibrils are arranged in a multidimensional net in the primary cell wall whilst the middle lamella acts as a plastic cementing layer that holds adjacent cells together thus acting as a protective layer to the stomach acidity.[10]

In conclusion, *H. trifoliata* accelerates the healing of acid induced peptic ulcer in rats which may validate its use in the treatment of peptic ulcer in humans. Further studies using human subjects and the extraction of the active principle in *H. trifoliata* are in progress.

References

1. Gangaidzo I, Kiire C, Mason P, Sitima J, Gwanzura Prospective endoscopic study of duodenal ulcer Zimbabwe Blacks. *Cent Afr J Med* 1992;38:392-4
2. Gelfand M, Mavi S, Drummond RB, Ndemera B. The traditional medical practitioner in Zimbabwe. Harare Zimbabwe: Mambo Press, 1985:199.

3. Kasilo OJ, Nhachi CFB. Drug and Toxicology Information Service 1992, Issue No. 29. Harare, Zimbabwe.
4. Wang IY, Kaji SY, Takeuchi K, Okabe S. Delayed healing of acetic acid-induced gastric ulcers in rats by Indomethacin. *Gastroenterology* 1989;96:393-402.
5. Parry O, Duri ZJ, Zinyama E. The effects of *Heteromorpha trifoliata* on gastro-intestinal smooth muscle of the guinea pig. *J Ethnopharmacol* 1996;54:13-7.
6. Gosh MN, Schild HO. Continuous recording of acid gastric secretion in rats. *Br J Pharm* 1958;13:54-61.
7. Amure B O, Ginsburg L. Inhibitors of histamine catabolism and the action of gastrin in the rat. *J Pharmacol* 1964;23:476-85.
8. Osim EE, Arthur SK , Etta KM. Influence of kola nuts (*Cola nitida alba*) on *in vivo* secretion of acid in cats. *Int J Pharmacognosy* 1991;29:215-20.
9. Ganong WF. Review of medical physiology. Stanford, Conneticut, USA: Appleton and Lange, 1997:460-1.
10. Hostettmann K, Chinyanganya F, Maillard M, Wolfender JL. Chemistry, biological and pharmacological properties of African Medicinal plants. Proceedings of the First International IOCD Symposium, Victoria Falls, Zimbabwe. 25-28 February 1996. Harare: University of Zimbabwe Press, 97.