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Gastro protective property of Amlapittagna syrup

Research Article

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Abstract

One of the lifestyle disorders that impacts 10% of the global population is gastric ulcer. H pylori infection, use of anti-inflammatory medications, cigarette smoking, chronic alcohol use, stress, and altered prostaglandin synthesis and E metabolism are the primary causes. Gastric ulcer signs are comparable to those of *amlapitta* (gastritis). Ayurvedic classics describe a variety of formulations, including *churna* (powder), *kwatha*(decoction), *asavarishta* (fermentation preparations), *rasayoga* (mineral preparations), and syrup, for the treatment of *amlapitta*. It is stated that *amlapittaghna arka* (distillate) can be used to treat *amlapitta*. *Guduchi* (*Tinospora cordifolia*), *nimba* (*Azadiracta indica*) and *patola* (*Trichosanthus dioca*) are the components of the *amlapittaghna arka*. Patients dislike the *tikta rasa* (bitter taste)of the medications on the above list. Syrup is the dosage form that is most commonly used across all patient age categories. *Arka* has been altered to take the form of syrup to increase the product's palatability and shelf life. Aspirin-induced gastric ulcers in Wistar albino rats were used in an experiment to measure the gastroprotective impact. pH, ulcer index, gastric juice volume, free acidity, total acidity, protein content, and carbohydrate content were among the biochemical indicators that changed.

Key Words: *Gastric ulcer, Amlapitta, Amlapittaghna arka, Syrup.*

Introduction

Gastric ulcer is one of the lifestyle disorders that impacts 10% of the global population. Stress, usage of anti-inflammatory medications, cigarette smoking, chronic alcohol use, h pylori infection, and altered prostaglandin synthesis and E metabolism are the primary causes(1). Aspirin, a popular NSAIDS used to treat gastric ulcers, damages the intestinal mucosa and may increase permeability or inhibit prostaglandin synthesis. Antacids, H2 receptor blockers, and proton pump inhibitors are some of the synthetic medications that are frequently used; taking them will cause side effects like headache, nausea, and constipation.(2) We have therefore embraced the ayurvedic system of medicine. the similar *amlapitta*-like signs of a gastric ulcer.

Ayurvedic classics describe a variety of formulations, including *churna, kwatha, asavarishta, rasayoga* and syrup, for the treatment of *amlapitta*. For the treatment of *amlapitta*, the *amlapittaghna arka* is described.(3) The *amlapittaghna arka* contains the herbs *guduchi* (*Tinospora cordifolia* (Willd.) Hook. f. and Thoms.), *nimba* (*Azadiracta indica* A.Juss.), and *patola* (*Trichosanthes dioca* Roxb.). The *tikta rasa* in the aforementioned medications makes them unpalatable to patients. One of the most popular dose forms among patients of all ages is syrup. *Arka* has been changed into syrup shape to improve the flavour and shelf life.

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Materials and methods

Table 1: Drugs & Chemicals used for the study

Sl No	Drugs
1	1.*Amlapittaghna arka-guduchi, nimba, patola*
2	2.*Amlapittaghna syrup-amlapittaghna arka*,
3	3.Reference standard drug (Ranitidine)
4	4.Aspirin (ulcer inducing agent)
	Chemicals used for experimental study
5	Pentobarbitone
6	Saline 9%
7	Formalin (10%)
8	Ether

Dose calculation

The dose for experimental study was calculated by extrapolating the human dose to animal dose based on the body surface area ratio (Paget's and Barnes-1964).(4)

Conversion of human dose to rat: Human adult dose x body surface area convertible Factor Rat dose=Human dose ×body surface area constant of rat×5

Table.2 : Showing calculation of animal dose

Drugs	Human dose	Rat dose
Amlapittaghna syrup:	48ml/day	48ml×0.018×5=4.32ml/kg body weight
Ranitidine Tablet	20mg/kg body weight	20mg×0.018×5=1.8mg/kg body weight
Aspirin	200mg/kg body weight	200mg0.018×5=18mg/kg body weight

Preparation of *Drug*

Amlapittaghna syrup was prepared at the Department of Rasashastra and Bhaishajya Kalpana S.D.M College. Hassan. 500ml *Arka* and 333 ml (66.7% v/v) of sugar was used for preparation of syrup. 250gm of drugs in the form of *Kalka* was added to 2500ml of water (1:10) and kept it for distillation till 240 ml of *amlapittaghna arka* (60%) was collected. *Arka* was warmed and sugar was added. Sugar has completely dissolved at 53.7 ^{0}C.

Preparation of *amlapittaghna* syrup

Sharkara* and *Amlapittaghna Arka	**Measurement of initial temperature**
	
Measurement of temperature of dissolving of sugar	**Measurement of temperature after formation of syrup**
	

Route of drug administration

Amlapittaghna syrup, reference standard drug, toxicant drugs were administered according to the body weight by oral route with the help of rat feeding needle attached to syringe.

Experimental procedure

Wistar strain albino rats of either species, weighing between 160g and 250g, were employed in the investigation. The animals were sourced from the S.D.M. Centre for Research in Ayurveda and Allied Science's animal home in Kuthpady, Udupi. Five sets of six albino rats each received a total of thirty albino rats, which were chosen. Each polypropylene enclosure with a top grill made of stainless steel could hold six animals. In order to prevent infections, desiccated paddy husk was frequently changed and used as bedding. The animals were subjected to 12-hour cycles of light and darkness, relative humidity of 50–70%, and an ambient temperature of 22–0.30 during the trial.

Animals were given normal laboratory rat pellet food from Sai Durga Feeds in Bengaluru, as well as unlimited access to water. The Institutional Animal Ethical Committee gave its approval to all experimental procedures in accordance with the CPCSEA's guidelines (IEC No. SDM/IEC/60/2017-2018 and Approval No. SDMCRA/IAEC/HSN-RS-09). Wistar albino rats were given aspirin and had their pyloric muscles tied using an adapted version of the Shay H et al. (1945) and Jainu M et al. (2006) protocols.

The chosen animals were randomly split into five groups the day before the dose, with six animals in each group. The test medications were given orally to the appropriate groups for ten days straight. Beginning on the fifth day of drug delivery, ranitidine was administered to the treatment group along with a vehicle. Rats were given a modified version of the procedure outlined by Jainu M et al. (2006) to cause gastric ulceration.(5,6) To prevent coprophagy, animals were transferred singly into metabolic cages at the conclusion of the dosing phase, or on the tenth day after dosing. Animals that were fasting for 24 hours were denied sustenance but were given unrestricted access to water. According to Shay et altechnique,.'s the pylorus was ligated on the eleventh day (1945).

The pylorus was ligated on the eleventh day using Shay et al (1945). Pentobarbitone (20 mg/kg body weight, IP) was used to anaesthetize the rats before the abdomen's layer-by-layer opening was performed. The cut was made just below and lateral to the xiphoid process.

In order to prevent traction on the pylorus or harm to its blood supply, the pyrogenic portion of the stomach was gently lifted out. With cotton thread, the pylorus was bound, and the stomach was delicately reinserted. Layers of interrupted sutures were used to seal the incision. During the time following surgery, the animals were denied access to food and drink.

After six hours of pyloric closure, all animals outside of group 2 received aspirin orally (200 mg/kg body weight). After receiving aspirin for 4 hours, animals are killed by an ether excess. After meticulously reopening the abdominal cavity, the stomach was removed after the oesophageal end was tied to stop the loss of gastric contents during the procedure. The contents of the stomach were emptied into tubes and centrifuged for 10 minutes at 2000 rpm. Gastric juice amount and pH were measured and used for biochemical calculation. The glandular region of the stomach is used for different biochemical parameters following the evaluation of ulcer score.

Biochemical parameters in gastric juice

Gastric Juice

Volume

The collected gastric juice was centrifuged at 2000 rpm for 10 min. The volume of supernatant was expressed as ml/kg body wt. which was used for further biochemical investigations.

Table.3: Grouping of animals

Sl.no.	Grouping of animals	Drug administered
1	Normal control	Normal tap water+diet+fasting
2	Pyloric ligation control	Normal tap water+diet+pyloric ligation
3	Aspirin+pyloric ligation control	0.5% CMC (5days) aspirin 200mg/kg + pyloric ligation
4	Reference standard	Ranitidine (20mg/kg 5 days), aspirin 200mg/kg+pyloric ligation
5	*Amlapittaghna* syrup	Test drug (4.32ml/10 days), aspirin 200mg/kg+pyloric ligation

pH of Gastric Juice

A drop of gastric juice was taken with a glass rod and placed on a strip of narrow range pH paper. The colour change was matched with the standard chart and the reading was noted.

Total and Free Acid (7)

It was estimated as described by Kulkarni.

Principle: The first indicator used in the titration i.e. Topfer's Reagent changes colour red to yellow at pH 2.9 to 4.6. The second indicator Phenophthalein changes the colour back to red at pH 8 to 10. The first titration to about pH 4.5 measures the amount of free Hydrochloric acid (free acid). Further titration from this point with Phenophthalein is against any organic acid and protein hydrolysate present in the gastric juice. The complete titration gives value for total acid

Reagents:

1. 0.01 N NaOH
2. Topfer's reagent-5% Dimethyl amino-azobenzene in absolute alcohol
3. Phenophthalein indicator-1% solution of Phenophthalein in 50% alcohol

Procedure

0.5ml of the supernatant of the gastric juice was pipette into a small conical flask. Two-three drops of Topfer's reagent added and titrated with 0.01N NaOH until all the traces of the red colour disappeared and the colour turned yellowish orange. The volume of NaOH added was noted. The first titration gives the value for free acidity and the latter value for total acidity.

If yellow colour was obtained on adding Topfer's reagent or Methyl orange, no free acid is considered to be present. In this case Phenophthalein was added and titration continued for the combined acid. This was equal to the total acid. The result was expressed as MEqHCL/100ml gastric juice.

Total Proteins (Modified Lowry S Method) (8)

Reagents required:

1. 1.Alkaline copper reagent: Add 10 ml of 0.5% Cuso4 with mixing to 20 ml of 10% NaOH. Then add 50 ml of 20% Na2co3 mix and make up to 100ml water.
2. 2.Phenol reagent: Dilute 1 volume of 2N Folin-Cocalteu's reagent with 16 volumes of distilled water just before use.
3. 3.Protein standard: Prepare Bovine Serum albumin stock solution with 1% Sodium Dodecyl Sulphate (SDS) to give a concentration of 1000mg l1000mewl and use it in the different range to plot a standard curve.

Procedure

To 0.5ml of sample, blank and standard were taken in duplicate,0.5ml of alkaline copper reagent added, mixed and let undisturbed for 10minutes.Then added 2.0 ml of Folin's reagent forcibly and rapidly to each tube. Mixed immediately and heated in a water bath at 55 0 C for 55minutes. Cooled in running water and read the absorbance of samples and standard at 650nm against blank calculated the protein content of sample by comparing with standard.

Total Carbohydrates (Dubois M et al 1956, Krishnaveni S et al 1984) (9,10)

Principle: In hot acidic medium glucose is dehydrated to Hydroxymethyl furfural. This forms a green coloured product with phenol and has absorption maximum at 490nm.

Reagent required

1. Phenol 5%: Redistilled (reagent grade) Phenol (50g) dissolved in distilled water and diluted to one litre.
2. Sulphuric acid 96% reagent grade
3. Standard Glucose: Stock 10 mg in 10ml of water

Procedure

1ml of 5% Phenol was pipetted into test tubes containing 1ml of the gastric juice and blank tube containing 1ml of distilled water and mixed. Then 5ml of 96% H_2SO_4 was added and mixed. Incubated at room temperature for 10minutes. The tubes were re-shaken and placed in a water bath at 28-30degree C for 20 minutes. Yellow orange chromophore was developed. Read the O.D AT 490nm in a Systronics UV-VISIBLE Spectrophotometer. Different concentrations of glucose standard solution were run similarly to prepare a standard curve.

Histopathology of Stomach Tissue (11)

A fragment of glandular part of stomach was transferred to 10% formalin and sent to a commercial laboratory for preparation of slides. The slides with sections obtained were scanned through Trinocular Carl Zeiss's scanned through Trinocular Carl Zeiss's microscope (Germany) under different magnifications. Changes if any in the cyto architecture were noted down.

Procedures followed to prepare histopathological studies:

Fixation

The tissues were excised out immediately after sacrificing the animals, cleaned of extraneous tissues, cut into pieces of appropriate thickness and were

transferred to10% formalin solution. The tissues were allowed to remain in it till they are taken for processing.

Tissue processing

The tissue processing involves dehydration, clearing and infiltration of the tissue with paraffin. The usual dehydrating agent is ethyl alcohol, acetone and isopropyl alcohol can also be used. Following dehydration tissue was transferred to a paraffin solvent, which is miscible with the dehydrating agent as well. These are known as clearing agent such as chloroform and xylene. The tissue were thoroughly washed by placing them under running tap water and then passed through a series of following solvents as per schedule for dehydration, clearing and paraffin infiltration.

Alcohol 70%	:	20 min
Alcohol 80%	:	20 min
Alcohol 90%	:	20 min
Alcohol 95%	:	20 min
Isopropyl Alcohol	:	20 min
Acetone (2 changes)	:	20min
Chloroform (3 changes)	:	20min
Melted paraffin wax (60degree C), (2 changes)	:	30 min.each

Next the tissues were embedded in paraffin wax to prepare tissue blocks, which are oriented so that sections are cut in desired plane of the tissue. Tissue blocks were fixed to a metal object holder after trimming them to suitable size.

Section cutting: The tissue section of the 5-6mm thickness were cut with the help of Spencer type rotating microtone and floated in a water bath between 50-550C for 30 minutes and then they were mounted on clear glass slides with a drop of Mayer's egg albumin dried on hot plate at 500C for 30 minutes.

Assessment of stomach ulcer

The stomach was removed, cleansed, and opened along its greater curvature. The interior surface was then carefully washed with cold saline solution, fixed on the wax board, and checked under a microscope for ulceration. For the purpose of calculating the ulcer index, the severity of each ulcer and the overall amount of ulcers in each rat were noted. The ulcer score was calculated using Suzuki et.al scoring's system (1976).

0- No visible ulcer
1-Maximum diameter of 1mm
2- Maximum diameter of 1-2mm
3- Maximum diameter of 2-3mm
4- Maximum diameter of 3-4mm

The severity of ulcer score is calculated by multiply the ulcer score with maximum diameter. The mean number of ulcer in each group is also recorded for calculating the ulcer index.

Fig.1-Stages of experimental study

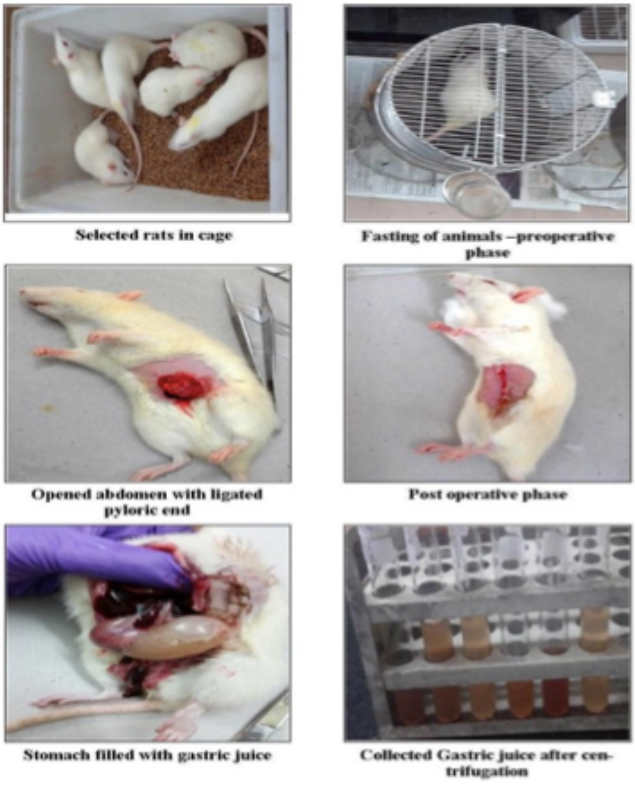

Table 4: Results of biochemical parameters

Group	pH	Volume	Ulcer index	Free acidity	Total acidity	Carbohydrate	Protein
Pyloric ligation group	1.71±0.28	3.5±0.85	35±10.06	2.04±0.13	3.44±1.28	350.57±63.84	7922.85±1160.0
Aspirin+ pyloric ligation group	2.5±0.34	2.91±0.63	16.66±2.62*	1.14±0.10	2.42±0.20	549.4±63.70	11134.2±981.31
Reference standard (Ranitidine)	3.83±0.65	2.66±1.10	5.66±1.80	0.8±0.15	2.12±0.25	720.4±162.06	9264±1230.4
Amlapittaghna syrup	3.16±0.54	10.5±1.32**	23±3.96	0.93±0.23	3±0.93	6416.66±399.21**	8790±1414.0

Discussion

The digesting effects of gastric juice that has been retained and disruption of gastric blood flow cause the induction of a gastric ulcer in the pyloric ligated animal.(12) Gastric ulcers can also be brought on by the collapse of the mucosal barrier and auto-digestion of the gastric mucosa brought on by elevated acid pepsin production.(13) Whether pepsin secretion is stimulated along with or without acid secretion, this is the main factor in the formation of stomach ulcers.(14)

Aspirin harms mucosal tissues and interferes with defence mechanisms like mucus and bicarbonate release, surface epithelial hydrophobicity, and mucosal blood flow by inhibiting the production of prostaglandins.(15) When aspirin was administered to pyloric-ligated rats, the glandular region of the stomach was badly damaged, which further increased the acidity and decreased the gastric mucosa's resilience.(16)

Gastric pH

Comparing the *amlapittaghna* syrup group (26.4%) to the Aspirin + pyloric ligation group (46.19%), there was no statistically significant difference. Although the pH of the *amlapittaghna* syrup group has not greatly increased, encouraging the release of bicarbonate from the gastric mucosa is beneficial due to its antiulcer properties.(17)

Gastric juice volume

When compared to the Aspirin + pyloric ligation group (16.85%), there was a very small increase in *amlapittaghna* syrup (260.825%). Because the unprotected lumen of the stomach is exposed to accumulating acid, the amount of gastric juice secreted has a big impact on how ulcers form. Additionally, it acts as a cue for the secretory processes that produce gastric juice.

Free Acidity

Comparing the *amlapittaghna* syrup group to the Aspirin + pyloric ligation group, there was a non-significant decrease (18.421% vs. 44.11%). Drugs must possess properties that lower acidity in order to be successful against stomach ulcers. Either an anti-secretory effect or a medication that neutralises stomach acid can decrease acidity.

Total acidity

Comparing the group receiving aspirin + pyloric ligation to the *amlapittaghna* syrup group, there was a nonsignificant increase in total acidity (23.967%).

Ulcer index

Amlapittaghna syrup had a non-significant increase (38.05%) when compared to the group receiving aspirin + pyloric ligation. gastric ulcer is caused by an increase in the ulcer score.

Protein estimation

Amlapittaghna syrup had a non-significantly lower decrease (21.05%) than the group receiving aspirin + pyloric ligation. A decline in the amount of protein in the gastric juice can be interpreted as a sign of less leakage and consequently less ulceration.

Carbohydrate Estimation

Amlapittaghna syrup had a significantly higher increase (1067.93%) when compared to Aspirin + pyloric ligation. Pyrus ligation-induced stomach ulcers are brought on by increased acid-pepsin buildup, which results in pyloric blockage and consequent mucosal damage.

A significant amount of mucus is released after minor injury, providing an ideal medium for repair. Mucin is a viscous, comparatively resistant glycoprotein that creates an acid barrier. It makes a sizeable contribution to the mucous, an essential pre-epithelial substance that acts as the body's first line of defense against ulcerogens. Total hexoses and mucopolysaccharide-like sialic acid, as well as mucin, are significantly increased, leading to a substantial increase in total carbohydrate.

Conclusion

In *amlapittaghna* syrup, very significant increase in gastric pH & volume, non-significant increase in total acidity and significant increase in ulcer index was observed. In Experimental study *amlapittaghna* syrup was shown non-significant effect in anti-ulcer activity.

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