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COMPARATIVE EVALUATION OF ANTI-ULCER ACTIVITY OF AAMALAKI MASHI PREPARED BY BAHIRDHUM AND ANTARDHUM METHODS IN EXPERIMENTAL RATS

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ABSTRACT – The anti-ulcer activity of Aamalaki Mashi prepared by Bahirdhuma and Antardhuma methods was investigated in "Ethanol induced mucosal damage in Rats" animal model. Common parameters determined were ulcer index and gastric pH. Aamalaki Mashi prepared by Bahirdhuma method at a dose of 1000 mg/kg produced significant inhibition of the gastric lesions induced by ethanol and showed significant ($p<0.05$) reduction in ulcer index as compared to standard drug Ranitidine 100 mg/kg and Aamalaki Mashi prepared by Antardhuma method at a dose of 1000 mg/kg. The present study indicates that Aamalaki Mashi prepared by Bahirdhuma method possess anti-ulcerogenic property which can be attributed to its gastro-protective and antioxidant activity.

Keywords – Aamalaki Mashi, Ethanol induced ulcer model, gastroprotective.

INTRODUCTION

Mashi Kalpana is one of the derived formulation mentioned in our ancient Ayurvedic texts which is obtained by incinerating the raw medicinal drug. Even though Acharya Charaka has mentioned about this Kalpana and its utility, nowadays there is very restricted use of this dosage form. Also, it is less known to common people and very few Ayurvedic practitioners use it regularly in their practice. Presently mainly Mashi formulations like Triphala Mashi, Aamalaki Mashi, Ela Mashi, Mayurpiccha Mashi are in use.

In todays date, it is really a virtuous approach in the field of Ayurvedic research that researchers should concentrate towards this overlooked dosage form. Aamalaki Mashi in which the main ingredient is Aamalaki, inspite of no textual reference is in use since a long time in ethnomedical system in treating gastric disorders like Amlapitta (hyperacidity), Adhmana and Aanaha (abdominal distension). It was first prepared by Late

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Vaidya B.V. Gokhale. At present, one of the proprietary medicines of Rasashala 'Charcosal (Vatanashak)' contains Aamalaki Mashi as the main ingredient and is mainly used in treating flatulence, dyspepsia, indigestion, nausea and vomiting. This particular symptom complex can be seen in various gastro-intestinal disturbances and one such condition is peptic ulceration. Presently Acid peptic diseases[1] are very commonly found of which gastric ulcers are predominantly seen. Gastric ulcers are a type of peptic ulcer disease which can be a serious gastrointestinal disorder requiring a well targeted therapeutic strategy. The goals of treating peptic ulcer disease are to relieve pain, heal the ulcer and prevent ulcer recurrence[2]. Currently there is no cost-effective treatment that meets all these goals. There are number of drugs in Modern medicine available for the treatment of these ulcers, but clinical evaluation of these drugs show higher incidence of relapse, side-effects and drug interactions. This has been the rationale for the development of new anti-ulcer drugs in which the herbal drugs can offer a better protection and decreased relapse.

Thus it is a logical approach to drug discovery by screening traditional herbal products instead of synthesized chemical molecules. So Aamalaki Mashi was undertaken as an archetype which may prove to be useful in treating acid peptic disorders. Taking into consideration this rationale, this hypothesis is put forth with an intention to prove its anti ulcer activity with animal experimentation. Scrutinizing the Ayurvedic drugs through established and universally accepted quality control and toxicological protocols will help us to achieve better results and strengthen the faith of people in this precious and timeless medicinal science globally[3].This can be useful in generating a systemic and scientific data of Aamalaki Mashi which can be further used as a reference for efficacy and clinical studies to describe newer therapeutic uses.

MATERIAL AND METHODS

Preparation of Aamalaki Mashi by Bahirdhuma method[4,5]

Principle – Open heating

Ingredients – Aamalaki Churna = 100 gms

Equipments – Rounded earthen pan, stirrer, glass thermometer, retort stand, L.P.G, weighing machine and watch.

Procedure –

1. An earthen pan containing Aamalaki Churna was kept on gas burner.
2. With the help of retort stand, glass thermometer was inserted in the

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Churna without touching the bottom of the pan.

3. The gas burner was ignited and the flame was adjusted to medium low.
4. Continuous stirring was done till the Churna became completely black colored and the fumes coming from the Churna were ceased.
5. At this stage, the pan was kept aside away from the flame and the final product which resembled a black ash was transferred to a clean plate.
6. The final product obtained weighed 56.69 gms.

Preparation of Aamalaki Mashi by Antardhuma method[6]

Principle – Closed heating

Ingredients – AamalakiChurna = 100 gms

Equipments –

a. Preparation of Sharavasamputa- Two Sharava, fuller's earth (multanimati), water and pieces of cloth.
b. Mashi preparation – Sharavasamputa containing Aamalaki Churna, 4.5 cow dungs, Laghu Puta, Til Tailam, match box, weighing machine and watch.

Procedure –

1. A Sharava containing AamalakiChurna was taken and another Sharava was placed on it upside down.
2. A mixture of fuller's earth and water was prepared and a piece of cloth was dipped in it so that the entire cloth was properly loaded with the mixture.
3. This mud cloth was wrapped around the area of contact of the Sharava.
4. After the mud cloth was completely dried, another mudcloth prepared in the similar manner was wrapped around it. Total seven mudcloths were used for wrapping and sealing.
5. Three cowdungs cut into small pieces were placed at the bottom of the Laghu Puta, the Sharavasamputa was placed over it and the remaining one and a half cowdungs pieces were placed covering the sides and the top of Sharavasamputa.
6. Some drops of Til Tailam were sprinkled over the cowdungs and with the help of a match stick the cowdungs were subjected to fire.
7. Sharavasamputa was kept for Swangashita and the observations were noted.
8. The yield of final product which was black like collyrium was 33 gms.

Experimental Animals used

Wistar albino rats of either sex, aged 10-12 weeks weighing between 150.0 – 180.0 gm were used for the present study. This

study was performed under the conditions recommended by the 'Committee for the Purpose of Control and Supervision of Experiments on Animals' (CPCSEA). The protocol was approved by Institutional Animal Ethics Committee (IAEC). 3 animals per cage were housed in polypropylene cages with stainless steel grill top, along with facilities for food and water bottle, and bedding of clean paddy husk was provided. The room temperature was maintained between 22-25°C, relative humidity 55-60 % and illumination cycle was set to 12 hours light and 12 hours dark. Pelleted feed was supplied by Nav Maharashtra Chakan Oil Mills Ltd., Pune, ad libitum during acclimatization and during the study. Potable water passed through 'Aquaguard' water filter ad libitum in plastic bottles with stainless steel sipper tubes was provided to these animals.

"Ethanol induced mucosal damage in Rats" animal model[7,8]

The rats were divided into respective groups of six animals each. Group I represented the Control group which received distilled water orally. Group II was Disease Control receiving ethanol. Group III was treated with Standard drug Ranitidine 100 mg/kg. Group IV was treated with Aamalaki Mashi prepared by Bahirdhuma method (drug T 1) at a dose of 1000 mg/kg and Group V was treated with Aamalaki Mashi prepared by Antardhuma method (drug T 2) at a dose of 1000 mg/kg. The rats were treated with the test and standard drugs prior to ulceration daily for seven days. On the day of ulceration, the rats were kept for fasting for 24 hours and then administered 1 ml of absolute ethanol orally. The animals were sacrificed after 1 hour of ulcerogen administration and their stomachs were excised and the gastric contents were aspirated. The contents were subjected to 1000 rpm for 10 minutes and then analysed for pH (digital pH meter). The stomachs were washed with normal saline and kept in 10 % formalin for the determination of ulcer index and histological studies.

Ulcer index was determined by the method of Andrade wherein,

Level I ulcer Area =<1mm^2

Level II Ulcer Area=1-3mm^2

Level III Ulcer Area=>3mm^2

The following parameters were determined

Ulcerative lesion Index (ULI) = 1 X (No. Of Ulcers in Level I) + 2 X (No. Of Ulcers in Level II) + 3 X (No. Of Ulcers in Level III)

Percentage Protective Ratio = 100-(ULI pretreated/ULI Control) *100

The results were expressed as Mean +_ SD and statistical significance between treated and control groups was analyzed using of one way analysis of variance (ANOVA), followed by Dunnett's t-test where P<0.05 was considered statistically significant.

RESULTS

1. Ulcer index and gastric pH–

It was observed that in the Ethanol induced ulcerated control group, the ulcer index was 42.83 +_ 10.02 and the maximum numbers of ulcers were of the score 2 and 3. Standard drug Ranitidine was found to produce a decrease in the ulcer index, the ulcer index being 33.50 +_ 7.23; protection percentage is 22 %. T1 was found to produce significant decrease in the ulcer index as compared to the standard drug, the ulcer index being 13.83 +_ 2.48 and there were less number of ulcers with score 2 and 3 as compared with standard drug, providing a protection of 68 %. The ulcer index in the group treated with T2 was higher than standard drug, the ulcer index being 37.50 +_ 18.03. In this group, there were maximum ulcers with score 2 and 3 and provided protection of 13 %.

Table 1. Effect of Aamalaki Mashi prepared by Bhairdhuma and Antardhuma methods on various parameters in ethanol induced gastric ulcer

Group	Treatment	Ulcer index	% Protection	pH of gastric juice
Group I	Normal Control	---	----	3.00
Group II	Disease Control	42.83 +_ 10.02	-----	2.77
Group III	Treated with Ranitidine 100 mg/kg	33.50 +_ 7.23	22 %	3.83
Group IV	Treated with Drug T1	13.83 +_ 2.48	68 %	4.00
Group V	Treated with Drug T2	37.50 +_ 18.03	13 %	2.90

2. Macroscopic evaluation

Ethanol-induced severe damage to the gastric mucosa are shown as elongated bands of hemorrhage in the disease control group. As compared with the disease control group, in standard Ranitidine group, area of the elongated bands of hemorrhages was less. T1 produced an

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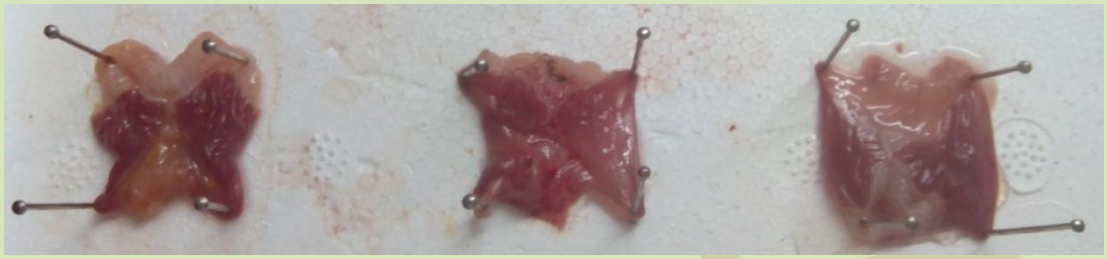
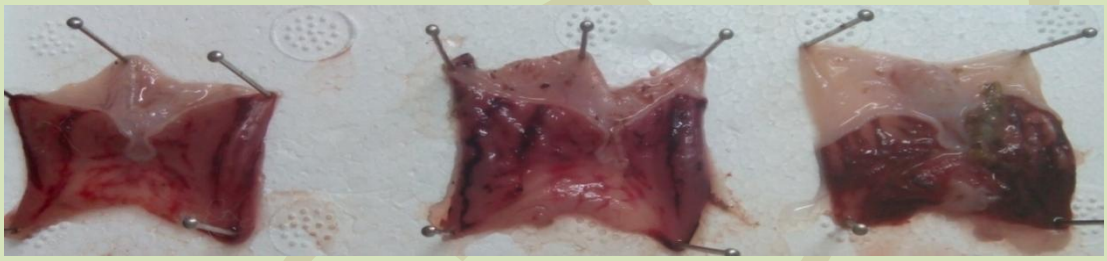
almost normal appearance of intact stomachs and very few elongated bands of hemorrhage were seen. T2 showed large numbers of elongated hemorrhagic bands as compared with the standard drug and T1.

Photo 1- Macroscopic view of gastric ulcers induced by Ethanol in all the groups

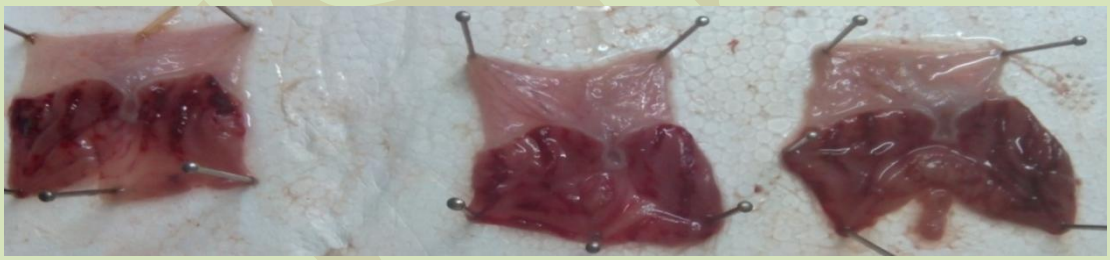
Control



Disease Control



Treated with Ranitidine 100 mg/kg



Treated with Drug T1 at a dose of 1000 mg/kg

Treated with Drug T2 at a dose of 1000 mg/kg

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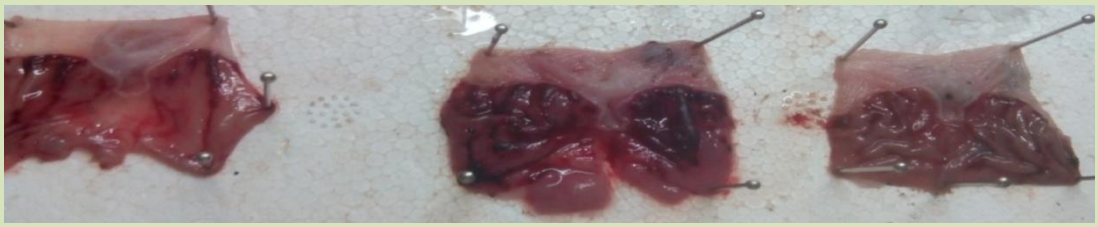
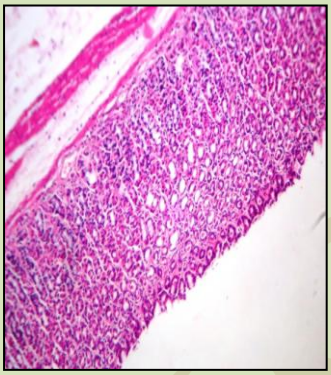


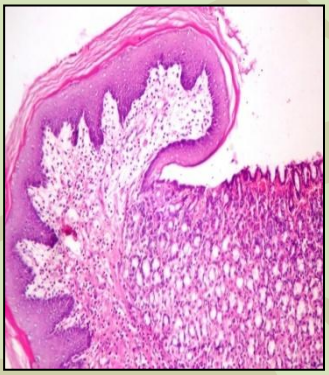
3. Histopathology of Stomach

The sections of stomach in disease control group showed hyper-keratinization, degenerative changes in non-glandular and glandular part, degenerative and necrotic changes of mucosa, hemorrhages in glandular part, chronic inflammatory changes of basement membrane and eosinophilic infiltration. Standard drug Ranitidine and drug T1 were helpful in reducing the inflammation and hemorrhages in the gastric mucosa. T2 drug imparted mild changes in the stomach mucosa.

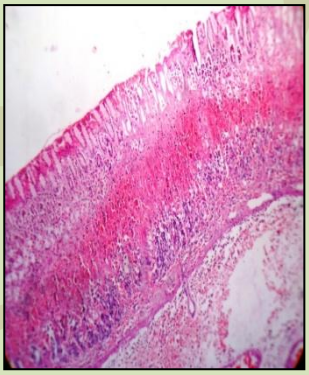
Fig 1. Histopathology of sections of stomach of Ethanol induced gastric ulcer model



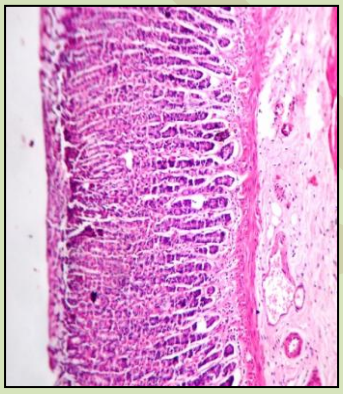
Normal Control



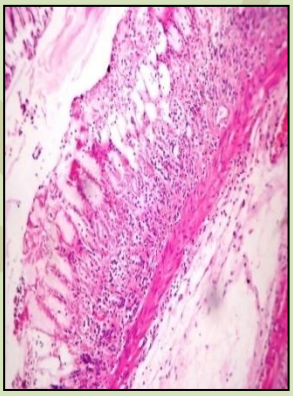
Disease Control



Treated with Ranitidine 100 mg/kg



Treated with T1



Treated with T2

DISCUSSION

The symptom complex mentioned in acid peptic diseases which are mostly seen in peptic ulcers like abdominal pain, nausea, water brash, vomiting, loss of appetite and weight loss. Complications include bleeding, perforation and obstruction in the digestive tract can be correlated with many such similar symptoms which are akin to those mentioned in ancient Ayurvedic texts like Pittaja Gulma, Parinam Shula, Urvadja Amlapitta, Chardi, Annadrava Shula, etc.Aamalaki Mashi in which the main ingredient is Aamalaki, inspite of no textual reference is in use since a long time in ethnomedical system in treating such gastric disorders where symptoms like flatulence, dyspepsia, indigestion, nausea and vomiting are particularly seen.

There are several models that are used to evaluate antiulcer medicines. Peptic ulcers can be induced by physiological, pharmacological or surgical manipulations in several animal species. 'Ethanol induced gastric mucosal damage in rats' animal model was used in the present study taking into consideration the prevention aspect. The ethanol-induced ulcer model is useful for studying the efficacy of potential drugs or testing agents that have cytoprotective and/or antioxidant activities. Acute exposure of the gastric mucosa of rats to ethanol can result in gastric lesions similar to those occurring in gastric ulcer; hence, ethanol-induced gastric ulcers have been widely used for the evaluation of gastroprotective activity. The ulcer index in group treated with T1 was less as compared with standard drug and drug T2 was less. Of all the groups, drug T2 had the least protection percentage and maximum ulcer index. Drug T1 has significantly protected the gastric mucosa against ethanol challenge as shown by reduced values of lesion index offering a better protection as compared to standard control group and drug T2 suggesting its potent cytoprotective effect. The increase in pH values indicates the effectiveness in reducing the gastric acidity, drug T1 is proved to have ph values higher than standard drug and drug T2. On histopathology of stomach, the sections of stomach in disease control group showed hyper-keratinization, degenerative changes in non-glandular and glandular part, degenerative and necrotic changes of mucosa, hemorrhages in glandular part, chronic inflammatory changes of basement membrane and eosinophilic infiltration. Standard drug Ranitidine was helpful in reducing the inflammation and hemorrhages in the gastric mucosa. Pre-treatment with Drug T1 was significant in reducing the inflammation, hemorrhages, degenerative and necrotic changes in the stomach tissues giving a better protection as compared with the standard drug. T2 drug imparted mild changes in the stomach mucosa and its protection activity was not as significant as that of drug T1. The ethanol administration to rats caused macroscopic gastric mucosal lesions

consisting of elongated dark bands generally parallel to the long axis of the corpus of the stomach, along with loss of normal color and mucus along with presence of petechiae, hemorrhage and edema. These lesions are most likely related to mucus depletion and a constrictive effect on veins and arteries of the gastric mucosal, producing congestion, inflammation and tissue injury. The reduction of gastric mucosal blood flow can result in hemorrhage and necrosis in damaged tissue. As compared with the disease control group, in standard Ranitidine group, area of the elongated bands of hemorrhages was less. T1 produced an almost normal appearance of intact stomachs and very few elongated bands of haemorrhage were seen. T2 showed large numbers of elongated haemorrhagic bands as compared with the standard drug and T1. The gastric mucosal protection against oxidative injuries caused by ethanol and this protection is most likely due to antioxidant and gastro protective properties of drug T1.

Thus, it can be concluded Aamalaki Mashi prepared by Bahirdhuma method has comparatively better efficacy than standard drug Ranitidine in anti ulcer activity. It was also observed that of the two pharmaceutical process of preparing Aamalaki mashi viz. 'Bahirdhuma' and 'Antardhuma' method, the Bahirdhuma method showed best results.

Hence newer therapeutic uses of Aamalaki Mashi can be evaluated by using different experimental models and its clinical efficacy can be tested in humans.

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