Original Article

A Comparative Evaluation of Various Therapies of Synthetic Drugs with Amla and Honey Combination for the Treatment of Gastroesophageal Reflux Disease

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ABSTRACT

Background: Gastroesophageal Reflux Disease (GERD) is disorder of oesophagus that causes ulcer and erosion. **Materials and Methods:** A comparison based on the *in-vivo* study was carried out to determine the therapeutic efficacy of herbal formulation using Amla and Honey in combination with Pantoprazole and Rebamipide for the treatment of GERD. The effects of all above mentioned medications were also checked on intestinal motility. *In vitro* studies were carried out to determine the antispasmodic activity of Amla and Honey in comparison to acetylcholine by isolated rat ileum. In pylorus ligation GERD model, mono-therapy was performed by giving herbal formulation of Amla and Honey. In combination therapy Pantoprazole and Rebamipide, Amla and Honey with Pantoprazole and Rebamipide were given and their therapeutic efficacy was observed at the ends of 14 days. In charcoal meal motility test the distance travelled by charcoal in intestine was measured. **Results and Discussion:** Combination therapy have highest therapeutic efficacy in comparison to mono-therapy of natural combination of Amla and Honey against Pantoprazole and Rebamipide. *In-vitro* antispasmodic activity revealed that Amla and Honey formulation decreased the contractility pattern in concentration dependent manner against acetylcholine. In charcoal meal motility test highest intestinal motility were observed in Amla and Honey and can be given in GERD for easily digestion of food from stomach. **Conclusion:** Herbal combination of Amla and Honey in comparative evaluation decreases the Gastroesophageal reflux disease, intestinal mobility,

and show antispasmodic activity in isolated rat's ileum. However, these combinations serve as a more potential agent for decreasing all the factors responsible for the reflux disease.

Keywords:

Combination therapy, Mono-therapy, Pantoprazole, Rebamipide, Mucosal.

INTRODUCTION

Gastroesophageal Reflux Disease (GERD) occurs in oesophagus due to reflux of stomach content. Major factors which increase refluxes of food are indigestion, obesity, and defect in valve of oesophago-gastric junction.[1,2] Abnormalities in peristaltic movement of oesophagus increase the GERD. Valve at the oesophago-gastric junction control the reflux of food toward oesophagus due to defect in valve or excess pressure of food in stomach increased due to certain reason and its causes GERD. Primary sign of GERD are frequent heartburn, regurgitation, chest pain. The severity of GERD increases if not treated at initial stages and choking, blotting and various other severe disorders like Barrett oesophagus which is cancerous form of oesophagus develops in GERD.[3] In GERD, mucosal membrane of oesophagus is damaged by high acid which regurgitate from stomach; higher the resistance of mucosal membrane lower is the level of GERD.[3,4] Antacids like Sodium bicarbonate, Sodium citrate, Magnesium hydroxide, Aluminum hydroxide, Aluminum phosphate are used in mild GERD to give symptomatic relief to patients.[5,6] Proton Pump Inhibitors (PPIs) are the first choice of drugs for treating GERD. Proton pump inhibitors include pantoprazole, omeprazole and ranaprazole.[7] PPIs decrease excess acid production in stomach resulted in relief phenomena by inhibiting the final acid production mechanism. PPIs decrease the function of proton pump which decrease the proton in stomach if fewer protons are available inside stomach there is less acidity.[8] Pantoprazole reduces excess acid secretion in stomach.[9] Rebamipide is another drug which has ulcer protective action. In GERD, PPIs are given once daily but resistance develop in few days. To overcome the resistance, dose of PPIs is increased to treat GERD. Amla possess certain wound healing action and is also used to treat ulcer. Honey possesses muco-protective action which prevents exposure of oesophagus mucous membrane to strong acid and there by decrease the severity of GERD.[10] Amla and Honey increase the digestion process which is abnormal in GERD. Obesity is reduced in GERD patients by taking Amla and Honey.[11]

Several herbs like Sini Zuojin decoction, ginger, Jovārish-e Jālīnūs, chamomile, liquorice root, marshmallow root, slippery elm are effecting in GERD treatment. GERD treatment by mono-therapy is not so effective and so currently combination therapies are in use to treat GERD.[12,13] PPIs are used in combination with Histamine 2 Receptor Antagonist (H2RAs) to prevent nocturnal acid breakdown which is one of the side effect of PPIs. Combination of antacids alginate formulation, domperidone and omeprazole, omeprazole and sustained released baclofen, aluminum hydroxide, magnesium and simethicone are effective in the treatment of GERD.[14] Combination therapy provides additional effect in comparison to mono-therapy in GERD. It reduces side effect, overcome tolerance and expedites the GERD treatment.[15]

MATERIALS AND METHODS

Amla is natural herbal medication also known as *Embilica officinalis* and it belongs to family Euphorbiacea.[16] Amla is known as gooseberry in India. Various species of Amla are grown in tropic and subtropical area like Sri Lanka, Pakistan, China, Malaysia and various other parts of the world. Gallic acid, ellagic acid, 1-O-galloyl-beta-D-glucose, 3, 6-di-O-galloyl-D-glucose are the major pharmacological active constituent of Amla.[17] Amla also contains various other pharmacological active constituents like quercetin, chebulagic acid, corilagin, 1, 6-di-O-galloyl beta D glucose, chebulinic acid, 3-ethylgallic acid (3-ethoxy-4, 5-dihydroxy benzoic acid) and isostrictiniin and various other present in Amla.[18] Pharmacological active constituents present in Amla provide immunity, give relief from bleeding disorder and when mixed with honey date provide relief from iron deficiency anemia in adolescent girls.[19] Amla also act as antioxidant, antibacterial, anti-aging and as a natural blood purifier as a skin health care products.[20]

Honey is made by honey bees from fruit nectar. Western Honey bees are also known as *Apis mellifera* used for honey production. Various type of honey bees are *Apis dorsata, Apis mellifera, Apis cerana, Apis mellifera caucasica, Apis mellifera liguistica, Apis mellifera scutellata, Apis mellifera mellifera, Apis mellifera iberiensis, Apis mellifera carnica* are present in different parts of world. Pharmacologically active constituents present in honey are flavonoids, alkaloids, glycosides, anthraquinones and volatile compounds. Other components present include acetic acid, citric acid, and formic acid, quercitin, galangin, kaempferol, luteolin, isorhamnetin, naringenin, hesperetin.[21] Honey also contains mostly Vitamin C as water soluble Vitamins with other Vitamins. Minerals like silicon, vanadium, zirconium, lithium, strontium, cadmium are present and in heavy metals lead, cadmium, arsenic is also present in honey.[22,23] Honey act as antioxidant, anti-inflammatory, anti-bacterial and anti-diabetic agents and also have important medicinal use in maintaining children health.[24] Old honey had highest antibacterial activity in comparison to fresh honey.[25] Syrian honey shows its prominent activity in antibiotic resistant bacteria severe infection caused by *Pseudomonas aeruginosa*.[26] Honey also used treat osteosarcoma, respiratory, gastrointestinal problem, cardiovascular, nervous system, asthma, throat infections, tuberculosis, hiccups, fatigue, dizziness and various other disorder.[27-30]

Dose Preparation

Amla and Honey combination were prepared by adding Amla juice and Honey in ratio of 1:1. Pantoprazole (5mg/kg) and Rebamipide (100mg/kg) dose was prepared by dissolving the drugs in 0.5% caboxymethyl cellulose solution.

In-vitro Anti-spasmodic Activity

Rat ileum preparation

Rats were euthanized by heavy dose of ether and then decapitated. After decapitation rat ileum was removed out and placed in double unit student organ bath aerated with 5% Co_2 and. 95% O_2. Double unit student organ bath were already properly set up.[31] Temperature and aeration of water in organ bath were properly maintained. After removing 1 to 2 cm rat ileum it was

properly tied in organ bath and kept in tyroid solution with appropriate aeration. The rat ileum is properly set up in double unit standard organ bath. Suspended ileums were put into equilibration period for 30min.[32] Antispasmodic activity of drug were observed by exposing rat ileum to different concentration of Amla and Honey combination in comparison to acetylcholine double unit standard organ baths were washed after exposing each concentration of Amla and Honey formulation. Different group of rats with different concentrations of Amla and Honey formulation are:

Group I: Acetylcholine treated rats with normal saline

Group II: Acetylcholine treated rats with Honey and Amla combination at dose of 25µg/mL

Group III: Acetylcholine treated rats with Honey and Amla combination at 50µg/mL dose

Group IV: Acetylcholine treated rats with Honey and Amla combination at dose of 100µg/mL

Rat ileum were placed in double unit student organ bath and change in the contraction pattern of test drugs with control were measured and compared in the kymograph. At the end, reduction in rat ileum contraction by Amla and Honey were expressed as mean ± SEM.

Charcoal Meal Motility Test

Animal were fastened overnight and dose of natural formulation of Amla and Honey were given orally. Standard drugs were given according to different group respectively. After 60 min charcoal meal with 1%w/v sodium caboxymethyl cellulose and 10% activated charcoal were given orally.[33,34] Animals were then euthanized and small intestine exposed.[35] Distance travelled by charcoal in different group was measured and compared.

Group I: Normal saline

Group II: Rebamipide and Pantoprazole

Group III: Amla and Honey

Group IV: Rebamipide, Pantoprazole with Amla and Honey

Experimental Model

Albino wistar rats were procured from animal house at Noida Institute of Engineering and Technology (Pharmacy Institute). Experiment on all animals were conducted in compliance with ethical principal and guideline set up by Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) with reference no. (1121\ac\CPCSEA\07). The study was approved by Institutional Animal Ethical Committee (IAEC) approval no IAEC/NIET/2020/01/21.

Healthy rats were acclimatized for one month. During one month albino wistar rats were given standard diet and water *ad libitium* and suitable environment. Rats were then fastened for 24 hr before surgery and then anesthetize with ketamine and xylazine hydrochloride.[36] After 24 hr midline incision were made and then corpus and fore stomach was tied and pylorus was

wrapped with O ring.[37,38] After surgery, rats were given normal saline, glucose and water for 24 hr.

Administration of Test and Standard Drugs

Control group were given normal saline daily. In second group Pantoprazole and Rebamipide was given orally and intravenously respectively. In third group Amla and Honey combination were given orally. In fourth group Amla and Honey combination was given with standard drugs Pantoprazole and Rebamipide.

Anti-GERD Treatment

After 14 days rats were then euthanized and whole oesophagus was removed and checked for mucosal damage, oesophagus erosion and body weight of all animals.

Statistical Analysis

After completion of experiment data were recorded and. results was evaluated by using In Stat Prism Graph pad Version 5.00 (Prism Graph Pad Software). Data were expressed as mean ± SEM.

RESULTS

In vitro Anti-spasmodic Activity

Contraction of rat ileum in comparison to acetylcholine was compared and evaluated. Amla and Honey formulation at negative log dose of acetylcholine (6.1650, 5.8640, 5.5629, 5.9609 and 4.6598) was plotted with increase in height at diffèrent dose. Amla and Honey combination reduced the contractile response of acetylcholine with increase in dose. At higher dose this increase in response and decreased in height was not consistent due to presence of limited number of receptor available to show the response.

Table 1:

Contractile response of rat ileum in organ bath by different groups when exposed to drug.

Dose of Ach. (µg)	Log dose (-Log M)	Contractile Height (mm)				
			Honey and Amla formulation			Ach
		Control	25µg/mL	50µg/mL	100µg/mL	10µg/mL
0.1	6.1650	8.4±0.5*	7.1± 0.1*	6.1± 0.1**	4.1± 0.8***	3.4 ± 0.1***
0.2	5.8640	10.1±0.3*	8.4± 0.3**	7.5±0.9**	6.7 ± 0.2***	5.2± 0.2***
0.4	5.5629	14.4±0.6**	13.1± 0.4*	11.0±0.5***	13.6±0.2***	7.1± 0.3***
0.8	5.2619	19.1±0.7**	19.5±0.4**	14.5± 0.1***	17.8±0.5***	7.2 ± 0.3***

1.6	5.9609	24.2±0.2*	23.2±0.8**	19.7± 0.8***	18.4±0.3***	9.0 ± 0.4***
3.2	4.6598	25.2±0.9*	23.1 ± 1.1*	22.5 ± 0.3**	18.6±0.4***	8.1 ± 0.4***

Values are expressed in Mean± SEM; ***p<0.001, **p<0.01, *p<0.05 when compared to control group (one way ANOVA followed by Dunnet's t-test), n=6.

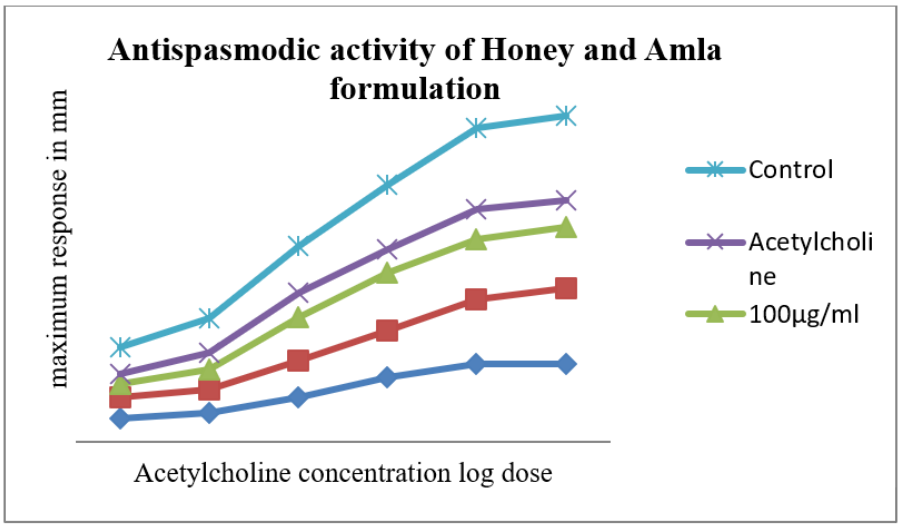

Figure 1:

Contractility pattern of Amla and Honey formulation against log dose of acetylcholine at different concentration.

Gastro Intestinal Motility Test by Charcoal Meal

In gastro intestinal motility there was a decreased in reflux action after administration of Pantoprazole and Rebamipide with Amla and Honey combination in different groups of rats. Highest decrease in gastrointestinal motility was observed in rats given Amla, Honey, Rebamipide, Pantoprazole in comparison to control whereas lowest decrease in intestinal motility observed in control groups of rats. Amla and Honey combination showed slight decrease in intestinal motility in comparison to rats given Rebamipide and Pantoprazole. It is found from above observation that Pantoprazole and Rebamipide had least effect on decrease in intestinal motility. Gastrointestinal motility of herbal combination of Amla and Honey with standard drugs Pantoprazole and Rebamipide showed prominent effect on decrease in gastrointestinal motility.

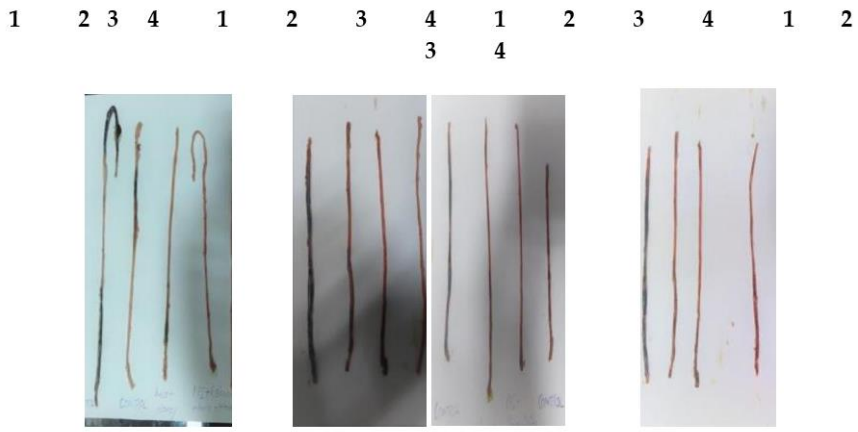

Figure 2:

Distance travelled by charcoal in intestine.

1-Control group; 2-Pantoprazole+ Rebamipide; 3-Amla+honey +Rebamipide+ Pantoprazole; 4-Amla+honey.

Table 2:

Distance travelled by charcoal in different groups of rats given standard and herbal drugs.

Drugs or herbal formulation given to rats	Mean distance travelled in intestine(cm)
Control group	35±1.85***
Amla+ Honey	28±1.78**
Pantoprazole+ Rebamipide	33±1.96*
Amla + Honey+ Pantoprazole+ Rebamipide	25±1.54*

Values are expressed in Mean± SEM; ***p<0.001, **p<0.01, *p<0.05 when compared to control group (one way ANOVA followed by Dunnet's t-test).

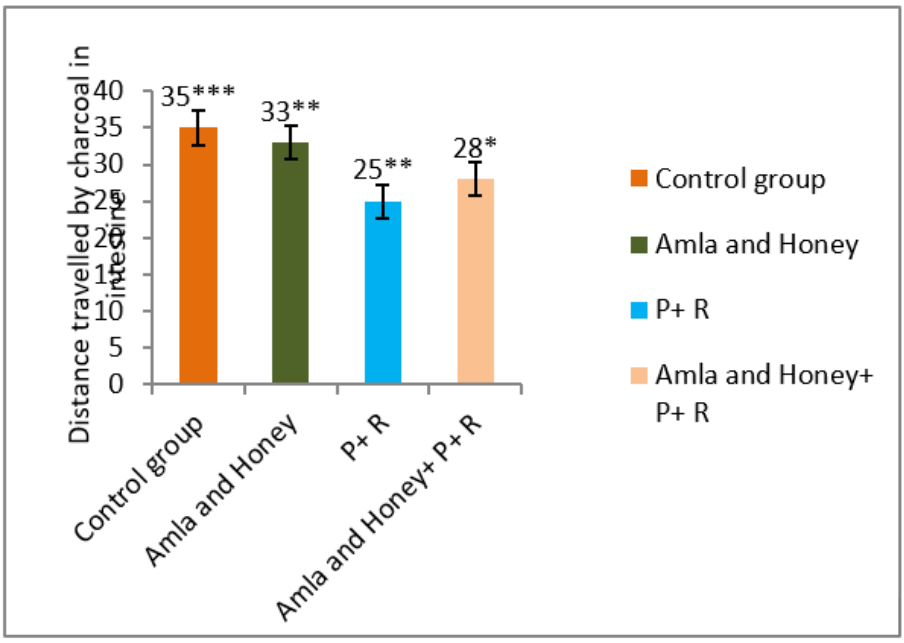

Figure 3:

Distance travelled by charcoal in intestine in different groups. Whereas P signify Pantoprazole and R represent Rebamipide Values are expressed in Mean± SEM; ***p<0.001, **p<0.01, *p<0.05 when compared to control group (one way ANOVA followed by Dunnet's t-test), n=6.

Anti-GERD model of pylorus ligation of Amla and Honey formulation and Rebamipide and Pantoprazole in mono-therapy and combination therapy

Histopathology of oesophagus

After 14 days of dosing of rats in GERD model by pylorus ligation it was observed that control group showed highest oesophagus erosion. By considering oesophagus erosion in control as maximum erosion i.e., poor histology of oesophagus mucosa, the percentage of oesophagus erosion in Amla and Honey were about half of oesophagus erosion in comparison to control. Histology of oesophagus erosion was improved then poorly damaged mucosa as in Amla with Honey group. In Pantoprazole and Rebamipide oesophagus erosion was reduced in comparison to Amla with Honey group and histology of oesophagus mucosa was good, In Amla, Honey and Pantoprazole, Rebamipide oesophagus erosion was reduced to one fourth in comparison to control group.

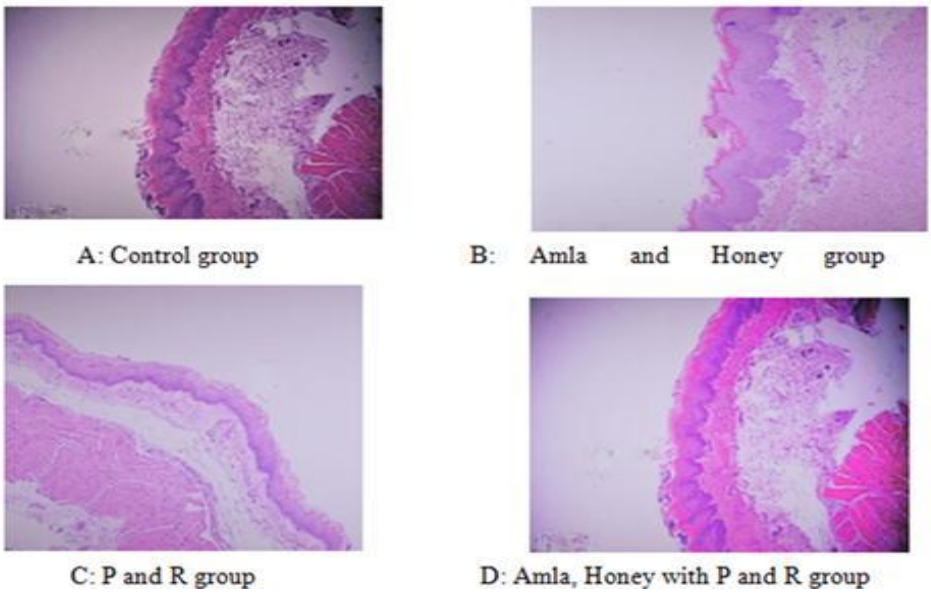

Figure 4:

Histology of oesophagus mucosal erosion in different groups of rats. Whereas P signify Pantoprazole and R represent Rebamipide Values are expressed in Mean± SEM; ******p*<0.001, *****p*<0.01, ****p*<0.05 when compared to control group (one way ANOVA followed by Dunnet's *t*-test), *n*=6.

Percentage of Esophageal Mucosal Erosion

Mean esophageal erosion were observed in all groups of rats. In control group oesophagus erosion was 80± 5% and in Amla and Honey group combination group 47± 8% erosion where as in Pantoprazole and Rebamipide group 35±10%. The lowest oesophagus erosion 25±12% was observed in rats treated with Amla, Honey, Pantoprazole and Rebamipide.

Mean Thickness of Oesophagus Mucosal Erosion

Mucosal thickness erosion in first group of rats served as control group, second group of rats treated with Amla and Honey combination and third groups of rats treated with Pantoprazole and Rebamipide, Fourth groups of rats given Amla and Honey, Pantoprazole and Rebamipide. Mucosal thickness of oesophagus erosion was 118.6±45, 65.54.± 23, and 50± 28, 21±10 micron in mentioned groups respectively.

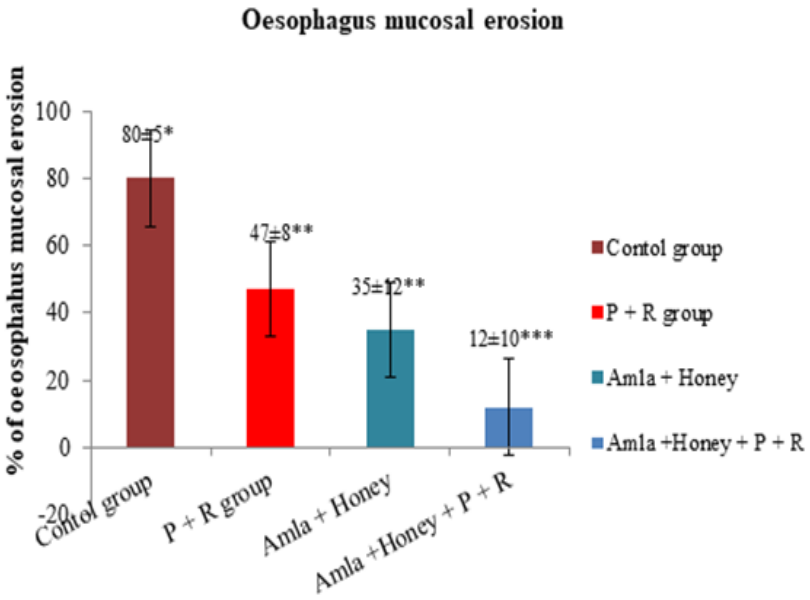

Figure 5:

Percentage of oesophagus erosion in different groups of rats. Whereas P signify Pantoprazole and R represent Rebamipide Values are expressed in Mean± SEM; ***p<0.001, **p<0.01, *p<0.05 when compared to control group (one way ANOVA followed by Dunnet's t-test), n=6.

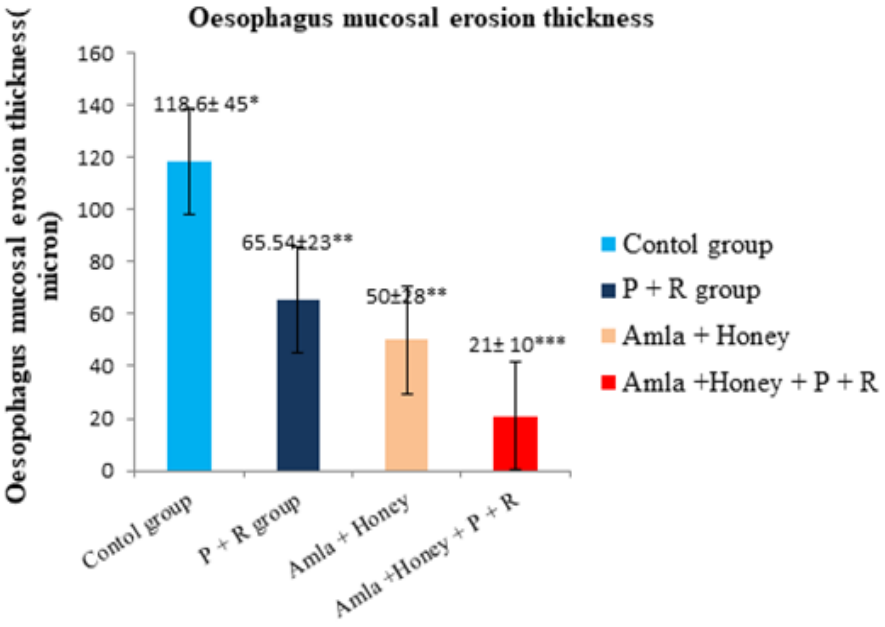

Figure 6:

Mean thickness of oesophagus mucosal erosion in different groups of rats. Whereas P signify Pantoprazole and R represent Rebamipide Values are expressed in Mean± SEM;

*****p<0.001, **p<0.01, *p<0.05 when compared to control group (one way ANOVA followed by Dunnet's t-test), n=6.**

Mean Body Weight

Mean body weight at the end of fasting was found to be 210.5 ± 8 gm. in control group, 214.5± 6gm in Amla and honey treated rats, 221.5±5gm in rats treated with Pantoprazole and Rebamipide, 234± 4.5gm in rats treated with combination of Amla, Honey and Pantoprazole and Rebamipide. Mean body weight at end of study was found to be 215± 5gm, 230.5± 5.5gm, and 239± 6gm and 257± 4gm in control, Amla with Honey, Pantoprazole with Rebamipide and Amla, Honey with Pantoprazole, Rebamipide group respectively. Statistical observation was made by measuring and comparing different body weight of rats in 14 days. In 14 days maximum increase in body weight was found in animal treated with Amla, Honey, Rebamipide and Pantoprazole groups whereas in control group of animals showed minimum increase in body weight. Amla and Honey combination showed little more increase in body weight as compared to control group whereas in standard drug Rebamipide and Pantoprazole exhibited little more increase in weight in comparison to Amla and Honey group. Maximum increase in body weight in Amla, Honey, Rebamipide and Pantoprazole might be due to mentioned drugs treat GERD at early stage and make albino wistar rat to take adequate diet and water, Control group shows minimum increase in weight due to absence of drugs in these groups of rats and they are unhealthy due to GERD.

DISCUSSION

In vitro spasmodic activity at different log dose of acetylcholine with Amla and Honey showed that there is an increase in contractile response with increase in dose but this increase was primary in consistent and goes toward stable state due to limited number of receptors available to perform the desired response.

In charcoal meal motility test, highest decrease in intestinal motility was found in rats treated with Amla, Honey and Pantoprazole, Rebamipide group as compared with other groups which might be due to combination of drugs which resulted in decrease of peristalsis movement of intestine and there by leads to decrease in distance travelled by charcoal. The control group showed highest intestinal motility due to absence of drugs whereas Amla and Honey combination show slightest decrease due to less action on intestine as drugs get absorbed in stomach before going to intestine.[39] Pantoprazole and Rebamipide show higher decrease in motility in comparison to Amla and Honey. This might be due to the fact that they act that their action of intestine motility on receptor underlying the gastro-intestinal tract is greater.

The protective action in GERD screening model by standards and herbal combination was maximum when combinations of drugs (Amla, Honey, Pantoprazole and Rebamipide) were administered orally and intravenously. Pantoprazole and Rebamipide were found to show additive effects together which protect oesophagus by reducing excess acid secretion by inhibiting proton pump action decreasing the final step of acid secretion.[40] Amla protect oesophagus mucus by cyto-protective and wound healing action, Honey also protect

oesophagus by forming a thin lining over the oesophagus and prevent exposure of acid to oesophagus. It was observed that highest therapeutic efficacy was found in Amla, Honey and Pantoprazole, Rebamipide followed by standard drugs Pantoprazole and Rebamipide. In herbal combination of Amla and Honey less therapeutic efficacy in comparison to standards drugs was observed whereas in control group no therapeutic efficacy was observed as normal saline was given orally.

Body weight was found to be increase in all groups but the highest increase in body weight was found in Amla, Honey and Pantoprazole, Rebamipide as compared with other groups and lowest in control group. From observation it was found that better therapeutically effective drugs treat GERD at early stage with a simultaneous increase in body weight was observed in different group of rats.

CONCLUSION

Standards drugs like Rebamipide and Pantoprazole showed highest therapeutic efficacy in comparison to herbal combination of Amla and Honey. In statistical comparison, it was seen that herbal combination of standard drugs showed significant improvement in therapeutic effect in comparison to mono-therapy by these drugs. In charcoal meal motility test, Amla, Honey, Rebamipide, Pantoprazole had the highest decrease in gastrointestinal motility as compared to all other groups. Amla and Honey showed decrease gastrointestinal motility but this decrease in motility is not prominent when compared to Amla, Honey, Rebamipide, and Pantoprazole. The contractile response of Amla and Honey showed significant decrease in contraction of rat ileum in concentration dependent manner when anti-spasmodic activity was conducted *in vitro* so it was then concluded that herbal and standard drug combination reduces all the parameter in GERD such as oesophagus erosion, mucosal damage, gastrointestinal motility and anti-spasmodic activity which might be due to combination of these drugs resulted in better therapeutic effect when combined together.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

GERD: Gastroesophageal reflux disease; **P:** Pantoprazole; **PPI:** Proton pump inhibitors; **R:** Rebamipide; **H2RAs:** Histamine 2 receptor antagonists; **mg:** milligram; **kg:** kilogram; **i.e.:** that is; **log:** Logarithm; **Gm.:** gram.

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