

Evaluation of Synergistic Activity of Ethanolic Leaf Extracts of *Tephrosia Purpurea* and *Bacopa Monnieri* in Ulcer Induced Rats

Ponnala Soumya^{1*}, Suvendu Saha², Saketha Ram Palakurthy³, Ravalva Pasupuleti³

¹Assistant Professor, Department of Pharmacology, Marri Laxman Reddy Institute of Pharmacy, Dundigal, Hyderabad-500043, India

²Associate Professor, Department of Pharmacology, Marri Laxman Reddy Institute of Pharmacy, Dundigal, Hyderabad-500043, India

³Jangaon Institute of Pharmaceutical Sciences, Jangaon, Warangal, Telangana, India

***Correspondence Author:**

Ponnala Soumya

E-mail: ramchettisoumya@gmail.com

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Abstract

Objective: To evaluate synergistic antiulcer activity of ethanolic extracts of *Tephrosia purpurea* and *Bacopa monnieri* in ulcer induced rats.

Methods: Ethanolic leaf extracts of both the plants were administered individually and in combination at a dose of 200mg/kg to ulcer induced male albino rats. Omeprazole 10mg/kg was used as standard. Pylorus ligation method, ethanol and indomethacin induced gastric ulcer models were the different gastric ulcer models selected for the induction of ulcer in rats. Ulcer index, ulcer score, total acidity, pH, percentage protection, volume of gastric juice were the parameters evaluated and compared in different groups in all the models.

Results: Decrease in the ulcer score, ulcer index, total acidity was observed and percentage protection was significant (* $p < 0.05$ and $p < 0.01$) with the combination extract compared to group received individual plant extracts.

Conclusion: Our results suggested that combination of two medicinal plants showed synergistic anti ulcer activity and decreased the formation of ulcer lesions in rats.

Keywords

Tephrosia purpurea; *Bacopa monnieri*; anti ulcer activity; Gastric ulcer; Ulcer index

1. INTRODUCTION

Peptic ulcer is rupture of inner wall or mucosa of stomach or duodenum. The pathophysiology involves imbalance between defensive (prostaglandins, mucus, bicarbonate, nitric oxide and growth factors) and aggressive factors (hydrochloric acid, pepsin, refluxed bile) in the stomach [1] [2]. Many antiulcer drugs

are used for ulcer treatment. The major drawback of ulcer therapy is their less efficacy in healing ulcers and their severe side effects. Medicinal herbs are quite effective in treating many diseases. Most of the synthetic compounds which are available currently show high side effect profile. In order to reduce the side effects and to improve the efficacy medicinal plants are widely used in treatment of many diseases.

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Medicinal herbs are indispensable part of the traditional medicine practiced all over the world due to low costs, easy access and ancestral experience[3]. Ayurvedic formulas contain many balancing herbs offering a high degree of safety and efficacy[4]. Single plant is effective in reducing the ulcers. Combination of two medicinal plants were selected and given to ulcer induced rats for treating ulcers. *Tephrosia purpurea* is a wild plant, known as 'Sharpunka' in sanskrit and 'Unhali' in Gujarati. It has been reported to possess hepatoprotective, mastcell stabilising and erythrocyte membrane integrity enhance effect in various experimental models[5]. *Tephrosia purpurea* is used traditionally to cure several types of external wounds and gastro-duodenal disorders. It cures kidney, spleen, heart and blood related disorders. Ethanolic extract of this plant has been reported as anticancer activity against in-vitro KB-cells culture[6]. Leaves of *Tephrosia purpurea* is used in dyspepsia, pectoral disease, haemorrhoid, syphilis, gonorrhoea[7]. *Tephrosia purpurea* is reported to have potent free radical scavenging property and free radical production may attenuate the reason for various diseases and disorders[8]. *Bacopa monnieri* is useful for improving mental clarity, confidence, memory and is effective against diseases like bronchitis, asthma, arthritis, constipation, digestive problems and is found to possess anticancer activity[9]. Antiulcer activity of ethanolic extracts of individual plants and the combination of ethanolic extracts of two plants was evaluated in ulcer induced rats. Our study was to observe the synergistic anti ulcer activity of two plants in ulcer induced rats. Pylorus ligation model, ethanol induced and indomethacin induced gastric ulcer models were selected for the study.

2. MATERIALS AND METHODS

2.1 Collection of plant and authentication

Tephrosia purpurea leaves were collected from Jangaon, Warangal district and *Bacopa monnieri* leaves were collected from Suvarnapuram, area of Khammam district. Both the plant specimens were authenticated by the Dr. Musthafa, professor in botany, KU, Warangal district, Telangana, India. One specimen of each was kept in the college for future reference.

2.2 Preparation of extraction

Leaves of both the plants were removed and shade dried. After drying the leaves were powdered mixed with ethanol and macerated for 7 days with occasional shakings. After extraction the powder was filtered into

china dish and kept for evaporation then the crude drug was subjected to evaluation of phytochemical constituents.

2.3 Phyto Chemical Evaluation

The chemical constituents present in the plants were screened using phytochemical evaluation studies using standard tests. Different tests for carbohydrates, fats, alkaloids, flavonoids, mucilages were conducted for both the plant extracts. Alkaloids, glycosides, flavonoids were present in both the plants which are responsible for anti ulcer activity[10].

2.4 Experimental animals

Experiments were conducted on male Sprague Dawley rats (150-200g) which were obtained from Hyderabad and housed in standard cages at room temperature (25 ± 2°) and fed with dry pellets and water ad libitum. Experiments on animals were conducted according to the guidelines of the CPCSEA (1322/ac/10/CPCSEA/2010).

2.5 Toxicity studies

In order to find out the minimum and maximum dose of the extract toxicity studies were performed on rats. Rats were administered with different doses of EETP and EEBM and the behavior of the animals were observed carefully for first two hours and then up to 24hrs and then observed for 14 days for its mortality[11-13]. After the observation, it was found that animals did not show mortality upto the dose level of 2000mg/kg body weight. Hence 2000mg/kg body weight was considered as maximum tolerated dose (MTD), 1/10th of the MTD (200mg/kg) was selected for the study.

2.6 Pharmacological studies

Antiulcer study in rats was conducted using three models pylorus ligation method, ethanol induced and indomethacin induced gastric ulcer model.

2.6.1 Pylorus ligation method

The anti ulcer activity was conducted on shay rat model. Animals were deprived of food 48 hrs before starting of the experiment, but had free access to water. In this model, rats were divided into six groups with six animals each. After the fasting period

Group I : Treated with 1% tween 80 (1ml/ kg, orally) and was kept as control.

Group II : Treated with omeprazole (10mg/ kg, orally) and was kept as standard

Group III : Treated with ethanolic leaf extract of

Tephrosia purpurea (200mg/kg, orally)

Group IV : Treated with ethanolic *Bacopa monnieri* leaf extract(200 mg/ kg, orally)

Group V : Treated with combination of leaf extract of *Tephrosia purpurea* (100mg/kg) and *Bacopa monnieri* (100mg/kg)

Group VI : Treated with ulcer inducing agent (kept as diseased control)

After 30min, rats were anesthetized with light ether, the abdomen was opened and the pyloric end was ligated and the abdomen wall is closed by sutures. After four hours, these animals were sacrificed and the stomach was isolated and cut open through its greater curvature. Gastric juice was collected and the volume of gastric juice was measured and centrifuged at 2000rpm for 10 min. pH of the gastric juice was determined and from the supernatant, aliquots (1ml

of each) were taken for the determination of total acidity[5].

Total acidity: an aliquot of 1ml gastric juice was taken into a 50 ml conical flask and two drops of phenolphthalein indicator was added to it and titrated with 0.01N NaOH until a permanent pink colour was seen. The volume of 0.01N NaOH consumed was noted. The total acidity is expressed as mEq/L by the following formula: [14]

$$\text{Acidity} = (\text{Volume of NaOH} \times \text{Normality of NaOH} \times 100 / 0.1) \text{ mEq. /L}$$

Ulcer score: The gastric mucosa was observed for ulcers using magnifying lens and the ulcer was scored according to its severity in comparison with that of standard. Ulcer score was given as:

0 – normal or no ulcer

1 – isolated haemorrhagic spot



Figure 1: GROUP I (CONTROL)



Figure 2: GROUP II(STANDARD)



Figure 3: GROUP III(EETP)



Figure 4: GROUP IV(EEBM)



Figure 5: GROUP V(EETP+EEBM)



Figure 6: GROUP VI (DISEASED CONTROL)

- 2 – dense haemorrhagic spot
- 3 – small ulcer
- 4 – large ulcer
- 5 – perforation[15]

Ulcer index : the ulcerated area was measured in mm under the microscope with the aid of a square grid and was noted as the ulcerative index.

$$\text{Ulcer index} = \frac{('n' \text{ lesion } 1^1 + 'n' \text{ lesion } 2^2 + 'n' \text{ lesion } 3^3)}{'n' \text{ animals}}$$

n = number of animals[16]

2.6.2 Ethanol induced gastric ulcers

Thirty six male albino Sprague dawley rats of either sex (150-200g) were taken. They were divided into six groups of six rats each. The animals were fasted for 24h before the experiment had a free access to water. After the fasting period

Group I : Treated with 1% tween 80(1ml/ kg, orally) and was kept as control.

Group II : Treated with omeprazole (10mg/ kg, orally) and was kept as standard

Group III : Treated with ethanolic leaf extract of *Tephrosia purpurea* (200mg/kg, orally)

Group IV : Treated with ethanolic *Bacopa monnieri* leaf extract(200 mg/ kg, orally)

Group V : Treated with combination of leaf extract of *Tephrosia purpurea* (100mg/kg) and *Bacopa monnieri* (100mg/kg)

Group VI : Treated with ulcer inducing agent (kept as diseased control)

The ethanol(90%) solution was given 1hour later. The animals were sacrificed after one hour using ether. The stomachs were removed and cut open using the greater curvature. Gastric juice was collected and



Figure 7: GROUP II(STANDARD)

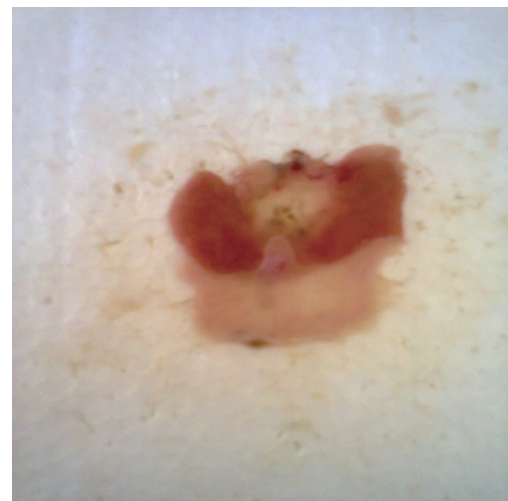


Figure 8: GROUP III (EETP)



Figure 9: GROUP IV(EEBM)



Figure 10: GROUP V(EETP+EEBM)



Figure 11: GROUP VI(DISEASED CONTROL)

centrifuged for 10 min at 2000rpm. pH of the gastric juice was determined and from the supernatant, aliquots(1ml of each) were taken for the determination of total acidity and ulcer was scored depending on its severity[5].

2.6.3 Indomethacin induced gastric ulcers

Thirty six male albino Sprague dawley rats of either sex (150- 200g) were taken. They were divided into six groups of six each. The animals were fasted for 24 hour before the experiment, but had free access to water. After the fasting period

Group I : Treated with 1% tween 80(1ml/ kg, orally) and was kept as control.

Group II : Treated with omeprazole (10mg/ kg, orally) and was kept as standard

Group III : Treated with ethanolic leaf extract of *Tephrosia purpurea* (200mg/kg, orally)

Group IV : Treated with ethanolic *Bacopa monnieri* leaf extract(200 mg/ kg, orally)

Group V : Treated with combination of leaf extract of *Tephrosia purpurea* (100mg/kg) and *Bacopa monnieri* (100mg/kg)

Group VI : Treated with ulcer inducing agent (kept as diseased control)

The test drug or reference or vehicle was administered in two doses with an interval of 15hrs. Then indomethacin (25mg/kg) solution was administered in two doses after 30 min of administration of each dose of the test compound. The rats were sacrificed after one hour using ether. The



Figure 12: GROUP II(STANDARD)



Figure 13: GROUP III(EETP)



Figure 14: GROUP IV(EEBM)



Figure 15: GROUP V(EETP+EEBM)



Figure 16: GROUP VI (DISEASED CONTROL)

stomach was removed and cut open along the greater curvature. Gastric juice was collected and the volume of gastric juice was measured and centrifuged for 10min at 2000rpm. pH of gastric juice, total acidity, ulcer score was determined[5].

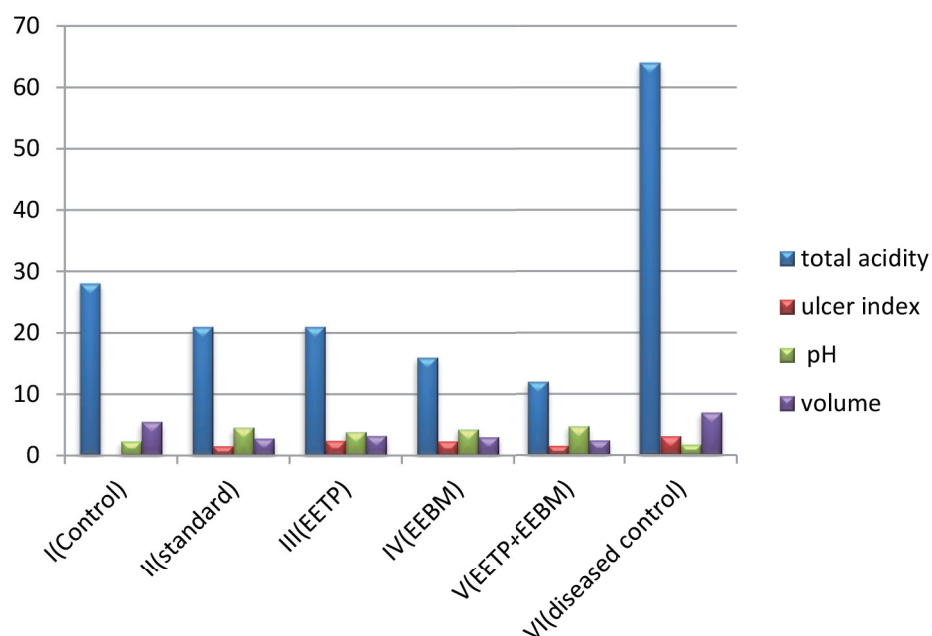
3. RESULTS

The effects of EETP, EEBM, and EETP+EEBM combination on ulcer induced rats is shown in tables 1,2 and 3. Ulcers were successfully induced by all the three models. This can be clearly shown by observing the stomach mucosa of the diseased control animals. In all the three models the combination of EETP with EEBM have shown reduction in the total acidity, gastric

Table 1: Effect of *Tephrosia purpurea* and *Bacopa monnieri* extracts on various parameters in pylorus ligation.

Treatment	Dose	Volume of gastric juice in ml	pH	Total acidity (mEq/L)	Ulcer score	Ulcer index (mm ²)	Percentage protection
Group I (1 % tween80)	1ml/kg	5.5±0.32	2.34±0.02	28±0.68	0	0	0
Group II (Omeprazole)	10mg/kg	2.79±0.02	4.55±0.03	21±0.7	1	1.53±0.05	50.65%
Group III (EETP)	200mg/kg	3.24±0.02	3.84±0.03	21±1.15	2	2.42±0.03	21.94%
Group IV(EEBM)	200mg/kg	3.03±0.06	4.24±0.02	16±1.01	1	2.30±0.08	25.81%
Group V (EETP+EEBM)	200mg/kg (100mg+100mg)	2.44±0.05	4.72±0.04	12±0.79	0	1.53±0.05	58.39%
GroupVI (Diseased control)	–	7.0±0.03	1.73±0.04	64±1.59	4	3.1±0.05	–

All values are expressed as Mean±SEM (n=6). p < 0.05 when compare to control. Statistical comparison was performed by using One-way ANOVA



Graph 1: Effect of *Tephrosia purpurea*, *Bacopa monnieri* and their combination extracts on different parameters in pylorus ligated rats.

juice, ulcer score and ulcer index and increased the pH of the gastric juice. The percentage protection of the combination extract is significant compared to individual extracts.

In all the three models the anti ulcer activity shown by the combination extract was significant. In pylorus ligation method, the percentage protection of gastric mucosa by the combination of EETP+EEBM

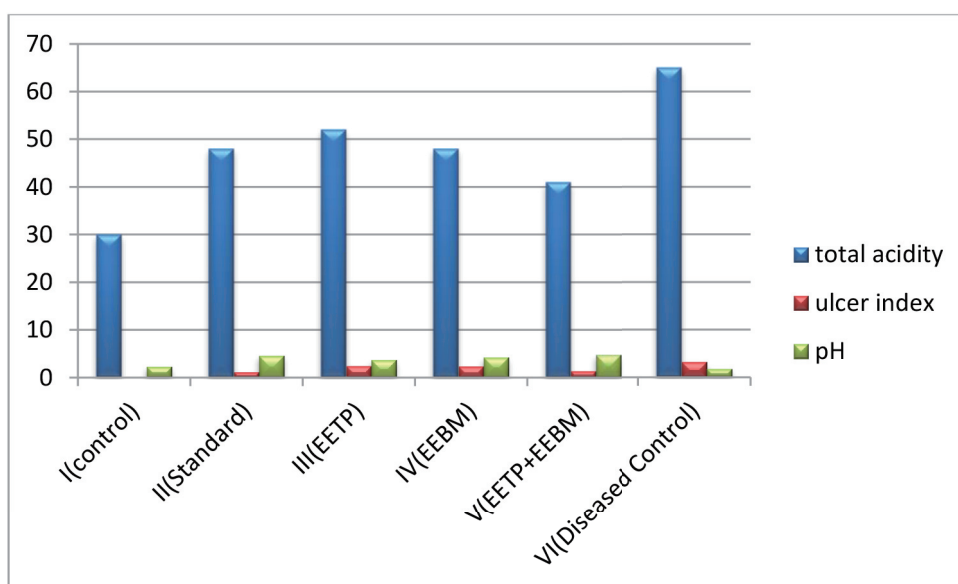
was 58.39% where as EETP alone showed 21.94% and EEBM alone showed 25.81%. there was significantly($p < 0.05$) decrease in total acidity, ulcer score and ulcer index (tables 1, 2 and 3).

In ethanol induced gastric ulcer model the EETP+EEBM extract showed 58.96% protection of gastric mucosa and EETP alone and EEBM alone showed 23.77% and 27.17% protection of gastric

Table 2: Effect of *Tephrosia purpurea* and *Bacopa monnieri* leaf extracts on various parameters on gastric ulcers induced by ethanol

Treatment	Dose	Ulcer score	Ulcer index (mm ²)	Percentage protection of ulcer	pH	Total acidity(mEq/L)
Group I (1 % tween80)	1ml/kg	0	0	0	2.34±0.02	30±0.77
Group II (Omeprazole)	10mg/kg	1	1.17±0.03	63.89%	4.61±0.03	48±1.06
Group III (EETP)	200mg/kg	2	2.47± 0.07	23.77%	3.74±0.03	52±1.78
Group IV(EEBM)	200mg/kg	1	2.36±0.04	27.17%	4.26±0.02	48±1.66
Group V (EETP+EEBM)	200mg/kg (100mg+100mg)	0	1.33±0.04	58.96%	4.80±0.03	41±0.87
Group VI (Diseased control)	–	3	3.24±0.08	–	1.79±0.04	65±2.3

All values are expressed as Mean±SEM (n=6). $p < 0.01$ when compare to control. Statistical comparison was performed by using One-way ANOVA



Graph 2: Effect of *Tephrosia purpurea*, *Bacopa monnieri* and their combination extracts on different parameters in ethanol induced rats.

mucosa.

In indomethacin induced gastric ulcer model, EETP+EEBM combination extract, EETP alone and EEBM alone showed 31.78%, 14.8%, 19.53% of stomach mucosa protection and significantly ($p < 0.05$) reduced all the parameters except the pH of the stomach.

4. DISCUSSION

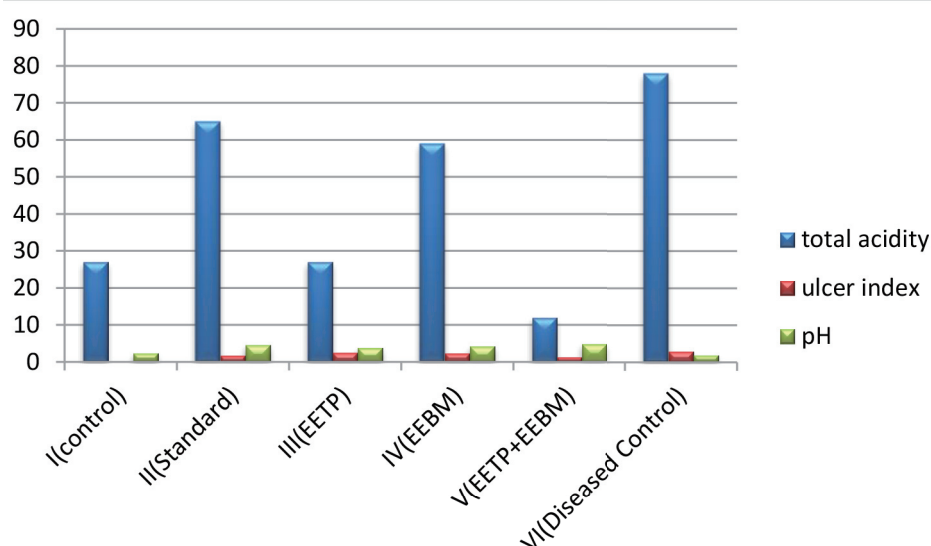
It is generally accepted that ulcer is resulted from an imbalance between aggressive and defensive factors. In order to balance them therapeutic agents like plant extracts are used to inhibit the gastric acid secretion or to improve the mucosal defense mechanism by stimulating mucus production and prostaglandin synthesis.

The present study was undertaken to evaluate the synergistic activity of ethanolic extracts of *Tephrosia purpurea* and *Bacopa monnieri* against ulcer induced

Table 3: Effect of *Tephrosia purpurea* and *Bacopa monnieri* leaf extracts on gastric ulcers induced by indomethacin in rats

Treatment	Dose	Ulcer score	Ulcer Index (mm ²)	Percentage protection of ulcer	pH	Total acidity(mEq/L)
Group I (1 % tween80)	1ml/kg	0	0	0	2.34±0.02	27±0.68
Group III (omepazole)	10mg/kg	1	1.72±0.12	10.52%	4.59±0.04	65±1.50
Group III (EETP)	200mg/kg	1	2.49±0.03	14.8%	3.81±0.06	27±1.20
Group IV(EEBM)	200mg/kg	2	2.35±0.01	19.53%	4.24±0.02	59±1.55
Group V (EETP+EEBM)	200mg/kg (100mg+100mg)	0	1.31±0.04	31.78%	4.88±0.04	12±1.90
Group VI (Diseased control)	–	3	2.92±0.05	–	1.96±0.07	78±2.03

All values are expressed as Mean±SEM (n=6). **p < 0.01; *p < 0.05 when compare to control. Statistical comparison was performed by using One-way ANOVA



Graph 3: Effect of *Tephrosia purpurea*, *Bacopa monnieri* and their combination extracts on different parameters in indomethacin induced rats.

rats. Anti ulcer activity of individual plant extracts was also evaluated.

The study was performed in male albino rats using three ulcer models-

1. Pylorus ligation method
2. Ethanol induced gastric ulcer model
3. Indomethacin induced gastric ulcer model

When the synergistic effect was evaluated astonishing results have been observed. In some instances the combination extracts of both the plants have shown potent action than that of the standard on various parameters.

Total acidity is one of the parameters which gives an idea about the intensity of the acidic environment of the stomach. It has been observed that there was significant decrease in total acidity than standard in pylorus ligation model than in ethanol and in indomethacin induced rat model.

Evaluation of pH of gastric juice determines the strength of acid, whether it is strong acid or weak acid. Stress plays an important role in pathogenesis of ulcers by vagal over activity and decrease in synthesis of prostaglandins which are involved in the inhibition of acid production. It is observed that EETP+EEBM increased the pH of gastric juice in all the three models.

Ulcer index used to measure the ulcerated area of stomach. The gastric lesions were measured. Rupture of the mucosa of the stomach leads to the formation of gastric lesions. Increased exposure of the gastric mucosa to acid results in rupture of mucosa. EETP decreased the ulcer formation, EEBM also decreased the ulcer index. The number of lesions was significantly decreased in EETP+EEBM. This effect is almost similar to the standard in the ethanol induced gastric ulcer model and ulcer index was decreased than the standard in indomethacin and pylorus ligation models.

Ulcer score is another parameter evaluated in all the 3 models. The score was given by measuring the intensity of gastric lesions. Gastric injury was induced by pylorus ligation method, and by administration of ethanol and indomethacin to each starved rat. Starvation leads to decrease in PG levels. (PGs-stimulates synthesis of mucus and have an antisecretory effect on gastric acid production). Increase in gastric mucosal lesions increases the ulcer score. EETP+EEBM decreased the formation of gastric lesions and decreased the ulcer score (Figure 5, 10 and 15).

Gastric volume is one of the parameters which gives an idea about the volume of gastric contents like HCl, bicarbonate, pepsinogen. Increase in acid secretion increases gastric volume. Inhibition of PG's increases

acid secretion. Pylorus ligation is the only stimulus for the increased secretion of gastric acid. In pylorus ligation model, more amount of acid gets accumulated in the stomach and increases gastric volume. EETP and EEBM individually decreased the gastric volume in pylorus ligation rats. EETP+EEBM significantly decreased the gastric volume than individual extracts and standard (Table 1).

5. CONCLUSION

The combination of EETP+EEBM was found to decrease the acid volume, total acidity and ulcer index more significantly than the individual extracts and the standard.

Total acidity was evaluated in all the three models. It is observed that, total acidity was significantly much more decreased in pylorus ligation model than in ethanol and indomethacin induced models in rats.

It is observed that the ulcer score in all the three models is decreased. The rats administered with EETP+EEBM showed '0' (zero) ulcer score in all the three models.

Ulcer index was evaluated in all the three models. It was observed that ulcer index was decreased in rats administered with EETP+EEBM, than the ulcer index of standard. In all the three models the decrease in ulcer index is almost similar compared with standard treated rats.

Ulcer protection percentage was evaluated in all the three models. It is observed that in indomethacin and pylorus ligation models the EETP+EEBM showed greater protection to ulcer than the standard and individual extracts. Where as in Ethanol induced ulcer model, the ulcer protection percentage is almost similar between the EETP+EEBM and the standard, and lesser than the individual test compounds.

Gastric volume is measured only in pylorus ligated rats but not in ethanol and indomethacin induced rats because pylorus ligation in the duodenum is the only stimulus for acid secretion in the stomach. Pylorus ligation increases the gastric volume. In the present study, the gastric volume is decreased in the rats pretreated with EETP+EEBM than the standard and individual plant extracts (Table 1).

On the basis of data it is concluded that, *Tephrosia purpurea* and *Bacopa monnieri* individually when they were administered to rats, reduced the incidence of gastric ulcer, gastric volume and total acidity. But these plants when they were used in combination, they significantly prevented the formation of gastric lesions.

Tephrosia purpurea and *Bacopa monnieri* when

combined and administered showed Synergistic antiulcer activity. Further research on this study with increased test doses may provide more effective compound with good efficacy and less side effects.

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Conflict of interest

There are no conflicting interests, as the authors have stated.

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