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**EVALUATION OF SYNERGISTIC ANTIULCER ACTIVITY OF AEGLE
MARMELOS AND OCIMUM SANCTUM IN ULCER INDUCED WISTAR RATS**

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ABSTRACT

Aim and objectives: The present study was done for the purpose of evaluating synergistic anti-ulcer activity of Aeglemarmelos and Ocimum sanctum in ulcer induced wistar rats. **Materials and methods:** Healthy 30 wistar rats of either sex were taken for this study. Rats were grouped randomly in study groups and ethanol extracts of Aeglemarmelos and Ocimum sanctum were given as per the study protocol. Ulcers were induced by forced swim methods. Results were calculated with the control group. **Results:** The ethanolic extracts of Aeglemarmelos and Ocimum sanctum showed significant anti-ulcer activity in forced swimming method. **Conclusion:** In the present study, combination of Aeglemarmelos and Ocimum sanctum at the dose of 300 mg/kg (150 gmeach of AM and OS) showed a significant anti-ulcer activity as compare to dose given of Aeglemarmelos (300mg/kg) and Ocimum sanctum (300mg/kg) individually. Ulcer index reduced as compared to control group and its results were similar to standard drug Omeprazole (20mg/kg), as evidenced by the decrease in gastric lesions.

KEYWORDS

Evaluating synergistic, Gastric mucosa and Aeglemarmelos.

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INTRODUCTION

It is assumed that it occurs due the imbalance between the defensive factors (i.e, mucin, prostaglandin, bicarbonate, nitric oxide and growth factors) and gastric aggressive factors (acid, pepsin, and H. pylori)¹. Peptic ulcers are assumed to be caused by the break in mucosal lining of the stomach. Peptic ulcer occurs when the thick layer of our mucus membrane that is responsible for protecting our stomach from the digestive juice (acid) is reduced. Due to its get thin, digestive juice

that is also called acid easily affects the tissue in the stomach. It leads to stomach ulcers. Peptic ulcer disease shows a serious medical condition due to H.pylori, alcohol, NSAIDs use, bad eating habits and changed life style of today generation has increased the frequency of ulceration in human. These are the painful sores occurs in the stomach or intestine. Ulcer is produced when the acid of our stomach get exposed to other areas this leads to a formation of ulcer.

Ocimum sanctum is mainly known as Holy basil and Tulasi. It is found in many parts India and it is native to Indian subcontinents and also cultivated throughout the Southeast Asian tropics. It is cultivated upto 1800m above the sea level. In India, plant is cultivated from Himalayas to Andman and Nicobar islands. It is commonly used as anti-ulcer agent, in memory enhancement², as mouthwash for plaque control³, antimicrobial⁴ antibacterial⁵, antioxidant⁶, hepato-protective⁷ Aeglemarmelos native to indian region. Aeglemarmelos comes to Rutaceae family. It is mainly known as Bael in Indian parts. Bael is a sacred tree of the hindus because its leaves are mostly used as in prayer of Lord Shiva and Parvati in India. Bael trees are native to the indian subcontinent, sub-himalayan region and Southeast Asia. Its presence in Sri Lanka, Thailand and Malaysia also. In India it is commonly found in foothills of Himalya, Bihar, Chhattisgarh, Uttaranchal, Jharkhand, Uttar Pradesh and Madhya Pradesh. This plant is used as immune-modulatory agent⁸, Used as anti-diabetic agent⁹, antimicrobial¹⁰, antidairrhoal¹¹, anti-inflammatory drug (leaves)¹², hypoglycaemic agent¹³, antioxidant¹⁴, cardioprotective¹⁵, hepatoprotective¹⁶.

MATERIAL AND METHODS

Plant Collection and Authentication

Plant and leaves of Ocimum sanctum (OS) and Aeglemarmelos (AM) were identified and collected from Sai planters, Meerut in the month of February. Leaves of Ocimum sanctum and Aeglemarmelos were authenticated by Dr. Vijay Malik, botany department, Chaudhary Charansingh University (CCSU), Meerut (UP). A reference no. was

provided by CCSU, Meerut after plant authentication (plant authentication no- REF.BOT./795/14/2/19).

Animals Maintenance

Healthy wistar rats (150-200 grams of weight) of either sex were issued from diseased free animal house of Translam Institute of Pharmaceutical Education and Research, Meerut, U.P. Animals were maintained and housed under suitable environment of controlled temperature ($24^{\circ}\text{C} \pm 5^{\circ}\text{C}$). Relative humidity was about ($55 \pm 10\%$) in a 12/12 h light/dark cycle. Four animals were kept together in a single polypropylene cage. Standard diet was given to the animals. All necessary facilities were provided to the animals such as food, water ad libitum etc. Protocols which were used in the study, were reviewed by the Institutional Animal Ethics Committee (IAEC) of T.I.P.E.R. and were as per the guidelines of the CPCSEA, Ministry of Forest and Environment, Government of India¹⁷.

Preparation of Plant extract

The leaves of AM and OC were collected after that they were dried. Leaves powder was prepared by using a mechanical grinder. The powdered material was soaked in ethanol (2Litre) separately with regular shaking (40 RPM) at 25°C for three days. After this process, it was filtered by using a filter paper. The ethanol extracts was evaporated under vacuum evaporator at 350°C temperature and in reduced pressure to obtain final semi-solid extracts¹⁸.

Phytochemical Screening

Phytochemical screening of ethanolic extracts of Aeglemarmelos (EEAM) and Ocimum sanctum (EEOS) was done for qualitative analysis of the various phyto constituents like alkaloids, carbohydrates, phenolics, tannins, saponins and flavonoids.

Phytochemical screening of the Aeglemarmelos and ocimum sanctum was done separately for detecting the activity chemical constituent present in both plants. The phytochemical screening was done after reviewing many articles.

Forced swimming method

30 healthy wistar rats (150-200gn) were arranged in 5 groups with 6 animals in each group of either sex. Following treatments were given to these groups:

Group I

Served as control group, treated with DW (5 ml/kg body weight)

Group II

Treated with omeprazole (20mg/kg), as a standard reference drug

Group III

Treated with EEAM (300 mg/kg)

Group IV

Treated with EEOS (300 mg/kg)

Group V

Treated with EEAM and EEOS (150mg/kg AM + 150mg/kg OS)

Animals were fasted for 1day before starting this method.

Induction of ulcer was done in rats by forced swimming method at normal temperature. Rats were forced to swim for 3-4 hours in tank of dimension (60cm x 90cm) and water was filled up to 30cm of height.

Specific treatments were given orally before forced swimming¹⁹.

Animals were sacrificed, stomachs removed and results of % protection and Ulcer index were calculated in this model¹⁸.

Determination of ulcer index

After cutting along the greater side, stomach tissues were washed with tap water, stretched with the help of all pins and then observed with the help of 10X magnifying glass. Ulcers were noted and the severity of ulcers were observed and reported by using given scores²⁰.

Severity of ulcer was calculated by using the following scale:

- 0 = Normal stomach
- 0.5 = Pink to red colored stomach
- 1 = Spot ulcer
- 1.5 = Hemorrhagic streaks
- 2 = Ulcers
- 3 = perforation

Mean ulcer score for each animal was expressed as UI (ulcer index).

By using the formula, UI can be calculated,

$$UI = UN + US + UP \times 10^{-1}$$

Where, UN = avg of number of ulcers per animal.

US = avg of severity score.

UP = % of animals with ulcers.

Percentage protection

% protection was calculated using the formula²¹,

$$\% \text{ protection} = \frac{(\text{Control mean ulcer index} - \text{Test mean ulcer index}) \times 100}{\text{Control mean ulcer index}}$$

Statistical Analysis

The statistical analysis of all the results was carried out using one- way ANOVA followed by Dunnet's test. All data have been expressed as the mean \pm standard error of mean (S.E.M) obtained results were compared with the control group with p-value ($p^{**} < 0.01$), which was accepted as significant.

RESULTS AND DISCUSSION

Preliminary Phytochemical Screening

Preclinical phytochemical screening was done for the detection of the chemical compounds. The chemical tests of EEAM and EEOS were done for the detection of chemicals that are present in these plants and have potential of treating the disease.

Effect of Ethanolic Extracts of Aegle Marmelos and Ocimum Sanctum in Forced Swimming Induced Ulcer in Rats

This study has showed significant ($p^{**} < 0.01$) results after evaluating anti-ulcer action of EEAM and EEOS in forced swimming induced ulcer in rats. There were significant results as compared to control group by using ANOVA followed by Dunnet's t-test as significant increase in the protection of ulcer and there was increase in the ulcer index and % protection after this evaluation.

In forced swimming induced ulcer model, EEAM and EEOS (individually) significantly reduce the ulcer Index and increased the %protection. Ulcer index (UI) reported by EEAM and EEOS extracts were 1.35 ± 0.63 and 1.26 ± 0.57 resp. UI of EEAM+EEOS and stndrd drug were 0.89 ± 0.21 and 0.73 ± 0.33 as compared to control group. EEAM and EEOS exhibited ulcer protection of 64.09% and 66.48% in 300 mg/kg of dose

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respectively while the % protection of EEAM and EEOS (150 mg/kg dose of both plant) was 76.32% whereas the standard drug omeprazole (20mg/kg) showed 80.58% protection. All these values were compared with control group.

This study has showed that the potential of treating ulcer by herbal drugs is effective as the synthetic drugs. On other hand, Synthetic drugs have many adverse effects on patient health and are target oriented while the herbal drugs have potential of treating ulcer and also have positive effect on curing other diseases (diabetes, arthritis, CV diseases etc. that have been proved by many researches) with minimal adverse effects. So EEAM+OS in combination has the therapeutic potential to control gastric ulcer. However further studies are necessary for its establishment and to find exact mode of action of these drug.

Values are express as mean \pm Standard error of mean (SEM), where n= 6/group observations, statistically comparisons as follows: Significant at $**p < 0.01$ Compared to Control group (ANOVA) followed by Dunnet`s t-test.

(*terms used EEAM- Ethanolic extract of Aeglemarmelos and EEOS- Ethanolic extract of Ocimum sanctum).

DISCUSSION

In this evaluation, various parameters were investigated for evaluating the synergistic antiulcer activity of EEAM, EEOS (individually and in combination). The purpose of this study was for revealing the new alternative for the treatment of ulcer that is the most common disease now days. This new alternative of herbal drug treatments can give patients a better drug after seeing the adverse effects of synthetic drug. So for this objective this study was done by using forced swimming model. Omeprazole at 20mg/kg was used as standard drug treatment.

This study showed its potential for treating the gastric ulcers effectively. In this study data were recorded which exhibits that the EEAM, EEOS and EEAM+OS, all these groups have gastro protective and anti-ulcer activity at 300mg/kg of doses in

Forced swimming model. Gastric parameters were taken for calculating the results of this study. Gastric lesions were also improved. %protection was observed and results were showing significant anti-ulcer and gastro-protective activity of EEAM, EEOS and EEOS+EEAM. %P was EEAM (64.09%), EEOS (66.48%) and EEOS+AM (76.32%) in forced swimming while stand. Drug showed 80.58% protection. So the overall observation is that the EEAM+OS showed better action potential against gastric ulcers as compared to individual action of AM and OS at 300mg/kg and these results were similar to the stand drug omeprazole.

The preliminary phytochemical evaluation of Aeglemarmelos showed that the coumerins, alkaloids, carbohydrates, phenolic comp., flavonoids, steroids, terpenoids, tanins, proteins and fixed oils were present during evaluation while saponins were absent. Ocimum sanctum showed the presence of alkaloids, carbohydrates, phenolic comp., flavonoids, steroids, terpenoids, tanins, saponins and fixed oils.

This antiulcer activity of AM and OS might be due to the coumerins, alkaloids, carbohydrates, phenolic comp., flavonoids, steroids, terpenoids, tanins, saponins and fixed oils. Terpenoids and glycosides are responsible for inhibiting gastric acid production and prevent gastric mucosa against ulcer models. Some studies has reported that the antiulcer action of Aeglemaremls is due to terpenoids, luvangetin, tannin, alkaloids, quercetin (flavonoid), and Eugenol which are present in it while OS antiulcer activity may be due to cytoprotective property, increasing prostaglandin synthesis and antioxidant action to prevent gastric mucosa.

Table No.1: Preliminary phytochemical screening of EEOS and EEAM

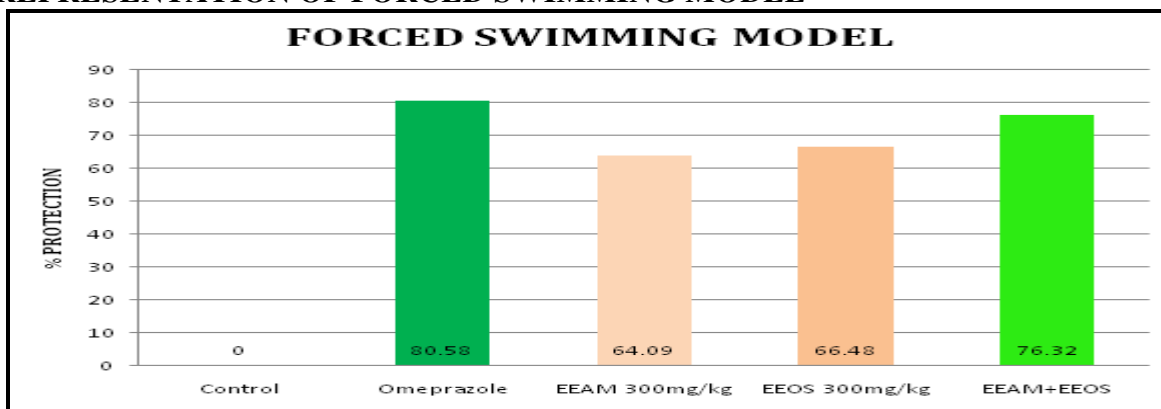
S.No	Constituents	Constituents	Inference	
			Aeglemarmelos	Ocimum sanctum
1	Alkaloids	Mayer`s test	(+)	(+)
		Dragendorff`s test	(+)	(+)
		Hager`s test	(+)	(+)
2	Carbohydrates	Molish`s test	(+)	(+)
		H ₂ SO ₄ test	(+)	(+)
3	Phenolic compounds	FeCl ₃ test	(+)	(+)
4	Flavonoids	Zinc + HCl test	(+)	(+)
5	Steroids	Liebermann and Burchard`s test	(+)	(+)
6	Terpenoids	Chloroform+acetic anhydride+ H ₂ SO ₄	(+)	(+)
7	Tanins	Gelatin test	(+)	(+)
8	Saponins	Foam test	(--)	(+)
9	Proteins and amino acids	Millon`s test	(+)	(--)
10	Coumerins	Powder + methanol + HCl	(+)	(--)
11	Fixed oils and fats	Ether + C ₆ H ₆ + CHCl ₃	(+)	(+)

Note + sign indicate presence and – sign indicates absence

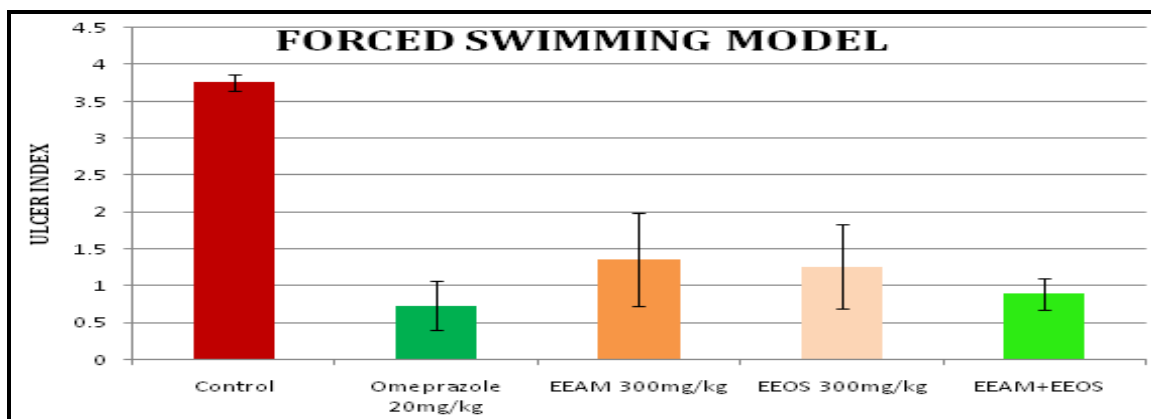
Table No.2: Ulcer index and % Protection of EEAM and EEOS in forced swimming induced ulcer in rats

Group No	Treatment	Ulcer index	Protection (%)
I	Control group (5ml/kg DW)	3.76 ± 0.11	-
II	Omeprazole (20mg/kg)	0.73 ± 0.33	80.58
III	EEAM (300mg/kg)	1.35 ± 0.63	64.09
IV	EEOS (300mg/kg)	1.26 ± 0.57	66.48
V	EEAM and EEOS (150mg/kg of EEAM+150mg/kg of EEOS)	0.89 ± 0.21	76.32

GRAPH REPRESENTATION OF FORCED SWIMMING MODEL



Graph No.1: Effect of treatments on percentage protection in forced swimming model



Graph No.2: Effect of treatments on ulcer index in forced swimming model

CONCLUSION

The results of this study showed that the EEAM+OS (in combination) have the significant potential of treating ulcer and protecting the gastric mucosa and these results are similar to standard drug in forced swimming model. After examining the data, present study shows that the Ethanolic extracts of Aeglemarmelos and Ocimum sanctum leaves in combination (150mg/kg each of AM and OS) have the potential for treating gastric lesions and can prevent gastric mucosa but further studies are required for identification of the active chemical co.

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CONFLICT OF INTEREST

We declare that we have no conflict of interest.

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