Panacea Journal of Pharmacy and Pharmaceutical Sciences 2019:8(2), 01-09
International Journal

Panacea Journal of Pharmacy and Pharmaceutical Sciences
ISSN: 2349 7025

PJPPS
Panacea Research Library
http://internationaljournal.org.in/journal/index.php/pjpps

Original Research Article **April-June 2019** **Volume 8 Issue 2**

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

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ABSTRACT:

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 Ethanolic extracts of AM and OS in combination has the therapeutic potential to control gastric ulcer. However further studies are required to established its exact mode of action and active.

Key Words: Antiulcer**,** Aegle Marmelos, Ocimum Sanctum

INTRODUCTION

In the present time the most widely spread and common disease is Peptic ulcer. It is assumed that it occurs due the imbalance between the defensive factors (mucin, prostaglandin, bicarbon-ate, nitric oxide and growth factors) and gastic aggressive factors(acid, pepsin, and H. pylori)[1]. Peptic ulcer disease is the breach or break in the inner lining of the stomach and the esophagus (that is the part of small intestine).

Approximately 10 out of 100 suffer from ulceration. It is most familiar disease now days which is disturbing day today life of many persons. Ulcers are cause of financial loss and responsible for health concern globally. In 2015, about 87,000000 were noticed for being affected by ulcers. There are about 350,000 to 500,000 new cases reported in a single year and more than 1000,00 patients hospitalizes in each year. It is most prevalent thing today [1] and it occurs mainly in the age of more than 60 years. Peptic ulcer may be of two types that might be of duodenal ulcers of may be of Gastric ulcers [2]. Peptic ulcer may be life threatening in case of if it gets chronic stage. According to reports, 267,500 people have been died from 1990 to 2015.

Major causes of ulcer are **H.pylori** and use of **NSAID**s. Other causes of Peptic ulcer are[3]

- Use of tobacco
- Excessive smoking
- Zollinger-Ellison syndrome it is rare condition. In this disease patient may be suffering from tumor in the pancreas of body.
- Crohn disease
- Disease of liver
- Stressing conditions can cause stress ulcers.
- Behcet disease
- Having radiation treatment
- Regularly intake of Aspirin, Ibuprofen, naproxen, or other Non- steroidal anti-inflammatory drugs(NSAIDs)
- Changed lifestyle
- Bad eating habits and others,

Treatment of PUs is use of proton pump inhibitors, H_2 receptor antagonists, antacids, and anti-H.Pyloric drugs like amoxicillin

The aim of this study was to evaluate the antiulcer potential of Aeglemarmelos and Ocimum sanctum when these are used together. Both plants are of common use and widely available in India, mostly found around a temple. Synthetic drugs are target oriented while herbal drugs not only treat that particular disease but also have positive health effects on other body organs (i.e. Aeglemarmelos and Ocimum sanctum is used as anti-inflammatory agent [4], antibacterial [5], anti-oxidant [6], cardio-protective[7]etc).

This study may be an alternative drug therapy after seeing the adverse effects of synthetic drugs in future.

MATERIALS AND METHODS

Plant collection and Authentication

Plant and leaves of Ocimum sanctum and Aeglemarmelos 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, ChaudharyCharansingh University, Meerut (UP). A reference no. was provided by CCSU, Meerut after plant authentication(plant authentication no- ***REF.BOT/795/14/2/19)***.

Animal maintenance

Animals were maintained and housed under standard conditions of controlled temperature (24˚C ± 5˚C). Relative humidity was about (55 ± 10%) in a 12/12 h light/dark cycle. They were caged with a maximum of four animals in each polypropylene cage and were fed with standard diet.All necessary facilities were provided to the animals such as food, water ad libitum etc. Protocols used in the study were reviewed by the Institutional Animal Ethics Committee (IAEC) of T.I.P.E.R. and were in accordance with the guidelines of the CPCSEA, Ministry of Forest and Environment, Government of India[1].

Toxicity study

Acute oral toxicity studies of ethanolicleaves extract of theAeglemarmelos and Ocimum sanctum were done according to the guidelines of OECD. Acute toxicity studies were done for determining LD50 for selection of dose as per OECD guideline no. 425. The healthy wistar rats of either sex, weighing 100-150 g were used for this study. Observation of the animals was done for 12 hours to detecting behavioral and autonomic changes. Observation of mortality was observed for 24 hours. The doses of 300mg/kg for AM, 300mg/kg for OS and 300mg/Kg for AMOS extract (150mg/kg of each plant) were selected based on the evaluation of preliminary toxicity testing **[8]**.

Animal grouping and Pylorus ligation method

30 healthy wistar rats (150-200gn) were divided into 5 groups with six 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 ethanolic extract of Aeglemarmelos leaves (300 mg/kg)

Group IV- treated with ethanolic extract of Ocimum sanctum leaves (300 mg/kg)

Group V - treated with ethanolic extracts of Aeglemarmelos and Ocimum sanctum (150mg/kg AE + 150mg/kg OS)

This is shay rat model. Before starting this method, Animals were fasted for 48 hours. Water was available for them. Treatment was provided for these groups (control/standard drug/extracts). After 30 minutes of the drug treatment, rats were anesthetized by using ether. After anesthesia abdomen was opened with small middle incision below xiphoid process. Ligation was done in the pyloric portion of the stomach after small lifted out without causing any type of harm to the blood supply of the rat stomach. Small incision was sutured carefully. Rats were sacrificed by using anesthesia abdomen was opened and a ligature was placed around the esophagus close to the diaphragm. The stomach was removed, along the greater curvature the stomach was opened and the contents were drained in a centrifuge tube for evaluation. Stomach was washed with tap water and further evaluations were done[9, 10].

Following parameters were evaluated and recorded after this model:

- Volume of gastric secretion
- pH of gastric content
- Total acidity
- Free acidity
- Ulcer index
- % protection

1. **Volume of gastric secretion**-

   After dissection of stomachs, gastric content was collected with help of syringe into graduated centrifuge tube through small nick along the great curvature.

2. **Evaluation of pH**: After removing stomach, gastric content was drained into graduated centrifuge tube through small nick along the great curvature. The tubes were centrifuged at 2000rpm for 10mins. 1ml of gastric juice was diluted with 1ml DW and evaluation for pH was done by using pH meter[11].

3. **Free acidity:** after gastric volume measurement and centrifugation, 1ml gastric juice (clear supernatant) was pipetted put and diluted with 10ml of distilled water into a 50 ml conical flask and 2-3 drops of Topfer's reagent as an indicator was added to it and titrated with 0.01N NaOH until a yellow to orange color was observed[12].

The volume of 0.01N NaOH consumed was noted. Free acidity was calculated by

Using the following formula:

$$\textbf{Free acidity} = \frac{\text{Volume of NaOH} \times \text{Normality} \times 100}{0.1}\ \text{mEq/L}$$

4. **Total acidity:** Titration was further continued using against 0.01N NaOH phenolphthalein as indicator, until it regained its permanent pink color. The volume of 0.01N NaOH consumed was noted. Total acidity was also calculated by using the same formula as that of free acidity **[12]**,

$$\textbf{Total acidity} = \frac{\text{Volume of NaOH} \times \text{Normality} \times 100}{0.1}\ \text{mEq/L}$$

5. **Determination of ulcer index**[13]

   After cutting along the greater curvature, stomach tissues were washed with tap water, stretched with the help of all pins and then observed with the help of 10X magnifying glass. The number of ulcer were noted and the severity recorded with the following scores

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,

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

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

US = average of severity score.

UP = percentage of animals with ulcers.

6. %Protection:

Percentage protection was calculated using the formula [14],

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

STATISTICAL ANALYSIS

The statistical analysis of all the results was carried out using one- way ANOVA followed by Dunnet'stest. All data have been expressed as the mean ± 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

This study has showed significant ($p^{**}<0.01$) results after evaluating anti-ulcer activity of ehtanolic leaves extracts of Aeglemarmelos and Ocimum sanctum in Pylorus ligation induced ulcer in rats. There were significant results as compared to control group by using ANOVA followed by Dunnet`s t-test as reduction in Gastric juice collection, total acidity and free acidity by the EEAM and EEOS extracts. pH increased towards alkaline when measured by pH digital meter.

In pylorus ligation induced ulcer model, ethanolic extract of A. marmelos leaves and Ocimum sanctum (individually) significantly reduces the ulcer Index, severity of ulceration and increases the percentage of protection.Ulcer index reported by EEAM and EEOS extractswere 2.01 ± 0.52 and 1.86 ± 0.33 resp. UI of EEAM+EEOS and stndrd drug were 1.11 ± 0.36 and 0.83 ± 0.4. The ethanolicextract of A. marmelosand ocimum sanctum leaves showed ulcer protection of 47.79% and 51.68% in 300 mg/kg of

doserespectively while the % protection of EEAM and EEOS (150 mg/kg dose of both plant) was *71.16%* whereas the standard drug omeprazole (20mg/kg) showed 78.44% protection. All these values were compared with control group[15].

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 Ethanolic extracts of AM and OS in combination has the therapeutic potential to control gastric ulcer. However further studies are required to established its exact mode of action and active.

Table no. 1: Ulcer index and % Protection of EEAM and EEOS in pylorus ligation induced ulcer in rats.

Group	Treatment	Ulcer index	Protection (%)
I	Control group (5ml/kg DW)	3.85±0.17	-
II	Omeprazole (20mg/kg)	0.83 ±0.4	78.44
III	EEAM (300mg/kg)	2.01 ±0.52	47.79
IV	EEOS (300mg/kg)	1.86 ±0.33	51.68
V	EEAM and EEOS (150mg/kg of EEAM+150mg/kg of EEOS)	1.11±0.36	71.16

Values are express as mean ±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)

Table no. 2: Effect of EEAM and EEOS in pylorus ligation induced ulcer in rats.

Group	Treatment	Volume of Gastric juice (ml)	pH of gastric juice	Free acidity (mEq/L)	Total acidity (mEq/L)
I	Control group (5ml/kg DW)	7.67±1.77	3.09±0.17	53.73±1.27	80.61±4.54
II	Omeprazole (20mg/kg)	2.03±0.35	5.13±0.06	30.71±0.88	42.83±2.23
III	EEAM (300mg/kg)	5.08±0.38	3.83±033	51.78 ±1.02	68.29±1.33
IV	EEOS (300mg/kg)	4.63 ±1.01	3.91±0.42	47.27±2.11	61.33±3.30
V	EEAM and EEOS (150mg/kg of EEAM+150mg/kg of EEOS)	2.39 ±0.17	4.87±0.17	35.17±0.88	48.09±1.5

Values are express as mean ±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)

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