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ANTI ULCER ACTIVITY OF THE LEAVES OF *RAPHNUS SATIVA* AND *LEUCAS ZEYLANICA*

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ABSTRACT

The present study was aimed at the investigation of the anti-ulcer activity of the ethanolic and petroleum ether-dichloromethane extracts of the leaves of the traditional Indian medicinal plants *Raphnus sativa* and *Leucas zeylanica* at different doses using ranitidine as standard drug. Two methods say alcohol induced ulcers and pyloric ligation were adapted to evaluate the biological potential of the plant extracts using male wistar rat experimental animal models. In both the methods, the extracts were found to possess dose dependant anti-ulcer activity. When compared, the petroleum ether dichloromethane extracts of the both the plants at the dose of 300 mg/kg exhibited significant activity comparable to ranitidine drug. Continuation of the research in this regard is highly recommended for the isolation and the characterization of chief chemical constituents responsible for this pharmacological potential of the extracts.

INTRODUCTION

For over a century, peptic ulcer has been one of the important gastro intestinal disorders across the world. Especially, both the duodenal and gastric ulcers severely affecting the people of western and southern parts of the world respectively. It requires a well targeted strategy for effective control as it effects the digestive health of the persons severely. It is believed to be due to imbalance between the protective and promoting factors of ulcer. Various external factors such as alcohol, use of nonsteroidal anti-inflammatory drugs, infection with *Helicobacter pyroli* followed by tissue necrosis and decreased defense mechanisms will aggravate the disorder that need special medical and health care.

Current anti-ulcer drugs like cimetidine, omeprazole, ulcer neutralizing agents, mucoprotective agents possess several limitations such as side effects, drug interactions, tolerance, relapses, non-affordability etc., necessitates the need for the discovery of new agents overcoming these limitations. Nature provided several resources such as plant products that are traditionally and scientifically proved to be safe and effective agents for the treatment of various disease and disorders in human beings.[1-5]

Several plant products from *Azadirachta indica*, *Allophylus serratus*, *Emblica officinalis*, *Ocimum sanctum* etc., have been reported for the anti-ulcer activities and proved scientifically as safe and effective agents.[6-9]

The objective of the present study is an attempt in the scientific development of the traditional medicines as safe and effective alternatives to current drugs. In this study, the anti-ulcer activity of the alcoholic and petroleum ether-dichloromethane (PED) extracts of the leaves of *Raphnus sativa* (RS) and *Leucas zeylanica* (LZ) the traditional Indian medicinal plants claimed in folklore for anti-ulcer activity and scientifically for several pharmacological activities but not for anti-ulcer activity. The plants also reported to contain several biologically active compounds such as alkaloids, terpenoids, steroids, flavonoids etc.; Therefore, the plants can be scientifically investigated for their anti-ulcer potential. Ranitidine was used as reference standard for the study.[10-16]

MATERIALS AND METHODS

PLANT MATERIALS: The plants were authenticated by the department of Botany, Kakatiya University, Warangal. Dried leaves of RS and LS were collected from local areas and were dried under sun light for 24 hours, then powdered and are subjected to maceration with alcohol and PED for 2 days with intermittent shaking. Then, these were filtered and dried to get the extracts. Finally, these extracts were dissolved in 0.5% (w/v) aqueous carboxy methyl cellulose and are used as test samples.

CHEMICALS: All the chemicals and reagents used for the study were of analytical grade and procured from authenticated sources.

ANIMALS: Healthy, adult, male wistar albino rats weighing 150-250 grams and obtained from authenticated sources were used as experimental models. All the animals were

maintained and treated as per the guidelines of the Institutional animal ethics committee of the college.

ACUTE TOXICITY STUDIES: The prepared plant extracts were tested for their toxic effect to determine the maximum dose of the study. It was done as per the OECD-423 guidelines in animals in 24 hours for Lethal dose (LD) 50 and behavioral changes.

PROCEDURE: Two methods were adopted for evaluation of the anti-ulcer activity of the plant extracts: 1. Ethanol induced gastric ulcers and 2. Pyloric ligation method.

Method 1**:** *Ethanol induced gastric ulcers:* Animals were divided into 10 groups 4 rats in each group. These were fasted for 36 hours prior to the administration of the samples. First one is the control group that received normal saline at the dose of 10 ml/kg orally. The second group given the standard drug ranitidine at the dose of 20 mg/kg orally. Third and fourth groups received the alcoholic extract (AE) of RS at the oral doses of 150 and 300 mg/kg respectively. Fifth and sixth groups were given PED extracts (PDE) of RS at the oral doses of 150 and 300 mg/kg respectively. Seventh and eight groups were administered the oral doses of 150 and 300 mg/kg of AE of LZ respectively.

Ninth and last group of animals were given the PDE of LZ at the oral dose of 150 and 300 mg/kg respectively. All the animals are fed with 1ml of 80% Ethanol orally and kept aside for 4 hours. Then, the animals were sacrificed by ether anesthesia and spinal nerve dislocation and their intact stomachs were isolated by surgical procedure through greater curvature and immediately washed under tap water.

(*Note: All the doses of the extracts were selected according to the acute toxicity studies.)

These isolated organs were used to score the ulcer index as a measure of ulcers formed with respect to ulcer area as follows.

0: Normal stomach

0.5: Red colored

1: Spot ulcers

1.5: Hemorrhagic streak

2: Moderate

3: Severe

Percentage of inhibition of the ulcer formation for reference and the extracts was calculated using following formula:

% Inhibition: [(Control-Standard (or) Test) / (Control)]

Method 2: *Pyloric ligation method:* Animals were grouped same as the above method and were fated for 24 hours. The animals were given the normal saline, standard ranitidine and the AE and PDE of the plants RS and LZ same as in the first method.

After 45 minutes of the administration of the samples, the pyloric ligation was done by ligating the pyloric end of the stomachs of the all animals after ether anesthesia without affecting the normal blood flow. The animals were allowed to recover depriving the supply of water in the post-operative period.

After 4 hours, the animals were sacrificed and ulcer scoring was done and their gastric juice used for analysis of different parameters like, pH, total gastric juice and total acid.

Total acid content determined by removing total gastric content of the stomachs into centrifuge tubes and centrifuged at 1000 rpm for 10 minutes. 1 ml of the supernant was diluted to 10 ml by distilled water and titrated against 0.01 N sodium hydroxide (NaOH) using Topfor's reagent and Phenolphthalein as indicator. The volume of NaOH consumed can be taken as a measure of acid present.

Total acid can be found by following formula.

Acidity = (Volume of NaOH consumed*Normality of NaOH)*100)

STATISTICAL ANLYSIS: All the values obtained were compared by Analysis of variance method and reported as mean +or- SEM and the probability $p<0.01$ considered as significant.[17-20]

RESULTS AND DISCUSSION

Table 1: Percentage yield of the extracts.

Extract	Percentage yield
AE of RS	9 %
PDE of RS	9.5%
AE of LZ	10%
PDE of LZ	10.5%

Table 2: Anti-ulcer activity of RS extracts in method 1.

Group / sample	Dose (mg/kg)	Ulcer area (mm ^{2}) Mean ±SEM	% Inhibition of ulcer
1. Control-normal saline	-	5.05±0.31	-
2. Standard-Ranitidine	20	0.35±0.20	91.23
3. AE	150	1.20±0.35	72.2
4. AE	300	1.02±0.57	79.34
5. PDE	150	0.90±0.14	82.23
6. PDE	300	0.49±0.52	89.7

All the values were found to be significant with p<0.01.

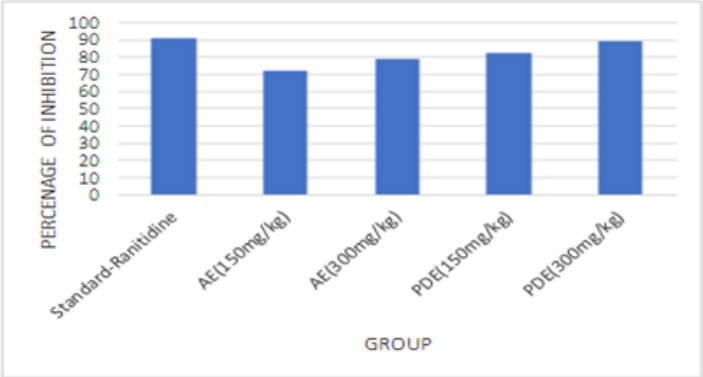

Figure 1: Anti-ulcer activity of RS method 1.

Table 2: Anti-ulcer activity of RS extracts in method 2.

Group / Sample	Dose (mg/kg)	Gastric pH	Volume of gastric juice (ml)	Total acid produced (µl)
Control-normal saline	-	2.67±0.06	2.5±0.06	44.1±0.09
Standard-Ranitidine	20	4.5±0.05	0.99±0.02	17.23±0.10
AE	150	3.5±0.04	1.5±0.07	35.5±0.23
AE	300	3.6±0.03	1.32±0.03	23.3±0.43
PDE	150	3.7±0.04	1.30±0.08	21.2±0.23
PDE	300	4.2±0.10	1.10±0.05	18.8±0.07

All the values were found to be within the limits with significance p<0.01.

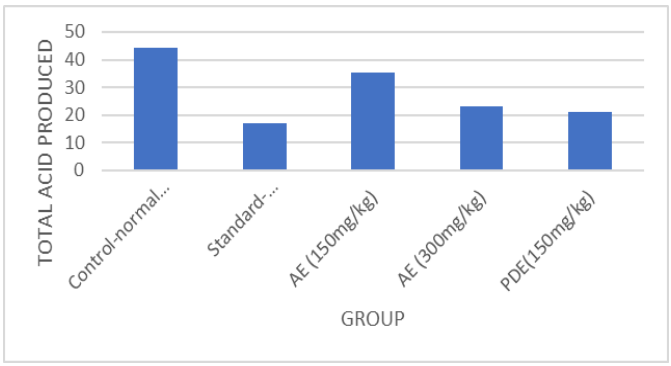

Figure 2: Antiulcer activity of RS method 2.

Table 4: Anti-ulcer activity of the extracts of LZ in method 1.

Group / sample	Dose (mg/kg)	Ulcer area (mm ^{2}) Mean ±SEM	% Inhibition of ulcer
1. Control-normal saline	-	4.90±0.32	-
2. Standard-Ranitidine	20	0.37±0.26	93.23
3. AE	150	1.90±0.40	70.45
4. AE	300	1.20±0.32	77.32
5. PDE	150	0.87±0.33	81.21
6. PDE	300	0.49±0.45	88.32

All the values were found to be significant with p<0.01.

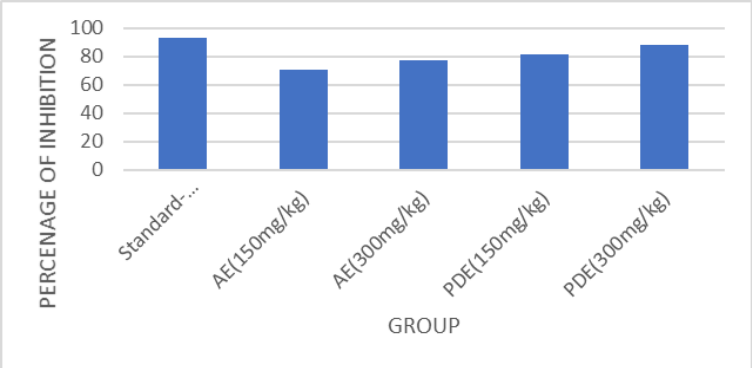

Figure 3: Antiulcer activity of LZ method 1.

Table 5: Anti-ulcer activity of the extracts of LZ in method 2.

Group / Sample	Dose (mg/kg)	Gastric pH	Volume of gastric juice (ml)	Total acid produced (µl)
Control-normal saline	-	3.20±0.04	2.9±0.07	48.4±0.10
Standard-Ranitidine	20	4.0±0.08	1.23±0.08	19.28±0.18
AE	150	3.30±0.07	1.43±0.09	37.2±0.21
AE	300	3.44±0.08	1.30±0.07	24.1±0.39
PDE	150	3.49±0.09	1.29±0.03	22.4±0.12
PDE	300	3.90±0.13	1.24±0.05	20.7±0.09

All the values were found to be significant with p<0.01.

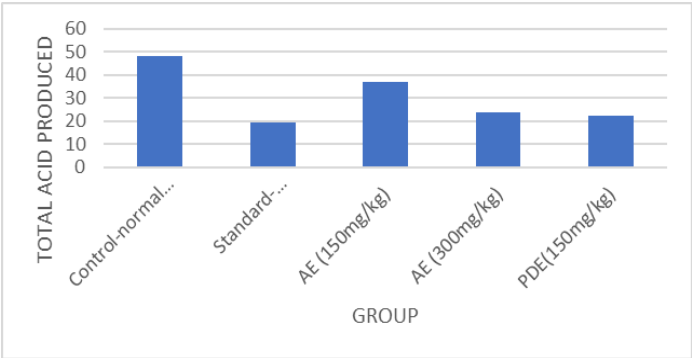

Figure 4: Antiulcer activity of LZ method 2.

From the above results, it was found that, in method 1, the plant extracts shown to possess dose dependent activity and the PDE extract of the both the plants RS and LZ at the dose of 300 mg/kg shown maximum activity comparable to the reference ranitidine.

In method 2 also, the plant extracts shown dose dependent activity and the PDE extract of the plants shown better activity with respect to all the parameters studied in comparison to standard drug.

The order of their potency can be expressed as follows.
Ranitidine(20mg/kg)>PDE(300mg/kg)>PDE(150mg/kg)>AE(300mg/kg)>AE(150mg/kg)

DISCUSSION

The results indicating that, the plants especially, the PDE extracts shown significant anti-ulcer activity that is comparable to standard drug ranitidine proved the traditional and scientific claims of the plants for Pharmacological activities and biologically active chemical constituents. These were also found to be safe in acute toxicity studies. The effect shown by the extracts also comparable to the scientific reports of the past reports for medicinal plants like *Ocimum sanctum* for this activity. However, the exact chemical compounds responsible for this biological activity still not known. Hence, offers greater scope for research and development in this regard.

CONCLUSION

All the extracts tested exhibited dose dependent activity. When compared among the extracts, the PDE of the plants had shown maximum antiulcer activity comparable to reference Ranitidine. More study is required for isolation and characterization of the chief chemical constituents responsible for this pharmacological activity.

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