WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 8.074
Volume 7, Issue 6, 514-523. Research Article ISSN 2277– 7105

IN-VITRO EVALUATION OF ANTIULCER ACTIVITY OF *MELIA AZEDARACH* LINN LEAVES ON WISTAR ALBINO RATS

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Article Received on
19 January 2018,
Revised on 11 Feb. 2018,
Accepted on 03 March 2018,
DOI: 10.20959/wjpr20186-11318

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ABSTRACT

Peptic ulcer disease (PUD) is considered as one of the common diseases in the world. Treatment of peptic ulcer with synthetic drugs such as proton pump inhibitors, H2 receptor antagonists and other non-steroidal anti-inflammatory drugs has shown adverse effects, relapses, drug interactions. Medicinal plants containing active chemical constituents are useful in prevention and treatment of various diseases. The present study shows the *Melia azedarach* Linn leaves have very good effect on pH of Gastric Contents. It lowers down the gastric content as compared to standard omeprazole. It is also shows the lowering ulcer index.

KEYWORDS: Peptic ulcer, Proton pump inhibitors, Ulcer Index, Omeprazole.

INTRODUCTION

Gastric ulcer, one of the most widespread, is believed to be due to an imbalance between aggressive and protective factors.[1] The gastric mucosa is continuously exposed to potentially injurious agents such as acid, pepsin, bile acids, food ingredients, bacterial products (Helicobacter pylori) and drugs.[2] These agents have been implicated in the pathogenesis of gastric ulcer, including enhanced gastric acid and pepsin secretion, inhibition of prostaglandin synthesis and cell proliferation growth, diminished gastric blood flow and gastric motility.[3] Drug treatment of peptic ulcers is targeted at either counteracting aggressive factors (acid, pepsin, active oxidants, platelet aggravating factor "PAF", leukotrienes, endothelins, bile or exogenous factors including NSAIDs) or stimulating the mucosal defences (mucus, bicarbonate, normal blood flow, prostaglandins(PG), nitric

oxide).[4] The goals of treating peptic ulcer disease are to relieve pain, heal the ulcer and prevent ulcer recurrence. Currently there is no cost-effective treatment that meets all these goals. Hence, efforts are on to find a suitable treatment from natural product sources.

MATERIALS AND METHODS

Animal Used: Wistar albino rats of either sex weighing between 150-200g are used for acute toxicity study to determine LD50 of various extracts. The rat stomach shows an obvious division into two parts. The upper 2/5th non-secretory portion (lumen) which is translucent and thinner than the lower 3/5th glandular secretory portion, which according to Shay et al. is analogous to the body of stomach in man both anatomically and functionally. The rat being omnivorous resembles man nutritionally and it is available to use adult rats of either sex for anti-ulcer studies.

Extracts used

- AlcE of leaves of *M. azedarach.*
- AqE of leaves of *M. azedarach.*

Acute toxicity study

The acute oral toxicity study was carried out as per the guidelines set by Organization for Economic Co-operation and Development (OECD), revised draft guidelines 423 B, received from Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Social Justice and Empowerment, Government of India. The "Up and Down" method was used for acute toxicity study. 1/10th of the LD50 cutoff dose was taken as therapeutic dose for antiulcer activity.

Preparation of animals

The animals were randomly selected and kept in their cages for at least 5 days prior to dosing to allow for acclimatization to the laboratory conditions.

Preparation and administration of doses

All the doses were prepared in distilled water using 2% tween 80 as suspending agent. In all cases the concentrations were prepared in 1 ml/100g of b.w. The test substances were administered in a single dose using a gastric intubation tube after fasting for 3 to 4 h.

Number of animals and dose levels

In each steps three animals were used. Since there was no information on the substance to be tested (i.e. extracts), starting dose was selected to be 200 mg/kg b.w.

Observations

Animals were observed initially after dosing at least once during the first 30 min, periodically during the first 24 h. In all cases death was observed within first 24 h. Additional observations like changes in skin and fur, eyes and mucous membranes, and also respiratory, circulatory, autonomic and central nervous systems, and somatomotor activity and behavioral pattern were also done. Attention was also given to observations of tremors and convulsions.

Evaluation of Antiulcer activity

Antiulcer studies

a) Aspirin induced ulceration for assessing anti-ulcer activity.

b) Pylorus-ligation method.

a) Aspirin induced ulcers in rats (Konturek et al. 1981)

Four groups of albino rats weighing 150-200g are used. Each group contains six animals. The test drugs are administered orally in 1% acacia solution 10min prior to oral aspirin in a dose of 200mg/kg (20mg/ml). Six hours later, the rats are sacrificed in ether anesthesia and their stomachs removed. Formal-Saline (2% v/v) is then injected into the totally ligated stomachs for storage overnight. The next day, the stomachs are opened along the greater curvature, then washed in warm water and examined under a 3-fold magnifier. The lengths of the longest diameter of the lesions are measured and summated to give a total lesion score (in mm) for each animal, the mean count for each group being calculated.

b) Pylorus ligation induced gastric ulcers

Twenty rats of either sex were randomly divided into four groups and fasted for 48 h with free access to water. Pyloric ligation was performed under light ether anesthesia to each animal.

Animals were given 1% CMC solution or leaves extracts 250mg/ kg or 20 mg/kg Omeprazole orally immediately after pylorus ligation. Animals were sacrificed 4 h later. The stomach was carefully removed and gastric contents were collected. The gastric juice was centrifuged at 3000 rpm for 30 min and the volume of the gastric juice was measured. Free and total

acidities in the supernatant were determined by titration with 0.1 N NaOH and expressed as mEq/L/100 g. The stomach was cut open along the greater curvature and pinned on a soft board for evaluating gastric ulcers and ulcer index was calculated. The percentage inhibition of ulcers was calculated as mean ulcer index of controlmean ulcer index of test/ mean ulcer index of control x 100.

a) Determination of Free Acidity and Total Acidity

The gastric contents were centrifuged at 1000 rpm for 10min. 1ml of supernatant was diluted with 9ml of distilled water. A volume of 2ml diluted gastric juice was titrated with 0.1N Sodium hydroxide run from a micro burette using 3-4 drops of Topfer's reagent as indicator until canary yellow color was observed. Volume of NaOH required was noted. This corresponds to free acidity. Further 2-3 drops of phenolphthalein was added and titrated with NaOH until pink color was restored. This gives total acidity. Free acidity and total acidity is expressed in terms of ml of 0.1N HCl per 100gms of gastric contents. This is the same as mEq/lit. To obtain this figure multiply the burette reading obtained from titration by 10.

b) Ulcer Scoring & Ulcer Index Determination

Each stomach was examined grossly and the ulcers were graded using the following system suggested by Kunchandy *et al*. 1985.

0 - Normal Mucosa

0.5 - Red coloration

1.0 - Spot ulcers

1.5 - Hemorrhagic streaks

2.0 - Ulcers >3 but <5

2.5 - Ulcer >5

Ulcer index was calculated using following formula:

Ulcer Index = 10/x

Where, X = Total mucosal area

Total ulcerated area

Table No. 1: Protocol for Antiulcer activity.

Group	Status	Induction	Treatment
I	Control	-----	-----
II	Standard treated	Aspirin, Pylorus ligation.	Omeprazole (20 mg/kg, p.o.)
III	AqE treated	Aspirin, Pylorus ligation.	AqE of leaves of *M. azedarach* (250 mg/kg, p.o.)
IV	AlcE treated	Aspirin, Pylorus ligation.	AlcE of leaves of *M. azedarach* (250 mg/kg, p.o.)

ANTIULCER ACTIVITY

1. Estimation of pH of gastric contents

In control animals, without any drug the mean is 2.45. Both the extracts showed rise in pH of gastric contents. Aqueous extract showed rise in pH (3.05) as compared to control. The rise in pH shown by Alcoholic extract is 2.87. Omeprazole, a standard drug raised the pH to 3.305, which is statistically significant ($p<0.001$). This is more potent than the extracts used. The results are shown in table no.1.

2. Estimation of free acidity of gastric contents in terms of ml of 0.1 N HCl/ 100ml of gastric contents

Gastric free acidity is increased to (27.23 mEq/litre) in control animals. Aqueous (15.18 mEq/litre) showed significant decrease in free acidity ($p<0.001$) as compared to control. The decrease in free acidity by Alcoholic extract was 17.95mEq/litre. When compared with Omeprazole, a known anti-ulcer drug, Aqueous extract is equipotent (12.90 mEq/litre), whereas other extracts are less potent in decreasing gastric acidity. The results are tabulated in table no.2.

Statistical Analysis

The data obtained by the various parameters was statistically evaluated by one way analysis of variance (ANOVA) followed by Dunnett 't' test for individual comparison using Graph Pad Prism software (Graph Pad software Inc., Version 4.0.0.255). The mean values ± SEM were calculated for each parameter. The differences in biochemical parameters between the ulcer induced group and standard drug treated group were considered as 100% and the changes in biochemical parameters by the plant extracts treated groups against the ulcer induced group were nalyzed accordingly. Level of significance was kept at $P<0.01$.

RESULTS

Table No. 2. Preliminary qualitative tests of various extracts of *Melia azedarach* Linn. Leaves.

Phytoconstituents	Present /Absent	
	AqE	AlcE
Alkaloids	+	+
Amino acids	-	-
Carbohydrates	+	+
Fats and oils	-	-
Flavonoids	+	-
Glycosides	+	+

Gums and mucilage	-	-
Proteins	-	-
Saponins	-	+
Steroids	+	-
Tannins	-	-
Triterpenoids	-	+
Vitamins	-	-
Organic acids	-	-

Table No.3 Estimation of pH of Gastric Contents.

Sr.No	Control	Standard (Omeprazole)	Aqueous Extract	Alcoholic Extract
1	2.60	3.20	3.02	3.27
2	2.41	3.01	2.93	2.28
3	2.50	3.10	2.98	3.41
4	2.30	3.40	3.95	2.09
5	2.50	3.40	2.80	3.50
6	2.40	3.72	2.64	2.40
S.E.	0.042	0.105	0.188	0.221
p value	--	<0.01** (S)	<0.05* (S)	>0.05 (IS)

All calculations are done by ANOVA & Dunnett 't' test. * (S) – Significant. IS- Insignificant.

Table No.4: Estimation of Free acidity of Gastric contents in terms of ml of 0.1N HCl/100ml of Gastric Contents.

Sr.No	Control	Standard (Omeprazole)	Aqueous Extract	Alcoholic Extract
1	26.0	12.5	16.5	17.9
2	27.5	12.5	14.0	18.0
3	27.9	13.5	15.3	18.7
4	27.5	13.5	15.0	18.6
5	27.0	12.8	16.2	18.2
6	27.5	12.6	14.10	17.6
S.E.	0.273	0.195	0.424	0.194
p value	--	<0.01**	<0.01**	<0.01**

Calculations are done by ANOVA & Dunnett 't' test. * (S) – Significant. IS- Insignificant

Table No.5 Estimation of Total acidity of Gastric contents in terms of ml of 0.1N HCl/100ml of Gastric Contents.

Sr.No	Control	Standard (Omeprazole)	Aqueous Extract	Alcoholic Extract
1	48.2	31.6	33.2	32.0
2	50.0	28.5	33.2	31.2
3	42.5	33.5	31.5	34.5
4	46.0	28.0	32.0	34.0
5	43.0	28.8	31.6	32.6
6	44.3	31.0	33.6	34.0
S.E.	1.215	0.879	0.376	0.2686
p value	--	<0.01**	<0.01**	<0.01**

Calculations are done by ANOVA & Dunnett 't' test. *(S) – Significant. IS- Insignificant

Table No.6: Determination of Ulcer index

Sr. No	Aspirin Control	Std. (Omeprazole)	Aqueous Extract	Alcoholic Extract
1	5.01	1.10	2.70	2.20
2	3.55	1.23	2.42	2.18
3	4.05	0	1.57	2.29
4	4.02	0	2.21	2.87
5	3.96	1.51	2.40	2.30
6	5.55	2.12	2.03	2.24
S.E.	0.359	0.346	0.1504	0.09
p value	--	<0.01**	<0.01**	<0.01**

Calculations are done by ANOVA & Dunnett 't' test. *(S) – Significant. IS- Insignificant

DISCUSSION

In the present study, dried powder of leaves of *M. azedarach* were subjected to extraction using 70%v/v alcohol and chloroform water I.P. Some part of both extracts was reserved for preliminary phytochemical investigation and rest was utilized for pharmacological screening. The preliminary phytochemical investigation showed presence of alkaloids, carbohydrates, glycosides, saponins, steroids and triterpenoids. The flavonoids being the major phytoconstituent was isolated from AlcE of leaves and subjected to qualitative TLC / HPTLC and FTIR analysis. It showed the presence of phenolic functional group in the glycoside. In FTIR spectrum of isolated flavonoids, the peak at 3684.68 cm-1 indicated the O-H stretching. UV spectra are supporting the results. However, further detailed study is necessary. The pharmacological screening included evaluation of antiurolithiatic activity using 0.75% ethylene glycol induced urolithiasis model in male Wistar albino rats. The kidney stone formation induced in rats, as a result of 14 days chronic administration of 0.75% ethylene glycol, was significantly inhibited by oral administration of AqE and AlcE of *M. azedarach* leaves. On prophylactic treatment, AqE and AlcE of *M. azedarach* leaves prevented the urinary stone formation. The study was extended to evaluate the antiulcer activity of *M. azedarach* leaves using Aspirin induced ulceration and Pylorus-ligation model in Wistar albino rats. AqE of *M. azedarach* leaves exhibited significant protection against Aspirin induced ulceration and Pylorus-ligation method as compared to standard drug Omeprazole.

CONCLUSION

In conclusion, the presented data indicate that administration of the AqE and AlcE of *M. azedarach* leaves to rats with ethylene glycol induced lithiasis reduced and prevented the

formation of urinary stones, supporting folk information regarding antiurolithiatic activity of the plant part. The mechanism underlying this effect is still unknown, but is apparently related to diuresis and lowering of urinary concentrations of stone forming constituents. The protective effect against oxalate induced lipid peroxidation may be contributory to the recovery of renal damage.

These effects could conclude the antiurolithiatic property of *M. azedarach*.

For assessing anti-ulcer activity Aspirin and Pylorus ligation induced ulceration model was used and various parameters like pH, free acidity, total acidity, ulcer index were determined. Finally detail histopathological observation was done on the basis of histopathological abdominal sections of photomicrographs of rat mucosa, stained with haemotoxylin-eosin. Aqueous extract significantly raised the pH of gastric contents. It lowered the free and total acidity and ulcer index as compared to Control group. The increased protection against Aspirin induced ulceration and Pylorus-ligation model by AqE of *M. azedarach* leaves concludes the prominent antiulcer activity of the plant part.

However, further research on detailed pharmacological screening, isolation of active phytoconstituents possessing the therapeutic activity and clinical study for evaluation of safety and efficacy of the drug needs to be assessed.

Hence, to put into a nutshell, more significant anti-ulcer activity of aqueous extract may be due to the presence of flavonoids. However, this claim demands further investigations. Till date, *M. azedarach* has been studied exhaustively; however, it can be evaluated for its other medicinal properties as per the literature.

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