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EVALUATION OF ANTI-ULCER ACTIVITY OF METHANOLIC EXTRACT OF *ANOGEISSUS LATIFOLIA* LEAVES IN ALBINO RATS

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ABSTRACT
Anogeissus latifolia (Roxb. ex DC.) Wall. ex Guill. & Perr. (Combretaceae) has been reported to be used in the treatment of various disorders including stomach and skin diseases. We studied the antiulcer potential of the methanolic extract in order to validate ethnobotanical claims regarding the plant use in the above-mentioned disorders. Gastroprotective potential of the methanolic extract of *Anogeissus latifolia* (ALE) (200 and 400 mg/kg/body weight) was studied on aspirin, and ethanol-induced ulcers. The results of the present study showed for the first time that the ALE possessed gastroprotective activity as evidenced by its significant inhibition in the formation of ulcers induced by chemical agents with a maximum of 88.95%. The parameters studied were ulcer index, gastric juice volume, pH, free acidity and total acidity. These findings could justify, at least partially, the inclusion of this plant in the management of gastric disorders in traditional medicine.
KEY WORDS: *Anogeissus latifolia*, gastric lesions, helicobacter pylori, balki, unani medicine.

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INTRODUCTION

Medicine of plant origin is based upon the premise that plants contain natural substances that can promote health and alleviate illness[1]. *Anogeissus latifolia* (Roxb. ex. Dc). wall. ex. Guill & perr. (Combretaceae) is a medium to large sized tree distributed throughout India in dry deciduous forests and in the subhimalayan region and hills of South India up to 1300 materials. Its common names are axle wood (English), balki, dhawa (Hindi). The plant has been already used in the Ayurveda system of medicine.

Ethnobotanically, the bark and leaves of *Anogeissus latifolia* has been extensively utilized in the treatment of various disorders like skin disease[2], Snake and Scorpio bite, stomach diseases[3],colic,cough and diarrhea, hepatoprotective[4], antioxidant potential studies, wound healing, Leaf of this plant is rich in gallotannins[5],the leaves have reported as anthelmintic activity[6]and gum has been reported as hypolipidemic activity[7].Gastric ulcer is among the major disease of GIT, for which a large number of traditional and modern medicines are being utilized. Among these, the medicines of plant origin are more popular because of their less adverse effect[8]. Previously, no scientific work has been reported on the anti-ulcerogenic activity of this plant leaves. The present study was designed to verify the claims of traditional and use in unani system of medicine. In this study, various parameters like ulcer index, gastric juice volume, pH, free acidity and total acidity were determined.

MATERIALS AND METHODS

Collection of plant

The fresh leaves of *Anogeissus latifolia* were collected in the month of September -2010 from forest area of Rajamandry district, Andhrapradesh, India, and authenticated by
Prof. P Jayaraman, Ph.D., Plant Anatomy Research Centre, Chennai, Tamil Nadu (Reg.No: PARC/2011/748). The voucher specimen was deposited at the department for future reference.

Extraction of plant material

Leaves were dry in shade and grinded in to coarse powder, and sieved through #40 meshes. About 400g of air dried powder was taken in 1000ml soxhlet apparatus and extracted with petroleum ether for 2 days. At the end of second day the powder was taken out and it was dried. After drying it was again packed and extracted by using methanol as solvent, till colour disappeared. The

temperature was maintained at 55ºC-65ºC. After that, the extract was concentrated by distillation and solvent was recovered. The final solution was evaporated to dryness and dry residue was obtained.

Animals

Male Albino rats both sex, weighing 150-200g were used. The experimental protocols were approved by institutional Animal Ethical Committee & a written permission from in house ethical committee has been taken to carry out (Reference no. JKKMMRFCP/2010/009) and complete this study.

EXPERIMENTAL PROCEDURE

Acute Oral Toxicity Study

Animals (Albino rats, 150-200gm) were selected for studies. The procedure was followed by using OECD guidelines 423 (Acute toxic class method).

Preliminary phytochemical analysis

The preliminary phytochemical analysis carried to find out the phytoconsituents present in the crude extracts.

Ethanol induced ulcer

Male Albino rats were divided into six groups of six animals per group and animals were fasted for 24 hrs prior to the experiment in perforated steel cages to avoid coprophagy. Six groups were made as below

Group I - animals served as normal controls.

Group II - received 1% CMC (1.0ml/kg p.o) as vehicle control.

Group III - received 200mg/kg, p.o methanolicextract of. *Anogeissus latifolia* leaves.

Group IV - received 400mg/kg, p.o methanolicextract of. *Anogeissus latifolia* leaves.

Group V - received 100mg/kg, p.o sucralfate as standard

One hour after the drug treatment the animals were treated with absolute ethanol [5ml/kg] to induce ulcers. The animals were sacrificed after 1hrs and stomach was opened and percentage inhibition of ulcer was determined [9].

Aspirin induced ulcer

Male Albino rats were divided into six groups of six animals per group and animals were fasted for 24 hrs prior to the experiment in perforated steel cages to avoid coprophagy. Six groups were made as below

Group I - animals served as normal controls.

Group II - received 1% CMC (1.0ml/kg p.o) as vehicle control.

Group III - received 200mg/kg, p.o methanolicextract of. *Anogeissus latifolia* leaves.

Group IV - received 400mg/kg, p.o methanolicextract of. *Anogeissus latifolia* leaves.

Group V - received 20mg/kg, p.o Omeprazole as standard.

One hour after the drug treatment the animals were treated with Aspirin [200mg/kg] to induce ulcers. The animals were sacrificed after 4hrs and the stomach was carefully excised keeping oesophagus closed and opened along greater curvature and luminal contents were removed. The gastric contents were collected in a test tube and centrifuged at 3000 rpm for 10 min. The pH of the supernatant was measured using digital pH meter[10]. The total and free acidity parameters were determined by using Topfers reagent and phenolphthalein indicator. The volume of total alkali consumed was noted which corresponds to total acidity.

The mucosa was flushed with saline and stomach was pinned on frog board. The lesion in glandular portion was examined under a 10x magnifying glass and length was measured using a divider and scale and gastric ulcer was scored. Ulcer index of each animal was calculated by adding the values and their mean values were determined and percentage inhibition was calculated[11].

- 0 – Normal coloured stomach
- 0.5 – Red colouration
- 1 – Spot ulceration
- 1.5 – Haemorrhagic streak
- 2 – ulcers
- 3 – Perforations

Statistical Analysis

All the values are expressed as mean ± S.E.M for groups of six animals each. Analyzed by one way ANOVA and compared by using Tukey- Kramer multiple comparison tests.

RESULTS AND DISCUSSION

Phytochemical analysis

The preliminary phytochemical analysis of the extract of *Anogeissus latifolia* leaves showed the presence of glycosides, tannins, saponins, alkaloids, terpenoids and flavonoids. The results of photochemical screening are given in **Table 1**.

Acute toxicity test

The LD_{50} estimated to be greater than 2000 mg/kg. Hence, the biological dose fixed 200mg/kg and 400mg/kg for the extract.

Ethanol induced ulcer

Effects of methanolic extract of *Anogeissus latifolia* wall on ulcer index induced by ethanol in rats are shown in **Table-2.**

Ethanol induced gastric damage showed gross mucosal lesion, including long hemorrhage bands and petechial lesion. Animals pretreated with methanolic extract of *Anogeissus latifolia* wall Roxb and standard drug sucralfate showed very mild lesions and sometimes no lesion at all, when compared to ulcer control group.

Ulcer index (UI) and acid parameters

Oral administration of methanolic extract of *Anogeissus latifolia* at doses of 200 and 400mg/kg exhibited dose dependent inhibition percentage of 74.18and 80.40 ($p<0.05$), ($p<0.001$) respectively compared to the ulcer control, proving the anti ulcer activity. The standard drug Sucralfate (100mg/kg) exhibited percentage inhibition of 88.95.

Aspirin induced ulcer model

Effects of methanolic extract of *Anogeissus latifolia* wall on ulcer index induced by aspirin in rats are shown in **Table 3** and its effect on acid parameters are shown in **Table-4.**

Ulcer index (UI) and acid parameters

Oral administration of methanolic extract of *Anogeissus latifolia* wall at doses of 200 and 400mg/kg exhibited dose dependent inhibition percentage of 50.90 and 71.43 ($p<0.001$) respectively compared to the ulcer control, proving the anti ulcer activity. The standard drug Omiprazole (20mg/kg) exhibited percentage inhibition of 84.86.

The effects of methanolic extract of *Anogeissus latifolia* wall on acid parameters were less significant at 200mg/kg dose. Methanolic extract of *Anogeissus latifolia* wall showed significant ($p<0.001$) effect at 400mg/kg dose compared to ulcer control animals. The volume of acid secretion, total and free acidity was decreased and pH of the gastric juice was increased compared to ulcer control group. But, in this gastric environment also able to induce ulcer, so it can be thought that the antisecretory activity might not be the main mechanism of action of these extracts.

Macroscopical Evaluation

Macroscopical changes of ethanol induced models were shown in figure 1, 2, 3, and 4.

CONCLUSION

The methanolic extract of leaf, showed the presence flavonoids and their glycosides, tannins, triterpenoids. These phytoconstituents present in the extract could be the possible agents involved in the prevention of gastric lesions induced by (chemical agents) aspirin and ethanol.

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Table 1: Preliminary Phytochemical Analysis of *Anogeissus latifolia* Roxb.ex.*DC* leaves

S.NO	PHYTOCHEMICAL TEST	RESULT
1	GLYCOSIDES	+
2	CARBOHYDRATES	-
3	SAPONINS	-
4	TANNINS & PHENOLIC COMPOUNDS	+
5	AKLOIDS	-
6	TERPENOIDS	+
7	FLAVANOIDS	+

+ indicates present and – indicates absent.

Table 2: Effect of *Anogeissus latifolia* leaf on ethanol induced ulcers

Group	Ulcer index (UI)	Percentage inhibition (%)
Normal Control	00.00 ± 0.00	-
Ulcer Control	62.16 ± 1.18	-
***Anogeissus latifolia* (200mg/kg)**	15.96 ± 0.47**	74.18
***Anogeissus latifolia* (400mg/kg)**	12.18 ± 0.67***	80.40
Sucralfate (100mg/kg)	6.88 ± 0.10***	88.95

All values are expressed as mean ± S.E.M.; (n=6) animals in each group. ***P<0.001, **P<0.01, Ulcer control group was compared with Normal control group. Sucralfate and Extract treated groups were compared with ulcer control group.

Table 3: Effect of *Anogeissus latifolia* Leaf on Aspirin induced ulcers

Group	Ulcer index (UI)	Percentage inhibition (%)
Normal Control	00.00 ± 0.00	-
Ulcer Control	28.40 ± 1.12	-
***Anogeissus latifolia* (200 mg/kg)**	13.95 ± 0.96**	50.90
***Anogeissus latifolia* (400 mg/kg)**	8.11 ± 0.43***	71.43
Omeprazole (100 mg/kg)	4.30 ± 0.10***	84.86

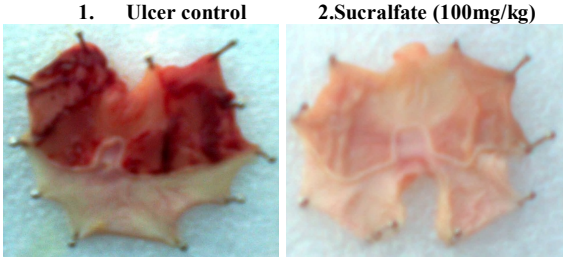
All values are expressed as mean ± S.E.M.; (n=6) animals in each group. ***P<0.001, **P<0.01, Ulcer control group was compared with Normal control group. Omeprazole and extract treated groups were compared with ulcer control group.

Table 4: **Effect of *Anogeissus latifolia* leaf on Gastric secretion, total and free acidity using aspirin induced ulcer**

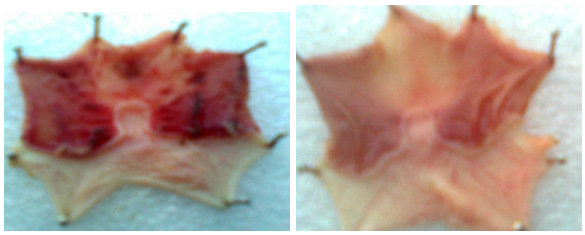
Group	Gastric volume (ml/100g)	pH of gastric juice	Total acidity	Free acidity
Normal Control	1.21 ± 0.06	2.01 ± 0.12	54.97 ±1.83	29.30 ±1.73
Ulcer control	2.46 ± 0.08	1.36 ± 0.10	73.42 ±3.14	42.35 ±1.66
***Anogeissus latifolia* (200mg/kg)**	1.80 ± 0.05**	1.75 ± 0.05**	60.54 ± 1.08**	34.88±2.14**
***Anogeissus latifolia* (400mg/kg)**	1.75 ± 0.07***	2.18 ± 0.15***	50.84±1.14***	28.24±1.75***
Omeprazole (20mg/kg)	1.11 ± 0.09***	2.8 ± 0.17***	45.72 ±1.39***	26.70 ±0.99***

All values are expressed as mean ± S.E.M.; (n=6) animals in each group. ***P<0.001, **P<0.01, Ulcer control group was compared with Normal control group. Omeprazole and Extract treated groups were compared with ulcer control group.

1. Ulcer control **2.Sucralfate (100mg/kg)**



Erosion of mucus layer Inhibition of gastric lesions



3. ALE 200 mg/kg **4. ALE 400mg/kg**

Treatment of *Anogeissus latifolia* Extract showed dose dependent inhibition

Fig 1-4: Effect of *Anogeissus latifolia* leaf on ethanol induced ulcers