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Comparative Evaluation of Gastroprotective Potential of *Dypsis lutescens* (H. Wendl.) and *Caryota urens*(L.) on Experimentally Induced Gastric Ulcer in Rats

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

The present study was carried out to find out the gastroprotective effects of the leaves extracts of *Dypsis lutescens* (H. Wendl.) and *Caryota Urens* (L.) in aspirin-induced gastric ulcer in rats. The ethanolic and aqueous extracts were prepared from the leaves of *Dypsis lutescens* and *Caryota urens* and were used for this study. All procured animals were divided into seven groups. Each group contain *n* = 6, all groups except the normal control treated with aspirin 150 mg/kg oral. Group, I served as the normal control group which received normal saline, group II served as negative control and group III as standard group receiving 20 mg/kg omeprazole and 4th to 7th groups treated with leaves extracts of *Dypsis lutescens* and *Caryota urens* respectively. Treatment was given to group II to group VII rats in every 24 h for seven days. After 24 h fasting, on the 8th-day stomach contents were aspirated under anaesthetic condition to check free and total acidity. Stomachs were opened in all sacrificed animals along the greater curvature to estimate ulcer index, percentage protection and histopathology studies.

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The results of the present study revealed that the severity of aspirin-induced ulceration was significantly ($P < 0.05$) decreased in *Dypsis lutescens* extracts treated groups in comparison with the control and *Caryota urens* treated groups. It was observed that the free and total acidity significantly decreased in the aqueous extract of *Dypsis lutescens* treated group when compared with the negative control and *Caryota urens* treated groups.

Keywords: Aspirin; Dypsis lutescens; Caryota urens; free acidity; gastric ulcer; total acidity.

1. INTRODUCTION

A peptic ulcer is a most common and genuine gastrointestinal problem which is caused due to the absence of equilibrium between the gastric secretions and the mucosal defensive components impacted by different aggressive and defensive factors such as acid-base balance and secretion, parietal cell activation, decrease in protective mucus secretion, decrease mucosal blood flow, the cellular recovery process, and endogenous protective factors like- prostaglandins and epidermal development factors [1-2]. Other variables like improper dietary habits, excessive use of non-steroidal anti-inflammatory drugs, stress, and infection with Helicobacter pylori also contribute to ulceration in the stomach [3].

The mortality rate due to ulcers is approximately 5% per 100,000 cases. For this reason, a few pharmaceutical products have been utilized reliably for the treatment of gastric ulcers aiming to decrease morbidity and mortality rates [4]. In spite of the advancement in ulcer treatment from vagotomy to anticholinergic medications, H2-receptor antagonists, acid neutralizers, proton pump inhibitors etc, in recent years the research has been reached out towards utilization of natural drugs, frequently named as comple-mentary and alternative medicines (CAM's). Indigenous drugs possessing fewer side effects with maximum therapeutic efficacy is the area of interest for the present-day research which aims for a better and safer approach for the management of Peptic Ulcer Disease [5].

The current research study was done to investigate the comparative evaluation of gastroprotective effects of *Dypsis lutescens* and *Caryota urens* in aspirin-induced ulcers in albino Wistar rats.

2. MATERIAL AND METHODS

2.1 Plant Material Collections and Authentication

The leaves of *Dypsis lutescens* (H.Wendl)) and *Caryota Urens* (L) required for the study were collected from in and around Hyderabad (Dist), Telangana; in the month of Dec 2019. The plant parts were authenticated by P. V. Prasanna, Scientist 'G' and HOD, Botanical Survey of India, Hyderabad and Dr A Vijaya Bhaskar Reddy, Head of the Dept. of Botany, Osmania University, Hyderabad and the voucher specimens were kept for further reference in the department.

2.2 Extraction

The leaves of *Dypsis lutescens* and *Caryota urens* were cleaned and shade dried for about 7 days. The shade dried leaves were then ground to a coarse powder using a mechanical grinder. The coarse powder of leaves of *Dypsis lutescens* and *Caryota Urens* were stored properly for further extraction procedures. The stored leaves were subjected to soxhlet extraction using Petroleum ether, Ethanol and water as per increasing the polarity order of solvents. The marc obtained was air-dried. The filtrate obtained was subjected to steam distillation, to concentrate the extract and the solvent was recollected and was used for further extraction process. A dark green residue was obtained on further concentrating and evaporating the extract on a water bath. The dried extract thus obtained was kept in the desiccator and was used for further pharmacological investigations [6].

2.3 Acute Oral Toxicity Testing

Ethanolic and Aqueous extracts of *Dypsis lutescens* and *Caryota urens* were administered at dose rate of 50, 500, 1000, 2000, 4000 and 5000 mg/kg to the test groups. Changes in behaviors of rats were observed for 14 days. The mortality rate was used to calculate median lethal dose (LD50) value.

2.4 Experimental Animals Setup

Forty-two healthy Albino Wistar rats of either sex were used for the gastro-ulcerogenic activity. We screened animals weighing between 140- 160 g for the study. All procured animals were kept in the animal houses for two weeks to adapt and

acclimatize the condition. During this period all animals received palatable uncontaminated and nutritionally adequate food according to CPCSEA and water *ad libitum.* After the adaptation period, rats were randomly divided into the following seven groups as per our experiment protocol [7]. All experimental procedures were conducted according to the guidelines of CPCSEA.

Group I Rats received Distilled water and no treatment, served as normal control

Group II Rats received 150 mg/kg aspirin, served as negative control

Group III Rats received omeprazole, orally at 20 mg/kg b.w. respectively for seven days, served as standard

Group IV Rats treated with ethanolic extract of *Dypsis lutescens* (400 mg/kg/day, p.o)

Group V Rats treated with aqueous extract of *Dypsis lutescens* (400 mg/kg/day, p.o)

Group VI Rats treated with ethanolic extract of *Caryota Urens* (400 mg/kg/day, p.o)

Group VII Rats treated with aqueous extract of *Caryota Urens* (400 mg/kg/day, p.o)

2.5 Induction of Ulcer

Aspirin was used as ulcer inducer at a dose of 150 mg/kg, p.o. The group II to group VII animals received oral administration of aspirin 1 hour prior to testing drugs administration, once daily for seven days [8].

Ulcer Index and Inhibition calculation The ulcer index was calculated in experimental animals by counting the lesions and scored as per below score.

0 = Normal coloured stomach
0.5 = Red colouration
1 = Spot ulcer
1.5 = Haemorrhagic streaks
2.0 = ulcers > 3 but < 5
3.0 = ulcers > 5

Ulcer index was calculated from mean ulcer score of each animal and ulcer protection formula calculated as per the given formula [9].

$$\% \text{ Ulcer protection} = \frac{\text{Ulcer Index in Control} - \text{Ulcer index in Test}}{\text{Ulcer Index in Control}} \times 100$$

2.6 Monitoring of Gastric Volume and pH

Monitoring of gastric volume done with gastric juice taken from stomach of animal. That gastric juice centrifuged at 800 rpm and superannuate portion taken into a pipette for estimation of volume and gastric pH [10-11].

2.7 Monitoring of Free Acidity and Total Acidity

Free acidity was measured in gastric juice of experimental rats with the help of sodium hydroxide. One ml of gastric juice and 0.01 sodium hydroxide used with topfer's reagent (As a marker) used till the red color changes to yellowish- orange.

Whereas, One ml of gastric juice and 0.01 sodium hydroxide was kept utilizing phenolphthalein as a marker until the yellowish-orange colour changed to red to calculate total acidity [12].

Acidity was determined by using the following formula and expressed as mEq/l/100g.

$$\text{Acidity} = \frac{\text{Volume of NaOH} \times \text{Actual Normality of NaOH} \times 100}{0.1}$$

2.8 Histological Examination of Gastric Tissues

All experimental animals used for the study were sacrificed at the end of the study and stomachs were opened and gastric juice was collected. The collected gastric juice was centrifuged for further biochemical estimations [13].

Stomach tissues were washed with normal saline to remove the debris and then processed for histological evaluation. Formalin was utilized as a tissue fixative agent. Stomach tissue was fixed in formalin solution (10% neutrally buffered) for 48hrs and afterwards processed further for histological examination. Formalin-fixed stomach tissue was dehydrated with ethanol and quickly cleaned with slightly warmed (45-47°C) 100% xylene solution [14]. After paraffin penetration, the tissues were embedded in stainless steel moulds for further use. Sections of gastric tissues 4 µm thick were cut on a microtome and histological assessment was performed with hematoxylin and eosin staining.

2.9 Statistical Analysis

This study results were analyzed statistically by one-way analysis of variance (ANOVA) followed by Dunnett's test. 'P' value less than 0.05 was considered as statistically significant. All results data expressed as mean ±SEM [15-16].

3. RESULTS

3.1 Acute Oral Toxicity Study

Acute toxicity studies of *Dypsis lutescens* and *Caryota urens* leaves extracts were done as per OECD guidelines. The results revealed the extracts did not show any significant fluctuations in behavioral or neurological responses up to 4000mg/kg body weight. There was a small changes observed in behavioral responses but no mortality or toxicity reaction observed up to 5000mg/kg body weight of ethanolic and aqueous extract of *Dypsis lutescens* and *Caryota urens* in all groups after 14 days, suggesting that LD_{50} of the extracts 4000 mg/kg b.w. So ED_{50} of herbal formulation was considered 1/10 of 4000 mg/kg.

3.2 Effect of Various Treatments on Biochemical Parameters

Values are expressed as mean ± SEM, (n = 6). Statistical analysis was performed by using ANOVA followed by Dunnett's test. Results were compared with Normal control [P < 0.0001*] negative control and Standard.

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3.3 Histopathology

Tissue samples of the stomach were collected and processed for histopathological examinations. Photomicrographs obtained after histopathological evaluation of stomach are presented below.

Table 1. Effect of various treatments on ulcer index in rats

Group	Treatments	Dose	Ulcer index	% protection
I	Normal control	-	-	-
II	Negative Control	-	12.33 ±2.14	
III	Standard group	20 mg/kg	3.55 ±1.42*	71.20 %
IV	Ethanolic Extract of *Dypsis lutescens*	400 mg/kg	8.71 ± 1.21	29.35 %
V	Aqueous Extract of *Dypsis lutescens*	400 mg/kg	4.43 ±0.92*	64.07 %
VI	Ethanolic Extract of *Caryota urens*	400 mg/kg	9.52 ±0.91	22.78 %
VII	Aqueous Extract of *Caryota urens*	400 mg/kg	7.71 ± 1.31	37.46 %

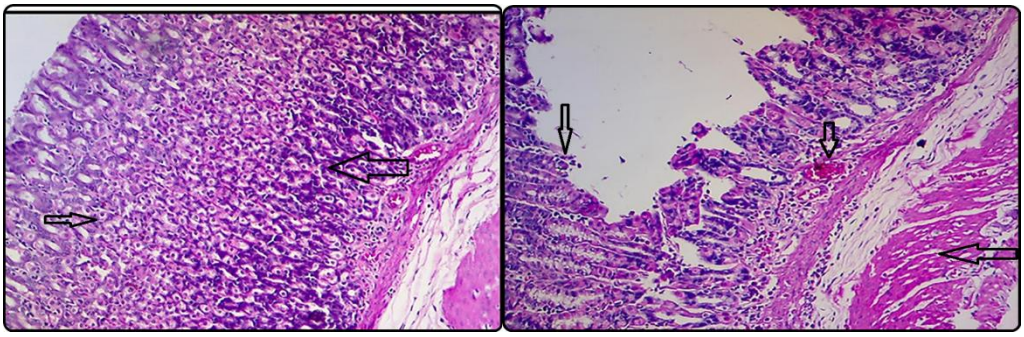
Table no.2. Effect of various treatments on Gastric Volume and pH in rats

Group	Treatments	Gastric Volume (ml/100g)	pH
I	Normal control	1.61 ± 0.55	3.98 ± 1.01
II	Negative Control	2.77 ± 0.98	1.29 ± 0.21
III	Standard group	1.61 ± 0.19*	4.12 ± 1.23*
IV	Ethanolic Extract of *Dypsis lutescens*	2.02 ± 0.95	1.87 ± 0.31
V	Aqueous Extract of *Dypsis lutescens*	1.68 ± 0.44*	3.82 ± 1.04*
VI	Ethanolic Extract of *Caryota urens*	2.14 ± 0.84	2.07 ± 0.42
VII	Aqueous Extract of *Caryota urens*	1.89 ± 0.75*	3.44 ± 1.02*

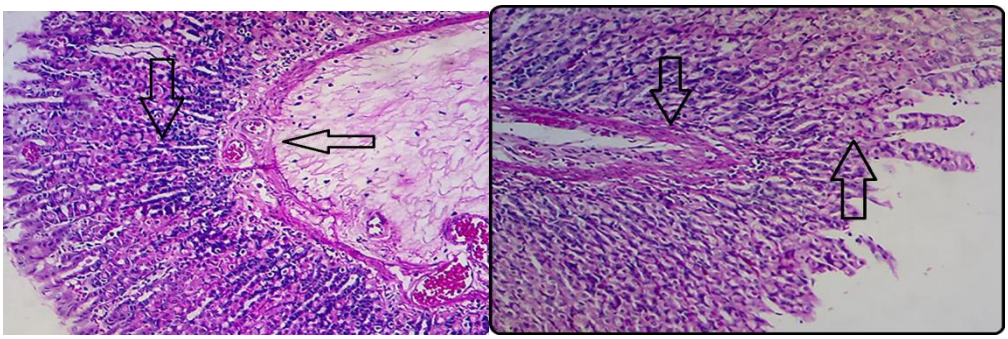
Table 3. Effect of various treatments on free acidity and total acidity in rats

Group	Treatments	Free Acidity (mEq/l/ 100g)	Total Acidity (mEq/l/ 100g)
I	Normal control	18.16 ± 2.77	23.44 ± 4.2
II	Negative Control	60.23 ± 5.21	82.18 ± 6.77
III	Standard group	21.44 ± 3.81*	33.12 ± 3.66*
IV	Ethanolic Extract of *Dypsis lutescens*	40.23 ± 3.42	52.88 ± 5.23*
V	Aqueous Extract of *Dypsis lutescens*	28.53 ± 2.84*	32.14 ± 3.02*
VI	Ethanolic Extract of *Caryota urens*	48.14 ± 3.77	56.14 ± 5.24
VII	Aqueous Extract of *Caryota urens*	32.11 ± 3.40*	38.23 ± 3.22*

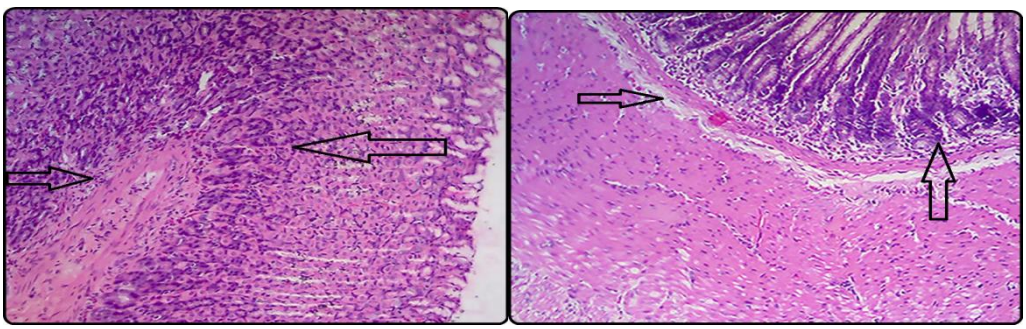
3.4 Histopathology of Stomach



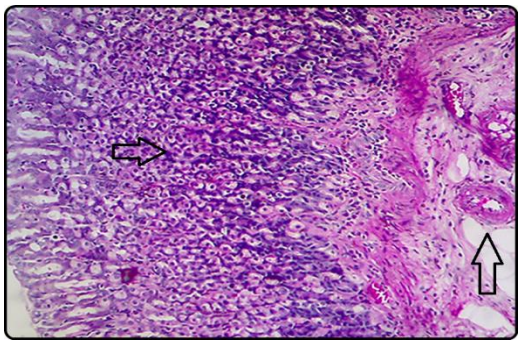
Normal control Negative Control



Standard Eth. Extract of *Dypsis lutescens*



Aq. Extract of *Dypsis lutescens* Eth. Extract of *Caryota urens*



Aq. Extract of *Caryota urens*

Fig.1. (A-G). Photomicrographs of stomach sections of Normal control Negative Control, standard, Eth. Extract of *Dypsis lutescens*, Aq. Extract of *Dypsis lutescens*, Eth. Extract of *Caryota Urens*, Aq. Extract of *Caryota urens* treated groups of rats (H&E staining 40X)

(A) Normal control group showing exhibited normal anatomy of the epithelial lining

(B) Negative Control group represent the disarrangement and erosion of mucosa, irruption of erythrocytes and lymphocytes in the submucosa representing necrotic debris in the lumen.

(C) Omeprazole treated group indicated gastric mucosa with complete epithelium membrane and showed regenerative cells.

(D) Eth. Extract of *Dypsis lutescens* treated group showed gastric mucosa with complete epithelium, lamina propria and muscularis mucosa whereas the upper layer appeared shaded off.

(E)Aq. Extract of *Dypsis lutescens* treated group showed gastric mucosa with completed epithelium, few degenerative changes indicates healing.

(F) Eth. Extract of *Caryota Urens* treated group showed mucosal ulceration consisting of necrosis, cellular debris, neutrophils and degenerated epithelial cells.

(G) Aq. Extract of *Caryota Urens* treated group showed mild inflammation and epithelium showed healing indicated by regenerative cells.

4. DISCUSSION

Non-steroidal anti-inflammatory drugs (NSAIDs) especially aspirin is incredibly hampered by their GIT distress propensity. Therefore, throughout the years, extensive research studies have been carried out to make aspirin free from ulcerogenic potential [17]. NSAIDs not only regulate prostaglandins, crucial inflammatory mediators but also control cell protection during the healing process. NSAIDs are acidic in nature and due to this reason, they produce localized erosion in stomach mucosa in acidic conditions [18].

We assessed the gastric ulcerogenic property in leaves extract of *Dypsis lutescens* and *Caryota urens.* The dose of aspirin 150 mg/kg was selected for the study on the basis of their possession of significant anti-ulcerogenic activity [19].

Results of the study indicated that percentage protection from aqueous and ethanolic extract treatment of *Dypsis lutescens* was 64.07%, 29.35 % respectively. The results from the data indicate that Aqueous extract of *Dypsis lutescens was* proven as more gastroprotective in comparison to the treatment with *Caryota Urens.*

Gastric juice volume and pH also play a crucial role in the development of ulcers. The present study results revealed that the gastric volume of the standard group was 1.61 ± 0.19 with 4.12 ± 1.23pH which was very similar to the gastric volume (1.68 ± 0.44) and pH (3.82 ± 1.04) of Aqueous extract of *Dypsis lutescens.* Free acidity and total acidity results proved that the gastroprotective potential of *Dypsis lutescens is* over *Caryota Urens.* pH analysis of the Aqueous extract of *Dypsis lutescens* revealed that they were basic in nature.

This may play a crucial role in gastric safety due to the unionized state in the stomach in a basic environment. This will prevent the damage in the

gastric mucosa, that cause gastric erosion and ulceration [20]. The presence of phytoconstituents like flavonoids, steroids, saponins in the extracts may be responsible for the activity which may be considered according to the literature from the previous studies [21-22].

Flavonoidal and polyphenolic compounds in dypsis lutescens like luteol, apigenin, vicenin ll, vitexin, orientin, gallic acid have been reported in the *Caryota urens* extracts and steroidal and terpenoidal compounds like beta sitosterol, lupeol, ursolic acid, myricadiol have also been reported in the extracts which may exhibit the proven pharmacological activity.[23-24].The quantitative estimation of leaf extract of Caryota Urens exhibited the presence of phenols, terpenoids and oxalic acid according to the literature and may be possible outcome for therapeutic activity [25].

Phytosterols like β-sitosterol and β-sitosterol-3-O-β-D-glucopyranoside proved to exhibit gastroprotective property from the latest research literature [26]. Rutin an isolated compound in the extracts may have been responsible for the exhibited activity [27].

Rich saponin content in the aqueous extract of *Dypsis lutescens* may have exhibited synergistic effect for the proved activity.

The test results were assessed based on significant changes with aspirin in the control, standard and test samples in the biochemical parameters [28-29].

5. CONCLUSION

The current investigation was performed to discover the ulcer protective impact of the plant extracts against aspirin. Significant changes were observed with aspirin in the mean ulcer score, free acidity, total acidity, gastric juice pH and gastric juice volume.

Histological assessment of the test animals indicated the more anti-ulcerogenic effect of *Dypsis lutescens* in comparison to *Caryota Urens* that might be due to COX-2 enzyme inhibition. However, further studies at the molecular level are required to establish these findings.

CONSENT

It is not applicable.

ETHICAL APPROVAL

Animal Ethic committee approval has been taken to carry out this study.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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