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Evaluation of antiulcer activity of aqueous extract of *Borassus flabellifer* (Linn.) Fruits.

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ABSTRACT

The fruit of ***Borassus flabellifer* Linn**. belonging to family Areaceae has been reported to be useful in treatment of gastric ulcer. In pharmacological screening the effect of different extracts (300mg/kg) p. o. of fruits of ***Borassus flabellifer* Linn.** was evaluated for their antiulcer profile by using aspirin Pylorus ligation and ethanol induced models using albino rats. Various parameters like volume of gastric content, pH of gastric content, free acidity, total acidity, ulcer index were determined. The results were comparable to that of standard drug (Ranitidine). Treatment with aqueous extract of fruits of ***Borassus flabellifer* Linn.** significantly showed the antiulcer activity as compared to control and other extracts . The histopathological study of stomach also supported the above results. The phytochemical analysis carried out revealed the presence of saponins, tannins, flavonoids, carbohydrates, amino acids and phenolic compounds in the extracts.

Keywords: Anti-ulcer, ***Borassus flabellifer* Linn**, Aspirin pylorus ligation, Ethanol induced ulcer, Ranitidine.

INTRODUCTION

Peptic ulcer disease (PUD) is a serious gastrointestinal disorder that requires a well targeted therapeutic strategy [1]. Pathogenesis of peptic ulcers involves disturbances in the acid–pepsin status of the gastric contents [2]. Pathophysiology of PUD involves an imbalance between offensive (acid, pepsin, and *H. pylori)* and defensive factors (mucin, prostaglandin, bicarbonate, nitric oxide and growth factors). Due to the high morbidity associated with this disease, there is a continuous need for newer anti-ulcer drugs [1]. A number of drugs are available for the treatment of peptic ulcer, but clinical evaluation of these drugs has shown incidence of relapses, side effects, and drug interactions. This has been the rationale for the development of new antiulcer drugs and the search for novel molecules has been extended to herbal drugs that offer better protection and decreased relapse. Drugs of plant origin are gaining popularity and are being investigated for a number of disorders, including peptic ulcer.

Borassus flabellifer Linn. (Arecaceae) is a tall tree (palm) growing in sandy soil and attaining a height of about 20-30 meters with a straight trunk [3]. The fruits are large and fibrous, containing usually three nuts like portions each of which encloses a seed [4]. The plant bears flowers and fruits during December to August [5]. The plant has been used traditionally as a stimulant, antilaprotic, diuretic and antiphlogistic. The fruits are stomachic, sedative, laxative and aphrodisiac in nature useful in hyperdipsia, dyspepsia, flatulence, skin diseases, heamorrhages, fever and general debility. The roots and juice of the plant are useful in inflammatory reactions. The ash obtained by burning the inflorescence is a good antacid antiperiodic, and is useful in heart burn and spleenomegaly [6]. Survey of literature revealed that the medicinal plant ***Borassus flabellifer* Linn**. have been used as antidiabetic, antidote, anti-inflammatory, wound healing, anthelmintic activity, analgesic and antipyretic. It has been reported that the methanolic extract from the male flowers of *Borassus flabellifer* Linn. inhibit the increase of serum glucose levels in sucrose-loaded rats which may be due to presence of spirostane-type steroid saponins. It also has been documented to possess immunosuppressant property.

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MATERIAL AND METHODS:

The fruits of *Borassus flabellifer Linn.* were collected from local areas of Pune, Maharashtra and authenticated by P.G. Diwakar Joint Director, at Botanical Survey of India (BSI), Govt. of India, Ministry of Environment and Forests, Pune, India. The fruits were then dried in shade at temperatures between 21-30^0 c for 15 to 30 days, after which these parts of plant were fine powered (40 size mesh) by hammer. Finally extraction was carried out by the following procedure.

Preparation of the extract

Around 400 gm of powder was subjected to successive hot continuous extraction (soxhlet) with petroleum ether, chloroform, methanol and chloroform-water. (Each time before extracting with next solvent the powdered material was dried at room temperature). After the effective extraction, the solvents were distilled off. The extract was then concentrated on water bath to avoid the decomposition of natural metabolites.

Experimental animals

Wistar albino mice of either sex weighing between 25 and 30 g were selected for acute toxicity studies and Wistar albino rats of either sex weighing between 150 and 200 g were selected for the antiulcer studies. The animals were acclimatized to standard laboratory conditions of temperature (22±3^{0}C) and maintained on 12:12 h light: dark cycle. They were provided with regular rat chow (Pranav Agro Industries LTD. Sangali.) and distilled water *ad libitum.*

Acute toxicity studies

Wistar albino mice of either sex weighing between 25 and 30 g were selected for acute toxicity The study was carried out as per the guidelines set by OECD and no adverse effects or mortality were detected in the mice up to 4 g/kg, p.o., during the 24 h observation period [7].Based on the results obtained from this study, the dose for anti-ulcer activity was fixed to be 300 mg/kg b.w.

Anti-ulcer activity

The anti-ulcer assays were performed using following protocols aspirin pylorus ligation and ethanol induced ulcer model. Since traditional uses of this plant is based on oral administration. Based on this traditional preparation and use, the extract was administered by oral route at 300mg/kg.

Aspirin Pyloric ligation-induced ulcer model: -

Adult Albino Wistar rats of either sex weighing between 150-200 g were used. They were housed in standard cages at room temperature (25 ± 2° C) and provided with food and water ad libitum. The animals were divided into five groups, consisting of five each. Methanolic and aqueous extracts of fruits of ***Borassus flabellifer* Linn.** and standard anti-ulcer drug Ranitidine were prepared in 1% tween 80 suspension as a vehicle. Group A received normal saline. Group B (control) received only 200 mg / Kg of Aspirin. Group C received Ranitidine orally at the dose of 20 mg/kg body weight. Group D received Methanolic extract of ***Borassus flabellifer Linn***. at the dose of 300 mg/kg body weight. Group E received aqueous extract of ***Borassus flabellifer Linn*.** at the dose of 300 mg/kg body weight [8]. All the animals received drug / extract treatment along with 200 mg / Kg of Aspirin once a day for four days. On the fifth day the 48 hr. fasted rats were sacrified after pyloric ligation. The stomach was carefully removed and contents were emptied into a graduated centrifuge. The gastric juice was centrifuged at 3000 r.p.m. for 30 min. and the volume of the gastric juice was measured [9]. 1ml of supernant was diluted with 9ml of distilled water. A volume of 2ml diluted gastric juice was titrated with 0.1N NaOH run from a microburette using 3-4 drops of Topfer's reagent as indicator until canary yellow colour was observed. Volume of NaOH required was noted. This corresponds to the free acidity. Further 2-3 drops of phenolphthalein was added and titrated with NaOH until pink colour was restored. This gives total acidity. Stomach was cut open along with the greater curvature and pinned on a soft board for ulcer Scoring. The number of ulcers was counted using a magnifying glass and the diameter of ulcer was measured using a vernier calipers [10].

Ulcer Score:

0-Normal ; 1-Scattered haemorrhagic spots ; 2- Deeper haemorrhagic spots: 3 -haemorrhagic spots and ulcers; 4- Perforation

The free acidity and total acidity were expressed as mEq/l.

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

Ulcer index was then calculated

$$\text{Ulcer Index (UI)} = \frac{10}{X}$$

$$\text{Where } X = \frac{\text{Total area of stomach}}{\text{Mucosal ulcerated area}}$$

Ethanol induced ulcer:

Adult Albino Wistar rats of either sex weighing between 150-200 g were used. They were housed in standard cages at room temperature (25 ± 2° C) and provided with food and water ad libitum. Rats were divided in five groups of five each. Animals in all groups were fasted for 36h before treatment. The standard drug and extract were administered 1 hr. before ethanol administration. Group A received normal saline. Group B (control) received only alcohol at the dose of 1 ml per rat. Group C received Ranitidine orally at the dose of 20 mg/kg body weight. Group D received methanolic extract of ***Borassus flabellifer Linn*.** at the dose of 300 mg/kg body weight. Group E received aqueous extract of ***Borassus flabellifer Linn***. at the dose of 300 mg/kg body weight [11]. Thirty minutes after drug administration, absolute ethanol was given orally to each rat at the dose of 1 ml/rat. 1h after ethanol administration the rats were killed by ether inhalation [12]. The stomach and duodenum were dissected out, inflated with 10ml of 2% formalin to fix both the inner and outer layers. The duodenum was opened along its anti-mesentric side and the stomach along the greater curvature. The damage area (mm) was measured under a microscope (10X). The sum of area of all lesions for each rat was calculated and served as the ulcer index.

Histopathological studies:

The stomachs from both the models were washed with saline and preserved with 10% formalin solution for histopathological examination. The central part of the damaged or ulcerated tissue was cut in half along the long diameter. After the standard processing, the wet tissue was embedded in paraffin and cut into thick section in a rotary microtome. The sections were stained with haematoxylin-eosin and mounted with Canada balsam. These were examined under the microscope and photographs for histopathological changes such as odema, inflammation, congestion, haemmorrhage and erosions were taken [13].

Statistical analysis

The data obtained by the various parameters was statistically evaluated by one way analysis of variance (ANOVA) followed by Dunnets't' test using Graph Pad Prism software. The mean values ± SEM were calculated for each parameter.

RESULTS:

The methanolic and aqueous extracts were employed for pharmacological screening. From the above extracts aqueous extract showed better anti-ulcer activity. The preliminary phytochemical screening carried out on aqueous extract of fruits of *Borassus flabellifer* Linn. revealed the presence of phytoconstituents such as carbohydrates, amino acids, flavonoids, tannins, saponins, vitamin C and phenolic compounds.

Aspirin pylorus Ligation ulcer model

Control animals showed volume of gastric content as (6.6ml) whereas animal treated with aqueous extract showed significant reduction in ($p<0.01$) volume of gastric content as(4.0ml). Ranitidine, a standard drug showed 3.48ml which is statistically significant ($p<0.01$). The results are shown in table 1.

The pH of gastric content in control animals was found to be 2.74 whereas animal treated with aqueous extract showed significant ($p<0.01$) rise in pH (3.77) as compared to control. Ranitidine, a standard drug raised the pH to 4.07, which is statistically significant ($p<0.01$). The results are shown in table 1.

Gastric free acidity is increased to (65.60 mEq/litre) in control animals. Aqueous extract (23.08 mEq/litre) showed significant decrease in free acidity ($p<0.01$) as compared to control. Ranitidine, a standard drug (20.92 mEq/litre), showed maximum decrease in gastric free acidity which is statistically significant ($p<0.01$). The results are shown in table 1.

Gastric total acidity was increased to (78.08 mEq/litre) in control animals. Animal treated with aqueous extract (34.52 mEq/litre) showed significant ($p<0.01$) decrease in total acidity as compared to control. Ranitidine, a standard drug (37.23mEq/litre), showed potent decreasing in gastric total acidity which is statistically significant ($p<0.01$). The results are shown in table 1.

Aspirin administration (200 mg/kg) resulted in the production of gastric mucosal damage. The ulcer index in control animals was found to be 3.24. It was significantly reduced to 2.37 in animal treated with aqueous extract.

Table No.1 Effect of *Borassus flabellifer* Linn. Fruits extract on Volume, PH, free acidity, total acidity, ulcer index in aspirin pylorus ligation and ethanol induced ulcer model.

Groups	Volume (ml)	PH	Free Acidity	Total acidity	Ulcer Index [APL]	Ulcer Index [Ethanol]
Normal	2.7 ± 0.3	3.726±0.2	24.32±0.2	34.23±0.2	2.748±0.3	2.836±0.2
Control	6.6 ±0.5	2.710±0.2	65.61±0.7	78.08±0.9	3.240±0.08	42.51±0.5
Ranitidine	3.4 ±0.4	4.074±0.1	20.93±0.9	37.23±0.6	1.198±0.1	9.094±0.3
Methanol Ex.	4.8±0.5	2.770±0.2	32.18±0.8	49.14±1.0	3.046±0.2	13.19±0.2
Aqueous Ex.	4.0±0.4	3.770±0.2	23.08±0.6	34.52±0.3	2.378±0.08	11.67±0.4

Fig.1 Aspirin pylorus-ligation ulcer model.

Fig 1.1: Normal

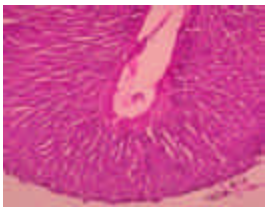


Fig 1.2 Control (Aspirin pylorus-ligation)

Fig 1.3: Standard (Ranitidine)

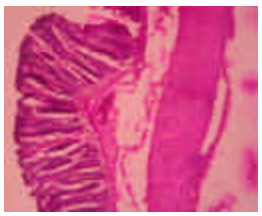


Fig 1.4 Methanolic extract treated group

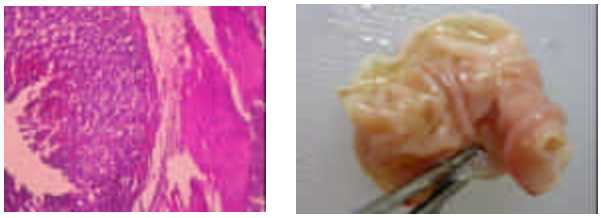


Fig 1.5 Aqueous extract treated group

Fig 2 Sections of ulcerated stomach obtained from rats of respective extract treated groups in Ethanol induced ulcer model.

Fig 2.1: Normal

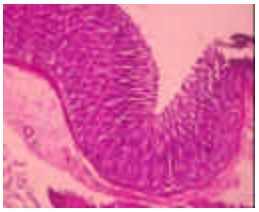


Fig 2.2 Control (Ethanol Induced)

Fig 2.3: Ranitidine (standard)

Fig 2.4Methanolic extract treated group

Significantly ($p<0.01$) reduced the ulcer index as compared to control. Ranitidine a standard anti-ulcer drug showed ulcer index of 1.19. The results are tabulated in table 1.

Ethanol induced ulcer model.

Ethanol administration (1 ml/rat) resulted in the production of gastric mucosal damage. The control animals showed ulcer index of 42.51. Whereas in animal treated with aqueous extract 11.67 showed significant ($p<0.01$) reduction in the ulcer index as compared to control. Ranitidine a standard anti-ulcer drug showed ulcer index of 9.09. The results are tabulated in table 1. Aqueous extract significantly raised the pH of gastric contents. It lowered the free and total acidity and ulcer index as compared to Control group in both the models.

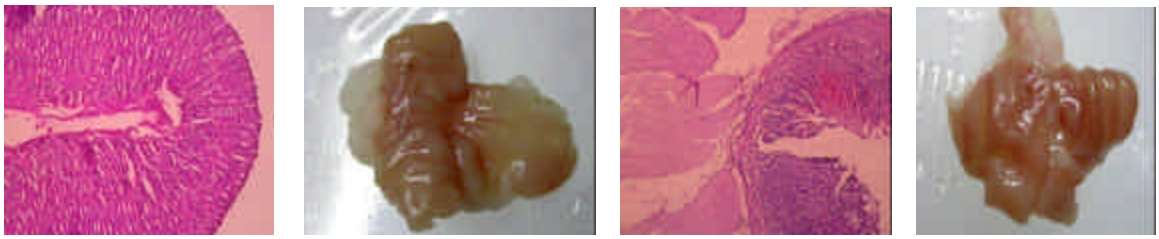
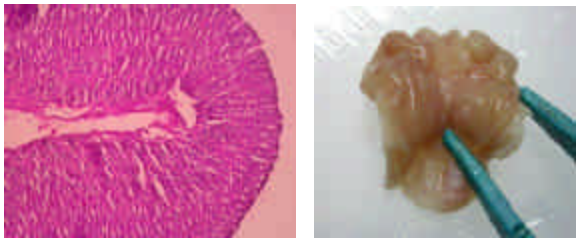


Fig 2.5: Aqueous extract treated group



Finally histopathological observation was done. Multiple aqueous extract treated section showed structure of stomach with less ulcer sight but mild congestion. Percentage of mucosal ulcer depth is less than control and methanolic extract group. The rats pre- treated with ethanol showed markedly extensive damage to gastric mucosa with the lesions extended deeply to mucosal layer, oedema and leucocytes infiltration of sub mucosal layer. Rats pre-treated with Ranitidine or *Borassus flabellifer* Linn. fruit extracts had comparatively better protection of the gastric mucosa as seen by marked reduction in ulcer area and absence of odema and leukocyte infiltration of sub mucosal layer.

DISCUSSION:

In most of the cases the etiology of peptic ulcer is unknown. Pathophysiology of PUD involves an imbalance between offensive and defensive factors. Many drugs are available for the treatment of peptic ulcer, including antacids, proton pump inhibitors, anticholinergics and histamine H_2- antagonists. Most of these drugs produce several adverse reactions such as hematopoietic changes, thrombocytopenia, nephrotoxicity and hepatotoxicity. Medicinal plants are amongst the most attractive source of new drugs, and have been shown to give promising results in treatment of peptic ulcer.

The anti-ulcerogenic activity of *Borassus flabellifer* Linn. was evaluated by employing aspirin and ethanol induced ulceration in rats. Aspirin (NSAIDs) is known to induce gastric ulceration. Ethanol-induced gastric lesion formation may be due to stasis in gastric blood flow, which contributes to the development of the haemorrhage and necrotic aspects of tissue injury. It has also been reported that leukotrienes antagonist and 5-lipoxygenase inhibitors are capable of inhibiting alcohol and NSAIDs- induced gastric ulceration in rats. Therefore the protection afforded by the *Borassus flabellifer* Linn. against aspirin and ethanol induced gastric ulceration could also be due to inhibition of 5- lipoxygenase pathway or to leukotriene's antagonistic activity.

In the present study *Borassus flabellifer* Linn. showed prevention of gastric lesions in the experimental models. *Borassus flabellifer* Linn. was found to decrease the acid volume and both free and total acid contents in rats. *Borassus flabellifer* Linn. affects the parameters that influence the initiation and perpetuation of ulceration. In addition, there is extensive experimental evidence, which indicates that certain substances through free radical scavenging action protect the gastric mucosa [15].

CONCLUSION:

The anti-ulcer activity could be attributed to the presence of high content of crude flavanoids, saponins and phenolic compounds. From literature survey phenolic compounds and saponins has been reported to possess anti-ulcer activity.

Hence, to put into a nutshell, more significant antioxidant activity and anti-ulcer activity of aqueous extract may be due to the presence of phenolic compounds, flavonoids or saponins.

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