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GASTRPROTECTIVE & ANTI-ULCER ACTIVITY OF *SACCHARUM OFFICINARUM* FRESH JUICE IN CHEMICAL INDUCED ULCER IN ALBINO RATS

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Keywords:

Saccharum officinarum, Fresh Juice, Ranitidine, Ethyl alcohol

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ABSTRACT: Objects: In the present study the anti-ulcer activity of *Saccharum officinarum* fresh juice was investigated in the chemical-induced ulcer. The efficacy of the plant based juice was compared with standard reference anti-ulcer drug Ranitidine. **Methods:** The fresh juice collected from the plant of *S. officinarum.* Firstly, washes the sugarcanes well and peel the outer layer of the cane with a big knife. Then cut them into small pieces and squeeze them for extraction. Take out the extract along with them in a big container. And then study with chemical-induced ulcer in healthy rats. **Result:** The modal of absolute 40% ethanol-induced ulcer, oral administration of fresh juice (20ml/kg/bw) dose showed that reduction in ulcer index, collection of gastric juice, free acidity, total acidity, and also shows the pH of gastric juice and all parameters compared with the control group. It was showing a protection index of 55.99% at the dose of 20ml/kg/bw. Ranitidine as reference standard drug and showing a protection index of 63.32% at the dose of 50mg/kg-bw. **Conclusion:** The result of the present study reveals that the plant juices are having efficiency in the gastroprotective activity. It is recommended that the above said plant-derived juices could be further studied for their anti ulcer efficacy in human subjects.

INTRODUCTION: The peptic ulcer refers to a spectrum of disorders that includes gastric ulcers, duodenal ulcers, or near the site of surgical gastrointestinal anastomosis [1].

Causes of Peptic Ulcer [2, 3] When the stomach's natural system is disturbed due to any obstruction, such as the damaging effects of digestive juices (including acid and pepsin, an enzyme that helps breakdown protein) stop working or the acid production is too overwhelming for these protective defenses to work properly, you can get an ulcer.

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And then, they are generated through an imbalance between mucosal aggressive & Protective factors [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.

Reduction of gastric acid production, as well as re-enforcement of gastric mucosal production, has been the major approach to cure peptic ulcer disease. As a result, more and more synthetic drugs are introduced and offering newer options for the treatment of peptic ulcers. The types of drugs vary from proton-pump inhibitor to H_2 antagonist or a cytoprotective agent. At the same time, each of these drugs confers simple or several side effects like arrhythmias, impotence, and gynaecomastia,

hyperplasia, and hematopoietic changes. Because of several side effects of synthetic medicines, there is a new thought of a better natural alternative for the treatment of peptic ulcers.

An alternative approach in recent days is the research of medicaments from Ayurvedic and traditional medicinal systems. The phyto-constituents available in the medicinal plants have proved to be clinically effective and relatively less toxic than the existing synthetic drugs and reducing the offensive factors and serving as a tool in the prevention of peptic ulcers. Several herbal plants are reported to have antiulcer activity, and several pre-clinical (animal) studies are reported on the efficacy of herbal medicines such as *Garcinia cambogia* [5], *Cissus quaddrangularis* Linn.[6], *Tephrosia populnea* [7], *Bambusa arundinacea* [8], *Ocimum sanctum*[9], *Emblica officinalis* [10], *Pterospermum acerifolium* [11], *Bauhinia variegate* [12], *Terminalia chebula* [13], *Spheranthus indicus* [14], polyherbal extract containing *Curcuma longa, Coriander sativum* and *Ocimum sanctum* [15] and Plant juices such as *Aloe vera*, banana stem juice and banana flower juice [16] and *Carica papaya* (papaya) fruit juice [17].

The present study evaluates the antiulcer and gastroprotective efficacy of Saccharum officinarum juice in alcohol-induced ulcerated rats. Ranitidine is used as standard reference drug.

Plant Profile:

Cultivation: *S. officinarum* is widely cultivated in India, mostly in Uttar Pradesh, Maharashtra, Punjab, Gujarat, Andhra Pradesh, Telangana, Karnataka *etc*. Sugar cane is also found in the tropics and south-east Asia.

Plant Profile – Sugarcane (*Saccharum officinarum)*

FIG. 1: SHOWN THE PLANT OF SUGARCANE

FIG. 2: SHOWN THE FRESH JUICE

Classification & Plant taxonomy:

Kingdom	**:**	Plants
Subkingdom	**:**	Tracheobionta
Super-division	**:**	Spermatophyta
Division	**:**	Magnoliophyta
Class	**:**	Liliopsida
Sub-class	**:**	Commelinidae
Order	**:**	Cyperales
Family	**:**	Poaceae
Genus	**:**	Saccharum – Sugarcane

Biological Source: It consists of the plant *Saccharum officinarum* L. belonging to the family Poaceae. Sugarcane is a large, strong-growing species of grass in the genus Saccharum. It originated in Asia and is now cultivated in tropical and subtropical countries worldwide for the production of sugar and other products. *S. officinarum*, a perennial plant, grows in clumps consisting of a number of strong unbranched stems. A network of rhizomes forms under the soil which sends up secondary shoots near the parent plant. The stems vary in color, being green, pinkish, or purple and can reach 5 m (16 ft) in height [18].

MATERIALS AND METHODS: Collection and Authentication of plant: The plant of *S. officinarum* was selected after the literature survey and collected from Gajraula, Amroha (U.P). The plant of *S. officinarum* was authenticated by the senior botanist Dr D.C Kasana; head of the Department of Botany, I.P College of Science, Bulandshahr (U.P), and India. Specification – IP College of Science - SOP- BVSO/09/1753.

Preparation of Juice:

1. Wash the sugarcanes (*S. officinarum*) well and peel the outer layer of the cane with a big knife.

2. Now cut them into small pieces and press them with a roller.
3. Take out the extract along with the peel in a big container.
4. Take another container and place a muslin cloth or strainer on it. Squeeze the juice out of the extract pressing through cloth or strainer.
5. And storage of juice at room temperature (12 to 20 ºC) in well-closed glass containers for future use.

Evaluation of Experimental Animals: Healthy adult Wister Albino rats and Albino mice were selected for the study. They were fed with standard pellet diet and water *ad libitum*. All animal protocols were approved by the Institutional Animal Ethical Committee (IAEC) of the organization (Reg. The Institutional Animal Ethical Committee of Janta College of Pharmacy Butana, (Sonepat) Haryana, India (CPCSEA-667/02/c/CPCSEA) approved the studies). All animals were maintained under standard conditions of humidity (50±10%), temperature (22±20 ºC) & light (12 hours light & 12 hours dark).

Experimental Design:

Ethanol Induced Ulcer Activity: Swiss albino mice were divided into 4 groups (n=6). The different groups of animals are assigned as follows.

TABLE 1: THE DIFFERENT GROUPS OF ANIMALS ARE ASSIGNED

S. no.	Groups	Treatments
1	Group 1	Received vehicle only
2	Group 2 (Control)	Served as control group and ulcer was induced with 10.0 ml/kg-bw of 40% alcohol (ethanol).
3	Group 3	Drug control animals- alcohol induced ulcerated animals treated with Ranitidine (50mg/kg-bw) for 21 days.
4	Group 4	Severed as treatment group and fresh juice of *Saccharum officinarum* (20ml/kg/bw) for 21 days.

Animals were starved for 12 h with access only to drinking water *ad libitum*. Gastric ulcer was induced with 10.0 ml/kg-BW of 40% alcohol (ethanol) induced in group II, III, and IV animals.

After 48 hours, an animal in Group II was sacrificed and checked for ulcer induction. Subsequently, from the same day, Group III Animals were given *S. officinarum* juice 20.0 ml/kg- body weight, and Group IV animals were treated with 50 mg/kg-bw of Ranitidine. The animals were anesthetized using ether. On the 22nd day, the animals were sacrificed. The abdomen was opened without causing any damage to its blood supply and an incision of 1cm long was made in the abdomen just below the sternum of the stomach was exposed. Passed a thread around the pyloric sphincter and applied a tight knot closed the abdomen wall by cervical decapitation, and the stomach was removed. The gastric fluid was collected in a graduated centrifuge tube and subject to analysis for total acid. Samples of stomach tissues were collected and stored for histopathological analysis [19].

Macroscopic Evaluation of Stomach: The abdomen was opened, the cardiac end of the stomach was dissected out & the content was drained into the glass tube. The volume of the gastric juice was measured, and its pH was determined. The isolated abdomen was examined by a 10X magnifier lens to assess the formation of an ulcer. The number of ulcers was counted [20].

Scoring of Ulcer: [21]

- 0 = Normal coloured stomach, 0.5 = Red colouration, 1 = Spot ulcer, 1.5 = Haemorrhagic streaks, 2 = Ulcers ≤ 3 but ≤ 5, 3 = Ulcers > 5

Calculation of Ulcer Index: [22]

- $U_1 = UN + US + UP \times 10^{-1}$

  Where,
  U_1 = Ulcer index, U_N = Average of number of ulcer per animal, U_S = Average of animal severity score, U_P = Percentage of animal with ulcer

Determination of Acid:

Acidity = Volume of NaOH × Normality of NaOH × Meq / Lit / 100g / 0.1

Determination of Percentage Protection: [23]

% Protection = Control mean ulcer index – test mean ulcer index × 100 / mean ulcer index

Biochemical Estimation: Gastric acid collected from ethanol-induced ulcer in rats. The gastric juice thus collected centrifuged, and the volume of gastric juice, as well as the pH of gastric juice was noted. The gastric juice subjected to biochemical estimations as follows:-

Determination of Free Acidity and Total Acidity [24]

1. Gastric juice (1 ml) was taken into a 100 ml conical flask, to this 2-3 drops of Topfer's reagent was added and titrated with 0.01N NaOH solution until all traces of red color disappears, and the color of the solution turns yellowish orange (endpoint).
2. The volume of alkali added was noted. This volume corresponds to free acidity,
3. 2-3 drops of phenolphthalein solution were added, and titration was continued until a definite red ting reappears.
4. The volume of alkali added was noted, which corresponds to total acidity.

Free Acidity was calculated by using the Formula:

Acidity = Volume of NaOH × Normality of NaOH × Meq / Lit / 100g / 0.1

Statistical Analysis: The data of results obtained were subjected to statistical analysis and expressed as mean ± SD. the data were statically analyzed by one-way analysis of variance (ANOVA) and compare the means of the studied groups with the standard. The data were statically analyzed by Graph pad prism Software version (7.1).

RESULTS AND DISCUSSION:

Ethanol Induced Ulcer Activity: The modal of absolute 40% ethanol-induced ulcer, oral administration of fresh juice (20ml/kg/bw) dose showed that reduction in ulcer index, collection of gastric juice, free acidity, total acidity, and also shows the pH of gastric juice and all parameters compared with the control group. It was showing a protection index of 55.99% at the dose of 20ml/kg/bw. Ranitidine as a reference standard drug and showing a protection index of 63.32% at the dose of 50mg/kg-bw. The results are shown in tables and figures for illustration **Tables 2-3** and **Fig. 3-11**.

TABLE 2: ANTI-ULCER ACTIVITY OF *S. OFFICINARUM* FRESH JUICE IN ETHANOL INDUCED ULCER MODEL

S. no.	Treatment	Ulcer Index	Ulcer Protection
1	Control	12.6833±0.3208	-
2	Ranitidine	4.6500±0.1166	63.32%
3	*S. officinarum* (fresh Juice)	5.5833±0.1376	55.99%
F, df value		401.053, (2/15)	
P-value		P<0.0001	

Value of mean SEM, n=6, p* p<0.01 when compared with control

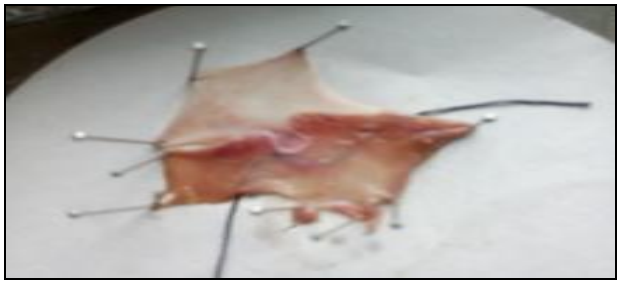

FIG. 3A: GROUP I: HEALTHY CONTROL ANIMAL

FIG. 3B: GROUP II: ULCER INDUCED ANIMAL

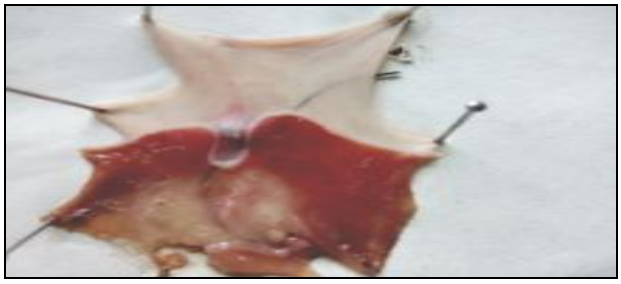

FIG. 3C: GROUP III: ULCER INDUCED ANIMAL TREATED WITH *S. OFFICINARUM* FRESH JUICE

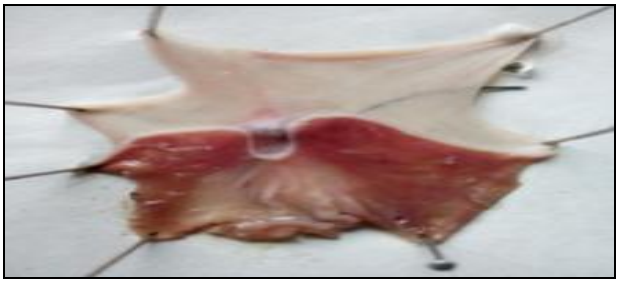

FIG. 3D: GROUP IV: ULCER INDUCED ANIMAL TREATED WITH STANDARD DRUG RANITIDINE

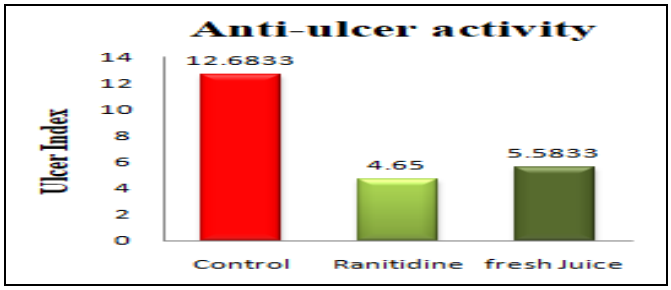

FIG. 3: REPRESENTING ULCER INDEX AND PERCENTAGE INHIBITION IN VARIOUS GROUPS

TABLE 3: ESTIMATION OF VOLUME OF GASTRIC JUICE pH, FREE ACIDITY, TOTAL ACIDITY OF GASTRIC JUICE

S. no.	Treatment	Volume of gastric Juice (ml)	pH of gastric Juice	Free acidity	Total acidity
1	Control	5.3000±0.0966	3.4166±0.2386	19.8333±0.4395	46.1167±1.8737
2	Ranitidine	3.2166±0.0737	5.3666±0.2403	10.7833±0.4422	30.8667±1.6177
3	*S. officinarum* (fresh Juice)	4.1500±0.0921	5.0333±0.1429	13.2867±0.2978	40.7700±1.01422
F, df value		140.3, (2,15)	24.15, (2,15)	137.2, (2,15)	25.10, (2,15)
P-value		P<0.0001	P<0.0001	P<0.0001	P<0.0001

Value of mean SEM, n=6, p* p<0.01 when compared with control

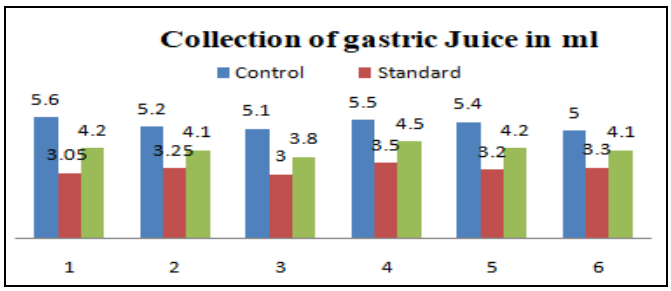

FIG. 4: REPRESENTING THE COLLECTION OF GASTRIC JUICE (ml) IN VARIOUS GROUPS

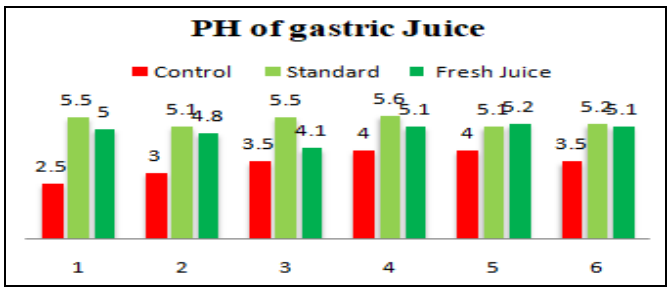

FIG. 5: REPRESENTING THE pH OF GASTRIC JUICE IN VARIOUS GROUPS

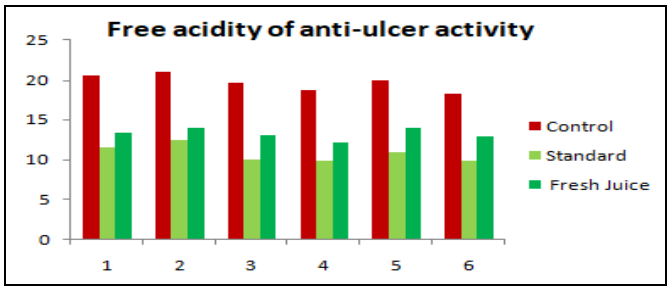

FIG. 6: REPRESENTING THE FREE ACIDITY OF GASTRIC JUICE IN VARIOUS GROUPS

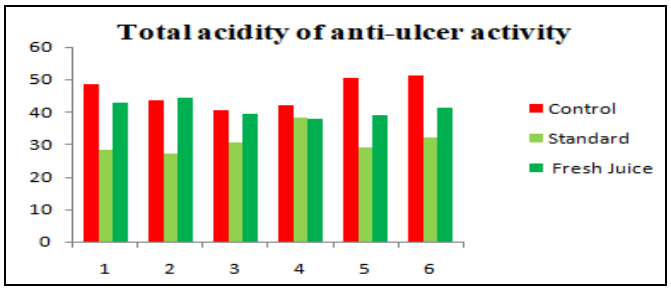

FIG. 7: REPRESENTING THE FREE ACIDITY OF GASTRIC JUICE IN VARIOUS GROUPS

Histopathology of Chemical Induced Ulcer Method Model (Hematoxin & Eosinx100):

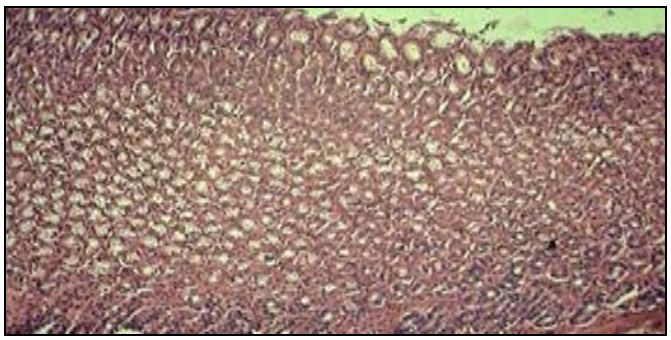

FIG. 8: SECTION OF GASTRIC MUCOSAL LAYER SHOWS NORMAL APPEARANCE CONTROL

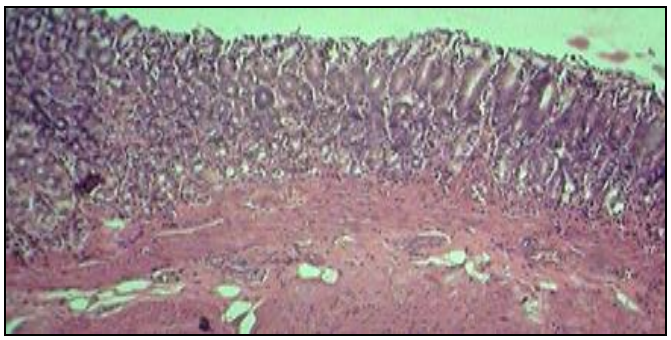

FIG. 8: CHEMICAL (40% ETHANOL) INDUCED ULCER METHODS SHOWS INFLAMMATION & MUCOSAL ULCERATION CONTROL

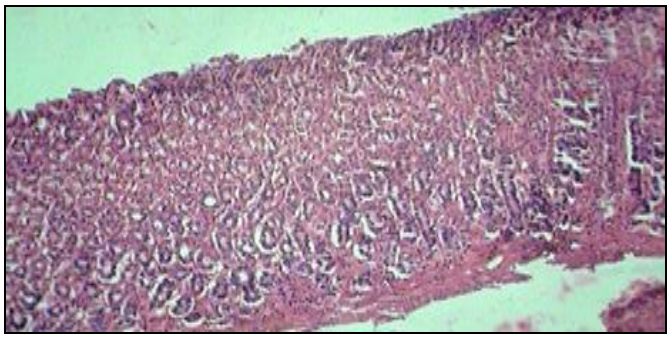

FIG. 10: *S. OFFICINARUM* L. FRESH JUICE SHOWS SOME SIGNIFICANCE CHANGE IN HISTOPATHOLOGY

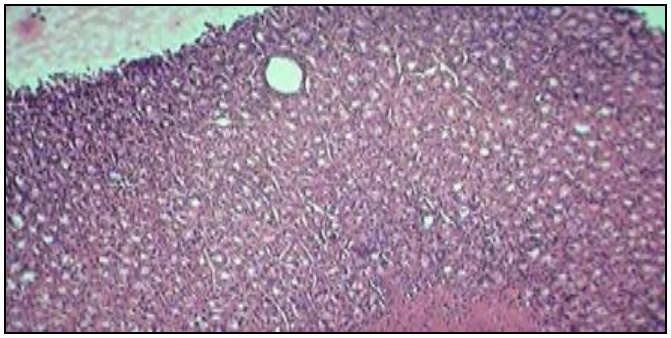

FIG. 11: STANDARD DRUG RANITIDINE (50mg/kg) SHOWS NO SINGNIFANCE CHANGE IN HISTO-PATHOLOGY ALMOST NORMAL APPEARANCE

CONCLUSION: *S. officinarum* fresh juice has been traditionally used for a number of disorders. The literature survey on the plant described that the plant possessed various traditional medicinal properties. The purpose of this research work was to study anti-ulcer activity of *S. officinarum* fresh juice and established the pharmacological characterization of the fresh juice of *S. officinarum* plant. The obtained plant juice was subjected to pharmacological study by a different experimental animal model to be used. *S. officinarum* fresh juice and exhibited better anti-ulcer activity using chemical (40% Alcohol) induced ulcer, comparable to standard ranitidine. Hence, it was concluded that the *S. officinarum* revealed more significant effect for anti-ulcer rather than individual fresh juice of plant when compared to the standard. Therefore it seems worthy to develop fresh juice of *S. officinarum* optimized affects in ulcer.

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Author Contribution Statement: Mr. Singh R. conceptualized and gathered the data with regard to this work. Dr. Shukla R. analyzed these data, and necessary inputs were given towards the designing of the manuscript. Both authors discussed the methodology and results and contributed to the final manuscript.

CONFLICTS OF INTEREST: We declare that we have no conflict of interest.

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