



## Evaluation of Anti-Nociceptive and Anti Diarrheal Activities of *SaccharumOfficinarum* Linn Fresh Juice on Albino Mice Model

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**Abstract:** *SaccharumOfficinarum* L. (Sugarcane) is an indigenous medicinal plant of India mostly in Uttar Pradesh and Maharashtra. The fresh juice has been used traditionally as folk remedies for the treatment of many diseases including diarrhea, dysentery, and potent diuretics. The juice of the plant is used in menstruation cycle problems. It is also used as a tonic, anti-periodic etc. However, there was no study on whole plant juice of *S. Officinarum* Linn. The present study was designed to investigate the anti-nociceptive and anti-diarrheal activities of *S. Officinarum*Linn on animal models at different doses such as 10 and 20 ml/kg. Various methods also were employed for investigating these activities such as castor-oil induced diarrhea, castor-oil induced entero pooling and gastrointestinal motility test, tail immersion, tail flick and hot plate methods. The diarrheal episode was inhibited by 31.88% and 40.95% for fresh juice at the doses of 10 and 20 ml/kg respectively. The juice significantly ( $p < 0.05$ ) reduced the intestinal volume ( $0.51 \pm 0.04$  ml) for 10 ml/kg and ( $0.46 \pm 0.02$  ml) for 20ml/kg for fresh juice compared to control ( $0.63 \pm 0.02$  ml) in castor-oil induced diarrhea and also decreased intestinal transit (54.58 – 60.12%) for fresh juice comparable with standard (loperamide 5 mg/kg). The fresh juice of *S. officinarum*L., increased latency in tail flick and tail immersion model and elevated the mean basal reaction time in hot plate model. The results of fresh juice of *S. officinarum*L. showed highly significant but dose dependent on anti-diarrheal and anti-nociceptive activities, which supports its use in traditional herbal medicine.

**Keywords:** *SaccharumOfficinarum*, Sugarcane, Loperamide, Anti-nociceptive, Anti-diarrheal, Morphine

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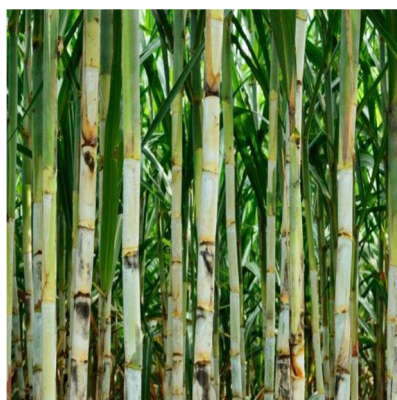


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## 1. INTRODUCTION

Since time immemorial, indigenous plants have been a major source of medicine. In folk medicine, they are used, in single or in combined forms for treating different types of pain and arthritic conditions. Pain is an unpleasant sensation localized to a part of the body. Pain usually occurs when peripheral nociceptors are stimulated in response to tissue injury, visceral distension, or other factors. In such a situation, pain perception is a normal physiologic response mediated by a healthy nervous system<sup>1</sup>. Pain is a sensory modality and primarily protective in nature, but often causes discomfort. It is the most important symptom that brings the patient to the physician. Analgesics relieve pain, without affecting its cause<sup>2</sup>. Currently available analgesic drugs such as opiates and NSAIDs are not useful in all cases due to their adverse effects. In this aspect, new compounds with improved pain management capacity and fewer side effects are being sought with urgency. Now-a-days, opiates and non-steroidal anti-inflammatory drugs are not useful in all cases, because of their side effects and low potency<sup>3</sup>. Diarrheal disease is the second leading cause of death in less than five children worldwide. According to the latest data every year, diarrhea kills around 760 000 children under five. There are nearly 1.7 billion cases of diarrhea disease year, globally<sup>4</sup>. Worldwide, diarrhea accounts for more than 5-8 million deaths in infants and small children less than 5 years each year. According to the World Health Organization (WHO) estimation for the

year 1998, there were about 7.1 million deaths due to diarrhea. In India, one third of the total child death burden is due to diarrhea<sup>6</sup>. WHO organized a Diarrhea Control Program where they emphasized use of traditional medicines to combat the episodes of diarrhea<sup>7</sup>. Diarrhea appeared by several mechanisms such as increasing the gut motility, along with increased secretion of ions, a decrease in the absorption of fluid, and thus a loss of electrolytes, particularly Na<sup>+</sup> and water<sup>8</sup>. Synthetic drugs as well as conventional treatments are failing to fulfill their objectives, due to their toxic and adverse side effects. For this reason it becomes necessary to search for other alternatives such as plants. For these consequences, herbal medicine has made a comeback to improve the fulfillment of our present and future health needs<sup>9</sup>. Considering the importance of plants as a vital source of medicine even today in the present study this plant *S. officinarum* Linn, which is popularly known as "Ganna" is one of the most versatile medicinal plants having a wide spectrum of biological activity. It belongs to the family Poaceae. *S. officinarum* is a persistent plant with juicy, thick, and stout stems; Clumps are pale. Leaves are broad and panicle. Spikelets are large, linear and oblong surrounded by hairs<sup>10</sup>. Rhizomes are formed under the soil; derived shoots are present near the parent plant<sup>11,12</sup>. And it is 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<sup>13</sup>. The plant of sugarcane (*Saccharum officinarum*) is shown in Figure 1.



**Fig 1: Plant of Sugarcane**

The phytochemical constituents of *Saccharum officinarum* have been extensively investigated. Alkaloids, flavonoids, reducing sugars, steroids, Saponins and tannins were documented as the chief chemical constituents. The stem of *S. officinarum* has laxative, diuretic, and cooling effect<sup>14</sup>. The pulp is used for covering wounds. Sugar cane is used by Borneo for the treatment of fractures<sup>15</sup>. Sugar cane extract is used by Chinese traditional medicine for promoting expulsion of phlegm from respiratory passages and stimulating gastric activity<sup>15</sup>. It is also used against various skin diseases such as abscess, ulcers, and wounds, and for other infectious diseases such as chest pain, eye inflammations, and sore throat. Juice of the stem is used in Ayurvedic Pharmacopoeia of India for hemorrhagic diseases and anuria and root is also used in dysuria<sup>15</sup>. It is also used in folk medicine as a remedy for arthritis, bedsore, boils, cold, cough, diarrhea, dysentery, fever, hiccups, sores, spleen, tumors and wounds<sup>15</sup>. The present study designed to investigate the anti-nociceptive and anti-diarrheal activities of *S. Officinarum* Linn on animal models at different doses such as 10 and 20 ml/kg. Various methods also employed for investigating these activities such

as castor-oil induced diarrhea, castor-oil induced entero pooling and gastrointestinal motility test, tail immersion, tail flick and hot plate methods.

## 2. MATERIALS AND METHODS

### 2.1. 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.

### 2.2. Preparation of Juice

The sugarcane stem is washed well and the outer layer is peeled with a suitable knife and pressed between two metal rollers. The cane extract was then collected in a big container and strained using a muslin cloth. It was later stored at room

temperature (12 to 20°C) in well-closed glass container for future use.

### 2.3. Evaluation of Experimental Animals

Healthy adult Wistar Albino rats and Albino mice were selected for the study. They were fed with a 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, (Sonapat) Haryana, India (CPCSEA-667/02/c/CPCSEA) approved the studies.). All animals were maintained under standard conditions of humidity (50±10 %), temperature (22±20°C) & light (12 hours light & 12 hours dark).

### 2.4. Castor oil induced diarrhea

This experiment was carried out by the method described by Awouters *et al*<sup>16</sup>. The experimental mice were kept fasting for 18 hours. Four groups of mice were taken for this experiment. Group I treated as control (saline 2 ml/kg body weight orally), Group II received standard drug (loperamide 5 mg/kg b. wt. i.p.) and Group III-IV received fresh juice of *S. officinarum* L. (10 and 20 ml/kg by oral route respectively). Then 1 h later, castor oil (0.4 ml/mice) was administered orally. The mice were then housed singly in cages lined with white blotting paper. The papers were changed every hour. The total number of both dry and wet feces excreted were counted every hour for a period of 4 h and compared with the control group. The total number of diarrheal feces of the control group was considered 100%.

### 2.5. Anti-nociceptive activity

#### 2.5.1. Eddy Hot plate test (Techno)

The anti-nociceptive activity of the *S. officinarum* Linn fresh juice was measured by hot-plate method<sup>17</sup>. The analgesic effect of the hot plate model was explained in the following steps.

1. Mice were divided into three groups of five animals each. Group I received normal saline (0.9% NaCl, 5 mL/kg b.w.) as control, group II received the standard drug morphine (5 mg/kg b.w.) subcutaneously, group III and IV *S. officinarum* Linn fresh juice (10 & 20 ml/kg) per oral route respectively.

2. The Basal reaction time was taken by observing hind paw

licking or jump response (whichever appears first) in animals when placed on the hot plate maintained at constant temperature (55°C). Normally animals showed such response in 6-8 sec. A 15 sec cut off period is observed to avoid damage to the paws.

3. Morphine (5 mg/kg) or *S. officinarum* Linn fresh juice (10 and 20 ml/kg) is orally administered to animals and the reaction time of animals on the hot plate at 15, 30, 60, and 120 min after the drug administration. As the reaction time with morphine or *S. officinarum* Linn fresh juice, 15 sec is taken as maximum analgesia and the animals are removed from the hot plate to avoid injury to the paws and basal reaction time at each time interval was calculated<sup>18</sup>.

### 2.6. Tail immersion method

Mice were divided into three groups of five animals each. Group I received normal saline (0.9% NaCl, 5 mL/kg b.w.) as control, group II received the standard drug morphine (5 mg/kg b.w.) subcutaneously, group III and IV *S. officinarum* Linn fresh juice (10 & 20 ml/kg) per oral route respectively. Latency of mice tail with-drawing from hot water was noted as the basal reaction time. The lower 3 cm portion of the tail of mice was dipped in a water bath maintaining a temperature of 55 ± 0.5°C. The reaction time was noted at 0, 30, 60, and 90 min. A maximum immersion time of 15 sec was maintained to prevent thermal injury to the animals<sup>19</sup>.

### 2.7. Tail flick Method

Mice were divided into three groups of five animals each. Group I received normal saline (0.9% NaCl, 5 mL/kg b.w.) as control, group II received the standard drug morphine (5 mg/kg b.w.) subcutaneously, group III and IV *Saccharumofficinarum* Linn fresh juice (10 and 20 ml/kg) per oral route respectively. Basal reaction time for radiant heat sensing was taken by placing the tip (last 1-2 cm) of the tail on the radiant heat source. The tail-withdrawal from the heat (flicking response) was taken as the end point. Normally a mouse withdraws its tail within 3-5 sec. A cut period of 10-12 sec is observed to prevent damage to the tail. Any animal failing to withdraw its tail in 3-5 sec is rejected from the study. Take at least 3-5 basal reaction times for each mouse at a gap of 5 minutes to confirm normal behaviour of the animal. The latency of reaction time at 5, 15, 30, and after the drug. As the reaction time reaches 10 sec it is considered maximum analgesia and tail is removed from the source of heat to avoid tissue damage. And calculated basal reaction time at each time interval<sup>20</sup>.

**Table 1: Groups subjected for Tail flick method**

S.No	Groups	Treatment
1.	Group 1 (Control)	Received normal saline (0.9% NaCl, 5 mL/kg b.w.)
2.	Group 2 (Standard)	Received the standard drug morphine (5 mg/kg b.w.) subcutaneously
3.	Group 3 (Test <sub>1</sub> )	<i>S. officinarum</i> L. fresh juice (10 ml/kg) per oral route respectively
4.	Group 4 (Test <sub>2</sub> )	<i>Saccharumofficinarum</i> Linn fresh juice (20 ml/kg) per oral route respectively

## 3. 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 various (ANOVA) and compare the means of the studied groups with standard. The data were statically analyzed by Graph pad prism

Software version (7.1). The ED<sub>50</sub> value was determined to be the best value fit regression line of a dose response curve.

## 4. RESULTS

The results are shown in tables and figure for illustration (Tables 1-4 and Figures 1-2).

## 4.1. Castor oil induced diarrhea in mice

Table 2: Effect of fresh juice of *S. officinarum* L on castor oil induced diarrhea in mice

S.No	Treatment	Dose (mg/kg)	No. of watery diarrhea	% Inhibition	F value	Dfn&Df Value	P value
1.	Control	-	22.33 ± 0.33	-	-	-	-
2.	Castor oil + Fresh Juice (test <sub>1</sub> )	10 ml/kg	15.21±0.44*	31.88	1242	1,3	<0.0002
3.	Castor oil + Fresh Juice (test <sub>2</sub> )	20 ml/kg	13.18 ± 0.35*	40.95	1056	1,3	<0.0002
4.	Loperamide (Standard)	5 mg/kg	5.6 ± 0.26*	74.92	692.0.	1,3	<0.0001

Values are mean ± SEM (n = 6) \*P < 0.05, the data were statically analyzed by one way analysis of various (ANOVA) and compare the means of the studied groups with standard.

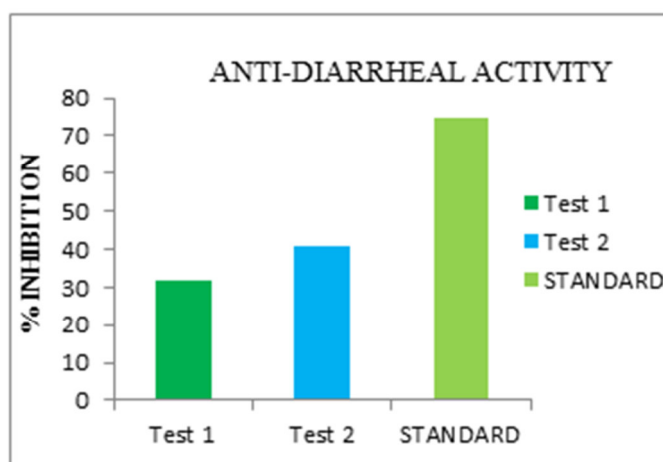


Fig 2: Graph representing percentage inhibition curve in various groups on castor oil induced Diarrhea

Table 2: Analgesic activity of *S. officinarum* L. fresh Juice in mice by Hot plate model

Groups	Drugs	Dose mg/kg	Reaction time in seconds				
			0 min	30 min	60 min	90 min	120 min
I	Control	-	3.74±0.24	3.86±0.33	4.04±0.19	4.05±0.24	3.76±0.28
II	Morphine	5	3.62±0.21	7.12±0.23*	10.15±0.18*	11.11±0.17*	9.22±0.17*
III	Castor oil + Fresh Juice (Test <sub>1</sub> )	10	3.54±0.23	4.87±0.34*	5.04±0.27*	5.66±0.25*	5.14±0.26*
IV	Castor oil + Fresh Juice (Test <sub>2</sub> )	20	3.80±0.11	5.94±0.12*	6.36±0.15*	6.58±0.14*	6.40±0.14*

Values are mean ± SEM (n = 6) \*P < 0.05, the data were statically analyzed by one way analysis of various (ANOVA) and compare the means of the studied groups with standard.

Table 3: Analgesic activity of *S. officinarum* L. fresh Juice in mice by Tail immersion model

Group s	Drugs	Dose mg/kg	Reaction time in seconds				
			0 min	30 min	60 min	90 min	120 min
I	Control	-	3.78±0.14	3.96±0.33	4.04±0.19	4.05±0.24	3.76±0.28
II	Morphine	5	4.99±0.27	8.12±0.23*	10.25±0.18*	12.11±0.17*	11.22±0.17*
III	Castor oil + Fresh Juice (Test <sub>1</sub> )	10	3.54±0.23	4.87±0.34*	5.14±0.27*	6.67±0.25*	5.45±0.26*
IV	Castor oil + Fresh Juice (Test <sub>2</sub> )	20	3.89±0.11	4.94±0.12*	6.86±0.15*	7.88±0.18*	6.40±0.15*

All values in terms of Mean ± SEM, (n=5) in each group \*P < 0.05 the data were statically analyzed by one way analysis of various (ANOVA) and compare the means of the studied groups with standard.

Table 4: Analgesic activity of *S. officinarum* L. fresh Juice in mice by Tail-Flick model

Groups	Drugs	Dose mg/kg	Basal reaction time in seconds				
			0 min	30 min	60 min	90 min	120 min
I	Control	-	2.78±0.14	2.96±0.33	4.04±0.14	4.10±0.34	3.76±0.27
II	Morphine	5	4.66±0.27	8.80±0.23*	9.25±0.18*	9.95±0.17*	9.22±0.17*
III	Castor oil + Fresh Juice (Test <sub>1</sub> )	10	3.50±0.23	4.77±0.34*	5.14±0.27*	5.67±0.25*	5.39±0.25*

IV	Castor oil + Fresh Juice (Test <sub>2</sub> )	20	3.89±0.14	4.94±0.16*	6.36±0.15*	6.88±0.18*	5.40±0.15*
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All values in terms of Mean ± SEM, n=5 in each group, \*P <0.05 statistically highly significant as compared with the control group.

## 5. DISCUSSION

Preliminary phytochemical screening showed the presence of catechin, tannins, alkaloids and flavonoids in the fresh juice of *S. officinarum* L., so the observed analgesic and anti-diarrheal activities may be attributed due to these compounds. The fresh juice of *S. officinarum* L the maximum significant anti-diarrheal ( $p < 0.05$ ) effect at the dose of 10 ml/kg and 20 mg/kg in compare to standard drug loperamide (5 mg/kg) and also possessed 31.88% and 40.95%, inhibitions of defecation respectively in the test of Castor oil induced diarrhea. Mechanism for the diarrheal effect of castor oil includes inhibition of intestinal Na<sup>+</sup> K<sup>+</sup> ATPase activity, thus reducing normal fluid absorption,<sup>21</sup> activation of adenylatecyclase or mucosal cAMP-mediated active secretion,<sup>22</sup> stimulation of prostaglandin formation and platelet activating factor,<sup>23</sup> However, it is well proved that castor oil produces diarrhea due to its most active component ricinoleic acid through a hyper-secretory response.<sup>24,25</sup> On the other hand, in castor oil induced method, the fresh juice depicted significant ( $p < 0.05$ ) effect at the dose of 10 and 20 ml/kg and also reduced the volume of intra-luminal contents respectively. These effects, which have direct consequences to reduced water and electrolytes secretion into the small intestine<sup>26</sup>, suggest that the fresh juice may enhance electrolyte absorption from the intestinal lumen consistent with inhibition of hyper-secretion. Hyper-motility characterizes diarrhea where the secretory component is not the causal factor.<sup>27-28</sup> Pre-treatment with the fresh juice suppressed the propulsive movement or transit of charcoal meal through the gastrointestinal tract which significantly indicates that the fresh juice may be able to reduce the frequency of stooling in diarrheal conditions. All these findings strongly suggested that the fresh juice of *S. officinarum* L should have anti-diarrheal activity and as per our best of knowledge which had never been explored before. On the other hand, in acetic acid induced writhing test the fresh juice of *Saccharumofficinarum*L. Showeda significant ( $p < 0.05$ ) inhibition such as 31.88% and 40.95% at the dose of 10 ml/kg and 20 ml/kg respectively, Table I. The response is thought to be mediated by the prostaglandin pathways, peritoneal mast cells and acid sensing ion channels<sup>29-30</sup>. In the hot plate method, the fresh juice of *S. officinarum*L. at a dose of 10 mg/kg & 20 mg/kg body weight showed significant anti-nociceptive activity .The results were found to be statistically significant (table 2). In tail immersion method, and tail- flick method the extent of activity shown by the fresh juice are less than that of the standard drug morphine but many fold more than that of the control group,

which justifies its activity. The results were found to be statistically significant, tables 2-3. This tail immersion method was used to evaluate the central mechanism of analgesic activity. Narcotic analgesics inhibit both peripheral and central mechanisms of pain, while non steroidal anti-inflammatory drugs inhibit only peripheral pain. This fresh juice inhibited both Narcotic analgesics inhibit both peripheral and central mechanism of pain.<sup>31,32</sup> Above observations suggest that the extract in graded doses reduces diarrhea by inhibiting peristalsis, gastrointestinal motility and castor oil induced enter pooling and inhibit both peripheral and central mechanism of pain. Earlier studies showed that antidiarrheal and antidiarrheal properties of medicinal plants were due to tannins, alkaloids, flavonoids, sterol and/or triterpenes and anti-nociceptive properties of medicinal plants due to alkaloid, flavonoids, steroids, glycoside etc. Hence, tannins, saponine, alkaloids, steroids and glycoside may be responsible for the mechanism of action of fresh juice of *S. officinarum*L against diarrheal and nociception.

## 6. CONCLUSION

From the above investigation it is quite apparent that a fresh juice of *S. officinarum* L. possesses the analgesic effect against different stimuli in small animals. This is evidenced by a significant increase in the reaction time by stimuli in different experimental models. And also Along with wide range of traditional uses such as treating diarrhea, as a tonic etc., In the present study,, *S. officinarum* L (fresh juice) showed dose dependent anti-diarrheal activity at the dose of 10 ml/kg and the inhibition rate was found at 31.88%; whether at the dose of 20 ml/kg, the inhibition rate increased up to 40.95%. It also depicted a significant success rate ( $P<0.05$ ). From the above point of view, it can be concluded that further investigation of fresh juice of *S. officinarum*L will help to develop noble antidiarrheal drugs and pain killers based on natural resources.

## 7. ACKNOWLEDGEMENT

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## 8. CONFLICT OF INTEREST

Conflict of interest declared none.

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