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# NEUROLOGICAL AND COMPULSIVE BEHAVIOUR ACTIVITY OF *Saccharum officinarum* Linn FRESH JUICE IN ANIMAL MODELS

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## ABSTRACT

*Saccharum officinarum Linn* (Poaceae) a persistent, juicy plant was evaluated for its neuroprotective effects in various animal models i.e. Compulsive behaviour (stereotypy), CNS Depressant Activity (Locomotor activity) and Forced swim test. Administration of fresh juice of *Saccharum officinarum Linn*, showed an increase in exploratory functions as compared to the control group in a dose dependant manner. Fresh juice of *Saccharum officinarum Linn*, significantly ($p < 0.01$) increase in CNS depressant activity of the animals in locomotor activity. A significant ($p<0.01$) dose-dependent antidepressant effect was observed in forced swim test. A dose dependent compulsive behaviour property was observed.

Phytochemical screening of fresh juice of *Saccharum officinarum Linn*, revealed the presence of alkaloids, glycosides, steroids, saponin, tannins, proteins, phenolic compounds and flavonoids. A number of researcher reported that the alkaloids, glycosides, and flavonoids rich plant fresh juice possess neurological and compulsive behavior properties properties mediated, through their affinity with benzodiazepine site of GABAergic complex system in the brain producing drowsiness and facilitating or maintaining sleep.

The results obtained in this study suggest that the fresh juice of *S. officinarum L.* possesses Neurological and Compulsive behavior properties. Thus, *S. officinarum L.* has potential clinical

applications in the management of anxiety and muscle tension disorders. Further investigations are warranted for elucidating the exact mechanism and bioactive compounds.

**Keywords: Neurological and Compulsive behavior properties, *Saccharum officinarum L.*, Diazepam, Rearing**

## INTRODUCTION

Learning is the process of acquisition of information and skills, while subsequent retention of that information is called memory. Learning and memory together called as Learning and memory is one of the most intensively cognition **[1]**. Memory is a fundamental mental process and without it we are capable of nothing. It is a faculty by which sensations, impressions, and ideas are stored and recalled. Learning and memory is one of the most intensively studied subjects in the field of neuroscience **[2]**. Dementia is a syndrome caused by disease of the brain, usually of a chronic or progressive nature, in which there is disturbance of multiple higher cortical functions, including memory, thinking, orientation, comprehension, calculation, learning capacity, language and judgement **[3]**.

Aging demographic transition is proceeding rapidly especially in India, China, and Latin America, where dementia is rapidly becoming the major public health problem **[4]**. Approximately 10% of the adults older than 65 years, and 50% of the adults older than 90 years have dementia **[5]**. Nootropic agents such as piracetam, pramicacetam, aniracetam and choline esterase inhibitors like donepezil are being primarily used to improve memory. However, the resulting adverse effects associated with these agents such as hepatotoxicity, nasal congestion, hypotension, gastro intestinal disturbances, rashes, constipation, tiredness, headache, drowsiness and systemic side effects upon chronic use have limited their use **[6]**.

The stem of *S. officinarum* has laxative, diuretics, and cooling effect **[7]**. The pulp is used for covering wounds. Sugar cane is used by Borneo for the treatment of fractures. Sugar cane extract is used by Chinese traditional medicine for promoting expulsion of phlegm from respiratory passages and stimulating gastric activity. 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. 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 **[8]**.

Therefore, in the present study *S. officinarum L* was evaluated as potential new remedial drugs with least reactions and maximum potency, which is accepted to be protected and financially viable.

## MATERIAL 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).

### Preparation of Juice:

Sugarcane (*Saccharum officinarum* L) juice was milled between two rollers and juice thus obtained was strainer on it. Squeeze the juice out of the extract pressing through cloth or strainer.

### Evaluation of Experimental Animals

Albino mice either sex (25–30 g) were used in the study. The animals were maintained in standard laboratory conditions (25 °C and light/dark cycles of 12/12 h) and fed with standard food and water ad libitum. All experimental procedures were reviewed and all animal protocols were approved by Institutional Animal Ethical committee (IAEC) of the organization (Reg. CPCSEA-667/02/c/CPCSEA)

### Experimental Estimation

### CNS Depressant activity (Locomotor activity)

### Principle

Most of the central nervous system acting drugs influence the locomotor activities in man and animals. The CNS depressant drugs such as barbiturates and alcohol reduce the motor activity while the stimulants such as caffeine and amphetamines increase the activity. In other words, the locomotor activity can be an index (alertness) of mental activity.

The locomotor activity (horizontal activity) can be easily measured using an actophotometer, which operates on photoelectric cells which are connected in circuit with a counter. When the bean of light falling on the photocell is cut off by the animal, a count is recorded. An actophotometer could have either circular or square arena in which the animal moves. Both rats and mice may be used for testing in this equipment.

### Procedure

Swiss albino mice were divided into 4 groups (n=5). The different groups of animals are assigned as follows.

| S.No | Groups | Treatments |
|---|---|---|
| 1. | Group 1 | Received vehicle only |
| 2. | Group 2 (Control) | Served as control group and depressant was induced with 2.0 mg/kg i.p |
| 3. | Group 3 | Severed as treatment group and fresh juice of *Saccharum officinarum* (10ml/kg/bw) for 10 minutes |
| 4. | Group 4 | Severed as treatment group and fresh juice of *Saccharum officinarum* (20ml/kg/bw) for 10 minutes |

1. Turn on the equipment (check and make sure that all the photo-cells are working for accurate recording) and place individually each mouse in the activity cage for 10 minutes. Note the basal activity score of all the animals.
2. Inject diazepam (3.0 ml/kg), & after 30 min re-test each mouse for activity scores for 10 min. Note the difference in the activity, before and after diazepam.
3. And inject Fresh juice of *Saccharum officinarum L.* (10, & 20 ml/kg/bw) respectively, & after 30 min re-test each mouse for activity scores for 10 min. Note the difference in the activity, before and after fresh juice.
4. Calculate percentage decrease in motor activity [9].

**Compulsive behaviour Activity**

Swiss albino mice were divided into 4 groups (n=5). The different groups of animals are assigned as follows.

| S.No | Groups | Treatments |
|---|---|---|
| 1. | I | Control received 0.9% NaCl |
| 2. | II | Standard received diazepam 2mg/kg |
| 3. | III | Severed as treatment group and fresh juice of *Saccharum officinarum* (10ml/kg/bw) |
| 4. | IV | Severed as treatment group and fresh juice of *Saccharum officinarum* (20ml/kg/bw) |

**Swim stress test**

CNS depressant activity was evaluated by the forced swimming test. All mice were first trained for swimming in a bath with dimensions (42 × 19 × 19 cm) as reported previously [10,11].Mice were placed individually for 6 min in a glass tub filled with water at room temperature (25 ± 2 °C) up to a marked level. Mice suddenly start to move their front and hind paws as soon as they are placed in water. The activity time was determined with the help of stop watch out of a total observation time of 6 min. Mice were considered immobile when they ceased struggling and started making the minimum movements necessary to keep afloat. This is the most commonly used method to evaluate depression **[12-14]**.

**Rearing Test**

A 1000-mL glass beaker lined with white paper on the bottom was used in this study. Upward movements of mice positioning the body in an erect position in the beaker were counted **[13-15]**.

**Traction Test**

This observation was made to determine the

time taken by the animal to travel on an iron rod of 1 m in length. Mice were first trained to walk on the iron rod. Any increase or decrease in the time taken by the drug-treated animals from that of the control animals to travel the rod describes the sedative or stimulant activity of the drug, respectively **[16-18]**.

**STATISTICAL ANALYSIS**

The results are presented as the mean ± standard error of the mean (SEM). One- way analysis of variance (ANOVA) was used for comparison tests of significant differences among groups, followed by Dunnett's't' post-test using Graph Pad Software, Inc., La Jolla, CA, USA.

**RESULT**

The results are shown in tables for illustration **(Tables 1-4)**.

**Locomotor activity**

The mean value in the control animals was 142.2 ±0.16.The mean values or *S.officinarum L.* at the dose of 10 ml/kg, & 20 ml/kg were 40±0.16, 98±0.43 and 114.4±0.42. The *S.officinarum L.* fresh juice managed to decrease the no of counts significantly ($p<0.01$) in a dose dependant manner as compared to control (**Table 1**). The mean value (40±0.16) in diazepam treated group were significantly lower as compared with control ($p<0.01$).

**Rearing Test**

The exploratory rearing activity observed for the control group was 38.8±1.4. The mean values for EECC at the dose of 10 ml/kg, and 20 ml/kg were 22±0.16, & 24.2±0.86. The *S.officinarum L.* managed to decrease the no of rearing significantly ($p<0.01$) in a dose dependant manner as compared to control (**Table 2**). The mean value (14.7±0.85) in diazepam treated group were significantly lower as compared with control ($p<0.01$).

**CNS Depressant Activity**

In forced swim test the immobility time was recorded. *S.officinarum L* at the dose of 10ml/kg, 20 ml/kg, increases immobility time (2.02±0.17, 2.56±0.16) respectively. The *S. Officinarum L* managed to increase the immobility time significantly ($p<0.01$) in a dose dependant manner as compared to control (04.14±0.12) (**Table 3**). The mean value (1.78±0.16) in diazepam treated group were significantly higher as compared with control ($p<0.01$). Increases in immobility times in this test indicate a decrease in swimming and struggling. This shows that *S. Officinarum L* has sedative diazepam like action.

**Traction Test**

The results of motor coordination activity in a traction test were noted for 30 min. The time for crossing the rod was observed and

compared with the control and standard drugs. The mean value in the control animals was 7.18±0.07. The mean values for *S.officinarum L* at the dose of 10ml/kg, 20 ml/kg were 8.24±0.18 and 9.57±0.76. The *S.officinarum L* managed to decrease the no of counts significantly ($p<0.01$) in a dose dependant manner as compared to control except at the dose of (10ml/kg, 20ml/kg) respectively, and show any significant activity (**Table 4**). The mean value (12.65±0.66) in diazepam treated group were significantly lower as compared with control ($p<0.01$).

**Table 1: Effect of *S. Officinarum L.* Fresh juice on locomotor activity**

| S.No | Groups | Treatment | No of counts (Mean±S.E.M) |
|---|---|---|---|
| 1. | I | Control (0.9% w/v NaCl) | 142.2 ±0.16 |
| 2. | II | Standard (Diazepam-2 mg/kg) | 40±0.16** |
| 3. | III | *S. Officinarum L.* (10 ml/kg-bw) | 98±0.43** |
| 4. | IV | *S. Officinarum L.* (20 ml/kg-bw) | 114.4±0.42** |

**Group I- Control (received 0.9 % w/v NaCl), Group II- Standard (received Diazepam 2mg/kg), Group III-IV- *S. Officinarum L.* Fresh juice with different doses; one-way ANOVA followed by Values are given as mean ± S.E.M. from five mice in each group, * p < 0.05 significant from control animals, ** p < 0.01 significant from control animals**

**Table 2: Effect of *S.officinarum L.* Fresh juice on rearing**

| S.No | Groups | Treatment | No of Rearing (Mean ± SEM) |
|---|---|---|---|
| 1. | I | Control (0.9% w/v NaCl) | 38.8±1.4 |
| 2. | II | Standard (Diazepam-2 mg/kg) | 14.7±0.85** |
| 3. | III | *S.officinarum L.* Fresh juice(10ml/kg) | 22±0.16** |
| 4. | IV | *S.officinarum L.* Fresh juice(20ml/kg) | 24.2±0.86** |

**Group I- Control (received 0.9 % w/v NaCl), Group II- Standard (received Diazepam 2mg/kg), Group III-V-ethanolic extract with different doses; one-way ANOVA followed by Values are given as Mean±S.E.M from five mice in each group, * p < 0.05 significant from control animals, ** p < 0.01 significant from control animals**

**Table 3: Effect of *S.officinarum L* fresh juice in forced swim test**

| S.No | Groups | Treatment | Time(Minutes) |
|---|---|---|---|
| 1. | I | Control (0.9% w/v NaCl) | 03.74±0.12 |
| 2. | II | Standard(Diazepam-2 mg/kg) | 1.78±0.16** |
| 3. | III | *S.officinarum L.* (10 ml/kg) | 2.02±0.17** |
| 4. | IV | *S.officinarum L.* (20 ml/kg) | 2.56±0.16 ** |

**Group I- Control (received 0.9 % w/v NaCl), Group II- Standard (received Diazepam 2mg/kg), Group III-IV- *S.officinarum L.* fresh juice with different doses; one-way ANOVA followed by Values are given as Mean±S.E.M from five mice in each group, *p < 0.05 significant from control animals, ** p < 0.01 significant from control animals**

**Table 4: Effect of *S.officinarum L.* fresh juice in Traction test**

| S.No | Groups | Treatment | Time(minutes) |
|---|---|---|---|
| 1. | I | Control (0.9% w/v NaCl) | 7.18±0.07 |
| 2. | II | Standard (Diazepam-2 mg/kg) | 12.65±0.66** |
| 3. | III | *S.officinarum L.* fresh juice (10ml/kg) | 8.24±0.18** |
| 4. | IV | *S.officinarum L.* fresh juice (20ml/kg) | 9.57±0.76** |

**Group I- Control (received 0.9 % w/v NaCl), Group II- Standard (received Diazepam 2mg/kg), Group III-IV *S.officinarum L.* fresh juice with different doses; one-way ANOVA followed by Values are given as Mean ±S.E.M. from five mice in each group, *p < 0.05 significant from control animals, ** p < 0.01 significant from control animals**

**DISCUSSION AND CONCLUSION**

Administration of *S.officinarum L.* fresh juice, showed an increase in exploratory functions as compared to the control group. *S.officinarum L.* fresh juice worked Based on this observation, it was suggested that the expression of an anxiolytic state in animals might be reflected by an increase in head-dipping behavior, while a decrease in the number of head dips was found to be correlated with the depressant effect. Likewise, our results demonstrated that *S.officinarum L.* fresh juice significantly ($p < 0.01$). These results taking together indicate that, in contrast to diazepam, *S.officinarum L.* fresh juice showed anxiolytic-like effects without affecting locomotor activity or without producing central nervous depression.

In rearing activity there was a dose-dependent stimulatory effect, while in rearing the activity was lower at the minimum dose but comparable to standard at a maximum dose of 20 ml/kg, such that no sedative effect was observed. In the rearing test, the activity was slightly standard reduced at 10 and 20 ml/kg respectively. There was a slight calming effect in rearing tests with an increase in dose.

The forced swimming test is frequently used for the assessment of antidepressant-like activity in animal models. Primilary phytochemical are Saponins, tannins, proteins, phenolic compounds and flavonoids. It is possible that the mechanism of anxiolytic action of *S.officinarum L.* fresh juice. The results obtained in this study suggest that the *S.officinarum L.* fresh juice possesses anxiolytic effect.

Thus, *S.officinarum L.* has potential clinical applications in the management of anxiety and muscle tension disorders. Further investigations are warranted for elucidating the exact mechanism and bioactive compounds in a dose dependant manner. Locomotor activity is considered as an index of alertness and a decrease in it indicates a sedative effect.

The shortening of immobility duration indicates antidepressant activity in this model, while prolonged immobility duration reflects a CNS depression-like effect. A significant ($p<0.01$) dose-dependent antidepressant effect was observed.

The traction test was performed in animals for determining the muscle-relaxant potency of the treatments. Results from this study revealed that *S.officinarum L.* fresh juice had mild muscle- relaxant activity. This mild effect was observed to be dose-dependent.

A number of researcher reported that the

alkaloids, glycosides, and flavonoids rich plant extracts possess sedative, anxiolytic, & antiepileptic properties mediated through their affinity with benzodiazepine site of GABAergic complex system or are direct or indirect modulators of this receptor's increases in GABA activity in the brain producing drowsiness and facilitating or maintaining sleep. Researchers concluded that the sedative and muscle- relaxant-like properties of benzodiazepines such as diazepam are mostly due to interference with the action of gamma aminobutyric acid (GABA).

**COMPETING INTEREST**

Authors have declared that no competing interests exist.

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