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SCREENING OF PHYTOCONSTITUENTS AND NEUROPROTECTIVE POTENTIALS OF HYDROALCOHOLIC EXTRACTS OF *SACCHARUM BENGHALENSE*

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Abstract

Psychoses refers disorders of the mind that cause a loss of reality awareness. *Saccharum benghalense* is synonym for *Tripidium bengalense* that is also called as munj grass that grows in desert regions and along river banks. The current research was based on the screening of phytoconstituents and neuroprotective potentials of hydroalcoholic extracts of *Saccharum benghalense*. The leaves of *Saccharum benghalense* was obtained from the Rohilkhand region, Uttar Pradesh. The plant was identified and authenticated by the botanist. The leaves were washed making dust-free and dried at room temperature or shade. The dried leaves were rendered into

coarse powders and then finally into fine ones. The powder is weighed and extracted through cold maceration process using hydro-alcoholic solution (water and ethanol; 1:1). The plant extract was performed its preliminary screening of phytoconstituents. The animal house, Department of Pharmacy, MJP Rohilkhand University, Bareilly provided Wistar albino rats of either sex weighing 120-140g. The animals were kept in good health, with room temperatures of 25°C and a 12-hour light/dark cycle. The rats were divided in 4 groups i.e., group 1: administered normal saline, group 2: administered Sodium arsenite (40mg/kg/day, p. o.), group 3: administered Sodium arsenite (40mg/kg/day, p. o.) + hydroalcoholic leaves extract of *Saccharum benghalense* (HLSB), (200mg/kg/day, p. o.) and group 4: administered Sodium arsenite (40mg/kg/day, p. o.) + hydroalcoholic leaves extract of *Saccharum benghalense* (HLSB), (400mg/kg/day, p. o.) for 21 days. Neuroprotective activity was observed through both behavioural (EPM, Light/dark arena, FST) and biochemicals (SOD, lipid peroxidation) parameters. In results, *Saccharum benghalense* significantly exhibited the antioxidant and neuroprotective effects in all the parameters when compared with sodium arsenite treated rats. It decreased lipid peroxidation that indicates its antioxidant action. SOD level was also found lowered in animals treated with the *Saccharum benghalense* herbal extract that indicates for their neuroprotective effect. In conclusion, hydro-alcoholic leaves extract of *Saccharum benghalense* is significant neuroprotective and antioxidant herbal drug. In future aspects, the responsible chemical constituents could be identified and isolated for an effective therapeutic moiety.
Keywords: *Saccharum benghalense,* phytoconstituents, neuroprotective activity, behavioral models, biochemical parameters.

INTRODUCTION

Psychoses refers disorders of the mind that cause a loss of reality awareness. Psychosis frequently manifests as delusions and hallucinations, or seeing or hearing things that are not there. Other signs include inappropriate behaviour for the circumstance and incoherent or nonsensical speech [1]. Adolescent mental disorders and anxiety are more common in women than in men. Anxiety problems affect women 1.5-2 times more frequently than they do men [2]. Delusions and hallucinations are examples of psychotic symptoms that might develop gradually over time or strike suddenly. Individuals experiencing psychosis might not even be aware that their feelings are strange. For them, the events occurring inside their minds are incredibly real. Psychosis can be brought on by a fundamental psychiatric problem, substance misuse, or another neurological or medical condition. First-episode psychotic diseases have been associated with reduced prefrontal, superior, and medial temporal grey matter along with other brain abnormalities [3]. Scientists have spent over a century studying schizophrenia, but they are still unable to determine its precise origin [4].

Saccharum benghalense is synonym for *Tripidium bengalense* that is also called as munj grass that grows in desert regions and along river banks. The grass is tall, with smooth, greenish brown panicles. The grass is overgrown and can reach heights of up to 7 feet. Leaf sheaths are straight, pale straw in colour, villous on the edges at the apex with long white hairs that are typically much

longer than the normal internode, and the highest sheath might occasionally reach beyond the base of the panicle. White blooms on it provide decorative significance [5].

Fig 1. *Saccharum benghalense* shrub

The plant is known by its common names, Kana, Sarkanda, and Moonja, and it is found in Pakistan and Afghanistan as well as northern and western India. Large tufted grass, the plant is of limited use as fodder because cattle and buffalo only consume the new leaves when there is a food shortage. The stem is used to make moorhas and chiks. It is native to Myanmar, Bangladesh, Nepal, Afghanistan, Pakistan, northern India, and Iran. Northeastern India, particularly Assam in the Terai-Duar grasslands at the foot of the Himalayas, is a major region of native distribution [6].
Common names: with the common names munj sweetcane, baruwa sugarcane or baruwa grass, bahupraja, bana, bhadramunja, Kana, Sarkanda and brahmanya.

Taxonomy [7]
Kingdom - Plantae
Class - Liliopsida
Order - Poales
Family - Poaceae
Genus - Saccharum (Tripidium)
Species - *benghalense*
The current study was based on the screening of phytoconstituents and neuroprotective potentials of hydroalcoholic extracts of *saccharum benghalense.*

MATERIALS AND METHODS

Materials

Dried leaves of *Saccharum benghalense*, Sodium arsenite, Wistar rats of either sex, Water bath, desiccator, ethanol, and distilled water.

Collection, Authentication and Extraction of plant

The leaves of *Saccharum benghalense* was obtained from the Rohilkhand region, Uttar Pradesh. The plant was identified and authenticated by the botanist. The leaves were washed making dust-

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free and dried at room temperature or shade. The dried leaves were rendered into coarse powders and then finally into fine ones. The powder is weighed and extracted through cold maceration process using hydro-alcoholic solution (water and ethanol; 1:1). The water-bath was used to dry the brownish, semisolid extract obtained at temperature not exceeding 40ºC [8].

PHYTOCHEMICAL SCREENING

The plant extract was screened for different phytoconstituents to evaluate their presence [9].

1. Detection of Alkaloids

Extracts were dissolved individually in dilute HCl and filtered.

Mayer's Test

Filtrates were treated with Mayer's reagent (Potassium Mercuric Iodide). Formation of a yellow-colored precipitate indicates the presence of alkaloids.

Wagner's Test

Filtrates were treated with Wagner's reagent (Iodine in Potassium Iodide). Formation of brown/reddish precipitate indicates the presence of alkaloids.

Hager's Test

Filtrates were treated with Hagers Reagent. Formation of yellow ppt indicates the presence of alkaloids.

2. Detection of Glycosides

Fehling's test

With distilled water dilution, Fehling's solutions A and B were heated for one minute. There were 8 drops of plant extract added to this transparent blue solution. It was then combined with 1 ml of Fehling's solution and heated for 5 minutes in a water bath. Brick red precipitation is an indication of glycoside content.

Test for cardiac glycosides

A millilitre of the leaf extract, two millilitres of glacial acetic acid, and a few drops of 5% FeCl3 were combined. A single millilitre of pure sulfuric acid was placed underneath this. When a brown ring forms at the interface, cardiac glycosides are present.

Detection of Saponins

Foam test

About 2g of the plant extract was mixed with 10ml of distilled water and shaken vigorously for a stable persistent froth. Appearance of froth indicates the presence of saponins.

3. Detection of Tannins

Ferric chloride test

0.5g of the dried powdered sample was boiled in 20ml of water in a test tube and then filtered. A few drops of 0.1% $FeCl_3$ was added and observed for brownish green-black or a blue-black coloration.

Lead acetate test

2ml of plant extract was combined with 2ml of distilled water. 0.01g lead acetate was added to this combined solution and shaken well. Development of white turbidity and precipitate indicates the presence of tannins [10].

4. Detection of Flavonoids

NaOH test

A small amount of extract was treated with aqueous NaOH and HCl, and observed for the formation of yellow orange color.

H_2SO_4 test

A fraction of the extract was treated with Conc.H_2SO_4 and observed for the formation of orange color.

5. Detection of terpenoids

5 ml of the aqueous plant extract were combined with 2ml of chloroform, which was then added, evaporated on the water path, and boiled with 3ml of concentrated H2SO4. As terpenoids took shape, a grey colour emerged.

6. Detection of Steroids

2 ml of chloroform and concentrated H_2SO_4 were added with the 5 ml aqueous plant crude extract. In the lower chloroform layer red color appeared that indicated the presence of steroids.

7. Test for Reducing Sugars and Carbohydrates

Molisch's test

To 2-3ml extract of individual solvents add few drops of α-naphthol solution in alcohol, shake and add concentrate H_2SO_4 from sides of test tube. Violet ring at the junction of two liquids.

Fehling's test

It is utilised to find decreasing sugars. Make a volume of 500 mL by dissolving 34.66 grammes of copper sulphate in distilled water (solution A). 50 grammes of sodium hydroxide and 17.3 grammes of potassium sodium tartrate should be dissolved in distilled water to a volume of up to 50 millilitres (Solution B). Prior to usage, combine two solutions in an equal volume. Fehling's A and B solution in a 1 mL mixture should be boiled for one minute. Add the test solution in an equal amount. Heat in a pot of boiling water for 5–10 minutes. A first yellow and then a brick red hue was seen.

Test for coumarins

1 mL of 10% NaOH was mixed with 1 mL of the leaf extract. The development of a yellow hue suggests the presence of coumarins.

Test for Anthocyanin and Betacyanin

A mL of the leaf extract was combined with a mL of 2N sodium hydroxide, and the mixture was heated to 100°C for five minutes. Anthocyanin is shown by the creation of bluish green colour, while betacyanin is indicated by the formation of yellow colour.

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Preparation of animals

The animal house, Department of Pharmacy, MJP Rohilkhand University, Bareilly provided Wistar albino rats of either sex weighing 120-140g. The animals were kept in good health, with room temperatures of 25°C and a 12-hour light/dark cycle. The relative humidity is kept at 44-56%, and the rats were provided a regular rodent diet and free access to water. The animals were kept on fasting but have free access to water until 1 hour before utilized in the study [11].

Group design

All the rats were divided into 4 groups (n=6) as followings-

Group 1: administered normal saline for 21 days.

Group 2: administered Sodium arsenite (40mg/kg/day, p. o.) for 21 days.

Group 3: administered Sodium arsenite (40mg/kg/day, p. o.) + hydroalcoholic leaves extract of *Saccharum benghalense* (HLSB), (200mg/kg/day, p. o.) for 21 days.

Group 4: administered Sodium arsenite (40mg/kg/day, p. o.) + hydroalcoholic leaves extract of *Saccharum benghalense* (HLSB), (400mg/kg/day, p. o.) for 21 days.

Experimental Protocols

A. Behavioural models

1. Elevated Plus Maze (EPM) Test

Elevated Plus Maze consists a 5 cm wide circular pathway; elevated 27 cm from the floor and diameter of maze kept 65 cm. The circular pathway is divided into 4 quadrants in which 2 are open and 2 are closed quadrants- where wall is 27 cm in height. Rats are placed facing towards anyone of the closed quadrants during each trial. Rats are allowed to explore the apparatus for 5 minutes only. No. of entries and time spent in open quadrants are recorded till 5 minutes [12].

2. Light-Dark Arena Model

In light-dark arena model, a 100Watt bulb is being placed 30 cm above to base of box. Rats are kept in centre of light arena (box) and must expose for 5 minutes. No. of entries and time spent in light arena segment are recorded till 5 minutes. It is cleansed every time before keeping a new rat [8].

3. Forced Swimming Test

Rats are dropped in glass (30×20cm) filled with water at depth of 15 cm and temperature maintained at approx. 30ºC. Rats are allowed to forcefully swim for the period of 5 min. The total mobility time is recorded each time in seconds in 5 min using stopwatch [13].

B. Biochemical parameters

❖ **Preparation of tissue homogenate**

An overdose of diethyl-ether was used to sacrifice the rats, and the liver was taken after decapitation. The liver was homogenized (1gm/10 ml PBS, pH 7.8) and centrifuged at 2-8°C for 10 minutes at 15000 rpm. The supernatant was used to calculate a variety of biological parameters.

1. Determination of SOD activity

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In a cuvette, 0.5ml carbonate buffer, 0.1ml EDTA, and 1ml epinephrine were mixed together. The optical density of produced adrenochrome was measured at 480 nm for three minutes at 30 second intervals. Standard solutions of 0.01 U/ml, 0.1 U/ml, 1 U/ml, and 10 U/ml were used to create the SOD calibration curve. The enzyme activity was measured in units per minute per milligramme of tissue [14].

2. Assay of lipid peroxidation

1 mL of material was combined with 0.2 mL sodium dodecyl sulphate, 1.5 mL acetic acid in hydrochloric acid, and 1.5 mL thiobarbituric acid in hydrochloric acid. The resulting mixture was heated for 1 hour in a hot water bath at 85°C. At 532nm, the intensity of pink colour generated was measured against a blank [15].

RESULTS AND DISCUSSION

Preliminary screening of phytoconstituents

Upon preliminary screening of phytoconstituents, *Saccharum benghalense* showed an excellent presence of different moieties. Alkaloids, terpenoids, flavonoids, phenols, coumarins and betacyanin were observed in abundance. While cardiac glycosides, tannins, steroids, were obtained in moderate quantity. The glycosides, saponins, and anthocyanin were found absent. After preliminary screening, *Saccharum benghalense* demonstrated for following phytoconstituents-

Table 1. Phytochemicals of *Saccharum benghalense* leaves extract

Phytoconstituents	**Hydroalcoholic leaves extract of *Saccharum benghalense***
Alkaloids	++
Glycosides	_
Cardiac glycosides	+
Tannins	+
Saponins	_
Terpenoids	++
Steroids	+
Flavonoids	++
Phenols	++
Coumarins	++
Anthocyanin	_
Betacyanin	++

Absent (-), Present (+), Abundance (++)

A. Behavioral models

1. Elevated Plus Maze (EPM) Test

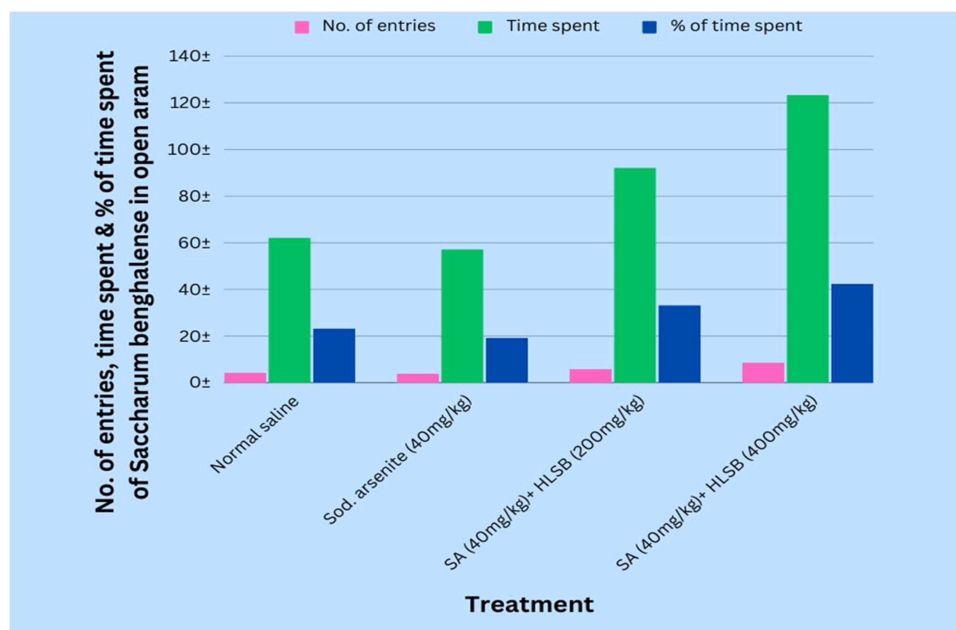
In EPM model, control group showed no. of entries, time spent and % of time spent in open arm as 4.19±0.32**, 62.18±0.20 and 23.31±0.39** respectively. Sodium arsenite (40mg/kg, p. o.) + HLSB (200mg/kg, p. o.) treated group showed no. of entries and % of time spent as 5.93±0.36*** and 33.18±0.24*** respectively. In contrast, Sodium arsenite (40mg/kg, p. o.) + HLSB (400mg/kg, p. o.) administered group exhibited no. of entries and % of time spent as 8.64±0.27*** and 42.39±0.19*** respectively. This model clearly confirms that the *Saccharum benghalense* leaves are effective in treatment of neurotoxicity.

Table 4.1 No. of entries, time spent & % of time spent of *Saccharum benghalense*

Treatment	**In open arm (sec)**		
	No. of entries	**Time spent**	**% of time spent**
Normal saline	4.19±0.32**	62.18±0.20	23.31±0.39**
Sodium arsenite (40mg/kg, p. o.)	3.79±0.24**	57.18±0.12	19.20±0.12**
Sodium arsenite (40mg/kg, p. o.) + HLSB (200mg/kg, p. o.)	5.93±0.36***	92.16±0.11	33.18±0.24***
Sodium arsenite (40mg/kg, p. o.) + HLSB (400mg/kg, p. o.)	8.64±0.27***	123.28±0.54	42.39±0.19***

Significance Level= *

Values were given in Mean ± S.E.M. and found statistically significant at P<0.05, compared to control (n=6)



2. Light/dark arena test

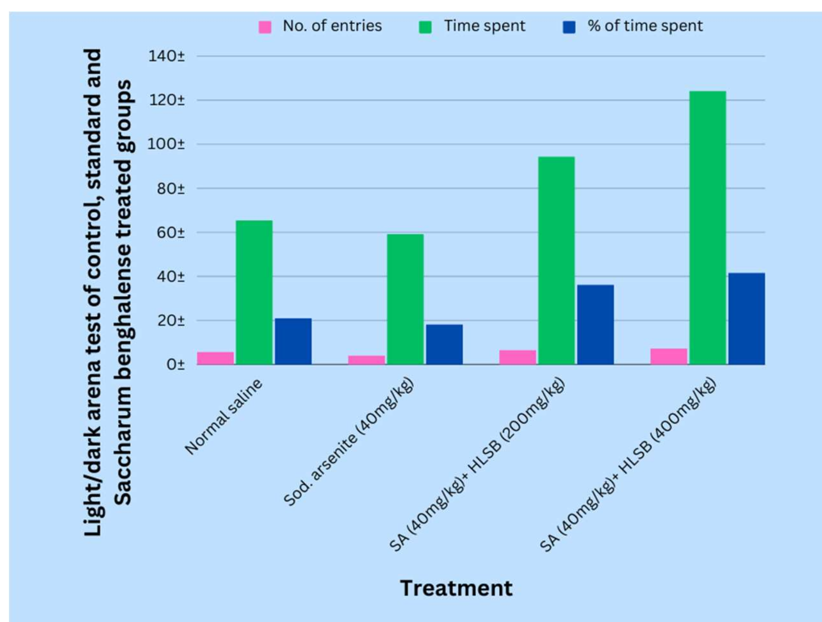
In light/dark arena model, no. of entries, time spent and % of time spent in light arena were recorded till 5 min. In sodium arsenite (40mg/kg, p. o.) + HLSB (200mg/kg, p. o.) treated rats, no. of entries in light arena was recorded as 6.62±0.10** and time spent 94.38±0.48 sec and thus % of time spent as 36.21±0.32** which was highest among all. The sodium arsenite (40mg/kg, p. o.) + HLSB (400mg/kg, p. o.) also showed increased no. of entries and % of time spent in light arena as 7.25±0.24** and 124.23±0.17 respectively.

Table 4.2 Light/dark arena test of control, standard and *Saccharum benghalense* treated groups

Treatment	Light arena (sec) No. of entries	Time spent	% of time spent
Normal saline	5.82±0.19*	65.41± 0.19	21.07±0.32**
Sodium arsenite (40mg/kg, p. o.)	4.12±0.27*	59.21± 0.61	18.20±0.13**
Sodium arsenite (40mg/kg, p. o.) + HLSB (200mg/kg, p. o.)	6.62±0.10**	94.38±0.48	36.21±0.32**
Sodium arsenite (40mg/kg, p. o.) + HLSB (400mg/kg, p. o.)	7.25±0.24**	124.23±0.17	41.62±0.34**

Significance Level= *

Values were given in Mean ± S.E.M. and found statistically significant at P<0.05, compared to control (n=6)



3. Forced swimming test

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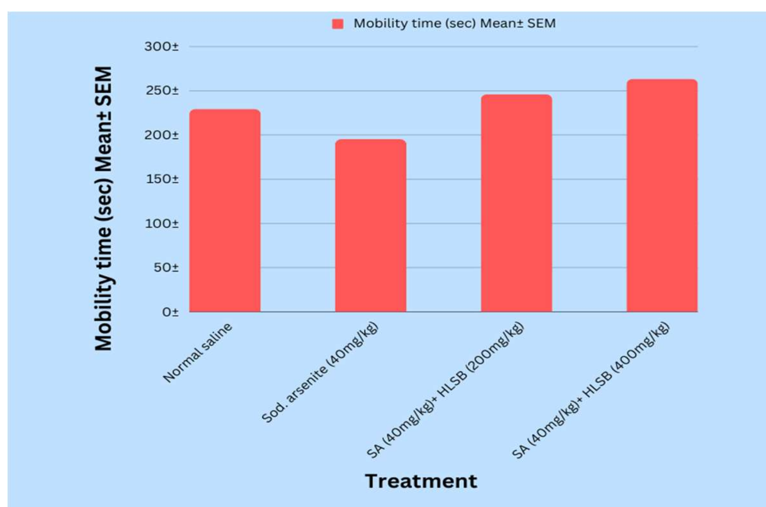
In Forced Swimming Test, mobility time was observed lowest in the case of control and highest in control which indicates for their neuroprotective activity. Sodium arsenite (40mg/kg, p. o.) + HLSB (200mg/kg, p. o.) exhibited increase in mobility time as 246.14±0.23** sec. While Sodium arsenite (40mg/kg, p. o.) + HLSB (400mg/kg, p. o.) treated rats showed mobilty score as 263.47±0.11*** sec. In both the doses, it significantly proved for its neuroprotective potential by facilitating the levels of neurotransmitters.

Table 4.3 Mobility time in FST of control, standard and *Saccharum benghalense* treated rats

Treatment	Mobility time (sec) Mean± SEM
Normal saline	229.38±0.26**
Sodium arsenite (40mg/kg, p. o.)	195.23±0.14**
Sodium arsenite (40mg/kg, p. o.) + HLSB (200mg/kg, p. o.)	246.14±0.23**
Sodium arsenite (40mg/kg, p. o.) + HLSB (400mg/kg, p. o.)	263.47±0.11***

Significance Level= *

Values were given in Mean ± S.E.M. and found statistically significant at P<0.05, compared to control (n=6)



B. Biochemical parameters

1. Determination of SOD level

In determination of SOD level, the control group exhibited SOD level 59.27±1.32* U/mg of protein in control group. Sodium arsenite (40mg/kg, p. o.) treated group exhibited SOD level 64.38± 0.26**U/mg of protein. Sodium arsenite (40mg/kg, p. o.) + HLSB (200mg/kg, p. o.) fed rats showed decreased SOD level as 52.20± 0.19**U/mg of protein. SOD level was seen as 38.32± 0.37***U/mg of protein. Thus, it might be used in the management of oxidation process.

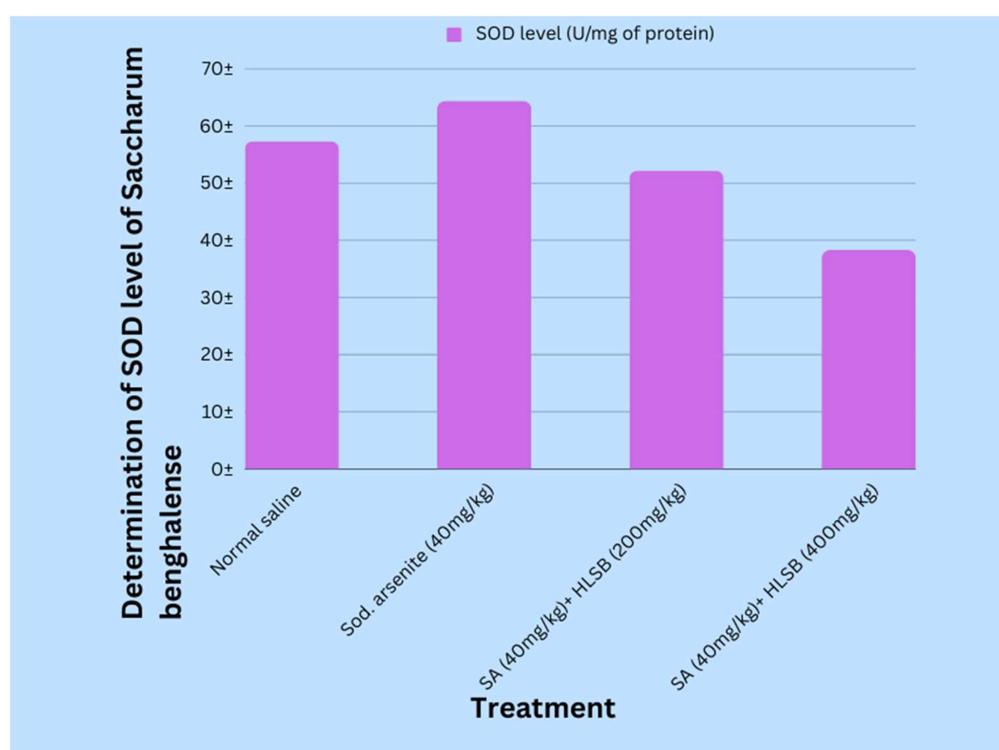
The following table depicts the SOD level of herbal extract-

Table 5. Determination of SOD level of *Saccharum benghalense*

Treatment	SOD level (U/mg of protein)
Normal saline	57.35± 0.64*
Sodium arsenite (40mg/kg, p. o.)	64.38± 0.26**
Sodium arsenite (40mg/kg, p. o.) + HLSB (200mg/kg, p. o.)	52.20± 0.19**
Sodium arsenite (40mg/kg, p. o.) + HLSB (400mg/kg, p. o.)	38.32± 0.37***

Significance Level= *

Values were given in Mean ± SEM and found statistically significant at $P<0.05$, compared to control (n=6)



2. Assay of lipid peroxidation

Normal saline treated animals showed lipid peroxidation as 29.47± 0.12* moles of MDA/g of liver whereas Sodium arsenite (40mg/kg, p. o.) fed rats showed lipid peroxidation 24.38± 0.33**moles of MDA/ g of liver. The sodium arsenite (40mg/kg, p. o.) + HLSB (200mg/kg, p. o.) and sodium arsenite (40mg/kg, p. o.) + HLSB (400mg/kg, p. o.) showed lipid peroxidation as 35.41± 0.35** moles of MDA/ g and 43.10± 0.52** moles of MDA/ g, respectively.

Following table depicts the assay of lipid peroxidation of herbal extract-

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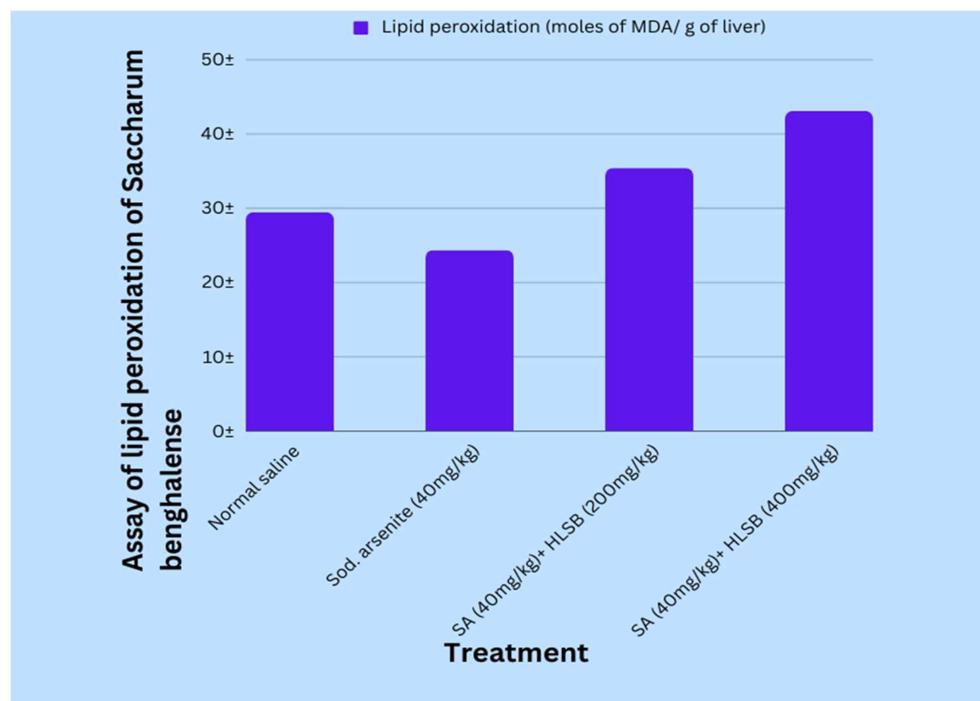
Table 6. Assay of lipid peroxidation of *Saccharum benghalense*

Treatment	Lipid peroxidation (moles of MDA/ g of liver)
Normal saline	29.47± 0.12*
Sodium arsenite (40mg/kg, p. o.)	24.38± 0.33**
Sodium arsenite (40mg/kg, p. o.) + HLSB (200mg/kg, p. o.)	35.41± 0.35**
Sodium arsenite (40mg/kg, p. o.) + HLSB (400mg/kg, p. o.)	43.10± 0.52**

Significance Level= *

Values were given in Mean ± S.E.M. and found statistically significant at $P<0.05$, compared to control (n=6)



In results, *Saccharum benghalense* significantly exhibited the antioxidant and neuroprotective effects in all the parameters when compared with sodium arsenite treated rats. It was found effective at both the doses 200mg/kg and 400mg/kg when tested in albino rats. It decreased lipid peroxidation that indicates its antioxidant action. SOD level was also found lowered in animals treated with the *Saccharum benghalense* herbal extract that indicates for their neuroprotective effect.

Stress induction results in elevated levels of MDA [16]. It has been discovered that oxidative stress-induced elevations in MDA can harm DNA [17]. Lipid peroxidation of polyunsaturated fatty acids brought on by ROS produces MDA. The advanced lipoxidation end products are produced

by this extremely reactive aldehyde [18]. Compared to those who survived, brain damage patients who did not survive had higher levels of MDA.

When delivered intranasally as opposed to intravenously, the quantity of chlorogenic acid in the brain was shown to be four times higher in a study conducted on Charles-Foster rats. Compound 2 can pass the blood-brain barrier, and 5β-carboxystrictosidine (1) and chlorogenic acid (2) are responsible for U. hirsuta's neuroprotective properties.

Another element linked to apoptosis is oxidative stress. Normally, antioxidants like glutathione, carotenoids, and ascorbic acid handle ROS and all superoxides. However, when these harmful molecules overwhelm antioxidant defences, oxidative stress conditions arise in cells, causing damage to DNA, lipid and protein degradation, and ultimately, apoptosis [18]. The apoptotic activation of mitochondria is the first step towards the oligomerization of procaspases and adapter proteins, which in turn triggers the auto-activation of initiator caspases and causes apoptosis [19]. This is the manner in which age-related intrinsic oxidative stress is typically associated with decreased antioxidant activity and mitochondrial malfunction. Postmortem investigations of the brains of patients with neurodegenerative diseases revealed the presence of several oxidative stress indicators [20]. Parkinson's disease patients' brains displayed signs of oxidative stress, such as lipid peroxidation and protein oxidation.

CONCLUSION

In conclusion, hydro-alcoholic leaves extract of *Saccharum benghalense* is significant neuroprotective and antioxidant herbal drug. It can be effectively used in the treatment of depression, mental agitation, and other neurotoxicity. Its efficacy might be due to presence of flavonoids, coumarins, and betacyanin in abundance.

Nowadays, as psychoses, anxiety and depression have become commonest form of mental disability, so it may demonstrate an economic and pharmacological impact in modulating the behaviour of neurons. In future aspects, the responsible chemical constituents could be identified and isolated for an effective therapeutic moiety.

FUNDING

Nil.

CONFLICT OF INTEREST

‘None’ conflict of interest was declared by the authors.

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