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Nootropic activity of ethanolic extract of Triticum aestivum in acute and chronic stress induced Wistar albino rats

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***Abstract*---**Objectives- The present study was designed to evaluate the nootropic activity of ethanolic extract of Triticum aestivum (TAE) on stressed wistar albino rats. Materials and methods- Effect of TAE was studied on acute restraint stress and chronic unpredictable stress induced rats. Chronic unpredictable stress was given for 10days. The reference standard drug (Piracetam-200mg/kg) and the test drug, TAE at doses of 150mg/kg and 200mg/kg b.w. were given to rats for 14 days. Learning and memory was assessed by using Elevated plus maze test. Animals sacrificed at the end of this experiment, the body weight adrenal and spleen weight, ulcer index as well as various biochemical parameters like malondialdehyde (MDA) and superoxide dismutase (SOD) were assessed. Results- Both acute as well as chronic stress induced a significant prolongation of learning time, whereas only with chronic unpredictable stress the memory or retention time was longer than that of the non-stressed control rats. Piracetam and TAE treated rats showed a significant reduction in acquisition and retention time in stress induced rats. Also, the stress induced ulcer scores were significantly reduced. The effects were comparable to normal control rats. Conclusion- In our study we found that TAE restored the stress induced impaired cognition which suggests its nootropic activity.

***Keywords*---**stress, triticum aestivum, transfer latency, ulcer index.

Introduction

Stress has become a regular feature of life today, yet there is a considerable ambiguity in the meaning of this word. In common usage, stress usually refers to an event or succession of events that cause a response, often in the form of distress. Stress targets nervous system along with the immune system, metabolic and cardiovascular system etc which are affected by both adaptive and mal-adaptive responses to stress [1].

Stress induced generation of reactive oxygen species (ROS) in brain is a contributing factor for alteration in motor, visceral, endocrine and behavioural performances. So, many researchers have explained that anti-oxidants ameliorate neurobehavioural and endocrine function by counteracting oxidative damage [2]. *Triticum aestivum*, commonly known as wheat grass has been used since ancient times in folk medicine for its medicinal properties. A number of scientific reports show that juice of wheat grass has potent anti-ulcer [3], antioxidant [4], anti-arthritic [5], antidiabetic [6] effects. Recently its neuroprotective effect on β-amyloid induced cell death and memory impairment has been studied in rats [7]. Hence the present study was undertaken to evaluate the nootropic effect of ethanolic extract of the test drug, *Triticum aestivum* against stress in wistar albino rats.

Methodology

In the present study, fifty four (54) wistar albino rats of either sex weighing between 100-150 gm were selected. The animals were randomly divided into nine (9) different groups of six in each (n=6). All animals were hygienically housed at room temperature and under standard laboratory condition of 12hr light and dark cycle in the animal house of department of pharmacology, M.K.C.G. Medical college, Berhampur, Odisha**.** The study was conducted after taking permission from Institutional Animal Ethical Committee (IAEC). The study period was 6 months.

Before the experiment all animals were acclimatized to standard laboratory conditions for 7 days and had free access to food and water throughout the period of experiment. They were used only once in the experiment. All experiments were carried out in the day time from 10:00hr and 16:00hr. In our study 1000 grams of Wheat grass powder in pure form was procured from Girme's Wheatgrass pvt. Ltd.The powder was subjected to soxhlet extraction with 99.99% ethanol for 24hrs.The alcoholic extract was then subjected to evaporation in a beaker on a water bath maintained at 50^0c till a thick paste of extract remained in the beaker. It was stored in refrigerator at 4^0C and used throughout the experiment. The yield obtained was 8.7% [2]. During the time of experiment fresh solution was prepared with 5% DMSO for daily administration.

For evaluating the nootropic activity, the test drug or reference standard drug or vehicle was administered to rats before the induction of stress as per the treatment protocol. One group of unstressed rats was treated as normal control group in each model of experiment and was considered for comparison. The stress was induced to the rats for short time (Acute) or for 10 days (Chronic) as per the

following schedule in each model of experiment and was treated as stress control group for comparison.

Stress Induction

Acute Stress [8] -Immobilisation of rats was done in a wire mesh for 2 hours called restraint stress.

Chronic Unpredictable Stress[9] - Chronic stress was induced in unpredictable manner as per the following schedule: Day-1- at 11.00am-50min cold room (4°c) ,and at 12.00pm-60min cage rotation. Day -2, at 1.00pm, 4hr wet bedding (400ml tap water in home cage), and at 6pm light on overnight. Day-3-at 12.00pm, 3hrs lights off and at 3.00pm-60min restraint stress. Day- 4, at 4.00pm-50, min cage rotation, and food-water deprivation overnight(15hr). Day-5, at 3.00pm-15min cold room isolation and at 4.00pm isolation housing overnight(17)hr.Day-6 at 11.00am-4hr wet bedding and at 3.00pm -2hr lights off. Day-7, at 1.00pm-30min cage rotation and at 6.00pm-1hr lights on. Day-8, at 10.00 am- 20min cage rotation and at 3.00pm for 60min restraint stress. Day-9, at 10.00am, 4hr wet bedding and at 6.00pm food and water deprivation. Day-10, at 6.00pm isolation housing and lights overnight.

The test drug doses were selected from a previous study in our laboratory on anti-diabetic activity of *Triticum aestivum* **[8]**.Among them the test dose of 150 and 200 mg/kg B.W were selected for further study on the basis of optimal response in the above tests. The dose of reference standard drug (Piracetam) was selected from different published article **[10]**.

Table 1: Experimental Design For Elevated Plus Maze Test

GROUPS(N=6)	STRESS GIVEN	DRUG TREATMENT	DOSE AND ROUTE
I	NIL	Vehicle-DMSO	5%,po.
II	Acute stress	Vehicle-DMSO	5%, po.
III	Acute stress	Piracetam	200mg/kg po.
IV	Acute stress	TAE	150mg/kg po
V	Acute stress	TAE	200mg/kg po.
VI	Chronic stress	Vehicle-DMSO	5%, po.
VII	Chronic stress	Piracetam	200mg/kg po.
VIII	Chronic stress	TAE	150mg/kg po
IX	Chronic stress	TAE	200mg/kg po.

For testing memory, laboratory model used is Elevated Plus Maze test (Exteroceptive behaviour model) [11] .The elevated plus maze for rats consist of two open arms (50cm x 10cm) and two close arm (50cm x10cm x40cm) facing each other with an open roof. The rats were allowed to move freely to explore the apparatus for 20sec. Rats were individually placed at the end of one open arm facing away from centre of plus maze .The time taken to move from open arm to either of the enclosed arm (Transfer Latency, TL) was recorded on the first day with cut off time of 90 sec. The TL following first trial served as acquisition (Learning) and TL was again recorded at 24hr and 48hr after first exposure and considered as retention/consolidation (Memory) time.

Functional biomarkers estimated in the study were Body weight, Adrenal and Spleen weight, Oxidative stress markers like Brain MDA and SOD, Protective effect against stress induced gastric ulcer, measured in terms of Ulcer index in Gastric mucosa. Following behavioural tests, rats from each groups were sacrificed by cervical dislocation. Whole brain was dissected then weighed and homogenized with 10ml of Normal saline. MDA and SOD estimated from brain homogenate.

Stomach was dissected out by dividing at gastro-esophageal junction and gastro-duodenal junction. Then stomach was opened along the greater curvature and washed gently in running water. The gastric mucosa was displayed on a wax platform and coded to eliminate bias. Using a magnifying glass the ulcer scores were recorded as Scoring: 0 - Normal coloured stomach, 0.5 - Red coloration,1-Spot ulceration,1.5 - Hemorrhagic streak, 2 – ulcers, 3-Perforations. Ulcer index was calculated for each rat by adding the scores.

The vehicle for reference standard drugs Piracetam was distilled water. For the test drug (Ethanolic extract of *Triticum aestivum)* Dimethyl-sulphoxide (DMSO) was the vehicle. From a pilot study it was observed that there was no difference [p>0.05] between the distilled water and DMSO treated rats with respect to different behavioural parameters.

Statistical Analysis

The statistical software Graphpad prism 5 was used for all the above statistical calculation.The parametric data were analysed by one way ANOVA followed by Tukey's multiple comparison 't'–test. The data of Non-parametric type were analysed using Kruskal wallis one way ANOVA followed by Dunn's multiple comparison for comparison of 3 or more group. P<0.05 was considered as minimum level of significance.

Results

Table-2: Effect of drugs on transfer latency (EPM) in rats exposed to acute stress

Group (n=6)	Time in second (Mean±SE)		
	Acquisition time	24 hr retention time	48 hr retention time
Normal control (Vehicle)	21±1.6	13±1.6	11±0.77
Stress control	39±1.9[a]	15±1.7	10± 1
Stress +Piracetam	20±1.2[b]	12±0.58	9±0.82
Stress +TAE-150 mg/kg	35±0.96	16±2.1	9.2±0.31
Stress +TAE-200 mg/kg	23±0.76[b]	13±0.88	9.5±0.62
F	43	1.1	1.2
P	<0.001	>0.05	>0.05

n=6, a: P<0.0001(Normal vs Stress control), b: p<0.001(Piracetam and TAE-200 mg/kg groups vs Stress control groups)

Table-3: ANOVA table showing analysis of data of table-2 (Aquisition time of groups)

ANOVA Table	SS	df	MS	F	P
Treatment (between groups)	1900	4	480	43	<0.001
Residual (within groups)	280	25	11		
Total	2200	29			

This ANOVA table compares the acquisition time of different treatment groups (from data of Table-II).The calculated F value is 43 at df 4, 25. Here the F value revealed that there is a highly significant difference among different treatment groups [p<0.001].

The post ANOVA Tukey's multiple comparison test showed that following acute restraint stress for 2hr, the acquisition time was significantly increased (39±1.9 sec) than that of normal control group (21±1.6 sec). Piracetam-200mg/kg significantly reduced the acquisition time (20 ± 1.2 sec), so also with TAE (200mg/kg) the acquisition time was significantly low (23±0.76 sec) [p<0.001] as compared to stress control group rats, again this effect was comparable to normal control rats (p >0.05).

Table 4: Comparison of effect of drugs on transfer latency (on EPM) in rats exposed to chronic unpredictable stress

Group (n=6)	Acquisition time	24 hr retention time	48 hr retention time
Normal control(Vehicle)	21±1.6	13±1.6	11±0.87
Stress control	50±2.1[a]	36±2.4 [a]	29±2 [a]
Stress +Piracetam	24±2.2[b]	15±0.87 [b]	8±0.73 [b]
Stress +TAE-150 mg/kg	34±1.3[b]	20±1.9 [b]	15±1.2 [b]
Stress +TAE-200 mg/kg	27±1.9[b]	16±1.3[b]	9.5±0.76 [b]
F P	39 <0.001	31 <0.001	50 <0.001

n=6 in each group, a: p<0.001(Normal vs Stress control), b: p<0.001(chronic stress control group vs Piracetam and TAE treated group)

In this table, following chronic unpredictable stress for 10days, the acquisition time was significantly increased (50±2.1sec) than that of normal control group rats (21±1.6 sec). Piracetam with 200mg/kg significantly reduced the acquisition time (24±2.2 sec), so also with pre-treatment of TAE (150mg/kg) and TAE (200mg/kg), the acquisition time was significantly reduced (35±1.6 and 27±1.9 sec) respectively [p<0.001] when compared with stress control group rats. Similarly the 24hr and 48hr retention time with stress control group was significantly higher (36±2.4 and 29±2.0 sec respectively) than that of the normal non-stressed rats at corresponding hours [p<0.001]. Piracetam-200mg/kg significantly [p<0.001] enhanced memory retention in terms of decreased transfer latency after 24hrs and 48hrs of first trial (15±0.87 and 8±0.73 sec) respectively

as compared to that of stress control group at corresponding hours. Similarly 150mg/kg B.w of TAE significantly lowered [p<0.001] the retention time at 24 hrs and 48hrs. i.e (20 ±1.9 and 15±1.2 sec respectively) in comparison to stressed rats. Also TAE (200mg/kg b.w) further reduced the 24 hr and 48 hr memory retention time (16±1.3 and 9.5±0.76 sec) to a highly significant extent as compared to stressed rats[p<0.001].

Table 5: Effect of different drugs on ulcer index of rats exposed to stress

Treatment groups	Mean ulcer index score ± SE	
	Acute stress	Chronic stress
Stress control	15±0.49	14±0.60
Stress +Piracetam	0.92±0.24[a]	0.83±0.11 [a]
Stress +TAE-150 mg/kg	12±0.83	1.7±0.21
Stress +TAE-200 mg/kg	0.75±0.11 [a]	0.83±0.11 [a]
K W statistics p	19.22 p<0.001	18.37 p<0.001

n=6, Acute and chronic stress- **a:** p<0.01(stress vs standard and TAE-200mg/kg).

Discussion

The report of study [12] states that, restraint stress for a brief period (2-6hr) can induce a series of dysfunction such as cognitive impairment, amnesia and insomnia. In the present study, the acute restraint stress in wire mesh for 2hrs and chronic unpredictable stress for 10days were adopted as stressor agent to induce behavioural disorder (dementia) and gastric ulcer in a rodent like rat. Wistar albino rats were selected for its easy availability, easy to handle and good experimental performance [13]. The test drug, selected for present study *Triticum aestivum,* has protective role of on Aβ-induced apoptosis in SH-SY5Y cells and cognitive dysfunctions in Sprague-Dawley (SD) rats as reported by Jung-Hee et al [7].

In EPM test, our results indicated that both acute restraint stress and chronic unpredictable stress induced a significant prolongation of learning time (Aquisition time) [Table-2 and 4] whereas with chronic unpredictable stress only the memory or retention time was longer than the control rats. This explains the memory retrieval was hampered at 24hr and 48hr of acquisition trial (1st trial) explaining the disturbed cognition as evidenced from increase in transfer latency time (Table-4). Our findings corroborate with study [14]. Piracetam, the reference standard drug, showed a significant reduction in acquisition time in both acute and chronic stress induced learning and memory impairment. The 24hr and 48hr retrieval time was reduced only in chronic stress model. It reveals its nootropic activity in stress induced impaired cognition (Table no-4).

Pre-treatment with Ethanolic extract of *Triticum aestivum* (200mg/kg) for 14days showed a significant decrease in transfer latency (acquisition and memory retention time) in both acute and chronic stressed rats. Our observation is consistent with study [15]. Thus *Triticum aestivum* showed reversed the stress

induced learning and memory disturbances and this effect was comparable to that of non-stressed control rats (Tables-2 and 4). Our result is strongly supported by the study of Jung-Hee et al [7].

In our study, the body weight and spleen weight before stress and after chronic stress for 10days were not significantly changed. But on exposure to chronic stress, the adrenal gland weight was increased to a significant extent than normal control rats similar to study [16]. Rats treated with reference standard drug and TAE (200mg/kg) attenuated the chronic stress induced increase in adrenal gland weight. Stressful life events adversely affect the Gastric ulcer formation, principally via acid secretions [17]**.** The rats exposed to acute restraint stress and chronic unpredictable stress showed a significant increase in scores of ulcer index and severe hemorrhagic gastric lesions. Pre-treatment with reference standard drug and TAE (200mg/kg) decreased the scores of ulcer index in comparison with stressed rats (Table-5) similar to study [3] .

Stress induced cellular generation of reactive oxygen species and subsequent neural tissue damage is proved to be a contributing factor for neurobehavioural alteration. In our observation, chronic unpredictable stress induced an increase in MDA and decreases SOD levels in brain to a highly significant extent when compared to that of normal control rats. Treatment of reference standard drug and TAE (200mg/kg) reduced the alterations in the brain MDA and SOD which explained the antioxidant property of *Triticum aestivum*.

Conclusion

In our present investigation, stress induction (Acute restraint stress and Chronic unpredictable stress) caused a remarkable alteration in behavioural parameters like learning and memory impairment, change in brain MDA, SOD, gastric ulceration. The test drug, *Triticum aestivum* restored stress induced all the above behavioural changes as well as inhibited the gastric ulceration. Even it restored the stress induced MDA and SOD changes in brain tissue.

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