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Antianxiety Activity of Ethanolic Extract of Triticum aestivum in Acute Stress Induced Wistar Albino Rats by Light and Dark Apparatus

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ABSTRACT

Objectives: The present study was designed to evaluate the antianxiety activity of Ethanolic extract of Triticum aestivum (TAE) on acute stressed wistar albino rats.

Materials and methods: Effect of TAE was studied on acute restraint stress induced rats. The reference standard drug (Diazepam 1mg/kg po) and the test drug, TAE at doses of 150mg/kg and 200mg/kg b.w. were given to rats for 14 days. Anti-anxiety activity was assessed by using Light and Dark Box test. Animals sacrificed at the end of this experiment. Then 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 Diazepam and TAE-200 mg/kg treated rats showed a significant prolonged stay in the light box when exposed to acute stress and this effect was comparable to that of normal no stressed rats.

Conclusion: The test drug, TAE-200mg/kg shows anxiolytic activity against acute stress in wistar albino rats.

Key words: Acute stress, Anxiety, *Triticum aestivum*, Light and dark box test, Transfer latency

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INTRODUCTION

Stress has become an integral part of day to day life. 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 neurobehavioral 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], and 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]. Basing on this, present study was undertaken to evaluate the anxiolytic effect of ethanolic extract of the test drug, *Triticum aestivum* against acute stress in wistar albino rats.

METHODOLOGY

In the present study, Thirty wistar albino rats of either sex weighing between 100-150 gm were selected. The animals were randomly divided into five (5) different groups of six rats in each (n=6) group. 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 three 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 300 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 500c till a thick paste of extract remained in the beaker. It was stored in refrigerator at 40C and used throughout the experiment. The yield [1] obtained was 8.7%. During the time of experiment fresh solution was prepared with 5% DMSO for daily administration.

For evaluating the antianxiety activity, the test drug or reference standard drug or vehicle was administered to rats before the induction of stress as per the treatment protocol for 14 days. Stress was induced to rats for short time (Acute stress) and these rats were treated as stress control group for comparison.

Acute stress induction [8]

Immobilization of rats was done in a wire mesh for 2 hours called restraint stress.

For testing anxiety, laboratory model used is Black & White Test Box /Light-Dark Box test [9]. It consists of a wooden box measuring 44X21X21 cm. One third of it, was darkened with black spray over its all surfaces. A partition containing a 13cm long X 5cm high opening separates the dark one third, from bright two third of the box. The light compartment was illuminated with a 60 watt bulb, located 40cm above the centre of it (Table 1). Rats were individually placed in light compartment of the box and loco motor activity was observed for 10min as follows.

- ✓ Number of exploratory rearing in the light and dark compartments.
- ✓ Number of transmissions between the two compartments.
- ✓ Time spent in the light and dark compartments.
- ✓ Latency of the initial movement from the light and dark compartments.

Following behavioural tests, rats from each laboratory test model groups, were sacrificed by cervical dislocation. Whole brain was dissected out and weighed individually .Then it was homogenized with 10ml of Normal saline. The brain homogenate was subjected estimation of MDA and SOD. 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.

Table 1: Experimental design for light and dark box 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	Diazepam	1mg/kg po
IV	Acute stress	TAE	150mg/kg po
V	Acute stress	TAE	200mg/kg po

The gastric mucosa was displayed on a wax platform and coded to eliminate bias. Using a magnifying glass the ulcer scores were recorded. 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 and recorded.

Statistical analysis

The statistical software Graphpad prism 5 was used for 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. The $p<0.05$ was considered as significant.

RESULTS

The latency of initial movement with acute stress control rats was significantly shorter [$p<0.01$] than normal control groups. [Acute:3.2 ± 0.48 vs 6.8 ± 1.0 esc]. Diazepam, the reference standard drug, prolonged the latency of initial movement in acute stress model rats [8.8 ± 0.70] significantly [$p<0.001$]. So, also with TAE-200 mg/kg treated stressed rats which is comparable to that of normal control rats [$p>0.05$] (Table 2).

The vehicle treated rats when exposed to acute stress, the time spent in light box was significantly reduced and stay in dark box was significantly longer than that of normal control group of rats in respective compartments. [Light box: 6.16 ± 0.47 vs 26.17 ± 3.83 sec; Dark box: 593.8 ± 0.47 vs 573.8 ± 3.83 sec respectively]. Diazepam treated group of rats showed a longer stay (77.17 ± 0.51) sec in the light box in comparison to stress control rats [$p<0.001$]. The stressed rats pre-treated with TAE-150 mg/kg also showed a significant change in time spent in light and dark boxes both in acute stress model [$p<0.05$]. On Pre-treatment with TAE-200 mg/kg, the time spent in light box in stressed rats was significantly longer [$p<0.001$] (43.17 ± 3.0). This reveals TAE-200 mg/kg has significant anxiolytic effect just like Diazepam (Table 3).

Stress control rats in dark box showed a highly significant increase in exploratory rearing [$p<0.001$] compared to normal control rats (18 ± 0.48 vs. 7.5 ± 0.56) (Table 4). Diazepam and TAE-200 mg/kg treated stressed rats

Table 2: Effect of drugs on latency of initial movement in rats exposed to acute stress.

Treatment groups	Latency of Initial movement in sec (Mean ± SE)
Normal control (Non-stressed)	6.8 ± 1.0
Acute Stress control	3.2 ± 0.48a
Stress +Diazepam	8.8 ± 0.70***
Stress +TAE-150 mg/kg	5.0 ± 0.37
Stress +TAE-200 mg/kg	6.5 ± 0.76*
F	9.1
p	<0.001

Latency of Initial movement- a-$p<0.01$(normal control vs stress control), and *: $p<0.05$, ***: $p<0.001$(stress vs drug treatment groups)

Table 3: Effect of different treatments on time spent in light and dark compartments.

Treatment groups	Time spent (sec) Mean ± SE	
	In Light Box	In Dark Box
Normal control	26.17 ± 3.83	573.8 ± 3.83
(Non-stressed)		
Acute Stress control	6.16 ± 0.47a	593.8 ± 0.47a
Stress +Diazepam	77.17 ± 5.1***	522.8 ± 5.1***
Stress +TAE-150 mg/kg	22.67 ± 2.5 *	577.3 ± 5.1*
Stress +TAE-200 mg/kg	43.17 ± 3.0***	556.8 ± 3.0 ***
F	64.39	64.39
p	<0.001	<0.001

Time spent: Acute stress: Light box and Dark box a: p<0.01 (stress vs normal control), *:p<0.001, ***: p<0.001 (stress control vs. Drug treatment groups)

Table 4: Effect of acute stress on exploratory behaviour in rats in light and dark test.

No. of rats	No. Of exploratory rearing in different compartments				No. of transmission in between compartments	
	Light compartment		Dark compartment			
	Normal	Stressed	Normal	Stressed	Normal	Stressed
1	2	4	9	17	3	3
2	4	5	6	18	2	5
3	3	4	6	17	2	5
4	1	5	8	18	3	6
5	2	6	7	19	2	4
6	3	8	9	20	2	4
Mean ± SE	2.5 ± 0.43	5.3 ± 0.61	7.5 ± 0.56	18 ± 0.48	2.3 ± 0.21	4.5 ± 0.43

Table 5: Effect of drugs on behaviour of rats exposed to acute stress.

Treatment groups	Mean ± SE		
	No. Of exploratory rearing		No. Of transmission
	Light box	Dark box	
Normal control (Non-stressed)	2.5 ± 0.43	7.5 ± 0.56	2.3 ± 0.21
Stress control	5.3 ± 0.61	18 ± 0.48a	4.5 ± 0.43
Stress +Diazepam	4.5 ± 0.99	8.2 ± 0.95**	3.5 ± 0.43
Stress +TAE-150 mg/kg	6.8 ± 1.0	14 ± 0.73	6.2 ± 0.48
Stress +TAE-200 mg/kg	5.5 ± 0.62	9.3 ± 0.42*	4.5 ± 0.43

No. Of exploratory rearing: n=6, Dark box; a- P<0.001(stress vs normal control); *: p<0.05, and **:p<0.01 (stress control vs. drug treatment groups)

Table 6: Effect of different drugs on ulcer index of rats exposed to acute stress.

Treatment groups	Mean ulcer index score ± SE
Stress control	15 ± 0.49
Stress +Fluoxetine	0.92 ± 0.24a
Stress +TAE-150 mg/kg	12 ± 0.83
Stress +TAE-200 mg/kg	0.75 ± 0.11 a
K W statistics	19.22
P	p<0.001

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

had a significantly higher number of exploratory rearing (8.2 ± 0.95 and 9.3 ± 0.42 respectively) in comparison to stress control rats [p<0.05] (Table 5). There was no significant change in the brain MDA and SOD levels of rats on exposure to acute stress [p>0.05] (Table 6).

DISCUSSION

The word stress has been associated with sensation of discomfort. It is a Psycho-physiological process induced by stressor agents and a physiological reaction that induces loss of homeostasis and rupture of psychological balance resulting in various physical and mental disorders [10,11]. Stress induces a variety of CNS disorders such as anxiety, depression, anorexia and elevated corticosterone level as well as plasma glucose concentrations in animals and humans [12,13] .Even It is reported that prolonged anxiety, emotional stress and trauma are known to cause severe gastric irritation [14]. The report of Imrana Tabassum et al, 2010 states that, restraint stress for a brief period (2-6hr) can induce a series of dysfunction such as cognitive impairment, anxiety, depression, amnesia and insomnia [12]. Hence in this study, the acute restraint stress was given in wire mesh for 2hrs. Wistar albino rats were selected in this research work because of its easy availability, easy to handle and good experimental performance [15].

Plants having antianxiety activity [16] are Abies pindrow, Achillea millefolium, Aloysia polystachya etc. The test drug, *Triticum aestivum*, has protective role of on Aβ-induced apoptosis in SH-SY5Y cells and cognitive dysfunctions in Sprague-Dawley (SD) rats [17]. Hence this study was undertaken to explore the neurological effects of ethanolic extract of *Triticum aestivum* against stress. Light and Dark box test is widely used tool to measure anxiety like behaviour in rodents and based on a conflict between the natural aversion of brightly illuminated area and on their spontaneous exploratory behaviour in novel experiment. This is a sensitive test to explore the anxiolytic effect of test drugs [18]. Diazepam[19] (1mg/kg) selected as reference standard drugs for anxiety test model.

The rats exposed to acute restraint stress when subjected to Light and Dark box test, they entered into dark compartment from light compartment quickly as compared to normal control rats (Table2). Again the time spent in the light box was brief in comparison to the non-stressed control rats (Table no-3). The number of exploratory rearing in both stress model rats were significantly more than non-stressed control rats (Table-5). These are the indications of high level of fear and anxiety. Also in comparison to the stress control rats, the time spent in Light box was longer and decrease in number of exploratory rearing in both compartments was observed with rats pre-treated with Diazepam and TAE-200mg/kg. These activities of the test drug are indices of its anxiolytic activity against stress. Similar observations were reported by a study in the plant Nymphaea alba in Light and Dark box test [20].

Stressful life events adversely affect the Gastric ulcer formation, principally via acid secretions [21]. The rats exposed to acute stress showed a significant increase in scores of ulcer index and severe hemorrha gic gastric lesions (Table-6). Pre-treatment with reference standard drug and TAE (200mg/kg) decreased the scores of ulcer index which is comparable to study [3]. Several studies have reported the anti-stress, gastro protective effects [12] of medicinal plants are due to their phenolic content, flavonoid etc. In this study [3] the test drug *Triticum aestivum* is rich in chlorophyll, minerals like magnesium, selenium, zinc, chromium, antioxidants like beta-carotene, Vit-E, Vit-C etc. These constituents might be responsible for its beneficial effects.

CONCLUSION

Triticum aestivum exhibited anti-anxiety effects in stress induced anxiety model and this effect may be mediated by central monoaminergic neurotransmitter system like 5-HT and Dopamine. In our study, Triticum also showed significant anti-ulcerogenic activity in stressed rats. Thus, *Triticum aestivum* may provide an alternative to conventional therapy for attenuating the behavioural impairments like anxiety in stress as well as it may come up with safe and effective treatment of ulcer as well.

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