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A study on effect of ethanolic extract of triticum aestivum in anxiety behaviour induced by chronic unpredictable stress in a rat model

Swapna Mahapatra
Assistant Professor, Department of Pharmacology, MKCG Medical College, Berhampur, Odisha, India

Prasanta Kumar Nayak
Assistant Professor, Department of Radio Diagnosis, MKCG Medical College, Berhampur, Odisha, India

Sanjeeb Kumar Pradhan
Assistant Professor, Department of General Surgery, MKCG Medical College, Berhampur, Odisha, India

Saroj Shekhar Rath
Associate Professor, Department of Pediatrics, MKCG Medical College, Berhampur, Odisha, India
Corresponding author email: drsarojrath@yahoo.com

***Abstract*---**Objectives- This study was undertaken to evaluate the effect of Ethanolic extract of Triticum aestivum (TAE) on different anxiety like behaviour induced by Chronic unpredictable stress in wistar albino rats. Materials and methods- Effect of TAE was studied on chronic unpredictable 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. Then the locomotor activity of rats was assessed as indicator of anxiety. Animals were sacrificed at the end of this experiment..The adrenal and spleen weight, ulcer index as well as various biochemical parameters like Malondialdehyde (MDA) and Superoxide dismutase (SOD) were assessed. Results- The stay in Light box was increased, rearing activity was increased to a significant level in both Diazepam and TAE-200 mg/kg treated rats and this effect was comparable to that of normal non-stressed vehicle treated rats. Chronic stress caused elevation of MDA and depression of SOD level which is reversed by Ethanolic extract of Triticum aestivum (TAE). Conclusion- The test

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drug Triticum reversed the stress induced anxiety behaviour and biochemical alterations as well as decreased the ulcer index.
***Keywords*---**Chronic Unpredictable Stress, Anxiety, Light and Dark Box test, Triticum aestivum, MDA, SOD, Ulcer index.

Introduction

In modern lifestyle stress is a common phenomenon and it has been realized that stress plays a major role in precipitating several diseases. Stress induces a variety of autonomic, visceral, immunological and neuro-behavioural responses such as anxiety, depression, impaired cognition in animals and humans. In folk medicinal practice many plants are used as brain tonic for different neurological disorders but most of them are not scientifically validated.

Triticum aestivum, commonly known as wheat grass has been used since ancient times in folk medicine for its medicinal properties. Many scientific reports show that juice of wheat grass has potent anti-ulcer [1], antioxidant [2], anti-arthritic [3], antidiabetic [4] effects. Its neuroprotective effect on β-amyloid induced cell death and memory impairment has been studied in rats [5]. Basing on this, present study was undertaken to evaluate the anxiolytic effect of ethanolic extract of the test drug, *Triticum aestivum* against chronic stress which is given in an unpredictable manner in wistar albino rats.

Methodology

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**.** 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. 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. The study was conducted after taking permission from Institutional Animal Ethical Committee (IAEC). The study period was three months.

All experiments were carried out in the day time from 10:00hr and 16:00hr. In our study, 300 grams of Wheat grass powder (test drug) 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 [6] obtained was 8.7%. During 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 were administered to rats for 14 days. Chronic unpredictable stress was induced to rats for 10 days i.e 4 days after drug treatment.

CHRONIC UNPREDICTABLE STRESS INDUCTION[7] **-** 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 10.00am - 20min of cage rotation and at 3.00pm for 60min restraint stress. **Day-9**, at 10.00am, 4hr of wet bedding and at 6.00pm food and water deprivation. **Day-10,** at 6.00pm, isolation and lights on overnight.

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	Chronic stress	Vehicle- DMSO	5% po
III	Chronic stress	Diazepam	1mg/kg po
IV	Chronic stress	TAE	150mg/kg po
V	Chronic stress	TAE	200mg/kg po.

The laboratory model used for anxiety testing was Black & White Test Box /Light-Dark Box test [8]. The Light and Dark Box 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 two sides. The light compartment was illuminated with a 60 watt bulb, located 40cm above the centre of it. Rats were individually placed in light compartment of the box and locomotor 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.

After behavioural tests, rats from each laboratory test model groups, were sacrificed by cervical dislocation. Whole brain was dissected out and weighed individually. The brain homogenate was subjected estimation of MDA and SOD. Functional biomarkers estimated in the study were Body weight, Adrenal and Spleen weight.

Protective effect against chronic stress induced gastric ulcer, measured in terms of Ulcer index in Gastric mucosa. Stomach was dissected out by dividing at gastro-esophageal junction and gastro-duodenal junction. 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. 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.

Results

Table 2: Time spent in light and dark compartments of different group of rats

Treatment groups	Mean±SE	
	Time spent (sec) in light box	Time spent (sec) in dark box
Normal control	26.17±3.83	573.8±3.83
Stress control	6.17±0.60[a]	593.8±0.60[a]
Stress +Diazepam	94.67±5.8***	505.8±5.8***
Stress +TAE-150 mg/kg	23.83±4.1*	576.2±4.1*
Stress +TAE-200 mg/kg	68.17±3.7***	531.8±3.7***
F p	83.18 <0.001	83.18 <0.001

Time spent-: *Chronic stress- Light box and Dark box* ***a****=p<0.05(stress vs normal control) , *:p<0.001, ***: p<0.001 (stress control vs Drug treatment groups)*

On exposure to chronic stress, time spent by the rats in the light box was brief to a significant extent [p<0.01] so also stay in the dark compartment was longer than that of the non-stressed rats [p<0.01] in comparison to the normal control rats. Diazepam treated group of rats showed longer stay in the light box in comparison to stress control rats [p<0.001] in the chronic stress models [94.67±5.8 sec]. Significant shorter stay in dark compartment in comparison to stress control rats in chronic stressed rats with Diazepam pre-treatment. The stressed rats pre-treated with TAE-150 mg/kg also showed a significant change in time spent in light and dark boxes in chronic stress models [p<0.05] so also with TAE-200 mg/kg, the time spent in light box both in chronic-stress rats was significantly longer [p<0.001] (68.17±3.7) sec.

Table 3: Effect of drugs on behaviour of rats exposed to chronic unpredictable stress

Treatment groups	Mean±SE		
	No. Of exploratory rearing		No. Of transmission
	Light box	Dark box	
Normal control	2.5±0.43	7.5±0.56	2.3±0.21
Stress control	7.3±0.67 [a]	28±3.4 [b]	4.2±0.48
Stress +Diazepam	2.8±0.48*	9.7±0.56*	3.7±0.33
Stress +TAE-150 mg/kg	6.7±0.67	17±1.3	5.5±0.43
Stress +TAE-200 mg/kg	3.7±0.71	9.7±0.92*	3.8±0.31

n=6: *Light box*: **a-**P<0.05 (stress control vs normal control), *Dark box*-**b:** p <0.001 (stress control vs normal control) *:p<0.05 (stress control vs drug treatment groups)
The rats showed a significant [p<0.05] increase in number of exploratory rearing both in light and dark compartment with that of normal control rats (7.3±0.67 and 28±3.4). Diazepam treated rats reduced the rearing number to a significant extent in both light as well as dark compartment. But TAE-200 mg/kg b.w showed a significant reduced number of rearing in dark box only (13±1.2) [p<0.05] compared to stressed rats.

Table 4: Effect of drugs on spleen and adrenal gland weights in chronic stressed rats

Treatment groups	Spleen weight (mg)	Adrenal weight (mg)
Normal control	290±25	11±0.41
Stress control	310±18	16±0.64[a]
Stress +Diazepam	320±11	13±0.34[b]
Stress +TAE-150 mg/kg	290±7.6	14±0.28
Stress +TAE-200 mg/kg	280±3.0	16±0.33[c]
F p	1.4 >0.05	19 <0.001

*n=6, **a**: p<0.001(stress control vs normal control); **b**:p<0.001(stress control vs standard), **c**:p<0.01(stress control vs TAE-200mg/kg)*

The Adrenal gland weights (average of left and right adrenals) of the stressed rats were significantly higher than that of normal control groups when exposed to chronic unpredictable stress (16±0.64 vs11±0.41 mg) [p<0.01]. Diazepam as well as the group treated with TAE-200 mg/kg showed a significant restoration of adrenal weight in comparison to stress control groups [p<0.01]. It was observed that there was absolutely no change in body weight and splenic weight of all treatment groups of rats on exposure to chronic unpredictable stress.

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

Treatment groups	Chronic stress
Stress control	14±0.60
Stress +Diazepam	0.83±0.11 [a]
Stress +TAE-150 mg/kg	1.7±0.21
Stress +TAE-200 mg/kg	0.83±0.11 [a]
K W statistics P	18.37 P<0.001

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

The mean ulcer index was significant in rats exposed to chronic unpredictable stress (14±0.60). The reference Diazepam group of rats on exposure to chronic stress had mean ulcer index (0.83±0.11). In comparison to that of stress control rats, ulcer indices were decreased to a significant extent [P<0.05]. Similar effects [p<0.05] obtained with pre-treatment of TAE-200mg/kg in chronic stressed rats (0.83±0.11).

Table 6: Effect of drugs on MDA and SOD in rats exposed to chronic stress

Groups	Brain MDA(nmole/gm) Mean ± SE	Brain SOD(IU/mg) Mean ± SE
Normal control	194±1.59	23.83±0.60
Stress control	231±6.60 [a]	16.33±0.42[b]
Stress +Diazepam	186.2±4.75 ***	23.17±0.47 ***
Stress +TAE-150 mg/kg	213.7±11.48	19.0±0.68 *
Stress +TAE-200 mg/kg	202.2±2.915 *	23.0±0.36***
F p	7.37 <0.001	38.85 <0.001

n=6,MDA and SOD Chronic stress, ***a:*** *P<0.01 and* ***b:*** *P<0.001 (stress control vs normal control); *: p<0.05****,*** *and* ******:****p<0.001 (stress control vs drug treatment groups)*

On exposure to chronic unpredictable stress, there was a significant reduction in brain SOD level and increase in brain MDA levels in comparison to that of normal vehicle treated non-stressed rats [P<0.001]. On pre-treatment with reference standard drug, TAE-200mg/kg, these changes are reversed significantly.

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 [9]. Depending upon the individual's response to adapt to stress, a negative stress or distress may induce development of various illness bringing a great harm to human being's quality of life [4]. The widely accepted chronic inescapable intermittent stress, particularly of an unpredictable pattern, is more likely to induce nervous, endocrine, biochemical and immune changes by chronic stress of a predictable manner [10]. Exposure of rodents to unpredictable stress brings a change in behavioural profiles indicative of human psychopathlogy [11].

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 experiments [12]. The time spent in the light box was brief in comparison to the non-stressed control rats (Table-2). The number of exploratory rearing in both stress model rats were significantly more than non-stressed control rats (Table-3). This indicates high level of fear and anxiety. In comparison to stress control rats, 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.Similar study done [13].

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. Our observations corroborate with study [14]. Rats treated with reference standard drug and TAE (200mg/kg) attenuated the chronic stress induced increase in adrenal gland weight (Table no-4).

Stressful life events adversely affect the Gastric ulcer formation, principally via acid secretions [15]**.** The rats exposed to chronic unpredictable stress showed a significant increase in scores of ulcer index and severe hemorrhagic gastric lesions. Pre-treatment with Diazepam and TAE (200mg/kg) decreased the scores of ulcer index in comparison with stressed rats (Table-5). A similar observation was found the anti-ulcer activity of *Triticum* [1].

Conclusion

The test drug, Ethanolic extract of *Triticum aestivum* possess significant antistress activity, as shown by its mitigating effects on chronic unpredictable stress induced neurological, behavioural perturbations in the form of anxiety like behaviours and biochemical alterations. It also decreased the ulcer indices induced by different type of stressful events like chronic unpredictable stress.

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