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Antidepressant Activity of Ethanolic Extract of *Triticum Aestivum* in Stress Induced Wistar Albino Rats

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ABSTRACT:

Objectives- The present study was designed to evaluate the antidepressant activity of ethanolic extract of Triticum aestivum (TAE) on stressed wistar albino rats. **Materials and methods**- Effect of ethanolic extract of Triticum aestivum (TAE) was studied on stress induced rats. Anti-depressant activity was assessed by using forced swimming test. Animals were sacrificed at the end of this experiment, the body weight adrenal and spleen weight, ulcer index and biochemical parameters like malondialdehyde (MDA) and superoxide dismutase (SOD) were assessed.**Results-** the rats exposed to either acute or chronic stress significantly increased the immobility time in forced swimming test. The TAE-200mg/kg treated rats of both acute and chronic-stressed models had the period of immobility significantly lower than that of stress control group rats.**Conclusion-** The results of the present study indicate that ethanolic extract of *Triticum aestivum* possess significant antistress activity, as shown by its mitigating effects on acute restraint stress and chronic unpredictable stress induced neurological, behavioural and biochemical perturbations.

Keywords: Stress, Depression, forced swimming test, Triticum aestivum.

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INTRODUCTION:

According to WHO, the proportion of the global population with depression in 2015 is around 4.4%. Depression is more common among females (5.1%) than in males (3.6%).Stress targets nervous system

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along with the immune system, metabolic and cardiovascular system etc.Stress induced generation of reactive oxygen species (ROS) in brain is a contributing factor for alteration in different motor, visceral, endocrine and behavioural performances. So, anti-oxidants ameliorate neurobehavioural and endocrine function by counteracting the oxidative damage [1]. *Triticum aestivum*(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 [2], antioxidant [3], anti-arthritic [4], antidiabetic [5] effects. Recently its neuroprotective effect has been studied in rats [6]. Hence the present study was undertaken to evaluate the antidepressant effect of Ethanolic extract of *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 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 six 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 500 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 50^0ctill 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% [1]. During the time of experiment fresh solution was prepared with 5% DMSO for daily administration.

For evaluating the antidepressant activity, the test drug or reference standard drug or vehicle was administered to rats before the induction of stress as per the treatment protocol. Stress was induced to rats for short time (Acute stress) or for 10 days (Chronic stress)and were treated as stress control group for comparison.

STRESS INDUCTION:Acute stress [7] -immobilisation of rats was done in a wire mesh for 2 hours called restraint stress.**Chronic unpredictable stress**: Chronic stress (like cage rotation, wet bedding,cold room, food and water deprivation etc) was given by at different times for 10 days as per the protocol l**[8]**.

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The test drug doses were selected from a previous study in our laboratory on anti-diabetic activity of *Triticum aestivum*[7]. 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 (Fluoxetine) was selected from different published articles [9].

Table 1: EXPERIMENTAL DESIGN FOR FORCED SWIMMING 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	Fluoxetine	20mg/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	Fluoxetine	20mg/kg po.
VIII	Chronic stress	TAE	150mg/kg po
IX	Chronic stress	TAE	200mg/kg po.

For testing depression laboratory model used was Forced swimming test [10]. Rats were individually forced to swim in an open cylinder of height 40cm, diameter 18 cm.It contained fresh water to a height of 15cm maintained at $25^0c \pm 2^0c$.The duration of immobility was recorded within 5min time period as cut off time. Rats were considered immobile when cessation of swimming occurs, with head floating just above the water level**.**

Functional biomarkers like Body weight, Adrenal and Spleen weight, Oxidative stress markers like Brain MDA and SOD, and stress induced gastric ulcer in terms of Ulcer index in Gastric mucosa were measured. Following behavioural tests, rats were sacrificed by cervical dislocation. The brain homogenate was subjected for estimation of MDA and SOD.

Stomach was dissected out by dividing at gastro-esophageal junction and gastro-duodenal junction then cut along the greater curvature and washed gently in running water. The gastric mucosa was

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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 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. The $p<0.05$ was considered as significant.

RESULTS: The observations of the study are as follows

Table 2:Period of immobility of Normal and Acute stressed rats on Forced swimming test

Rat No.(n=6)	Immobility period(sec)	
	Normal rats	Acute restraint stressed rats
1	20	65
2	12	35
3	30	40
4	15	48
5	12	42
6	18	38
Mean±SE	18±2.8	45±4.4

The time period of immobility in acute restraint stressed rats is increased

Table 3: Effect of drugs on Period of immobility of Acute and Chronic stressed rats on Forced Swimming Test

Group(n=6)	Time in sec.(Mean±SE)	
Normal control	**18±2.8**	
	ACUTE STRESS	**CHRONIC STRESS**
Stress control	45±4.4[a]	93±2.5[a]
Stress +Fluoxetine	26±1.6[b]	29±2.2[b]
Stress +TAE-150 mg/kg	36±1.7	30±2.2[b]

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Stress +TAE-200 mg/kg	26±2[b]	30±1.5[b]
F	14	180
p	0.001	0.001

n=6, ***a****: p<0.001(Normal vs Stress control) and* ***b****: p<0.001(drug treatment groups vs respective stress control group)*

Rats exposed to acute or chronic stress significantly increased immobility time (45±4.4 and 93±2.5sec respectively) compared to normal control (18±2.8 sec) [p<0.001].The post–ANOVA multiple comparison test showed Fluoxetine-20mg/kg treatment reduced immobility [*p*<0.001] both in acute and chronic stressed rats (26±1.6and 29±2.2sec respectively).Similarly TAE-200mg/kg treated rats had period of immobility (26±2and 30±1.5sec) significantly lower than that of stress control group rats [*p*<0.001].

Table 4: 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 +Fluoxetine	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 Fluoxetine and TAE-200mg/kg).*

Table 5: Effect of drug treatment on MDA and SOD in rats exposed to acute and chronic stress

Groups	**Brain MDA(nmole/gm) Mean ± SE**		**Brain SOD(IU/mg) Mean ± SE**	
Normal control	**194±1.59**		**23.83±0.60**	
Treated group	**Acute stress**	**Chronic stress**	**Acute stress**	**Chronic stress**
Stress control	187.7±5.31	231±6.60 [a]	21±1.31	16.33±0.42[b]
Stress +Fluoxetine	189.7±4.19	186.2±4.75 ***	21.83±1.01	23.17±0.47 ***

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Stress +TAE-150 mg/kg	199.3±6.40	213.7±11.48	21.17±1.24	19.0±0.68 *
Stress +TAE-200 mg/kg	195±3.08	202.2±2.915*	21.50±0.76	23.0±0.36***
F **p**	1.06 >0.05	7.37 <0.001	1.24 >0.05	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 Fluoxetine)*

This table shows that, there was no significant change in brain MDA and SOD levels in acute stressed rats [p>0.05]. But a significant increase in brain MDA levels in rats on exposure to chronic stress. With pre-treatment of Fluoxetine and TAE-200mg/kg b.w, the rise in brain MDA level was reduced to a significantly [p<0.05] in stressed rats.On exposure to chronic unpredictable stress, there was a significant reduction in brain SOD level in comparison to normal vehicle treated non-stressed rats [p<0.001]. On pre-treatment with Fluoxetine and TAE-200mg/kg, there was significant increase in SOD levels compared to that of stressed rats [p<0.05].

DISCUSSION:

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.

The advantages of Forced swimming test (FST) is that it is a fast, reliable, low costing tool, easy to handle and has proven its reliability across laboratories for testing anti-depressant activities of novel drugs with a strong predictive validity [11] . It is hypothesized that the immobility during the forced swimming test in a narrow space reflects the animal's behavioural despair i.e. escape was impossible, which is similar to human depression [12].

From forced swimming test it was observed that both acute restrained stress and chronic unpredictable stress increased the immobility time to a highly significant extent. The reference drug Fluoxetine (20mg/kg) and test drug TAE (200mg/kg) significantly reduced this stress induced change in immobility time (Table no-3) in both acute and chronic stressed rats. Our observations show to our knowledge for the first time that the test drug, *Triticum aestivum* given orally for 14days is effective in

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producing significant anti-depressant effect similar to that of antidepressant activity of some medicinal plants like Ocimum sanctum, Cammelia sinensis, Morus alba etc[13,14].

Stressful life events adversely affect the Gastric ulcer formation, principally via acid secretions[15]**.** 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-4) similar to study [2].

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 decrease in SOD levels in brain to a highly significant extent compared to that of normal control rats. Treatment of reference standard drug(Fluoxetine) and TAE (200mg/kg) reduced the alterations in brain MDA and SOD which explained the antioxidant property of *Triticum aestivum*.

The antidepressant effect in stress induced behavioural disorders may be mediated by central monoaminergic neurotransmitter system (5-HT and Dopamine) [14]. Our test drug showed significant antidepressant effect in the Forced swimming test model, which might be due to this anti-seroternergic or dopaminergic action.

Several studies have reported the anti-stress, gastroprotective effects [14] of medicinal plants are due to their phenolic content, flavonoid etc. In study[2] the test drug 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* exhibited antidepressant effects in stress induced depression model and this effect may be mediated by central monoaminergic neurotransmitter system (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 depression in stress as well as it may come up with safe and effective treatment of ulcer as well.

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