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Research Article

Exploring The Therapeutic Potential Of Banaba Extract On Immobilized Restrainer Induced Stress Model In Mice

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ABSTRACT

Effect of Banaba Extract on Restrainer-Induced Stress Model in Experimental Mice. The mice were divided into five different groups. Each group contains six animals. The mice were treated with Banaba extract up to 15 days. Restrainer-induced stress models were evaluated. Restraint the animal for 6 hours/day up to 15 days. Various parameters were evaluated using different behavioral models like an elevated plus maze (EPM), light and dark test (LDT), open field test, and tail suspension test (TST). Biochemical parameters were evaluated to measure the cortisol level and antioxidant parameters (Lipid Peroxidation, Nitric Oxide, Superoxide Dismutase, and Catalase). This investigation showed the beneficial effect of Banaba Extract on current conditions. This study showed significant ($p<0.05$) increased time spent in the open arm in EPM, time spent in the light area in LDT, Significant ($p<0.05$) increased in the No. of square crossing in OFT, and Significant ($p<0.001$) reduced immobility duration in TST as compared to restrainer induced stress group and control group. Also, there was a significant ($p<0.001$) decrease the cortisol level in blood, a Significant ($p<0.05$) reduced the level of Lipid Peroxidation, Nitric Oxide and a Significant ($P<0.05$) increase in the level of Superoxide Dismutase, Catalase as compared to restrainer induced stress in stress group and control group. In conclusion- Our study suggests that the preclinical restrainer model is effective to induced stress in laboratory mice. Stress assessment in animals is done at various levels i.e. behavioral level, biochemical (cortisol level), and antioxidant level. Also, we report that the administration of Banaba Extract may prevent restrainer-induced stress.

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INTRODUCTION

The most prevalent neurodegenerative disorder worldwide is stress. which negatively impacts on quality of life. Neurodegeneration is oxidative stress (1). According to a survey, 77% of Indians suffer at least one symptom of stress regularly. Stress can be defined as 'external events or conditions that affect the organism. Stress affects health directly by influencing autonomic and neuroendocrine responses (2). Immobilization stress as also been reported that to increase the cortisol level (3,4). The hypothalamus also controls the secretion of the stress hormone cortisol via the hypothalamic-pituitary-adrenal axis (5). The stress response is characterized by the activation of the hypothalamus-pituitary-adrenal (HPA) axis and the subsequent rise in glucocorticoid (GC) secretion. HPA axis activation is predominant in adaptive and defensive responses to stress. However, in the chronic stress process, the HPA axis is usually in a constant high-response level, culminating in increased GC and functional disorders of the nervous, endocrine, and immune systems among others (6,7). In psychology, stress is evident in such feelings as anxiety, pressure as well as pain and also stress induced changes which are associated with oxidative damage, namely, free radical damage (8,9,10). Oxidative stress characterizes probably the most typical denominators of toxicity. oxidative mediated reactions are engaged in numerous basic factors of living processes such as processes of cell respiration (mitochondria), lipid synthesis, metallic metabolism, lysosomes, phagocytosis of foreign bodies (inflammation and immunity), and xenobiotic biotransformation of organic and natural compounds (11). In normal functioning of eukaryotic cell for mitochondria are the major source of energy or adenosine triphosphate (ATP) .(12).Dysfunctioning of mitochondria are target to ROS and to increase the oxidative damage, reduced the mitochondrial ATP production, increase the mitochondrial DNA mutation then ultimately affects the neurons and accelerats the neurodegenerative process (12,13). Antioxidants protect our entire body from free radical damage. Free radical damage within the cells has been associated with a range of disorders such as cancer, arthritis, atherosclerosis, stroke, Alzheimer's disease, diabetes, and emphysema in smokers, etc. (14). Free radicals can respond in an indiscriminate fashion leading to damaging almost every cellular constituent. A broad range of antioxidant defences, both endogenous and exogenous, do exist to protect cellular elements from free radical-caused damage. These can be divided into three main groups' viz., antioxidant enzymes (Catalase, Superoxide dismutase, Lipid peroxidation, etc.) chain-breaking antioxidants, and transition metal-binding proteins. (15). To demonstrate the difficulty of the stress reaction and the multifaceted manifestations are the leading ideas of experimental models used for stress induction in wet lab animals. Restraint stress is an established animal model for stress (16,17). The present study was designed to investigate effect of banaba extract against restrainer induced stress-like behavior, oxidative damage and cortisol level in mice.

MATERIAL AND METHOD

Animal

Male albino mice (35-50 g) were used in the experiment. The animals were procured from Laxmi Biotech, Pune, India. Animals were kept in cages at a normal laboratory temperature of 25±2°C, with a relative humidity of 45–55% and a 12–12 h light/dark cycle. All mice were placed separately in cages made of polypropylene. Animals had free access to water and standard laboratory feed (Nutrivet Lab, Pune India). The Institutional Animal Ethical Committee (IAEC) approved the protocol of this study.

Study Design

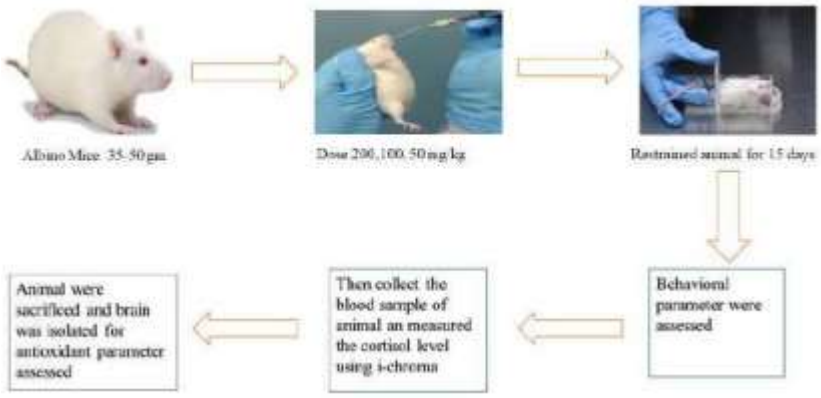
1. Grouping of Animal:

Five groups of mice were created, with six mice in each group.

Group I: Control,

Group II: Stress-Restrainer induced stress upto 15days, Group III: S+T1(50mg/kg) upto 15days, Group IV: S+T2(100mg/kg) upto 15 days, Group V: S+T3(200mg/kg) upto 15 days.

2. Induction and Assessment of Stress in Mice:



Assessment of behavioural parameters:

Elevated Plus Maze

EPM is widely used for behavioral tests for research on anxiety initially developed for mice and rats (18). The EPM apparatus was constructed of two open arms (20×5 cm) across from each other and perpendicular to two closed arms (20×5×25 cm) (19). The EPM apparatus was placed 50 cm off the ground (20). The mice were put in the center, facing an open arm, at the start of each test. The mice were given 5 minutes to explore the EPM to record the duration of stay in each arm and the entries (21).

Light and Dark Test

The light and dark tests may be behavioral tests useful to predict anxiety-like activity in mice (22). There are two compartments on the LDT device. Two-thirds of the box comprises a light compartment and one-third of the box is a dark compartment (22). The mice were first put in the center of the light. The transitions and the time spent in the dark box and light box were recorded for five minutes (23).

Tail Suspension Test

The tail suspension test may be the behavioral test most widely used model for assessing antidepressant-like activity in mice (23). The animal was suspended by the tail on the edge of a hook 50 cm above the floor using adhesive tape placed approximately 1cm from the tip of the tail. Total immobility time was recorded during 6min (22).

Open Field Test

The open field test may be a behavioural test used models for assessing locomotor activity in mice The open field apparatus was made from wood having dimensions (56 x 56 x 40cm). The floor was divided into 16 equal squares. Animal were put at one corner of the square of an open field. The number of square crossings and the number of grooming were recorded during 5min (24).

Assessment of biochemical parameters

1. Cortisol level in the blood (using ichroma)

Cortisol levels were measured blood samples were collected making a small incision in the tail. 150 units of detector diluent were taken using a pipette and dispensed to the detector tube containing a granule. 50 units of sample (whole blood) were

taken using a pipette and is pensed to the tube. 75 units of the sample mixture were taken and dispensed into the cartridge. the cartridge was inserted into the slot of the i-chamber for incubation for 10 minutes.

To scan the sample-loaded cartridge, it was inserted into the cartridge holder of the instrument or the chroma test. To start the button on instrument chroma and record the result display on screen (25).

2. Antioxident parameter

Tissue homogenate mice were sacrificed and isolated the brain and washed with ice-cold tris hydrochloric buffered saline. The brains were cross-chopped with fine slices, suspended in chilled 0.25M sucrose solution. The tissues were homogenized in chilled tris hydrochloric buffer (10mM, pH 7.4) to a concentration of 10% w/v. The homogenate was centrifuged at 10,000 rpm at 0°C for 15 minutes using high-speed cooling centrifuged. The cleared supernatant was used for the determination of lipid peroxidation (LPO), nitric oxide (NO), superoxide dismutase (SOD), and catalase (CAT) for antioxidants (26).

Assay of lipid peroxidation

The 2.0ml supernatant was mixed with 2.0ml freshly prepared 10%w/v TCA and placed in an ice bath for 15 minutes. The precipitate was separated by centrifugation and in 2.0 ml of clear supernatant solution 2.0 ml of freshly prepared TBA was added. The resulting solution was heated in a boiling water bath for 10 minutes. It was then immediately chilled in ice for 5 minutes. The pink color developed was measured at 532nm against a reagent blank (27).

Assay of nitric oxide

In 1ml of homogenate, 1ml of Griess reagent and incubated for 15min at 37°C. Were Measured the absorbance of the above solution at 540nm against a Griess reagent blank (28).

Assay of superoxide dismutase

0.5ml of tissue homogenate was diluted with 0.5ml of distilled water, to which ice-cold 0.25ml ethanol and 0.15ml chloroform was added and centrifuged at 2500rpm for 15 minutes. Then 0.5ml of supernatant was mixed with 1.5ml of carbonate buffer and 0.5ml of EDTA solution. The reaction was initiated by adding 0.4ml of Epinephrine and the change in optical density/minute was measured at 480nm against a blank (29).

Assay of catalase

Mixed 2ml supernatant with 1ml of Hydrogen peroxide to initiate the reaction. Prepared the blank by adding 2ml of diluted sample in 1ml of phosphate buffer (50 mM, pH 7.0). Measured the absorbance at 240nm (30).

Statistical Analysis:

The mean and SEM for each group's data is shown. A one-way ANOVA was used for statistical analysis, followed by Dunnette's test utilizing Graph-Pad Prism version 5.0 (USA). Statistics were found to be significant at p values < 0.05, $P<0.01$, $P<0.001$.

RESULTS

Behavioral Parameters

Effect of Banaba Extract administration on restrainer induced stress by using EPM Fig.1,2 shows restrainer-induced stress in the stress group, as indicated by significantly ($p<0.01$) more time spent and no. of entries in close arm as an anxiogenic effect. Contrarily, administration of Banaba Extract was observed significantly ($p<0.01$) more time spent and no. of entries in open arm as a reduced anxiety.

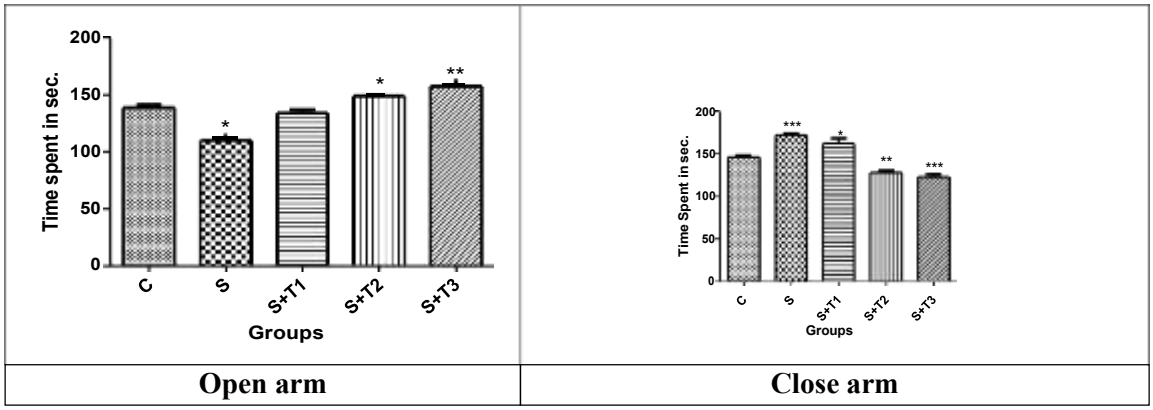


Fig.1: Effect of BE administration on Time Spent in EPM

Whereas, T1: Banaba Extract 50mg/kg, T2: Banaba Extract 100mg/kg, T3: Banaba Extract 200mg/kg, S: Stress animal and C: Control animal

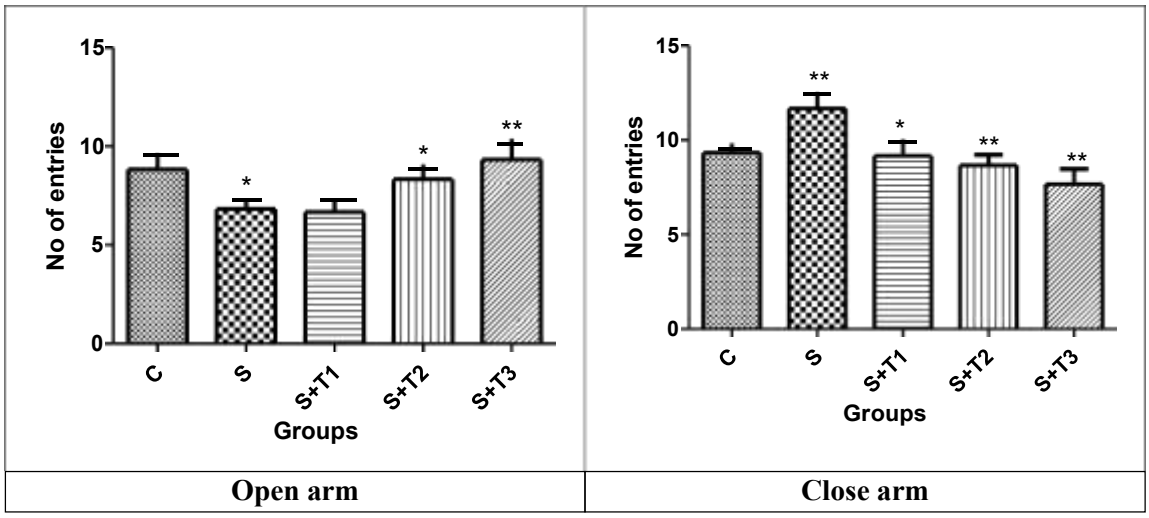


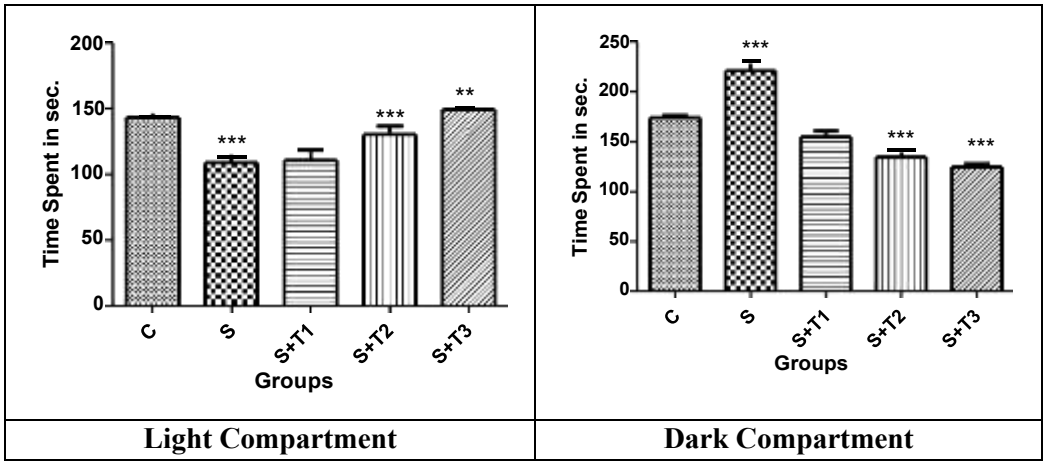
Fig. 2: Effect of BE administration on No of entries in EPM

Table 1: Effect of Banaba Extract administration on restrainer induced stress by using EPM

Sr. No	Groups	Open arm		Close arm	
		Duration	Entries	Duration	Entries
1	Control	138.8±2.400	8.833±0.792	146±2.028	9.333±0.494
2	Stress	110.2±6.024*	6.833±0.477*	172±2.129***	11.67±0.889**
3	S+T1	134±3.109	6.667±0.614	162±6.023*	9.167±0.872*
4	S+T2	149±4.297*	8.333±0.760*	128.2±2.386***	8.667±0.557**
	S+T3	157.2±6.685**	9.333±1.054**	123±3.286***	7.667±0.802**

Effect of Banaba Extract administration on restrainer-induced stress by using LDT

Fig.3,4 shows restrainer-induced stress in the stress group, as indicated by significantly ($p<0.01$) more time spent and no entries in the dark compartment as compared to the light compartment. Contrarily, administration of Banaba Extract was observed significantly ($p<0.01$) more time spent and no. of entries in the light compartment as an antistress effect.



Light Compartment	Dark Compartment

Fig. 4: Effect of BE administration on No of entries in LDT

Table 2: Effect of Banaba Extract administration on restrainer-induced stress by using LDT

Sr No.	Group	Light Compartment		Dark Compartment	
		Duration(sec.)	Entries	Duration(sec.1)	Entries
1	Control	143.3±3.323	10.33±0.667	174±2.176	10.50±0.763
2	Stress	109.2±5.770***	8.167±1.014*	221±7.700***	12.50±0.763**
3	S+T1	110.8±7.670	8.500±0.118	155±5.983	10.50±0.428
4	S+T2	130.5±6.201***	9.000±0.517*	134.7±5.308***	9.333±0.881**
5	S+T3	149.2±1.833**	10.33±0.421*	124.2±3.169***	8.667±0.714**

Effect of Banaba Extract Administration on Restrainer stress by using TST

Fig.5 shows that restrainer-induced stress in the stress group significantly($p<0.001$) increases the duration of immobility as compared to the control group. In the drug-treated group S+T2, S+T3 significantly($p<0.01$) decreased the duration of immobility as compared to the control group and stress group as an antistress effect.

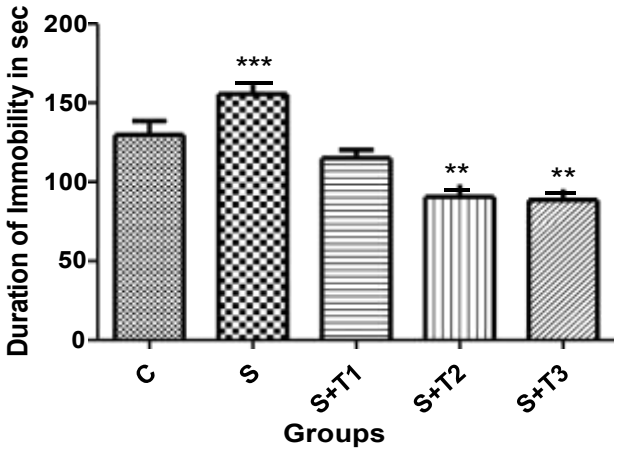


Fig. 5: Effect of BE administration on Duration of Immobility in TST

Table 3: Effect of Banaba Extract administration on restrainer-induced stress by using TST

Sr. No.	Groups	Duration of Immobility (sec)
1	Control	129.8±8.507
2	Stress	155.5±6.244***
3	S+T1	115.0±5.190

4	S+T2	90.50±7.991**
5	S+T3	88.50±7.065**

Effect of Banaba Extract administration on restrainer-induced stress by using OFT

Fig.6,7 shows restrainer-induced stress in the stress group has significantly ($p<0.01$) decreased the no. of the square crossing as compared to the control group. conversaly administration of Banaba Extract as more no of square crossing and grooming as compared to the control group.

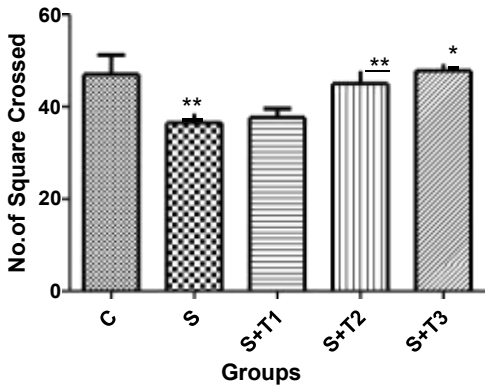


Fig.6: Effect of BE administration on no of square crossing in OFT

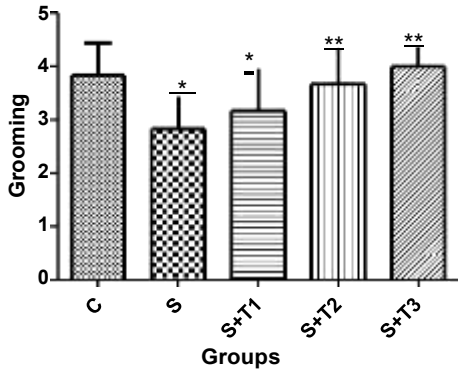


Fig. 7: Effect of BE administration on Grooming in OFT

Table. 4: Effect of Banaba Extract administration on restrainer-induced stress by using OFT

Sr. No.	Groups	No. of square crossing	Grooming
1	Control	47±4.211	3.833±0.600
2	Stress	36.50±2.045**	2.833±0.600*
3	S+T1	37.67±1.926	3.167±0.792*
4	S+T2	45±2.757**	3.667±0.666**
5	S+T3	47.83±1.493*	4.000±0.365**

Biochemical parameter

Effect of Banaba Extract administration on restrainer stress to Measure the cortisol level

Fig.8 shows that the cortisol level after 15 days of restraint stress was significantly ($p<0.001$) increased in the stress group when compared to the control group. Cortisol level was significantly($p<0.001$) decreased in drug-treated groups containing S+T3 when compared to the control group.

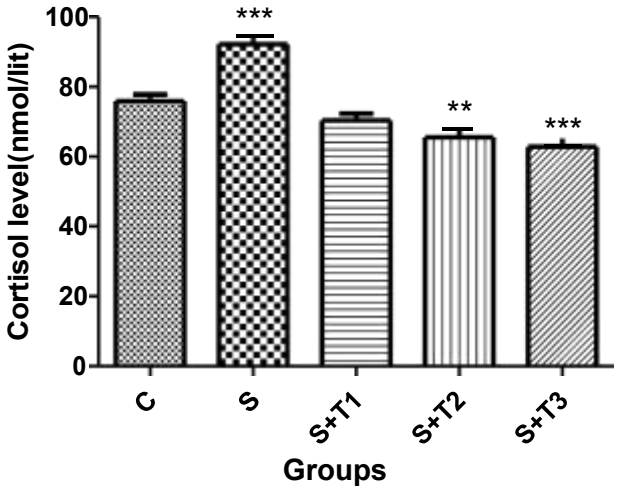


Fig. 8: Effect of BE administration on cortisol level

Table 5: Effect of BE administration on restrainer-induced stress to measure the cortisol level

Sr. No.	Groups	Cortisol Level
1.	Control	75.89 ±1.883
2.	Stress	92.25± 2.103***
3	S+T1	70.35±1.976
4	S+T2	65.52±2.508**
5	S+T3	62.91±2.285***

Antioxidant Parameter

Effect of Banaba Extract Administration on Restrainer induced stress to Measure the LPO Level Fig.9 shows restrainer restrainer-induced stress in the stress group has significantly($p<0.01$) increased the LPO level as compared to the control group. In drug drug-treated group S+T2, S+T3 has significantly($p<0.01$) decreased the LPO level as compared to the control group and stress group.

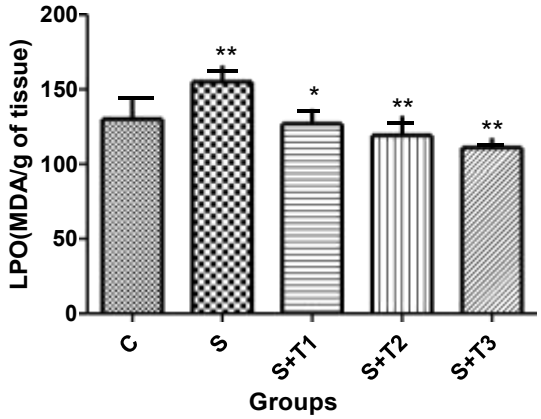


Fig. 9: Effect of BE administration on LPO Level

Table 6: Effect of Banaba Extract administration on restrainer induced stress to Measure the LPO Level

Sr. No.	Groups	LPO (MDA/g of tissue)
1	Control	133.03±13.50
2	Stress	155.2±10.92**
3	S+T1	127.1±9.668*
4	S+T2	119.2±13.26**
5	S+T3	111.0±6.582**

Effect of Banaba Extract Administration on Restrainer induced stress to Measure the NO Level

Fig.10 shows restrainer restrainer-induced stress in the stress group has significantly($p<0.01$) increased the NO level as compared to the control group. In drug drug-treated group S+T2, S+T3 has significantly($p<0.01$) decreased the NO level as compared to the control group and stress group.

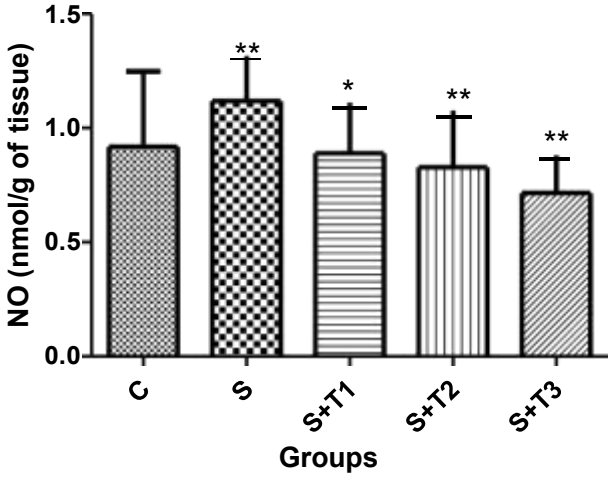


Fig. 10: Effect of BE administration on NO Level

Table 7: Effect of Banaba Extract Administration on Restrainer induced stress to Measure the NO Level

Sr. No.	Groups	NO (nmol/g of tissue)
1	Control	0.918±0.329
2	Stress	1.118±0.199**
3	S+T1	0.889±0.221*
4	S+T2	0.827±0.249**
5	S+T3	0.715±0.164**

Effect of Banaba Extract Administration on Restrainer induced stress to measure the SOD Level

Fig.11 shows restrainer-induced stress in the stress group has significantly($p<0.001$) decreased the SOD level as compared to the control group. In the drug-treated group S+T2, S+T3 has significantly($p<0.01$) increase the SOD level as compared to the control group and stress group.

Effect of Banaba Extract Administration on Restrainer induced stress to measure the SOD Level

Fig.11 shows restrainer-induced stress in the stress group has significantly($p<0.001$) decreased the SOD level as compared to the control group. In the

drug-treated group S+T2, S+T3 has significantly($p<0.01$) increase the SOD level as compared to the control group and stress group.

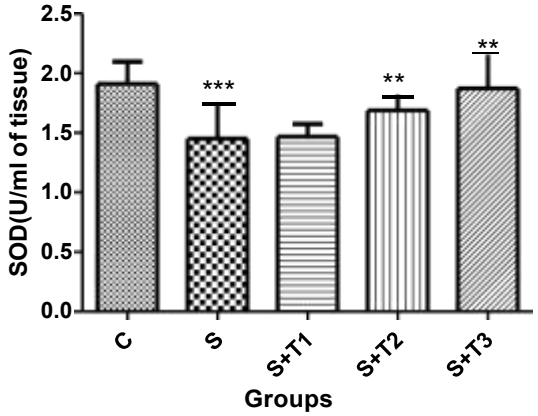


Fig. 11: Effect of BE administration on SOD Level

Table 8: Effect of Banaba Extract Administration on Restrainer induced stress to measure the SOD Level

Sr. No.	Groups	SOD (U/ml of tissue)
1	Control	1.912±0.184
2	Stress	1.452±0.301***
3	S+T1	1.468±0.106
4	S+T2	1.688±0.134**
5	S+T3	1.872±0.285**

Effect of Banaba Extract Administration on Restrainer induced stress to Measure the CAT Level

Fig.12 shows restrainer restrainer-induced stress in the stress group has significantly($P<0.01$) decreased the CAT level as compared to the control group. In drug drug-treated group S+T2 has a significant ($P<0.001$) increase in the CAT level as compared to the control group and stress group.

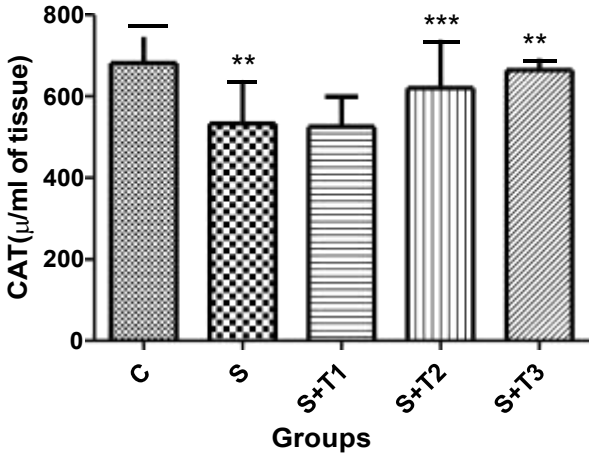


Fig. 12: Effect of BE administration on CAT Level

Table 9: Effect of Banaba Extract Administration on Restrainer induced stress to Measure the CAT Level

Sr. No.	Groups	CAT (µ/ml of tissue)
1	Control	681.6±64.65
2	Stress	533.1±104.8**
3	S+T1	525.6±73.61
4	S+T2	620.1±117.7***
5	S+T3	664±28.76**

DISCUSSION

In the current investigation, we assessed the effect of Banaba Extract on restrainer-induced stress in mice. provide information about the medicinally protective effect of Banaba Extract on restrainer-induced stress in mice. Specific hormone flow forms the hypothalamic-pituitary-adrenal (HPA) axis and is the primary system underlying stress physiology (31). In blood circulation, adrenal glucocorticoids reach vast levels of total plasma concentrations approximately 30 min after activation of the HPA axis (32). To demonstrate the difficulty of the stress reaction and the multifaceted manifestations are the leading ideas of experimental models used for stress induction in wet lab animals. Restrainer stress is an established animal model for psychological stress. Restraint stress induces oxidative stress through the elevation of corticosterone. The development of oxidative stress can be understood from the changes in the status of Antioxidant enzymes and lipid peroxidation particularly in the hypothalamus and hippocampus. Restraint stress-induced oxidative stress disrupts functions of the hypothalamus and hippocampus particularly disruptions in special recognition, memory, and anxiety were observed. Moreover, Restraint stress-induced oxidative stress altered the cytokine profile of animals, namely IL6, and IL10 which are associated with psychological stress (33). In this study, various behavioral parameters are assessed observed locomotor activity, anxiety, and depression. The elevated plus maze and light and dark area are the most commonly employed tests for assessing anxiety-like behavior after restrainer-induced stress. (34). In the elevated plus maze open arm/light compartment is more fear-provoking than the closed arm. The ratio of entries and time spent in open arms/closed arms reflects the animal perception of safety towards closed arms and fearfulness towards open arms (35). Changes in the emotional state of mice were evaluated in terms of changes in exploratory activity, i.e. total locomotor activity of square crossing, and grooming. (36). The elevated plus maze is the most validated test for assessing anxiety-like behavior in rodents. (37). In present study shows that EPM test restrainer-induced stress has increased the time spent and no. of entries in the closed arm. After the drug (BE) treatment significantly increases the time spent and no.of entries in open arms. The light-dark test is an exploration-driven test that relies on the voluntary locomotion of rodents. In general, rodents present a tendency to stay in a relatively safe area (the dark chamber of the box) versus a more aversive area (the light chamber of the box) (38). In present study shows that 15 days of restrainer-induced stress has significantly increased the time spent and no. of entries in the dark compartment. In drug (BE) treatment significantly increases the time spent and no. of entries in the light compartment as an antistress effect. The tail suspension test for assessing depression-like behavior in rodents. In general, rodents have increased the duration of immobility. In present study shows in mice restrainer induced stress to increase the duration of immobility. In

drug treatment to significantly decrease the duration of immobility, this is indicated as the anti-depressive activity. The open-field test is used to evaluate the animal's emotional state (39). In present study shows restrainer-induced stress in mice has decreased the no. of crossing and grooming, as stress rodent. After drug (BE) treatment significantly increase no. of square crossing and grooming as an antistress effect. The restrainer induced stress which in turn increased the spontaneous production of free radicals resulting in oxidative stress. Excessive ROS can cause oxidative damage to the brain, especially the hypothalamus and hippocampus. Damage to hippocampus cells ultimately can cause a deficiency in cognitive functions including memory and neuro-behavioural changes. In addition to that, the possible role of Elevated IL6 in mood disorders and depression has already been reported. (40,41) Hence, collectively abnormal elevation of corticosterone, IL6, and excessive ROS might have affected the memory function of stressed mice. This study shows an elevation of cortisol levels after restraint stress indicating the actuation of the hypothalamic-pituitary-adrenal (HPA) axis and to increase in the cortisol level after stress. In drug (BE) treatment 15 days to decrease the cortisol level. In addition, the hypothalamus also controls the secretion of the stress hormone cortisol via the HPA axis. Stress affects the nitric oxide level as an antioxidant enzyme. The present study shows that restrainer-induced stress increases the level of Nitric Oxide. In treatment of drug (BE) significantly decreases the level of NO. Oxidative stress induced by restrainer stress affects Lipid Peroxidation. High LPO observed in the hypothalamus and hippocampus is due to oxidative stress induced by restraint stress. The present study shows an increase in the level of lipid peroxidation by restraint-induced stress. In-drug (BE) treatment significantly decreases the level of LPO.

Superoxide Dismutase is an antioxidant enzyme. The decrease in the SOD observed in the hypothalamus and hippocampus is due to oxidative stress induced by restraint stress. In-drug (BE) treatment significantly increases the SOD level. The present study shows restraint restraint-induced stress produces oxidative stress and decreases the level of CAT. In-drug (BE) treatment significantly increases the level of CAT.

CONCLUSION:

Our study suggests that the preclinical restrainer model is effective to induced stress in laboratory mice. Stress assessment in animals is done at various levels i.e., behavioral level, biochemical (cortisol level), and antioxidant level. Also, we report that the administration of Banaba Extract may prevent restrainer-induced stress.

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