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Original Article

Synergistic Anti-Stress Effect of Selected Medicinal Plants in Mice

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ABSTRACT

Background: The discovery of efficacious anti-stress therapies is imperative, as chronic stress plays a substantial role in the development of numerous physical and mental health issues. **Materials and Methods:** The present study evaluates the synergistic anti-stress effects of co-administration of *Terminalia tomentosa* and *Withania somnifera* in mice. In present study, aqueous extracts of *Terminalia tomentosa* and *Withania somnifera* were administered orally to mice individually and in combination. The mice were then subjected to chronic stress models, including the Restraint stress, Elevated Plus Maze (EPM), Open Field apparatus (OF), Light and Dark model (LD) to evaluate the efficacy of the treatments. The combined treatment of *Terminalia tomentosa* and *Withania somnifera* resulted in a significant increase in time spent in open arm, number of square travelled time in combination as compared to individual treatments. **Results and Discussion:** Biochemical assays revealed that the combination therapy markedly reduced plasma corticosterone levels, a key stress hormone. Additionally, the antioxidant defense system was also enhanced, as evidenced by elevated activities of Glutathione (GSH) and Lipid Peroxides (LPO). **Conclusion:** These results suggest that the combination of *Terminalia tomentosa* and *Withania somnifera* exerts a synergistic effect in mitigating stress, highlighting their potential as a natural therapeutic option for stress management.

Keywords: Anti-stress activity, LPO, Stress, *Terminalia tomentosa*, *Withania somnifera*.

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INTRODUCTION

Stress can be characterized as any circumstance that upsets the homeostasis of the body. The body's homeostatic mechanisms become defective when stress levels are too high, posing a threat to the organism's ability to survive (Lakshmi and Sudhakar 2009). It is believed that stress plays a significant role in the development of various diseases. Social issues like hypertension, peptic ulcer illness, diabetes, immunodeficiency, regenerative disappointment, and uneasiness are brought about by the association of the focal sensory system (CNS), endocrine, and metabolic frameworks (Rai *et al.*, 2003).

Terminaria tomentosa is a member of the family *Combretaceae*. Steroids, carbohydrates, flavonoids, triterpenoids, tannins, and saponins are all found in the bark (Saggu *et al.*, 2007). It has different therapeutic properties including hostile to leukemia, against glucose, against oxidant, hostile to contagious and against loose bowels. This plant's bark is useful for bronchitis, wounds, bleeding, burns, broken bones, and diarrhea (Jitta *et al.*, 2019). It also has astringent properties. Flavonoids, polyphenols, and tannins were found in T. tomentosa skin, which was the subject of a phytochemical investigation. As far as anyone is concerned, no investigations have been finished on the calming and against rheumatic impacts of *T. tomentosa*. Its anti-inflammatory and anti-rheumatic effects are the subject of this study (Gupta, 2003; Jitta *et al.*, 2019).

Withania somnifera is member of the family *Solanaceae*, an Ayurvedic wonder tree regularly found in the Indian subcontinent (Widodo *et al.*, 2007). It is a significant native restorative plant to treat many problems connected with pressure and the focal sensory system (CNS) like pressure, a sleeping disorder, uneasiness, joint inflammation, and Alzheimer's Illness (Kataria *et al.*, 2013). It also contributes significantly to the treatment and prevention of drug addiction (Kulkarni and Dhir, 2008; Prakash *et al.*, 2013). *Withania somnifera* has antioxidant and free radical scavenging properties that were demonstrated in a MPTP-treated mouse model of Parkinson's disease (Rajasankara *et al.*, 2009).

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Researchers have recently focused their attention on synergy-based herbal formulations made up of several plant extracts. The enhanced efficacy of a formulation based on polyherbal extract is linked to its synergistic effect. Synergy-based formulations take use of the fact that different diseases have complex underlying pathophysiologies and are caused by a multitude of factors. As a result, it is thought that these formulations can increased the active ingredients' bioavailability, enhanced their therapeutic effects, and lessen their harmful consequences (Parveen *et al.*, 2021).

Withania somnifera and *Terminaria tomentosa* synergistic anti-stress effects on mice were the focus of this study. Frequently, compounds with against stress movement prompt enemy of stress impacts through changes in the degrees of different biochemical markers, Such as, corticosterone, GSH, LPO (Sandhya *et al.*, 2011).

This study aims to develop and implement suitable animal models that replicate stress conditions for assessing the anti-stress effects of the selected medicinal plants, to administer precise doses of selected medicinal plant extracts to the experimental animals to evaluate their individual and synergistic anti-stress effects, to measure and analyze physiological parameters such as hormonal levels, immune responses, and oxidative stress markers to quantify the anti-stress effects of the medicinal plant extracts, and to investigate and quantify any synergistic anti-stress effects that may arise from the combination of selected medicinal plants, providing insights into potential additive or enhanced benefits.

MATERIALS AND METHODS

Materials

Animals

The basic biological material in our study was the white mice *(Mus musculus*) from the strain Albino in the number of 30 mice, obtained from LACSMI Bio farms (Laboratory Animal Centre for Safe Medical Innovations),12, Rachana Blossom, Jagdish Nagar, Aundh, Pune, India was house in polypropylene cages and maintained at 16 days light\dark cycle under controlled temperature (22-25ºC) and relative humidity (50-60%). The animal house was registered under the CPSCEA with registration no.1566/PO/Re/S/11 CPSEA as of 23/11/2011The experiment was conducted in accordance with the Committee for the Purpose of Control and Supervision of Experimental Animals, and the animals were given unrestricted access to normal pellet food and water. (CPCSEA) under the study protocol no KBH/IAEC/2023/12-6. The mice were kept in plastic cages at a steady temperature of 23±1ºC with a 12-hr. light/12-hr dark cycle to adapt them to the natural photoperiod standards. The cages were cleaned and the bedding was changed once every two days. The mice had full access to conventional rodent food and water.

Plant materials

Organic *Withania Somnifera* (WS) root and *Terminalia tomentosa* (TT) bark was obtained from Pandit Jawaharlal Nehru Botanical Garden - Butterfly Garden, Nashik, Maharashtra. The plant materials authenticated from Botany department of M S G College Malegaon, have been used for future reference with a voucher specimen number is MSG/PG/BOT-83-16/01/2024.

Methods

Preparation of aqueous extract of Terminalia tomentosa and Withania somnifera by decoction

For this extraction, we followed herbalists' recommendations and used the decoction method. 100 mL of distilled water were combined with 5 g of crushed dried plant. After the mixture boiled, the temperature was lowered until 50 mL, or half of the original volume, of liquid remained. Whatman paper was then used to filter the mixture and then was dried individually.

Experimental design

Thirty Swiss albino mice of both sexes aged 6-8 weeks and weighing 20-25 g. Over the course of the trial, which lasts for the 16 days, the mice were divided into 6 groups each including 6 animals. Group I (VC) received distilled water at a dose of 5 mL/kg orally. Group II (Stressed) was subjected to stress only. Group III (Standard) was administered Diazepam at a dose of 2 mg/kg intraperitoneally. Group IV (TT) received *Terminalia tomentosa* at a dose of 200 mg/kg orally. Group V (WS) was given *Withania somnifera* at a dose of 100 mg/kg orally. Group VI (TT+WS) received a combination of *Terminalia tomentosa* and *Withania somnifera* at doses of 50 mg/kg and 100 mg/kg, respectively, orally each day from day 1 to day 16.

Behavioral tests

Every behavioral test was conducted between 09:00 and 16:00, which is the light phase of the circadian cycle. Every behavioral test was spaced out by at least one day from the next. The order of the mice's tests was random. After testing, the equipment was cleaned with 70% ethanol and water containing superoxidized hypochlorous to eliminate any bias resulting from smell cues. The following order was followed for conducting the behavioral tests. The oral method was used to deliver the aqueous extract of TT and WS half an hour before to the behavioral test. Diazepam was administered ip to the standard group mice. A comparable volume of vehicle was given to the control mice (Yang *et al.*, 2011).

Stress

Mice in the experimental groups were subjected to restraint stress once per day for 7 days. TT and WS were given to the experimental groups 30 min before the restraint stress in order to evaluate the anti-stress effects. The mice were kept in a tightly fitted ventilated plastic restrainer tube for 6 hr without access to food or water.

This process was repeated for 7 days. The control mice were kept in a separate room, with no contact with the stressed mice, and were administered the vehicle (Ueno *et al.,* 2019).

Locomotor activity

The animal moves in a square arena on an actophotometer. A photoelectric cell coupled in circuit with a counter power an actophotometer, which makes it simple to measure the horizontal activity, or locomotor activity. Counts are kept when an animal breaks the light beam falling on the photo cell. After 10 min of each animal being in the Actophotometer, the activity score for each animal was tallied. The lower activity score was seen as a stress indicator (Raj *et al.*, 2018).

Elevated plus maze

Carobrez *et al.*, 2005 gave the method of Elevated plus maze test. The device had two open arms measuring 8 x 25 cm and two closed arms measuring the same with transparent walls that rose to a height of 30 cm. The arms was 40 cm above the ground and composed of white plastic plates. The identical type of arms was positioned across from one another. For 10 min, each mouse was let to roam freely between the two closed arms while facing one of the squares in the center of the maze. Video recordings and analysis were done on the quantity of arm entries, distance traveled (cm), latency to enter the open arms (s), and amount of time spent in the arms (Carobrez and Bertoglio 2005).

Open field apparatus

Chen *et al.*,2024 studied the method for Open field apparatus. Every mice was positioned in the middle of an open field apparatus measuring 60 × 60 × 40 cm. Both the number of rearings and the number of squares traveled were noted. The 30×30 cm rectangle in the field's middle was designated as the central area. Each mice were recorder for the 5 min. The video recordings and analysis were done (Chen *et al.,* 2024).

Light and dark model

The light/dark transition test studied by Sasibhushana *et al.*, 2024. The device was a cage (60×60×40 cm) split into two rooms of the same size, each with a tiny rectangular entrance (5×3 cm) that allowed entry to a chamber. There was 400 lux of strong illumination in one chamber and 10 lux of darkness in the other. For 10 min, mice were placed in the dark chamber and given full reign to wander between the two rooms. Video recordings and analyses were made of the following: the length of time spent in the light chamber(s), the total number of transitions, the latency to enter the chamber(s), and the distance traveled within the chamber (in centimeters) (Chaouloff *et al.,* 1997).

Biochemical test

At the end of the study the animals were anesthetized using ketamine and the blood was withdrawn by retro-orbital plexus. The blood was settled for some time by keeping it ideal and was kept in centrifuge (remi) to separate serum. The separated serum was sent to Shri bios innovations laboratory, Pune for the estimation of Serum-cortisol level. After this the animals were euthanized using excess dose of ketamine. The tissue was excised washed with normal saline and was further sent to Shri bios innovations laboratory, Pune for the estimation of antioxidants (GSH, MDA) (Sasibhushana *et al.*, 2024; Tiwari *et al.,* 2017).

Statistical Analysis

The Mean±Standard deviation (SD; n=6) is the data's expression. A statistical analysis was performed with the software Graph Pad Prism 5.0. One-way Analysis of Variance (ANOVA) was performed, followed by Bonferroni's multiple comparison t-test, respectively, to determine statistical differences. Significance levels were indicated as $^{*}p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$ compared to the disease/vehicle control group, and $\#p<0.05$ compared to the vehicle control.

RESULTS

Locomotor activity

Restraint stress reduced the activity of animal. It was measured in actophotometer as locomotor activity Stress group (DC), Standard group (STD), *Terminalia tomentosa* (TT), *Withania somnifera* (WS) and TT+ WS compared with Vehicle control shows significant decrease in locomotor activity that indicates the groups rather than VC mice had stress shown in Figure 1 and Table 1.

Mice Behavior on Elevated Plus Maze Model

The effects of TT and WS was demonstrated in Figure 2 and Table 2 demonstrates both alone and in combination, on the time spent in the open arm, number of open arm entries, and anxiety index in the elevated plus maze model on Day 16. The Vehicle Control group (VC) exhibited 33.17±4.916% time spent in the open arm, 14.33±3.559 open arm entries, and an anxiety index of 0.41±0.05514. The Disease Control group (DC) showed

Table 1: Locomotor Activity in Actophotometer.

Group	Treatment	Locomotor Activity in Actophotometer
VC	Vehicle control	142.0±4.69
DC	Disease Control	125.2±4.30$^{\#\#\#}$
STD	Diazepam	122.0±5.29$^{\#\#\#}$
TT	*Terminalia tomentosa*	121.8±6.55$^{\#\#\#}$
WS	*Withania somnifera*	124.0±7.48$^{\#\#\#}$
TT+WS	*Terminalia tomentosa* + *Withania somnifera*	127.2±4.26$^{\#\#}$

Values in results are expressed as Mean± SD, (n=6), $\#\#\#p<0.001$ and $\#\#p<0.01$ significantlydifferent in comparison to vehicle control).

a significant decrease in these measures, with 10.17±2.317% time spent in the open arm (#p<0.05), 1.333±1.033 open arm entries (#p<0.05), and an anxiety index of 0.9417±0.06047. Diazepam (STD) at a dose of 2 mg/kg significantly increased the time spent in the open arm to 32.17±6.274% (p<0.001), the number of open arm entries to 11.5±2.168 (p<0.001), and decreased the anxiety index to 0.43±0.09423 (p<0.001). TT at 200 mg/kg resulted in a significant increase in time spent in the open arm to 19.5±4.037% (p<0.001), the number of open arm entries to 7.333±1.633 (p<0.001), and an anxiety index of 0.7183±0.04708 (p<0.001). WS at 100 mg/kg showed similar significant effects, with 20.83±6.047% time spent in the open arm (p<0.001), 6.5±2.429 open arm entries (p<0.001), and an anxiety index of 0.7517±0.05913 (p<0.001). The combination treatment of TT+WS at doses of 100 mg/kg and 50 mg/kg, respectively, further improved the outcomes with 30±2.757% time spent in the open arm (p<0.001), 12.67±2.50 open arm entries (p<0.001), and an anxiety index of 0.565±0.1019 (p<0.001). These results

Table 2: Effect of TT and WS alone and in-combination on (a)Time Spent in Open Arm, (b)No of Open arm Entries, (c)Anxiety Index of Elevated Plus Maze Model.

Group	Treatment	a) Time Spent in Open Arm	b) No of Open arm Entries	c)Anxiety Index
VC	Vehicle control	33.17±4.916	14.33±3.559	0.41±0.05514
DC	Disease Control	10.17±2.317#	1.333±1.033#	0.9417±0.06047#
STD	Diazepam	32.17±6.274***	11.5±2.168***	0.43±0.09423***
TT	*Terminalia tomentosa*	19.5±4.037*	7.333±1.633*	0.7183±0.04708**
WS	*Withania somnifera*	20.83±6.047**	6.5±2.429**	0.7517±0.05913**
TT+WS	*Terminalia tomentosa* + *Withania somnifera*	30±2.757***ƐΨΨ	12.67±2.50***ƐƐΨΨ	0.565±0.1019***ƐƐΨΨ

Values in the results are expressed as Mean±SD, (n=6), # p<0.05 significantly different in comparison to vehicle control. *p<0.05, **p<0.01, ***p<0.001, significantly different in comparison to disease control. Ψ significantly different in comparison to *Terminalia tomentosa*. Ɛ significantly different in comparison to *Withania somnifera*.

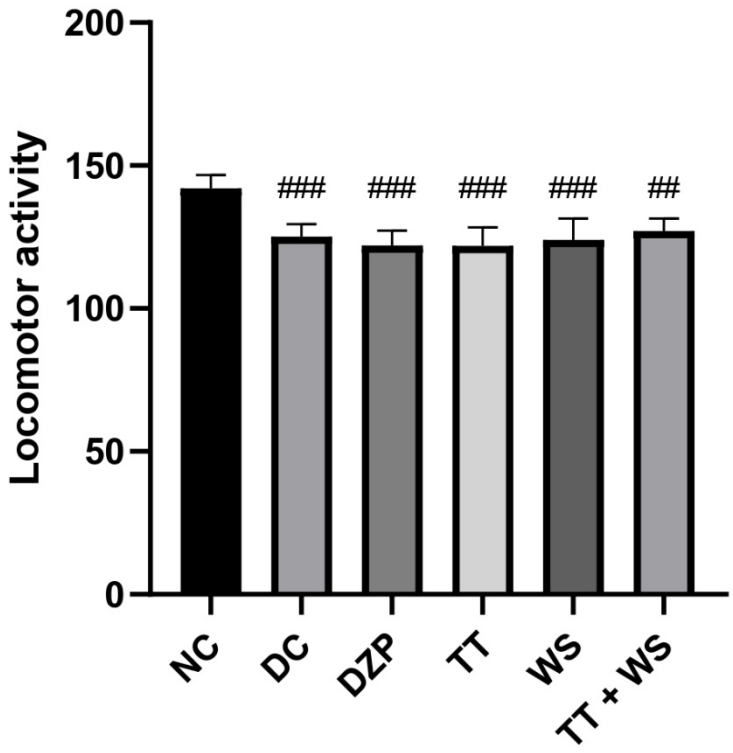


Figure 1: Locomotor activity in actophotometer. Values in the results are expressed as Mean±SD, (n=6), ###p<0.001 and ##p<0.01 significantly different in comparison to vehicle control.

indicate that TT and WS, both individually and in combination, significantly affect anxiety-related behaviors in the elevated plus maze model.

LIGHT AND DARK MODEL

The effect of TT and WS shown in Figure 3 and Table 3, both alone and in combination, on the time spent in the light area of the plus maze model on Day 16. The Vehicle Control group (VC) spent 38.5±6.317% of the time in the light area. The Disease Control group (DC) showed a significant increased, spending 13.17±1.722# of the time in the light area (#p<0.05). Diazepam (STD) at a dose of 2 mg/kg significantly increased the time spent in the light area to 30.67±4.082% (p<0.001). TT at 200 mg/kg resulted in an increased to 22.67±2.805% (p<0.01), while WS at 100 mg/kg led to a similar increased to 21.67±2.733% (p<0.05). The combination of TT+WS at doses of 100 mg/kg and 50 mg/kg, respectively, produced a significant increase in time spent in the light area to 32.5±3.937% (p<0.001, ƐΨ). These results suggest that both TT and WS, individually and in combination, effectively increase the time spent in the light area of the plus maze model, indicating potential anxiolytic effects.

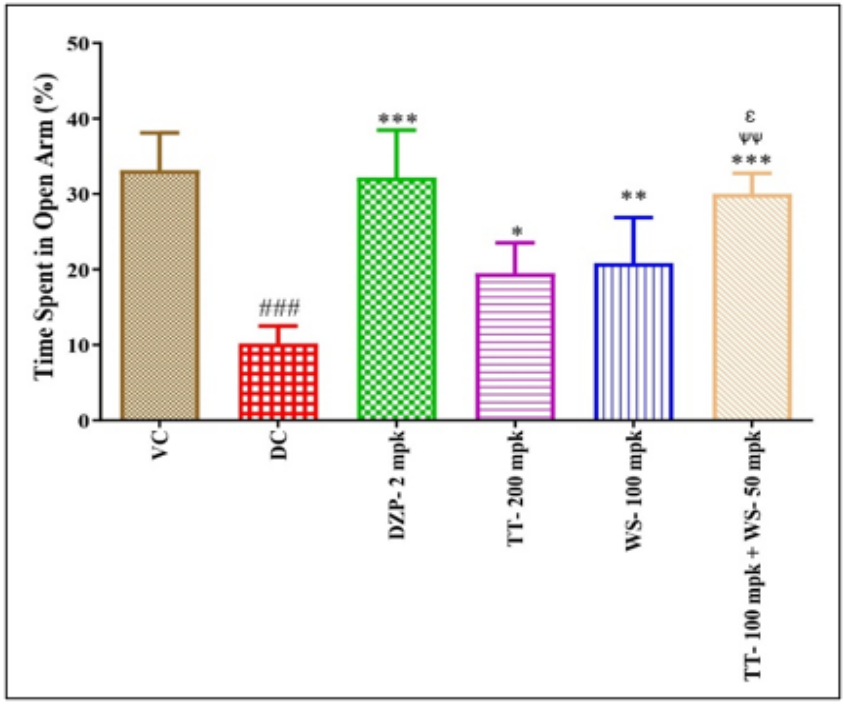
Open Field Test

The effects of TT and WS illustrated in Table 4 and Figure 4, both alone and in combination, on the number of crossings and rearings in the open field test on Day 16. The Vehicle Control group (VC) displayed 30.5±6.348 crossings and 12.33±3.011 rearings. The Disease Control group (DC) showed a significant decrease in the number of crossings to 6±2.366 (p<0.05) and a significant increase in the number of rearings to 36.17±2.229 (p<0.05). Diazepam (STD) at 2 mg/kg significantly increased the number of crossings to 29±6.387 (p<0.001) and significantly decreased the number of rearings to 14±3.033 (p<0.001). TT at 200 mg/kg resulted in a significant increase in the number of crossings to 15.83±4.262 (p<0.001) and a significant decrease in the number of rearings to 26±4.05 (p<0.01).WS at 100 mg/

Table 3: Effect of TT and WS alone and in-combination on Time Spent on Light area of Light and Dark Model.

Groups	Treatment	Time Spent on Light area (%)
VC	Vehicle Control	38.5±6.317
DC	Disease Control	13.17±1.722#
STD	Diazepam	30.67±4.082***
TT	*Terminalia tomentosa*	22.67±2.805**
WS	*Withania somnifera*	21.67±2.733*
TT+WS	*Terminalia tomentosa* + *Withania somnifera*	32.5±3.937***ƐƐƐΨΨ

Values in the results are expressed as Mean± SD, (n=6), # p<0.05 significantly different in comparison to vehicle control. *p<0.05, **p<0.01, ***p<0.001, significantly different in comparison to disease control. ƐΨ significantly different in comparison to *Terminalia tomentosa*. Ɛ significantly different in comparison to *Withania somnifera*.



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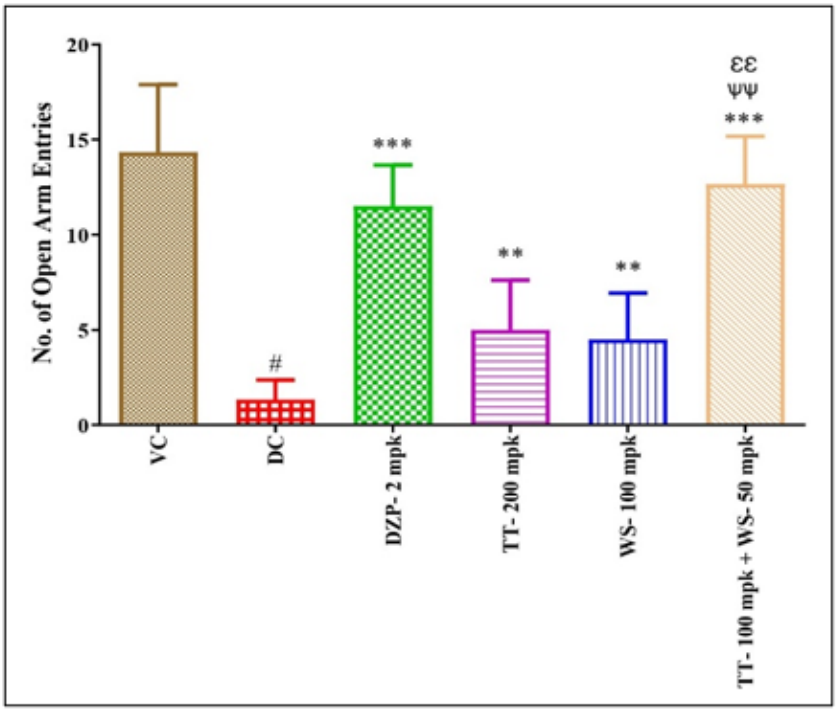

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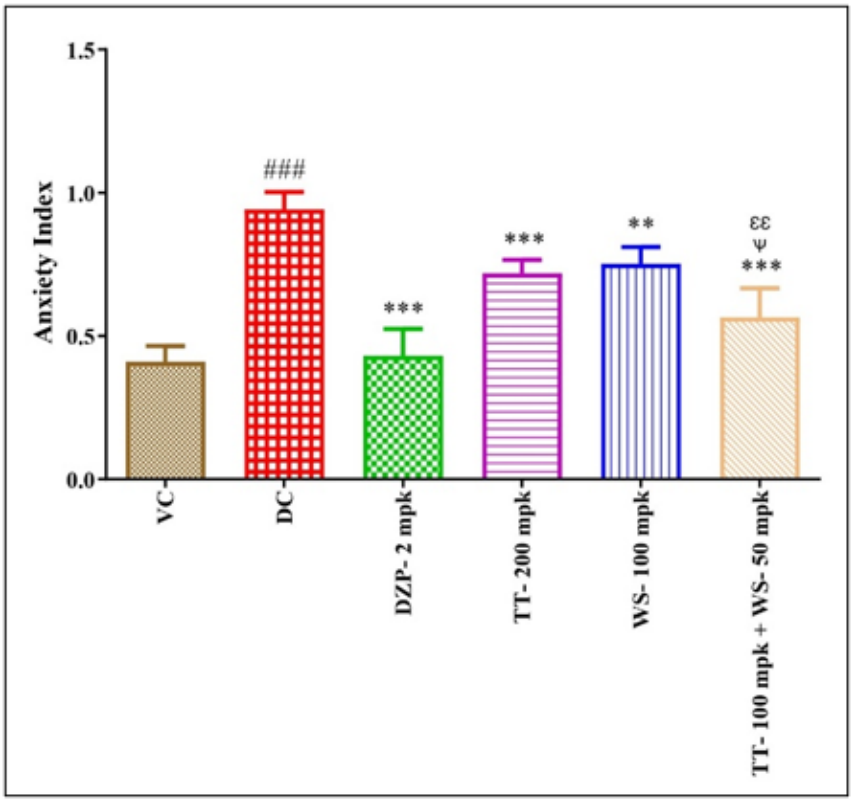


(c)

Figure 2: Effect of TT and WS alone and in-combination on (a)Time Spent in Open Arm, (b) No of Open arm Entries, (c)Anxiety Index of Elevated Plus Maze Model.

kg significantly increased the number of crossings to 16.83±3.817 (p<0.001) and decreased the number of rearings to 24.5±3.674 (p<0.01). The combination treatment of TT+WS at doses of 100 mg/kg and 50 mg/kg, respectively, led to a significant increase in the number of crossings to 26.67±3.777 (p<0.001, Ψ, Ɛ) and a significant decrease in the number of rearings to 17.00±3.74 (p<0.001). These results indicate that both TT and WS, individually and in combination, significantly affect exploratory behavior in the open field test on Day 16.

GSH Level in Brain Tissue

The study assessed the effect of TT and WS was demonstrated in Figure 5. Effect of TT and WS, both individually and in combination, on the GSH (Glutathione) levels in brain tissue. The findings are summarized in Table 5. The Vehicle Control group (VC) had a GSH level of 13.01±1.98 mmol/mg protein. The Disease Control group (DC) showed a significant reduction in GSH levels to 5.84±1.20 mmol/mg protein (p<0.05 compared to the vehicle control). Treatment with Diazepam (STD, 2 mg/kg) significantly restored the GSH levels to 12.43±1.99 mmol/mg protein (p<0.001 compared to the disease control) as shown in Figure 5. TT, 200 mg/kg resulted in a GSH level of 8.43±1.16 mmol/mg protein, while WS, 100 mg/kg increased the GSH levels to 8.97±1.13 mmol/mg protein (p<0.05 compared to the disease control). The combination treatment TT+WS, 100 mg/kg +50 mg/kg) significantly increased the GSH levels to 11.85±1.46 mmol/mg protein (p<0.001 compared to the disease control. Additionally, the GSH levels in the combination group were significantly higher compared to the TT group (p<0.05) and the WS group (p<0.05). These findings suggest that both TT and WS help in restoring GSH levels, with a more pronounced effect when used in combination.

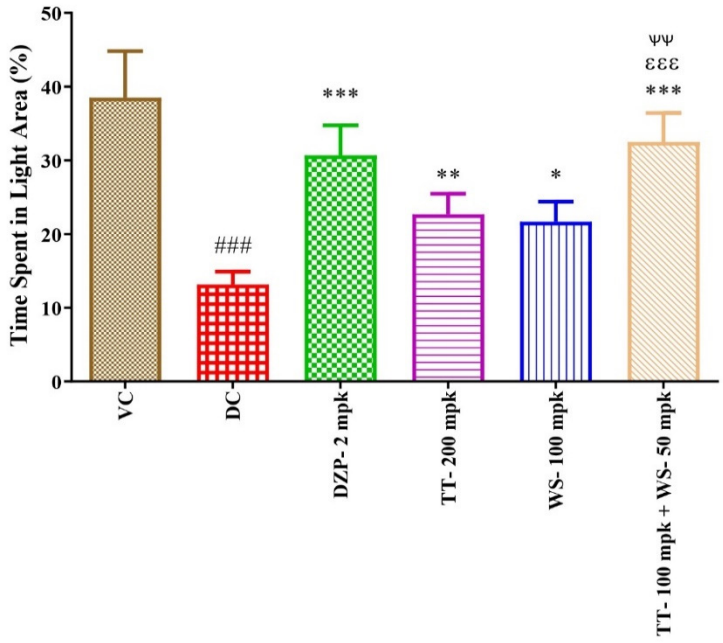
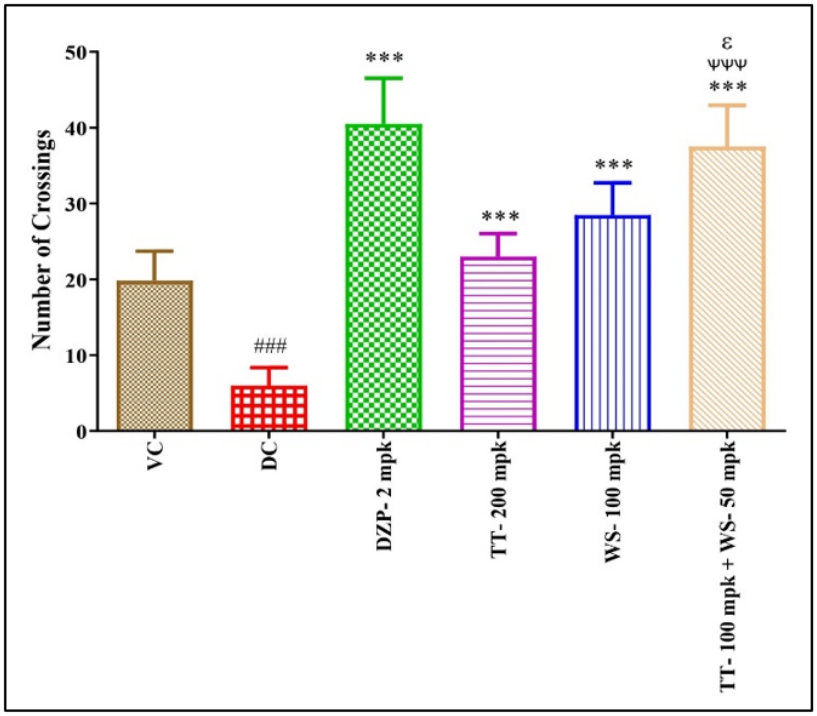


Figure 3: Effect of TT and WS alone and in-combination on Time Spent on Light area of Light and Dark Model.

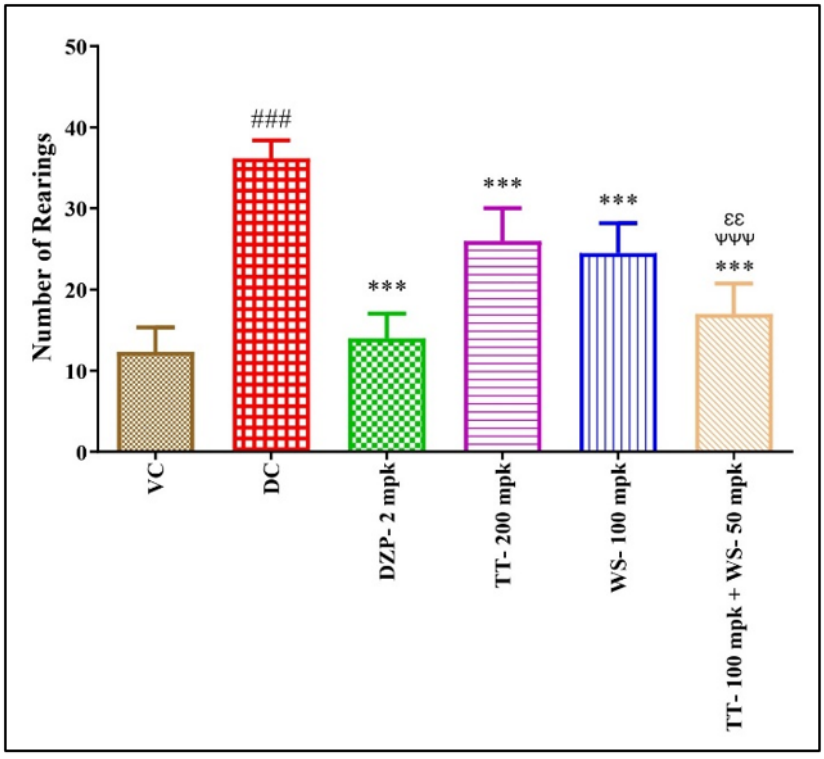
Table 4: Effect of TT and WS alone and in-combination on (a) Number of Crossings and (b) Number of Rearings of Open Field Test.

Group	Treatment	Number of Crossings	Number of Rearings
VC	Vehicle control	30.5±6.348	12.33±3.011
DC	Disease Control	6±2.366#	36.17±2.229#
STD	Diazepam	29±6.387***	14±3.033***
TT	*Terminalia tomentosa*	15.83±4.262***	26±4.05**
WS	*Withania somnifera*	16.83±3.817***	24.5±3.674**
TT+WS	*Terminalia tomentosa* + *Withania somnifera*	26.67±3.777***ΨΨΨ^{67}	17±3.742***ΨΨΨ^{4}

Values in the results are expressed as Mean±SD, (n=6), # p<0.05 significantly different in comparison to vehicle control. *p<0.05, **p<0.01, ***p<0.001, significantly different in comparison to vehicle control. Ψ significantly different in comparison to *Terminalia tomentosa*. Ɛ significantly different in comparison to *Withania somnifera*.



(a)



(b)

Figure 4: Effect of TT and WS alone and in-combination on (a) Number of Crossings and (b) Number of Rearings of Open Field Test.

Table 5: Effect of TT and WS alone and in-combination on GSH and MDA Level.

Groups	Treatment	GSH level in brain Tissue (mmol/mg protein)	MDA Level (nmol/mg protein)
VC	Vehicle control	13.01±1.98	15.72±2.30
DC	Disease control	5.84±1.20#	36.34±5.25#
STD	Diazepam	12.43±1.99***	19.00±2.15***
TT	*Terminalia tomentosa*	8.43±1.16	28.62±5.16*
WS	*Withania somnifera*	8.97±1.13*	27.89±4.11**
TT+WS	*Terminalia tomentosa* + *Withania somnifera*	11.85±1.46***ψ1	21.15±2.07***ψ

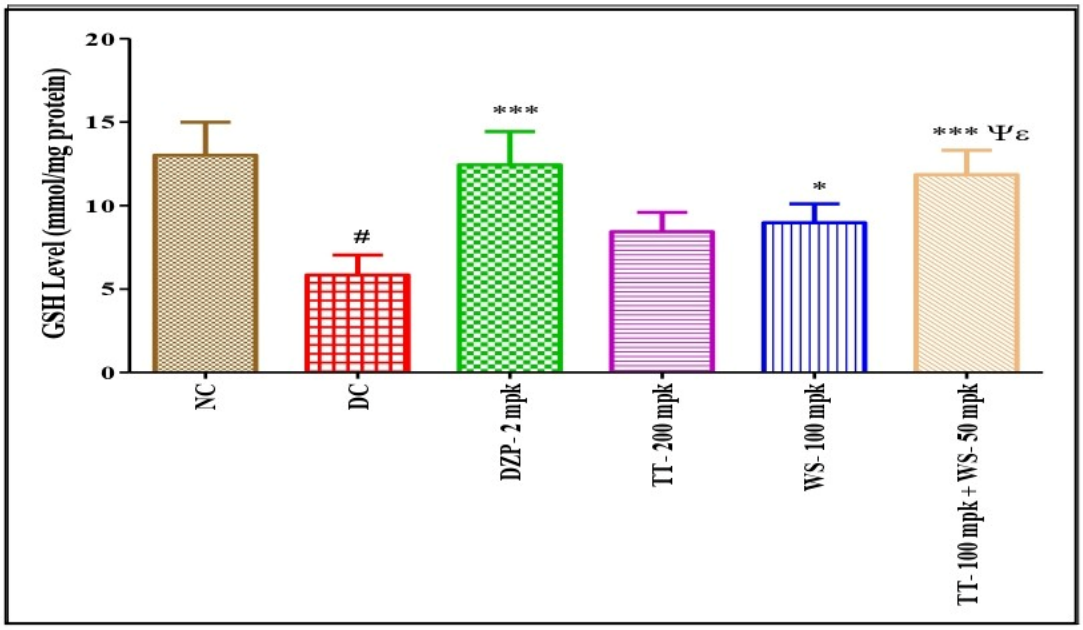


Figure 5: Effect of TT and WS alone and in-combination on GSH Level.

MDA Level in Brain Tissue

The study examined the effect of TT and WS was demonstrated in Figure 6. Effect of TT and WS, both individually and in combination, on MDA (Malondialdehyde) levels in brain tissue. The findings are summarized in Table 5. The Vehicle Control group (VC) had an MDA level of 15.72±2.30 nmol/mg protein. The Disease Control group (DC) showed a significant increased in MDA levels to 36.34±5.25 nmol/mg protein (p<0.05 compared to the vehicle control). Treatment with Diazepam (STD, 2 mg/kg) significantly reduced the MDA levels to 19.00±2.15 nmol/mg protein (p<0.001 compared to the disease control). TT, 200 mg/kg reduced the MDA levels to 28.62±5.16 nmol/mg protein (p<0.05 compared to the disease control), while WS, 100 mg/kg lowered the MDA levels to 27.89±4.11 nmol/mg protein (p<0.01 compared to the disease control). The combination treatment TT+WS, 100 mg/kg+50 mg/kg) significantly reduced the MDA levels to 21.15±2.07 nmol/mg protein (p<0.001 compared to the disease control) as shown in Figure 6. Additionally, the MDA levels in the combination group were significantly lower compared to the TT group (p<0.05). These results indicate that both TT and WS are effective in reducing MDA levels, with a more pronounced effect when used in combination.

Cortisol Level in Blood Serum

The study investigated the effects of TT and WS, both individually and in combination, on serum cortisol levels. The findings are summarized in Table 6. The Vehicle Control group (VC) had a serum cortisol level of 1.25±0.05 µg/mL. The Disease Control group (DC) showed a significant increased in serum cortisol levels to 11.80±1.83 µg/mL (p<0.05 compared to the vehicle control). Treatment with Diazepam (STD, 2 mg/kg) significantly reduced the serum cortisol levels to 2.99±1.08 µg/mL (p<0.001 compared to the disease control). TT, 200 mg/kg lowered the serum cortisol levels to 9.31±0.85 µg/mL (p<0.05 compared to the disease control), while WS, 100 mg/kg significantly reduced the cortisol levels to 6.98±1.49 µg/mL (p<0.001 compared to the disease control) as shown in Figure 7. The combination treatment TT+WS, 100 mg/kg +50 mg/kg) produced a significant reduction in serum cortisol levels to 4.73±1.19 µg/mL (p<0.001 compared

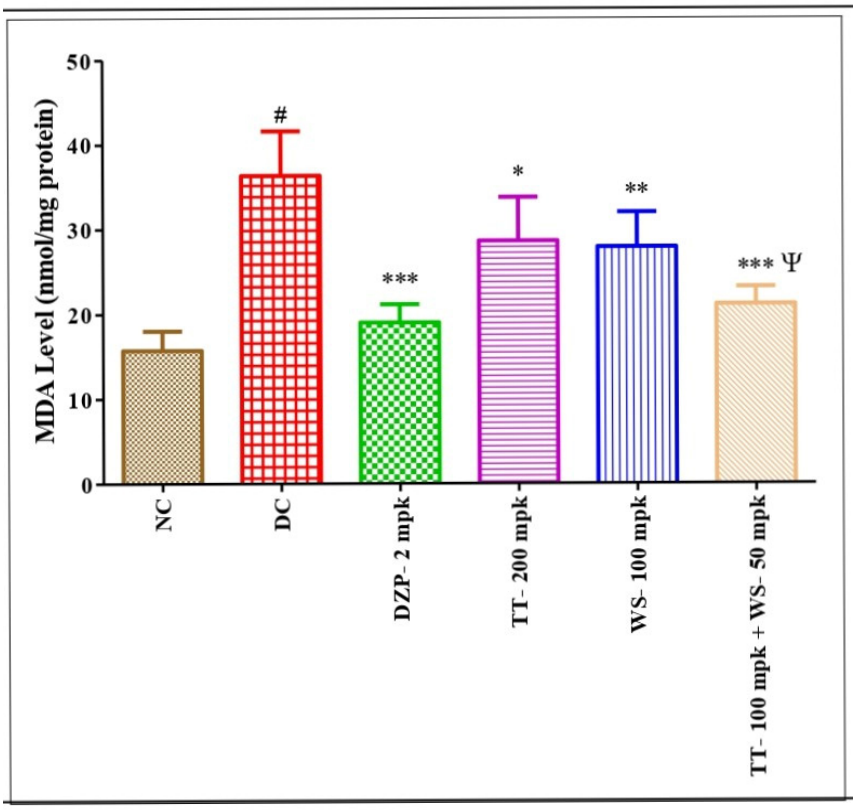


Figure 6: Effect of TT and WS alone and in-combination on MDA Level. Data are shown as mean± SD, (*n*=6), # *p*<0.05 significantly different in comparison to vehicle control. **p*<0.05, ***p*<0.01, ****p*<0.001, significantly different in comparison to vehicle control. Ψ significantly different in comparison to TT. Ɛ significantly different in comparison to WS.

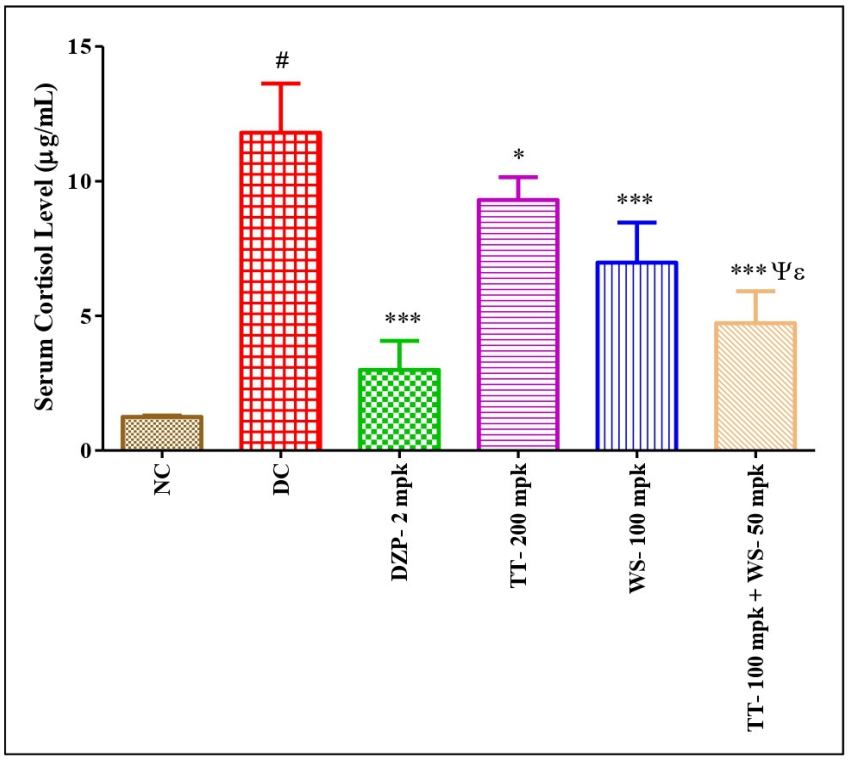


Figure 7: Effect of TT and WS alone and in-combination on Serum Cortisol Level. Data are shown as mean±SD, (*n*=6), # *p*<0.05 significantly different in comparison to vehicle control. **p*<0.05, ***p*<0.01, ****p*<0.001, significantly different in comparison to vehicle control. Ψ significantly different in comparison to TT. Ɛ significantly different in comparison to WS.

Table 6: Effect of TT and WS alone and in-combination on Serum Cortisol Level.

Groups	Treatment	Serum Cortisol Level (µg/mL)
VC	Vehicle control	1.25±0.05
DC	Disease Control	11.80±1.83#
STD	Diazepam	2.99±1.08***
TT	*Terminalia tomentosa*	9.31±0.85*
WS	*Withania somnifera*	6.98±1.49***
TT+WS	*Terminalia tomentosa* + *Withania somnifera*	4.73±1.19***ψƐ

to the disease control). Additionally, the serum cortisol levels in the combination group were significantly lower compared to the TT group ($p<0.05$) and the WS group ($p<0.05$). These findings suggest that both TT and WS are effective in reducing serum cortisol levels, with a more pronounced effect when used in combination.

DISCUSSION

The synergistic anti-stress effect of *Terminalia tomentosa* and *Withania somnifera* in mice was assessed through a series of behavioral and biochemical assays, including the Elevated Plus Maze (EPM), Open Field Apparatus (OF), Light and Dark (L and D) Method, Glutathione (GSH), Lipid Peroxidation (LPO), and cortisol levels. In the EPM test, mice treated with the combination and individual of these two herbs spent significantly more time in the open arms and made more entries into the open arms compared to controls and individual herb, indicating reduced anxiety-like behavior. The OF results showed that treated mice exhibited increased locomotor activity and spent more time in the central area, reflecting decreased anxiety and enhanced exploratory behavior. In the L and D test, the combination treatment led to a significant increase in the time spent in the light compartment and the number of transitions between light and dark areas, further suggesting reduced anxiety levels.

Biochemically, the combination therapy resulted in higher GSH levels, demonstrating enhanced antioxidant defense, and significantly lower LPO levels, indicating reduced oxidative stress. Additionally, treated mice showed a notable decrease in cortisol levels, a key biomarker of stress, compared to the control group. The observed reductions in both oxidative stress markers and cortisol levels underscore the potent synergistic effects of *Terminalia tomentosa* and *Withania somnifera* in mitigating stress. The combined anxiolytic and antioxidant properties of these herbal extracts contribute to their overall efficacy in reducing stress and anxiety, highlighting their potential as a natural therapeutic approach for stress management.

CONCLUSION

The present study evaluated the synergistic anti-stress effect of *Terminalia tomentosa* and *Withania somnifera* in mice. The results demonstrated that the combination of these two herbs produced a significant reduction in stress-induced biochemical and behavioral changes compared to the control and individual treatments. The combination therapy showed improved adaptation to stress, as evidenced by normalized levels of stress markers such as cortisol, GSH, LPO and enhanced performace in behavioral tests such as Elevated plus maze, Open field apparatus and Dark and light model.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

GSH: Glutathione Reductase; **LPO:** Lipid Peroxidation; **TT:** *Terminalia tomentosa*; **WS:** *Withania somnifera*; **EPM:** Elevated Plus Maze; **OF:** Open field apparatus; **LD:** Light and Dark model.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

All the animal experimental protocols employed in this study received thorough review and approval from the Animal Ethics Committee of the department of Pharmacology, KBHSS Trust Institute of Pharmacy, Malegaon.

REFERENCES

Carobrez, A. P., & Bertoglio, L. J. (2005). Ethological and temporal analyses of anxiety-like behavior: The elevated plus-maze model 20 years on. Neuroscience and Biobehavioral Reviews, 29(8), 1193–1205. https://doi.org/10.1016/j.neubiorev.2005.04.017

Chaouloff, F., Durand, M., & Mormède, P. (1997). Anxiety- and activity-related effects of diazepam and chlordiazepoxide in the rat light/dark and dark/light tests. Behavioural Brain Research, 85(1), 27–35. https://doi.org/10.1016/s0166-4328(96)00160-x

Chen, L., Lu, Y., Hua, X., Zhang, H., Sun, S., & Han, C. (2024). Three methods of behavioural testing to measure anxiety-A review. Behavioural Processes, 215, Article 104997. https://doi.org/10.1016/j.beproc.2024.104997

Gupta, A. K. (2003). Quality standards of Indian medicinal plants, 1. Indian Council of Medical Research.

Jitta, S. R., Daram, P., Gourishetti, K., Misra, C. S., Polu, P. R., Shah, A., Shreedhara, C. S., Nampoothiri, M., & Lobo, R. (2019). *Terminalia tomentosa* bark ameliorates inflammation and arthritis in carrageenan-induced inflammatory model and Freund's adjuvant-induced arthritis model in rats. Journal of Toxicology, 2019, Article 7898914. https://doi.org/10.1155/2019/7898914

Kataria, H., Wadhwa, R., Kaul, S. C., & Kaur, G. (2013). *Withania somnifera* water extract as a potential candidate for differentiation-based therapy of human neuroblastomas. PLOS One, 8(1), Article e55316. https://doi.org/10.1371/journal.pone.0055316

Kulkarni, S. K., & Dhir, A. (2008). *Withania somnifera*: An Indian ginseng [*Withania somnifera*: An Indian ginseng]. Progress in Neuro-Psychopharmacology and Biological Psychiatry, 32(5), 1093–1105. https://doi.org/10.1016/j.pnpbp.2007.09.011

Lakshmi, B. V. S., & Sudhakar, M. (2009). Screening of *Psidium guajava* Leaf extracts for anti-stress activity in different experimental animal models. Pharmacognosy Research, l(6), 359–366.

Parveen, A., Zahiruddin, S., Agarwal, N., Siddiqui, M. A., Ansari, S. H., & Ahmad, S. (2021). Modulating effects of the synergistic combination of extracts of herbal drugs on cyclophosphamide-induced immunosuppressed mice. Saudi Journal of Biological SciencesVCes, 28(11), 6178–6190. https://doi.org/10.1016/j.sjbs.2021.06.076

Prakash, J., Yadav, S. K., Chouhan, S., & Singh, S. P. (2013). Neuroprotective role of *Withania somnifera* root extract in maneb-paraquat-induced mouse model of Parkinsonism. Neurochemical Research, 38(5), 972–980. https://doi.org/10.1007/s11064-013-1005-4

Rai, D., Bhatia, G., Sen, Sen, T., & Palit, G. (2003). Anti-stress effects of *Ginkgo biloba* and *Panax ginseng*: A comparative study. Journal of Pharmacological Sciences, 93(4), 458–464. https://doi.org/10.1254/jphs.93.458

Raj, P. V., Rani, M. R., Nayak, R. P., & Rao, S. N. (2018). A study on anxiolytic activity and locomotor behavior of *Curcuma amada* rhizomes using *Wistar albino* rats. International Journal of Basic and Clinical Pharmacology, 7(8), 1557. https://doi.org/10.18203/2319-2003.ijbcp20183023

RajaSankar, S., Manivasagam, T., Sankar, V., Prakash, S., Muthusamy, R., Krishnamurti, A., & Surendran, S. (2009). *Withania somnifera* root extract improves catecholamines and physiological abnormalities seen in a Parkinson's disease model mouse. Journal of Ethnopharmacology, 125(3), 369–373. https://doi.org/10.1016/j.jep.2009.08.003

Saggu, S., Divekar, H. M., Gupta, V., Sawhney, R. C., Banerjee, P. K., & Kumar, R. (2007). Adaptogenic and safety evaluation of seabuckthorn (*Hippophae rhamnoides*) leaf extract: A dose dependent study. Food and Chemical Toxicology, 45(4), 609–617. https://doi.org/10.1016/j.fct.2006.10.008

Sandhya, K., Desai, M., Desai, N., Arya, P., & Pooja, T. (2011). Antistress activity of Boerhavia diffusa root extract and a polyherbal formulation containing *Boerhavia diffusa* using cold restraint stress model. International Journal of Pharmaceutical Sciences, 3(1), 130–133.

Sasibhushana, R. B., Shankaranarayana Rao, B. S., & Srikumar, B. N. (2024). Anxiety- and depression-like behavior following short-term finasteride administration is associated with impaired synaptic plasticity and cognitive behavior in male rats. Journal of Psychiatric Research, 174, 304–318. https://doi.org/10.1016/j.jpsychires.2024.04.029

Tiwari, S., Gupta, S., Laeeq, S., & Mahdi, A. A. (2017). Anxiety and depression as risk factor for the development of oral lichen planus and its association with blood antioxidant level. Journal of Oral and Maxillofacial Pathology, 5, 2.

Ueno, H., Shimada, A., Suemitsu, S., Murakami, S., Kitamura, N., Wani, K., Matsumoto, Y., Okamoto, M., Fujiwara, Y., & Ishihara, T. (2019). Anti-stress effects of the hydroalcoholic extract of *Rosa gallica* officinalis in mice. Heliyon, 5(6), Article e01945. https://doi.org/10.1016/j.heliyon.2019.e01945

Widodo, N., Kaur, K., Shrestha, B. G., Takagi, Y., Ishii, T., Wadhwa, R., & Kaul, S. C. (2007). Selective killing of cancer cells by leaf extract of Ashwagandha: Identification of a tumor-inhibitory factor and the first molecular insights to its effect. Clinical Cancer Research, 13(7), 2298–2306. https://doi.org/10.1158/1078-0432.CCR-06-0948

Yang, X., Wang, S., Rice, K. C., Munro, C. A., & Wand, G. S. (2008). Restraint stress and ethanol consumption in two mouse strains. Alcoholism, Clinical and Experimental Research, 32(5), 840–852. https://doi.org/10.1111/j.1530-0277.2008.00632.x

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