Bulletin of Environment, Pharmacology and Life Sciences
Bull. Env. Pharmacol. Life Sci., Vol 12 [10] September 2023 :299-304

Online ISSN 2277-1808
Journal's URL:http://www.bepls.com
CODEN: BEPLAD

ORIGINAL ARTICLE **OPEN ACCESS**

Adaptogenic Activity of D-Pinitol against Diverse Stressors Induced in Swiss Albino Mice

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ABSTRACT
D-Pinitol is evaluated for its adoptogenic activity/anti-stress activity through forced swimming test along with post-motor performance, tail suspension test, and elevated maze test. Four groups consisting of six Swiss albino mice (20–30 g each) were used for this investigation. Mice without administering any drug were kept as Control. Standard (Diazepam 2 mg/kg i.p.), test drug low dose (D-Pinitol 100 mg/kg) and test drug high dose (D-Pinitol 400 mg/kg) were administered to mice for assessing adaptogenic activity. Compared with the control group, all doses of D-Pinitol showed significant reduction in the immobility time for both tail suspension test and forced swimming test, increased swimming endurance time, and improved post-motor performance, including spontaneous motor activity and Rota rod falling time. In comparison to the control group, D-Pinitol (100 mg/kg and 400 mg/kg) significantly increased the amount of time that was spent in the open arm present in the Elevated Plus Maze. The previous study found that stress results in the production of free radicals. In animals, stress also causes hyperlipidemia, hypoglycemia, and a rise in serum cortisol levels. Based on previous research, capacity of D-Pinitol as an antioxidant, and lowering abnormal blood levels of glucose, cortisol, and cholesterol can be utilized to correct various pathological conditions aforementioned to treat and prevent stress. This study intended to show that D-Pinitol can prevent stress disorders at in vivo levels because it showed adaptogenic activity or anti-stress effect.
Keywords: *D-Pinitol, Diazepam, adaptogenic activity, forced swimming test, tail suspension test, elevated maze test.*

Received 23.07.2023 Revised 21.08.2023 Accepted 13.09.2023

INTRODUCTION

Adaptogens are stress-response modulators which improve a person's generalised resilience to stressful conditions by enhancing the capacity for survival and adaptation (1)(2). Adaptogens fall into two primary categories. Plant adaptogens fall under the first category, whereas synthetic adaptogens (also known as actoprotectors) fall under the second category (3). According to scientific evidence, high or continuous psychological stress can cause persistent disorders of neurological function, some of which entail the destruction of neuronal cells. Recent research is currently underway exploring the biological mechanisms of psychological stress activates or inhibits. In psychological stress, mechanism of stress induction involves activation of hypothalamic-pituitary-adrenal axis leading to release of excessive cortisol and occurrence of inflammation in the brain because of cytokines release leading to oxidative stress (4).
According to previously published research, people with psychological issues caused by stress could benefit from antioxidant supplement treatment given as an adjunct to conventional therapy. Therefore, antioxidant supplement therapy is beneficial for patients with stress-induced oxidative stress as an adjuvant therapy or therapy (6).
D-Pinitol, 3-O-methyl ether of D-Chiroinositol, is occurring in various plant sources prevalently including Gliricidia sepium, Bougainvillea spectabilis, Glycine max L Merr., and Tamarindus Indica Linn (7). D-Pinitol has been documented in more than 30 medical uses, including anti-diabetic, insulin-regulator, anti-Alzheimer's, anti-cancer, antioxidant, anti-inflammatory properties and hepatoprotective properties (8). As a result, this research study aimed to assess the adaptogenic activity of D-Pinitol using Swiss Albino mice.

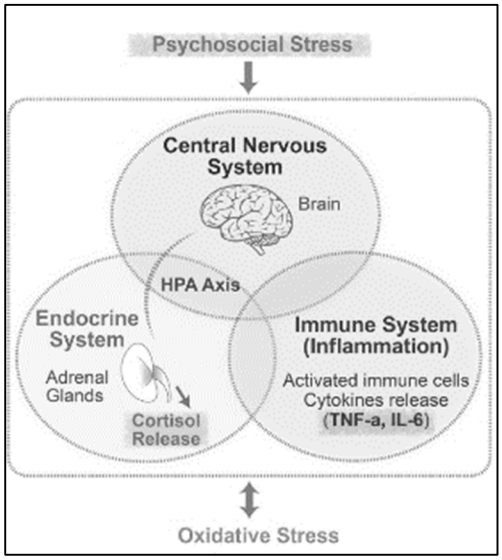

Figure.1. Psychological stress induced inflammatory oxidative stress (5)

MATERIAL AND METHODS

In vivo Adaptogenic Activity

Animal Care and Handling

Both sex of Swiss Albino Mice (Weight: 25–30 g) were housed with a 12-hour light/dark cycle in cages. The animals were acclimated in cages and provided with a clean and controlled environment in accordance with the Committee for Control and Supervision of Experiments on Animals (CCSEA) specifications before the experiment began (9). Dose selections for Diazepam and D-Pinitol were based on Ghias, M, et al. (10) and Navaaro et al. respectively (11).

Materials required

D-Pinitol (TCI Chemicals), Diazepam injection (NEON Laboratories Limited), Glass cylinders 2 numbers (height 25 cm, diameter 10 cm), Tap Water, Burette Stand with clamp, Micropore Adhesive tape, and Elevated Plus Maze Model.

Methodology

I. Forced swimming endurance test and Post-swimming motor function test

The untrained mice for swimming were used in the forced swimming test. The glass cylinder with the height of 25 centimeter and with the diameter of 10 centimeter was poured with 10 cm of water having normal temperature. Each mouse was placed inside a glass cylinder and to measure the period of time during which the animals were mobile and immobile, they were left in the cylinder for 6 minutes. The final 4 minutes of the 6 minutes testing session were used to calculate the total duration of immobility. The mouse was believed to be immobile when it stopped its attempts to move and remained unmoved within the water, moving only to maintain its head above the surface. Antistressor will shorten the period of immobility (12). Next, the mice were allowed to swim until they became too exhausted, and the time it took for them to drown was recorded (13). The animals were taken away and given about five minutes to recover and dry. On a Rota rod, the animals were evaluated for muscular coordination, and the duration of time spent on the rod was recorded. For about ten minutes, they were then monitored for spontaneous motor activity in a photoactometer (14).

II. Tail suspension Test

Individual animals were suspended 50 cm from the bottom on the burette stand clamp with micropore adhesive tape (approximately 1 cm) at the tail end. For a total of six minutes, mice were suspended in the stand. The last 4 minutes of the test were used to assess the duration of immobility. Only when mice are hanging passively and without any movement, they are believed to be immobile. In these experiments, anti-stressor reduces the immobility of the mice (15).

III. Elevated Plus Maze Test

The elevated plus maze was made up of two open arms (each measuring 50 cm by 10 cm) intersected by two closed arms (each measuring 50 cm by 10 cm by 40 cm). A center square (10 cm by 10 cm) joined the arms together. The maze was constructed with a height of 70 cm. Following sixty minutes after administration of the medication, each mouse was placed separately in the middle of the maze with their heads toward the open arm. Subsequent behavioral parameters were taken for a duration of five minutes. The length of time spent in the open arm was recorded (16).

Statistical Analysis:
Statistics were evaluated to be significant at P values under 0.05 (P<0.05). One-way ANOVA followed by Tukey test as post hoc test was performed statistically using GraphPad Prism software version 8.01 for this research.

RESULTS AND DISCUSSION
During the forced swimming test, the control animals continued to be immobile for the majority of the time. The immobility time of control, standard (Diazepam 2 mg/Kg) and test (D-Pinitol 100 mg/kg and D-Pinitol 400 mg/Kg) were depicted in Table.2. The immobility time of the standard diazepam and test drugs, viz., D-Pinitol in low and high doses were significantly (***P<0.001) reduced compared to the control. Diazepam, D-Pinitol 100 mg/Kg, and D-Pinitol 400 mg/Kg significantly (**P<0.01) lengthened the time that mice spent swimming before drowning compared to control animals (Table 3). Hence, mice pre-treated with D-Pinitol in both doses were shown to have longer swimming endurance. The duration spent on the Rota rod was considerably prolonged by Diazepam (***P<0.001), D-Pinitol 100 mg/Kg (**P<0.01), and D-Pinitol 400 mg/Kg (***P<0.001) compared with that of control. These findings are shown in Table 4. The photoactometer measurement for spontaneous motor activity was significantly (***P<0.001) improved by diazepam, D-Pinitol 100 mg/Kg, and D-Pinitol 400 mg/Kg (Table 5) compared with that of control. In the tail suspension test, which was depicted in Table 6, Diazepam and both of the D-Pinitol test doses significantly (***P<0.001) reduced the amount of time spent immobile compared to the control. In elevated plus maze test, mice receiving the standard dose of diazepam and D-Pinitol (100 mg/Kg and 400 mg/Kg) increased their time spent in the open arm significantly (***P<0.001) compared to the control group. These findings are given in Table 7.
Stress, which is brought on by free radicals, is the root cause of many human disorders. Continuous stress raises free radical levels, which causes an aberrant physiological condition, the emergence of psychological dysfunction, and the decline in cognitive performance (17). The other pathological findings involved in stress was activation of hypothalamic-pituitary-adrenal axis leading to the release of cortisol (14), which subsequently causes the mobilization of fats, carbohydrates, and lipids from storage and elevates blood sugar, triglyceride, and cholesterol levels (18). In earlier studies conducted by Rengarajan et al., the effect of D-Pinitol in reducing free radical scavenging and its antioxidant capacity were proved (19). D-Pinitol has previously been shown to be able to lower blood sugar levels in diabetic animals by changing the levels of the hormones that regulate blood sugar, including glucagon, and insulin and to reduce elevated plasma level of cortisol (20). Because of its aforementioned properties, D-Pinitol may be accountable for the observed anti-stress/adaptogenic action in this study using diverse stressor models in mice.

Table.1. Treatment Protocol

GROUP	LABELLED	TREATMENT
I	Control	-
II	Standard Drug	Diazepam (2 mg/Kg) i.p.
III	Test Drug 1– Low Dose	D-Pinitol (100 mg/Kg) p.o.
IV	Test Drug 2 – High Dose	D-Pinitol (400 mg/Kg) p.o.

Table 2: Effect of D-Pinitol on mice in immobility time in swimming endurance test

GROUPS	LABELLED/DOSE	FORCED SWIMMING ENDURANCE TEST
		IMMOBILITY TIME (IN SECONDS)
I	CONTROL	139.2 ± 19.33
II	DIAZEPAM 2 mg/Kg	65.17 ± 9.131 ***
III	D-PINITOL 100 mg/Kg	94.33 ± 15.02 ***
IV	D-PINITOL 400 mg/Kg	108 ± 3.742 ***

N=6; mean ± S.E.M; Where Group II, Group III & Group IV were compared with Group I; *p < 0.05, **p < 0.01, ***p < 0.001

Table 3: Effect of D-Pinitol on mice in swimming endurance in minutes

GROUPS	LABELLED/DOSE	DURATION OF SWIMMING ENDURANCE (TILL DROWNING) IN MINUTES
I	CONTROL	7.061 ± 2.02
II	DIAZEPAM 2 mg/Kg	14.30 ± 4.293 **
III	D-PINITOL 100 mg/Kg	21.3 ± 1.981 **
IV	D-PINITOL 400 mg/Kg	21.02 ± 1.35 **

N=6; mean ± S.E.M; Where Group II, Group III & Group IV were compared with Group I; *p < 0.05, **p < 0.01, ***p < 0.001

Table 4: Effect of D-Pinitol on mice in Rota Rod falling off time

GROUPS	LABELLED/DOSE	ROTA ROD FALLING OFF TIME (IN SECONDS)
I	CONTROL	12.5 ± 1.871
II	DIAZEPAM 2 mg/Kg	45.5 ± 6.565 ***
III	D-PINITOL 100 mg/Kg	23.67 ± 4.412 **
IV	D-PINITOL 400 mg/Kg	80.67 ± 8.618***

N=6; mean ± S.E.M; Where Group II, Group III & Group IV were compared with Group I; *p < 0.05, **p < 0.01, ***p < 0.001

Table 5: Effect of D-Pinitol on mice in Spontaneous Motor Activity done (Photoactometer) in post-swimming motor function test

GROUPS	LABELLED/DOSE	SPONTANEOUS MOTOR ACTIVITY SCORES (IN MINUTES)
I	CONTROL	83 ± 5.215
II	DIAZEPAM 2 mg/Kg	181 ± 7.321 ***
III	D-PINITOL 100 mg/Kg	202.1 ± 5.636 ***
IV	D-PINITOL 400 mg/Kg	238.7 ± 23.47 ***

N=6; mean ± S.E.M; Where Group II, Group III & Group IV were compared with Group I; *p < 0.05, **p < 0.01, ***p < 0.001

Table 6: Effect of D-Pinitol on mice in immobility time in Tail suspension test

GROUPS	LABELLED/DOSE	TAIL SUSPENSION TEST IMMOBILITY TIME IN SECONDS
I	CONTROL	174.8 ± 9.13
II	DIAZEPAM 2 mg/Kg	105 ± 26.89 ***
III	D-PINITOL 100 mg/Kg	114.5 ± 30.41 ***
IV	D-PINITOL 400 mg/Kg	90.67 ± 15.16 ***

N=6; mean ± S.E.M; Where Group II, Group III & Group IV were compared with Group I; *p < 0.05, **p < 0.01, ***p < 0.001

Table 7: Effect of D-Pinitol on mice in Elevated plus Maze Model

GROUPS	LABELLED/DOSE	TIME SPENT IN OPEN ARM (IN SECONDS)
I	CONTROL	75 ± 4
II	DIAZEPAM	257.8 ± 18.65***
III	D-PINITOL 100 mg/kg	165.2 ± 2.787 ***
IV	D-PINITOL 400 mg/kg	184.3 ± 6.976***

N=6; mean ± S.E.M; Where Group II, Group III & Group IV were compared with Group I; *p < 0.05, **p < 0.01, ***p < 0.001

CONCLUSION

The prospective behavioral effects of D-Pinitol were examined in this study using a variety of acute stress methods. D-Pinitol (100 mg/Kg and 400 mg/Kg) has considerably proved its capacity to stop the changes brought on by stress when administered to mice. Therefore, it can be said and inferred from all stressor studies that D-Pinitol possesses adaptogenic property (antistress effect) and thereby gives protection for stress induction. D-Pinitol's adaptogenic action was possibly due to its antioxidant property and its ability to ameliorate abnormally elevated plasma glucose, cortisol, and fatty acid levels.

ACKNOWLEDGMENT

The authors thank the authorities of Faculty of Pharmacy, Dr.M.G.R. Educational and Research Institute, Deemed to be University, Chennai, Tamil Nadu for providing the necessary facilities to do this research.

FUNDING SUPPORT

The authors declare that they have no funding support for this study.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest for this study.

INFORMED CONSENT

The Institutional Animal Ethics Committee (IAEC) of A.C.S. Medical College, Dr. M.G.R. Educational and Research Institute (Deemed to be University), approved the experimental protocol. The approval number was VI/IAEC/Dr MGR/2053/PO/ReBi/S/19/CPCSEA/28.01.2023/05.

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CITATION OF THIS ARTICLE

Sudha M, Annapoorna D, Athira M Nair, Harshavardhini R, Janani Priya C, Nishanth S. Adaptogenic Activity of D-Pinitol against Diverse Stressors Induced in Swiss Albino Mice. Bull. Env.Pharmacol. Life Sci., Vol 12 [10] September 2023: 299-304