International Journal of Allied Medical Sciences and Clinical Research (IJAMSCR)

ISSN:2347-6567

IJAMSCR |Volume 10 | Issue 2 | Apr - Jun - 2022
www.ijamscr.com

Research Study *Medical research*

EVALUATION OF ANTIOXIDANT EFFECT OF *PISONIA ALBA* ROOT IN ANXIOUS RATS

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ABSTRACT

The goal of this study was to see if an ethyl acetate extract of Pisonia alba root (PAE) might help nervous Sprague–Dawley rats relax. To test the preventative and curative effects of PAE, animals were separated into six groups (n = 6) and given 200 mg/kg and 400 mg/kg p.o. for 21 days. Animals were socially isolated for 21 days to generate anxiety. Animal anxiety was measured using the elevated plus maze (EPM) and the light and dark model. After 21 days of social isolation in rats, oxidative stress markers such as lipid peroxidation (LPO), superoxide dismutase (SOD), catalase (CAT), and reduced glutathione (GSH) were measured in blood and brain tissue homogenate. PAE 200 mg/kg and 400 mg/kg p.o. were given to rats. When compared to the negative control, both treatments demonstrated a substantial (P 0.001) increase in the frequency of open arm entries and time spent in open arm in EPM. When compared to the negative control, there was a substantial (P 0.001) increase in the number of lightbox entries and time spent in the lightbox in the light and dark models. When compared to the negative control, there was a substantial (P 0.001) improvement in endogenous anti-oxidants such as SOD, CAT, reduced GSH, and lower levels of LPO in blood and brain tissue. The current study implies that PAE may play a role in anxiety therapy via controlling oxidative stress.

Keywords: Antioxidant, anxiolytics, *Pisonia alba*, oxidative stress.

INTRODUCTION

The most common mental condition is anxiety. [1] Depression, obsessive-compulsive disorder, generalised anxiety disorder (GAD), panic attacks, phobias, and post-traumatic stress disorder are all addressed. [2] In many countries, including the United States, it is the most prevalent neuropsychiatric condition. [3],[4] In India, the prevalence of anxiety is quite high, at 8.5 percent, which is nearly 1 percent of the worldwide incidence of 7 percent to 9 percent. [5] Anxiety disorders impact over 16.6 percent of the world's population. [6] GAD is the most prevalent anxiety condition, but it's not as severe as panic disorder, and it frequently coexists with mental illness. Females have around a 2:1 anxiety ratio versus males. Anxiety is controlled by the serotonin, noradrenergic, dopaminergic, and histaminergic systems. Diazepam (DZM) is the most commonly prescribed anxiety medication, however it comes with a slew of drawbacks, including tolerance, dependency, reduced alertness, and cognition. When you stop taking DZM, you'll get withdrawal symptoms.

Pisonia alba also known as *Pisonia alba spanoghe*, *pisonia umbellifera*, belongs to the family of Nyctaginaceae. It is found on many of the Seychelles Islands that have had habitat restoration and subsequently is a key part of the habitat associated with high biodiversity and a complex food web. It is therefore not as easy as replacing Pisonia with other native tree species; it was discovered by(7) that Pisonia is the most common nest tree for the Seychelles warbler an endemic land bird brought back from near extinction by careful habitat management and translocation, thus showing that careful consideration of the entire island ecosystem is essential. Uses: The leaves are edible. Young leaves are used as a vegetable. Leaves make good cattle feed

too and are mostly used to treat rheumatism or arthritis. In traditional Indian medicine, they are used as an anti-diabetic; Leaves , of course , are used by natives as cattle feed; They are cooked and eaten for arthritis; The leaves are also carminative; Leaves are an antidote for snake bites. (8) but no data is available for its anti-anxiety activity.

MATERIALS AND METHODS

Plant Extract

Pisonia alba root was harvested in the Erode district's Gobi neighbourhood. During the month of January 2018, plant gathering took place. Dr.P.Jayaraman, professor at PARC in West Tambaram, Chennai, confirmed the root's authenticity. Roots were dried in the shade and finely pulverised, weighing 1 kg, before being macerated for 7 days with ethyl acetate. The extract of P. alba root (PAE) weighed 10.7 g and yielded 1.070 percent w/w in percentage practical yield.

Animal

For the pharmacological testing, healthy male Sprague–Dawley rats (200–250 g) were employed. For the duration of the animal study, the animals were housed in polypropylene cages with wire mesh tops and husk bedding and kept under standard environmental conditions (25°C 2°C, relative humidity 60% 5%, light-dark cycle of 12 h each) and fed a standard pellet diet (Trimurti feeds, Nagpur) and water ad libitum. During the day (08:00–16:00 h), the trials were carried out. The Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) and the Institutional Animal Ethics Committee governed the housing and treatment of the animals (IAEC).

Experimental

The animals were divided into six groups, each with six animals: Animals in the control group were neither exposed to social isolation or given any drugs. Animals in the negative control group were isolated in a dark room for 21 days without receiving any therapy. Animals in the low dosage group (PAE 200 mg/kg p.o.) were socially isolated and given 200 mg/kg of P. alba extract once daily p.o. Animals in the high dosage group (PAE 400 mg/kg p.o.) were socially isolated and given 400 mg/kg of P. alba extract once daily p.o. DZM group: Animals were socially isolated and treated with DZM (2 mg/kg) alone i.p. 1 hour before the experiment, VE group: Animals were socially isolated and treated with DZM (2 mg/kg) alone i.p. before the experiment, VE group: Animals were socially isolated and treated with DZM (2 mg/kg) alone i.p Animals were socially isolated and given a single dose of Vitamin E (100 mg/kg) p.o. once daily for 21 days. In socially isolated rats and normal rats, behavioural and oxidative stress parameters (in blood and tissue) were investigated (social isolation for 21 days). Animals were studied using several behavioural models such as the elevated plus maze (EPM) and the light and dark paradigm after 21 days of social isolation.

Behavioral Study

Elevated plus maze

The percent number of open arm entries, duration spent in open arms, and percent number of closed arm entries were all investigated. A decrease in apprehensive behaviour was indicated by an increase in the % number of open arm entrances and the amount of time spent in open arms. [9]

Light and dark model

This model is divided into two halves, each of which is joined by a tiny aperture. One part is small, dark, and unlit, while the other is huge, highly lit, and white. The number of entrances and the amount of time spent in the light compartment were used to calculate anxiety. [10]

Determination of Oxidative Stress Parameters in Blood and Tissue

Blood sample preparation

Phosphate buffer saline (PBS) (8 ml) was added to packed cells to make a 5 percent suspension of red blood cells (RBCs). A total of 0.5 ml of 5% RBC was combined with 5 ml of distilled water, agitated for 5 minutes, and then maintained at 4°C for 5 minutes. After that, 0.15 ml of distilled water was added, followed by 0.4 ml of 3:5 chloroform–ethanol solutions, which were forcefully agitated to precipitate haemoglobin. A clean erythrocyte lysate was obtained by centrifuging the mixture.

Tissue preparation

Animals were slaughtered using deep ether anaesthesia after receiving the treatments for 21 days. The brain was removed and washed extensively in 0.1 M PBS with 0.1 mmol/L phenyl methanesulfonyl fluoride. To make a 10% solution, this tissue was blotted dry and homogenised in 0.1 M PBS in an ice bath. In a cooling centrifuge at 0°C, this suspension was spun for 1 hour at 16,000 rpm. After measuring the protein content, the supernatant was used to analyse the parameters of oxidative stress. [11]

The molar extinction coefficient of malondialdehyde (MDA) (1.56/105) was used to calculate lipid peroxidation (LPO), which was represented in nanomoles of MDA/gHb. [12] The activity of the catalase (CAT) enzyme in erythrocyte lysate was assessed by measuring the reduction in absorbance spectrophotometrically at 240 nm for 1 minute. [13] The activity of superoxide dismutase (SOD) in erythrocyte lysate was tested spectrophotometrically; the rise in absorbance was recorded at 420 nm for 3 minutes. The rate of auto-oxidation of pyrogallol is 50% inhibited by one unit of enzyme activity, as measured by change in absorbance/minute at 420 nm. [14] The amount of blood-reduced glutathione (GSH) was determined by mixing 0.2 ml whole blood with 1.8 ml distilled water, then adding 3.0 ml of precipitating mixture. It was centrifuged for 5 minutes at 2000 rpm, then 1 ml of the supernatant was added to 1.5 ml acid, 5 ml phosphate solution, and 0.5 ml Dithionitrobenzoic, 5'-Dithiobis (2-nitrobenzoic acid) reagent. At 412 nm, the absorbance was measured. [15]

RESULTS

Table 1 shows that in the negative control group, there was a significant (P 0.001) increase in closed arm entries and a decrease in open arm entries when compared to the control group. When compared to the negative control group, PAE 200 mg/kg p.o., PAE 400 mg/kg p.o., and DZM group exhibit a substantial (P 0.001) drop in closed arm entries and a significant (P 0.001) rise in open arm entries. When compared to the control group, there was a substantial (P

0.001) increase in time spent in the closed arm and a reduction in time spent in the open arm in the negative control group. When compared to the negative control group, the PAE 200 mg/kg p.o., PAE 400 mg/kg p.o., and DZM groups exhibit a substantial (P 0.001) decrease in time spent in the closed arm and a significant (P 0.001) increase in time spent in the open arm.

Table 1: Effect of P.alba root extract on anxiety by elevated plus maze test after 21 days of social isolation

Groups	0 day				21 days			
	Number entries in closed arm (%)	Number entries in open arm (%)	Time spent in closed arm (s)	Time spent in open arm (s)	Number entries in closed arm (%)	Number entries in open arm (%)	Time spent in closed arm (s)	Time spent in open arm (s)
Control groups	68.73±0.55	29.05±0.67	41.49±0.53	51.30±8.41	67.81±0.47	30.07±0.80	38.49±0.80	52.60±6.24
Negative control groups	62.26±0.11	36.62±0.41	43.14±0.21	49.32±0.21	71.83±1.23	26.07±1.11	47.08±1.41	37.89±1.42[a]
Low dose group	64.56±0.84	33.33±2.11	47.24±5.21	42.16±2.10	59.75±5.21**	39.32±1.54**	38.01±2.00**	42.92±1.21*
High dose group	60.77±1.85	37.21±2.10	50.21±0.87	49.11±2.22	52.71±2.01**	45.29±3.10**	36.31±5.21**	47.77±2.30**
DZM group	66.04±0.39	31.84±5.20	50.22±9.21	41.41±5.21	50.51±2.01**	47.29±3.10**	33.81±2.01**	48.61±3.01**

*P<0.001 when compared with normal control group, *P<0.05 when compared with negative control group, **P<0.001 when compared with negative control group. All values are shown as mean±SD and n=06. SD=standard deviation, DZM=Diazepam*

Table 2 demonstrates that the dark box entries of the negative control group increased significantly (P 0.001) when compared to the control group. When compared to the negative control group, PAE 200 mg/kg p.o., PAE 400 mg/kg p.o., and DZM group exhibit a substantial (P 0.001) drop in dark box entries and a significant (P 0.001) rise in light box entries. When compared to the control group, there was a substantial (P 0.001) increase in time spent in the dark box and a reduction in time spent in the light box in the negative control group. When compared to the negative control group, the PAE 200 mg/kg p.o., PAE 400 mg/kg p.o., and DZM groups exhibit a substantial (P 0.001) decrease in time spent in the dark box and a significant (P 0.001) increase in time spent in the light box.

Table 2: Effect of P.alba root extract on anxiety by light and dark model after 21 days of social isolation

Groups	0 day				21 days			
	Number entries in dark box (%)	Number entries in light box (%)	Time spent in dark box (s)	Time spent in light box (s)	Number entries in dark box (%)	Number entries in light box (%)	Time spent in dark box (s)	Time spent in light box (s)
Control groups	59.49±0.21	38.49±0.24	50.14±0.52	243.25±6.32	60.83±0.64	37.37±0.71	59.49±0.74	234.60±5.31
Negative control groups	62.64±0.12	35.61±0.33	55.34±0.34	252.34±0.33	69.87±1.24	30.54±1.43	119.06±1.39	153.54±1.12[a]
Low dose group	61.76±0.54	36.21±2.75	49.74±5.54	223.24±2.45	62.34±5.40**	35.07±1.34**	89.48±5.24**	186.22±1.67*
High dose group	62.86±1.42	35.18±2.04	77.00±0.67	234.21±2.14	60.55±1.61**	37.06±3.45**	82.27±4.20**	203.6±3.61**
DZM group	61.33±0.24	36.21±5.48	45.49±6.30	252.36±5.24	57.49±3.01**	40.22±2.11**	65.38±2.04**	230.5±2.12**

*P<0.001 when compared with normal control group, *P<0.05 when compared with negative control group, **P<0.001 when compared with negative control group. All values are shown as mean±SD and n=06. SD=standard deviation, DZM=Diazepam*

Table 3 shows that in the negative control group, there was a significant (P 0.001) increase in LPO and a decrease in SOD, GSH, and CAT levels, whereas in the PAE 200 mg/kg p.o., PAE 400 mg/kg p.o., and VE group, there was a significant (P 0.001) decrease in LPO and an increase in SOD, GSH, and CAT levels in blood.

Table 3: Effect of P.alba root extract on oxidative stress parameters in blood after 21 days of social isolation

Groups	0 day				21 days			
	LPO (nMMDA/ gHb)	SOD U/mg protein	CAT U/mg protein	GSH (❍M/ mg protein)	LPO (nMMDA/ gHb)	SOD U/mg protein	CAT U/mg protein	GSH (❍M/ mg protein)
Control groups	5.16±0.26	442.07±13.01	35.38±4.34	40.12±1.21	5.029±0.11	435.3±5.70	34.08±0.29	40.07±0.73
Negative control groups	4.26±0.33	353.20±0.45	31.78±0.55	34.73±0.53	7.28±2.00	272.14±1.30	24.89±1.29	27.14±1.10[a]
Low dose group	4.87±0.62	331.76±18.42	29.77±4.35	32.64±1.32	4.51±4.30**	345.36±18.21**	31.53±4.32**	30.04±1.58*
High dose group	5.18±1.28	355.74±20.24	38.30±3.34	35.30±2.21	4.371±10.51**	375.06±17.36**	36.84±4.64**	32.3±3.31**
VE group	6.06±0.84	355.17±5.84	34.54±7.20	38.47±7.23	6.147±2.14**	416.14±1.35**	36.41±2.33**	37.31±2.69**

*P<0.01 when compared with normal control group, *P<0.05 when compared with negative control group, **P<0.01 when compared with negative control group. All values are shown as mean±SD and n=06. SD=standard deviation, VE=vitamin E, LPO=lipid peroxidation, CAT=Catalase, GSH=Glutathione.*

Table 4 reveals that in brain tissue, the negative control group had a substantial (P 0.001) rise in LPO and a significant (P 0.001) drop in SOD, GSH, and CAT levels as compared to the control group. There was a substantial (P 0.05) drop in LPO and an increase in CAT level in brain tissue in the PAE 200 mg/kg p.o. treated group. There was a substantial (P 0.001) drop in LPO and an increase in SOD, GSH, and CAT levels in brain tissue in the PAE 400 mg/kg p.o. and VE groups.

Table 4: Effect of P.alba root extract on oxidative stress in brain after 21 days of social isolation

Groups	LPO activity (nMMDA/g protein)	SOD activity U/mg protein	CAT activity U/mg protein	GSH activity (❍M/ mg protein)
Control groups	23.16±3.31	133.0±8.07	137.4±10.61	43.81±1.66
Negative control groups	37.29±2.59	82.49±0.87	52.33±1.24	26.56±0.53
Low dose group	22.15±1.65	103.0±3.46	74.24±3.64	34.56±1.21
High dose group	16.16±1.36	121.4±1.44	93.36±1.02	44.45±2.74
VEgroup	23.08±2.46	132.2±6.36	155.2±6.25	41.22±0.34

*P<0.01 when compared with normal control group, *P<0.05 when compared with negative control group, **P<0.01 when compared with negative control group. All values are shown as mean±SD and n=06. SD=standard deviation, VE=vitamin E, LPO=lipid peroxidation, CAT=Catalase, GSH=Glutathione.*

DISCUSSION

The antioxidant potential of PAE is used to assess its anxiolytic efficacy in this study. Anxiety was created in the rats in this study via social isolation for 21 days. Novelty-induced hyponeophagia, social engagement, and open field exploration are some of the numerous models utilised to generate anxiety. [16] Social isolation raises oxidative stress levels, altering and causing anxiety-like behaviour. In the current study, it was shown that socially isolated animals have a significantly higher degree of oxidative stress and anxiety. For the investigation of anxiogenic or anxiolytic effects of drugs in rats, the EPM and dark and light models are the most often utilised models.[17],[18],[19] The current study found that PAE had a substantial (P 0.001) anxiolytic effect on socially isolated animals, as evidenced by an increase in the percent of entries and time spent in open arms and a decrease in the percent of entries and time spent in closed arms when compared to the negative control. When administered at 200 mg/kg p.o. and 400 mg/kg p.o. dosages, PAE therapy resulted in a substantial (P 0.001) increase in the number of entries and time spent at the light box in the dark and light test. According to the literature, social isolation affects glucocorticoid production and the neurotransmitter system, both of which cause stress. [17] Stress changes catecholamine metabolism, resulting in an increase in free radical generation. [20] It was also discovered that anxiety increases the production of reactive oxygen species, and that a drug with antioxidant properties effectively manages anxiety-like behaviour. This has fueled research into finding antioxidants in foods and medicinal plants, resulting in the discovery of over 4000 antioxidants. [21] This study demonstrated that social isolation raises LPO levels considerably (P 0.001) and lowers SOD, CAT, and GSH levels in the blood and brain of nervous rats when compared to normal rats. The treatment of nervous rats with PAE reduces LPO levels while increasing SOD, CAT, and GSH levels considerably (P 0.001).

CONCLUSION

The findings support the theory that oxidative stress plays a role in anxiety. The findings imply that under anxiety, PAE increases the intracellular activity of several antioxidant enzymes. It's also possible that these plant compounds can protect against oxidative damage. P. alba's positive antioxidant benefits might be due to suppression of certain pathways that are triggered as a result of increasing oxidative stress as anxiety progresses. As a result, antioxidant therapy may be beneficial in the treatment of anxiety and its consequences.

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