Antidepressant activity of Mucuna pruriens in prolonged chronic mild stress induced depression in Drosophila melanogaster

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Antidepressant activity of *Mucuna pruriens* in prolonged chronic mild stress induced depression in *Drosophila melanogaster*

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Abstract
Oxidative stress is known to impact neurological changes in the brain, predisposing an individual to brain disorders. This study explores the antidepressant potential of *Mucuna pruriens* (MP) through modulation of oxidative stress and cholinergic pathways, suggesting its potential as a therapeutic drug for mitigating depression induced by prolonged chronic mild stress (CMS) in *Drosophila melanogaster*. The efficacy of MP methanolic (MP-Meth) and aqueous (MP-Water) extracts was compared with two commercial antidepressants, Fluoxetine (FLX) and Amitriptyline (AMP). CMS was induced by exposing the flies to varying adverse conditions for 24 h. Fifty flies per group, with three replicates for each of the four regimens, were fed a standard feed and treated with 5 mg/ml/body weight for 5 days. Survival rate analysis was done and the antidepressant effect of *M. pruriens* was evaluated by measuring biochemical parameters. Under heat stress, AMP reduced catalase activity (90.8%) and G-S-T levels significantly (96.5%), whereas MP-Meth had the most ameliorating effect on catalase activity (94.2%). MP-Water notably increased AchE activity under both heat (61.8%) and cold stress (97%). AMP showed the highest decrease in lipid peroxidation under heat stress (2.7%), and MP-Water had the most significant decrease under cold stress (19.3%). However, the analysis indicated that both extracts of MP have their peculiarity and efficacy across biochemical markers and stress variations. These findings emphasize the significance of novel, innovative treatments to address the multifaceted nature of depression, therefore, recommending comprehensive exploration of aqueous and methanol extracts from M. pruriens as promising therapeutics for enhancing depression management.

Keywords Depression · *Mucuna pruriens* · Drosophila melanogaster · Chronic Mild Stress (CMS) · Antidepressant · Oxidative stress · Acetylcholinesterase

Introduction

Chronic mild stress (CMS) is a primary cause of depressive mood disorders, leading to various physiological brain changes such as altered corticosterone regulation via the hypothalamic–pituitary–adrenal HPA axis, impaired neurogenesis, dysfunctional synapses, and altered gene expression [1]. CMS also induces depressive-like behaviour, reduced reward response, and sleep disruptions, with decreased sucrose consumption indicating anhedonia [2]. Animal models of chronic mild stress have been instrumental in studying depressive neuropathology and testing potential therapeutic targets [3].

Extended author information available on the last page of the article

Depression is a debilitating mental illness affecting approximately 20% of the global population, often presenting asymptomatic in its early phases [4]. It is a leading cause of mortality and disability worldwide, associated with increased risk of criminal behaviour, substance abuse, and suicide [5]. In Nigeria, depression is prevalent, particularly among young adults, with approximately 7.1% incidence rates reported in various regions [6, 7].

Oxidative stress plays a crucial role in the pathogenesis of depression, linked to processes like brain lipid peroxidation, nitric oxide, and cyclooxygenase-2 (COX-2) activity [8]. High levels of reactive oxygen species (ROS) contribute to neuroinflammation, neurotransmitter imbalance, and disrupted neurogenesis/synaptic plasticity [9]. Depression has also been associated with changes in acetylcholinesterase (AChE) activity. Some studies suggest increased AChE activity as a compensatory mechanism for excess acetylcholine, while others report decreased AChE activity, highlighting the complexities of the AChE-depression relationship [10, 11].

Antidepressants, while effective, often require prolonged administration and are recommended alongside psychotherapies for moderate to severe depressive illnesses [12, 13]. Mucuna pruriens has shown neuroprotective action by restoring endogenous monoamine levels, including dopamine, through its L-dopa content, which crosses the blood–brain barrier and converts to dopamine [14, 15]. The findings from Karege et al. [16] imply that Mucuna pruriens can facilitate neuron growth and survival, which provides insight into some of its antidepressant properties.

Drosophila melanogaster, with its 60% genomic similarity to humans [17], is a valuable model organism for studying human diseases due to its short lifespan, prolific reproduction, and extensive genetic tools [18]. Despite anatomical differences, fundamental molecular pathways are highly conserved [19], making it a viable disease model [20]. Given the side effects and limitations of current antidepressant treatments, this study investigates the antidepressant activity of methanolic and aqueous extracts of Mucuna pruriens spicules. This herbal treatment approach aims to offer a more accessible option with potentially fewer side effects compared to pharmacological antidepressants. The study evaluates the ability of M. pruriens extracts to reverse CMS-induced depression in Drosophila melanogaster by examining their interaction with the nervous system through survival rate and biochemical assays.

Materials and methods

Chemicals

Standard drugs (Fluoxetine and Amitriptyline), Cornmeal, Sucrose, Yeast, Agar–Agar, Methyl paraben, Methanol, Potassium Phosphate Monobasic ($KHPO_4$), Potassium Phosphate Dibasic (K_2HPO_4), Reduced glutathione (GSH), 1-Chloro-2, 4-dinitrobenzene (CDNB) hydrogen peroxide (H_2O_2), butylhydroxytoluene, thiobarbituric acid, O-phosphoric acid, 5,5′-dithiobis-2-nitrobenzoic acid (DTNB), Acetylthiocholine, Lead acetate (10%), Wagner's reagent, Chloroform, Conc. sulphuric acid (H_2SO_4), Ferric chloride (10%), NaOH (1 M, 10%), Biuret reagent, Tannic acid, Folin-Ciocalteu's reagent, Sodium carbonate (7.5%), Acetic acid (10%), Ethanol, Ammonium hydroxide, Aluminum chloride (10%).

Fly stocks

The wild-type (Harwich strains) of male and female Drosophila melanogaster were acquired from Prof. Lucia Prieto-Godino in Neural Circuits and Evolution Laboratory, The Francis Crick Institute, London, England. The breeding environment maintained a temperature of 24 ± 2 °C, and 60% humidity, with 12 h day/night cycles, the experiment and breeding of the flies took place at the Clinical Biochemistry Research Laboratory of the Department of Chemical Sciences at Fountain University Osogbo, Osun State, Nigeria. To culture and nourish the flies, a cornmeal-based medium was utilized, with hygienic adaptations based on the protocols of Folarin et al. [21].

Plant source

Dry pods containing Mucuna pruriens seeds were harvested from Gbolasere farm, Mowe, Ogun State, Nigeria. The spicules from the pods of the Mucuna pruriens plant were identified and authenticated by the laboratory technician in the Clinical Biochemistry Research Laboratory of the Department of Chemical Sciences at Fountain University Osogbo, Osun State, Nigeria.

Preparation of *Mucuna* pruriens extracts

The bioactive metabolites of Mucuna pruriens spicules was extracted using the maceration method. 1 g of Mucuna pruriens spicules was weighed into two separate conical flasks. 100 ml of absolute methanol and distilled water were added respectively. Both were then subjected to intermittent shaking at 150 rpm for 72 h to yield a brownish extract. The resulting mixture was filtered through a cell strainer (40 µM), and the solvent was removed using a freeze dryer [22]. The extracts were stored in sterile bottles at 4°C until further use. The net yield for the aqueous extract was 2%, while that of the methanol extract was 13%.

Phytochemical screening

The liquid extracts were used for qualitative phytochemical screening for compounds, including tannins, alkaloids, terpenoids, phenols, steroids, flavonoids and protein content following the described methods:

Test for tannins using lead acetate

100 µl of both extracts were picked using a micropipette into a microcentrifuge tube, and then 3 drops of lead acetate (10%) were added. A yellow precipitate indicates the presence of tannins [23].

Test for alkaloids

300 µl of both extracts were picked using a micropipette into a microcentrifuge tube, and then a few drops of Wagner's reagent were added. A brownish-to-yellowish precipitate indicates the presence of alkaloids [24].

Test for terpenoids

500 µl of the extracts were mixed with 200 µl of chloroform and 300 µl of concentrated sulphuric acid, which was gently added to form a layer. A reddish-brown colour at the interface indicates the presence of terpenoids [24].

Test for phenols

200 µl of the extracts were picked, and 200 µl of distilled water was added. Then, 200 µl of 10% ferric chloride was also added. A blue black colour indicates the presence of phenols [24].

Test for steroids

100 µl of the extracts were picked inside a test tube and dissolved with 100 µl of chloroform. An equal volume of concentrated sulphuric acid was added to the test tube side by side. The upper layer of the test tube turned red, and the sulphuric layer turned yellow with green fluorescence, indicating the presence of steroids in the plant [24].

Test for flavonoids

200 µl of the extracts were picked inside the test tube, and 100 µl of 10% NaOH was mixed gently. A yellow colour indicates the presence of flavonoids. Few drops of diluted acid was then added and the solution turned colourless [23].

Test for protein using biuret test

200 µl of the extracts were picked, and 200 µl of biuret reagent was added. The mixture was allowed to stand for 5 min. A violet colour indicates the presence of protein [25].

Induction of depression in *Drosophila* melanogaster

The procedure for prolonged CMS-induced depression in D. melanogaster was adopted by Araujo et al. [26], with slight adjustments. For cold stress, a cold bath of ice was prepared in a styrofoam and a group of about 50 flies were placed in 10 plastic vials containing standard feed and immersed in the cold bath. The temperature was monitored regularly, and the flies were left in the cold bath for 24 h. After 24 h, the vials were removed from the cold bath, and the flies were allowed to recover at room temperature. For heat stress, a group of approximately about 50 flies was transferred into 10 vials containing standard feed, then placed in a water bath at a constant temperature of 36◦C and the flies were monitored for 24 h. After the stress induction process, the flies were subjected to survival rate analysis and biochemical assays.

Survival rate analysis

This was carried out to determine the efficacy of the different antidepressants (M. pruriens extracts, Fluoxetine, and Amitriptyline) employed for the treatment of CMS Induced depression in D. melanogaster. To achieve this, 1 to 3-day-old control and CMS Induced flies were divided into groups containing fifty (50) flies, with three (3) replicates for each group. Throughout the experiment, the survival of the flies was monitored by recording the number of dead flies daily during the entire 5-day exposure period. The treatment groups of flies were fed the standard diet mixed with the different treatments, while the control group was fed only the standard diet. After the treatment period, the data was analyzed, and the results were plotted using line graphs as the percentage of surviving flies.

Preparation of samples for biochemical assays

The CMS and Control fly groups were subdivided into sets of fifty (50) flies, each in three (3) replicates, and subjected to a 5-day treatment with Mucuna pruriens extracts and the standard drugs. During the 5-day treatment, the survival rate of each fly group was monitored and recorded after which the flies were then anaesthetized using ice, followed by weighing and homogenization in a 0.1 M phosphate buffer at pH 7.0 (in a ratio of 1 mg: 10 μl). This homogenate was subsequently centrifuged in an ultracentrifuge at 4000 rcf for 10 min at 4°C. The resulting supernatants were carefully separated from the pellets and then transferred into labelled microfuge tubes for further analysis of biochemical parameters.

Determination of catalase activity

Catalase activity was assessed using the Aebi method and the loss in absorbance was monitored using the UV/Visible spectrophotometer. The protocol was adapted from Adedara et al. [27] with little modifications,the reaction mixture comprised 50 mM phosphate buffer (pH 7.0), 300 mM H_2O_2, and the sample (diluted 1:50). The decrease in H_2O_2 absorbance was observed at 240 nm over 2 min at 10 s intervals using a UV/Visible spectrophotometer. The results were used to calculate catalase activity and expressed as μmol of H_2O_2 consumed/mins/mg protein.

Determination of Glutathione-S-Transferase (GST) activity

The glutathione-S-transferase activity was determined using the method of Habig and Jacoby with 1-chloro-2, 4-dinitrobenzene (CDNB) as the substrate. The assay mixture containing 500 μL of 0.1 M potassium phosphate buffer, pH 7.0, 200 μL of 10 mM GSH, 50 μL of the sample (diluted 1:5), and 150 μL of 10 mM CDNB was prepared. The reaction mixture was then observed at 340 nm for5 minutes at 10-s intervals using a UV/Visible Spectrophotometer reader. The results were subsequently expressed as μmol/min/mg protein [27].

Determination of acetylcholinesterase (AChE) activity

The method of Ellman et al. was used to determine the activity of acetylcholinesterase (AChE). The assay mixture comprised 650 µL 100 mM potassium phosphate buffer (pH 7.4), 100 µL of 10 mM 5,5′-dithiobis-2-nitrobenzoic acid (DTNB), 100 µL of sample supernatant, and 10 µL of 0.075 M acetylthiocholine as the initiator. Subsequently, the reaction was observed for 5 min at 15 s intervals and absorbance was measured at 412 nm using a UV/Visible spectrophotometer. The enzyme activity was estimated as µmol of acetylthiocholine hydrolyzed/min/mg protein [27].

Lipid peroxidation assay

The assay was performed as described by Ohkawa et al. and the absorbance was measured using a UV/Visible spectrophotometer after incubation of the reaction solution. The reaction mixture was composed of 5 µL of 10 mM butylhydroxytoluene, 200 µL of 0.67% thiobarbituric acid, 600 µL of 1% O-phosphoric acid, 105 µL of distilled water, and 90 µL whole fly homogenate. This mixture was incubated at 90 °C for 45 min. Subsequently, absorbance measurements were taken at 600 nm and 535 nm for each sample, and changes in absorbance were used to calculate lipid peroxidation and expressed as µmol of malondialdehyde (MDA) formed/ml [27].

Statistical analysis

The datasets acquired were analysed using Microsoft Excel and GraphPad Prism 5.0. For survival rate, line graphs provided visual representations of how the flies responded to the different antidepressant treatments. In contrast, bar plots with error bars showed the variation in biochemical markers using the standard error of the mean (SEM). Analysis of Variance (ANOVA) compared differences between the groups. A confidence interval of 95% and a significance threshold with a p-value of 0.05 determined statistical significance.

Results

This study shows a comprehensive examination of the phytochemical analysis of *Mucuna pruriens* extract and the effects of standard antidepressant drugs, Amitriptyline (AMP) and Fluoxetine (FLX), in comparison to plant extracts, MP-Water and MP-Meth, on *Drosophila melanogaster* under different stress-induced depression conditions. The results encompass phytochemical analysis, survival rates, catalase activity, G-S-T activity, AChE activity, and lipid peroxidation levels.

Phytochemical analysis of *Mucuna* pruriens extract

The qualitative analysis showed that saponins were absent in both extracts. Tannins, alkaloids, terpenoids, steroids, phenol, and protein were present in both extracts. However, only the aqueous extract contained flavonoids, as shown in Table 1.

Survival rate

Fig. 1

Catalase activity

- **Under heat stress (A)**, the initial catalase activity was **0.1596 µmol of H_2O_2/min/mg protein**. AMP significantly reduced activity to **0.0796 µmol of H_2O_2/min/mg protein**, showing the most pronounced reduction among treatments. MP-Meth followed with **0.095 µmol of H_2O_2/min/mg protein**, while FLX exhibited a moderate reduction

Table 1 Qualitative Profiling for Crude Methanolic and Aqueous *M. pruriens* Macerate

PHYTOCHEMICAL PARAMETERS	METHANOLIC EXTRACT	AQUEOUS EXTRACT
Tannin	Present	Present
Alkaloids	Present	Present
Terpernoids	Present	Present
Saponin	Absent	Absent
Phenol	Present	Present
Steroid	Present	Present
Flavonoid	Absent	Present
Protein	Present	Present

to **0.1537 µmol of H_2O_2/min/mg protein**. MP-Water also resulted in **0.1561 µmol of H_2O_2/min/mg protein**, indicating similar efficacy to FLX.

- **Under cold stress (B)**, the initial catalase activity was **0.0759 µmol of H_2O_2/min/mg protein**. MP-Meth had the highest ameliorating effect with **0.0406 µmol of H_2O_2/min/mg protein**, while MP-Water reduced catalase activity to **0.0443 µmol of H_2O_2/min/mg protein**. AMP reduced catalase activity to **0.0511** µmol of H_2O_2/min/mg protein and FLX resulted in a slight decrease to **0.0603 µmol of H_2O_2/min/mg protein** Fig. 2.

Glutathione-S-Transferase Activity

- **Under heat stress (A)**, G-S-T activity was initially **0.8616 µmol/min/mg protein**. AMP reduced G-S-T activity significantly to **0.030 µmol/min/mg protein**, while FLX decreased it moderately to **0.4688 µmol/min/mg protein**. MP-Meth caused a smaller reduction to **0.5016 µmol/min/mg protein**, and MP-Water had a strong effect, lowering it to **0.0347 µmol/min/mg protein**.
- **Under cold stress (B)**, the initial G-S-T activity was **0.7013 µmol/min/mg protein**. AMP led to a reduction to **0.4256 µmol/min/mg protein**, while FLX decreased G-S-T activity significantly to **0.0788 µmol/min/mg protein**. MP-Water reduced the activity to **0.3544 µmol/min/mg protein**, and MP-Meth resulted in **0.1294 µmol/min/mg protein** Fig. 3.

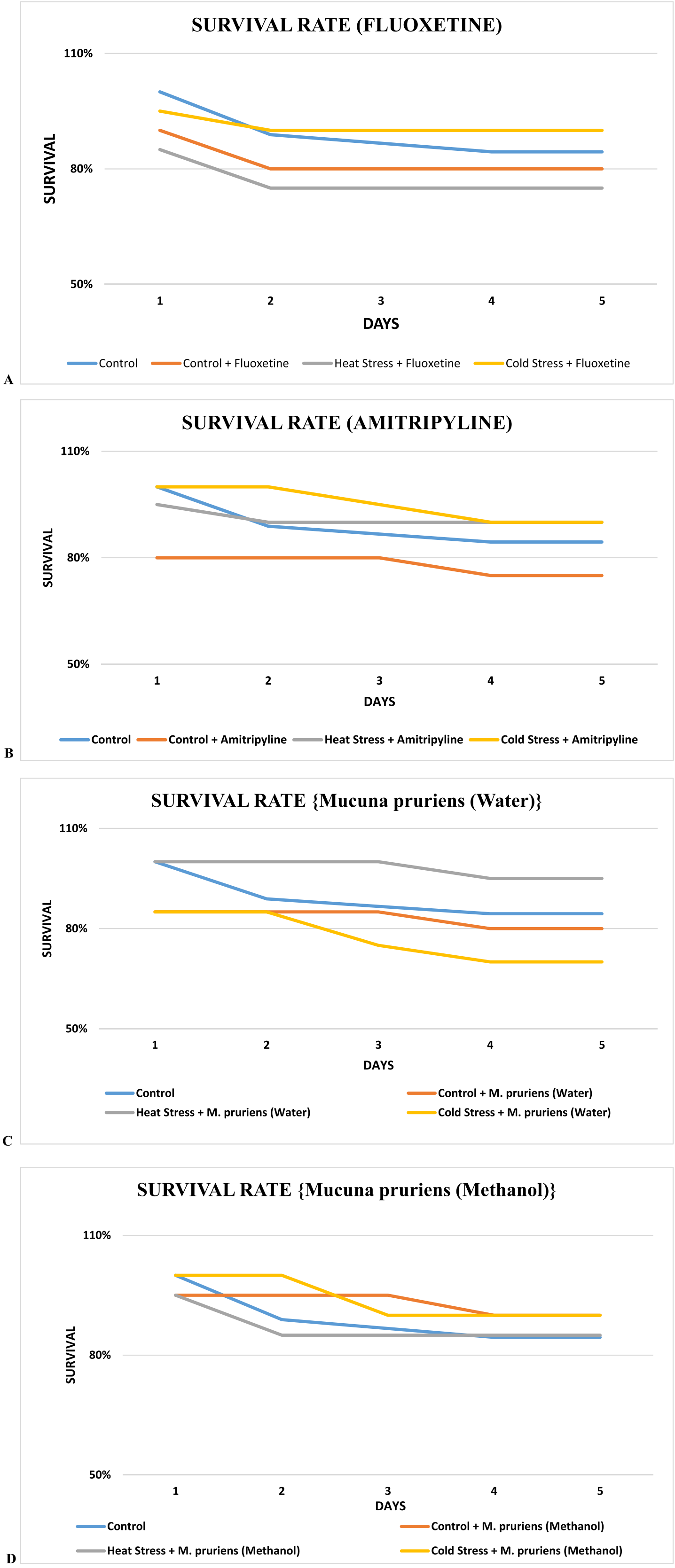
Acetylcholinesterase (AChE) activity

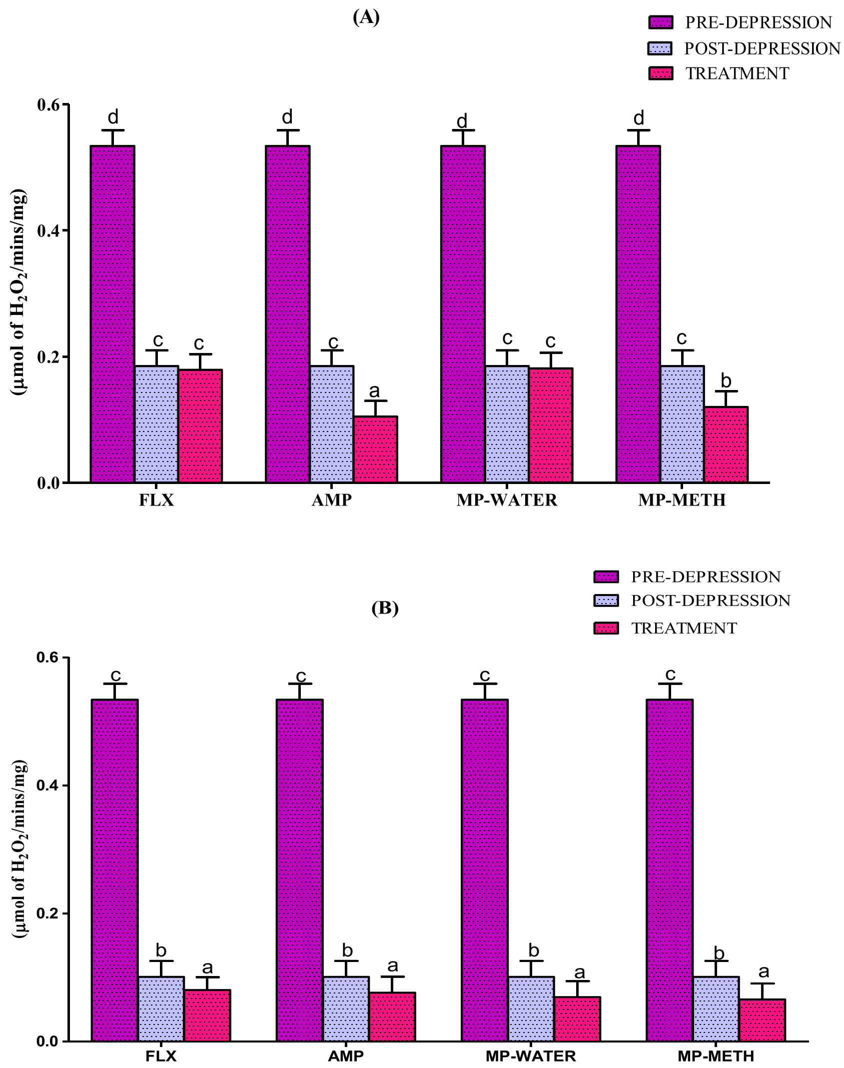
- **Under heat stress (A)**, AChE activity was initially reduced to **0.0087 µmol/min/mg protein**. MP-Water led to a significant increase, restoring AChE activity to **0.0228 µmol/min/mg protein**. AMP also elevated AChE activity to **0.0197 µmol/min/mg protein**, while FLX had a smaller effect, increasing it to **0.0106 µmol/min/mg protein**. MP-Meth raised AChE activity slightly to **0.0108 µmol/min/mg protein**.
- **Under cold stress (B)**, AChE activity was reduced to **0.0004 µmol/min/mg protein**. AMP restored AChE activity to **0.0226 µmol/min/mg protein**, close to baseline levels. FLX increased AChE activity to **0.0126 µmol/min/mg protein**, while MP-Water led to **0.0132 µmol/min/mg protein**, and MP-Meth resulted in **0.0128 µmol/min/mg protein** Fig. 4.

Lipid peroxidation

- **Under heat stress (A)**, lipid peroxidation was initially **5.48×10^{-9} µmol/ml**. AMP reduced lipid peroxidation to **5.33×10^{-9} µmol/ml**, while FLX slightly increased it to **5.80×10^{-9} µmol/ml**. MP-Water showed a higher increase to **6.88×10^{-9} µmol/ml**, and MP-Meth exhibited the highest level at **8.18×10^{-9} µmol/ml**.

Fig. 1 Survival rate analysis of CMS induced depression in *D. melanogaster* after 5 days of treatment. **(A)** Effect of FLX on Cold and Heat Induced depression in flies compared to Control. **(B)** Effect of AMP on Cold and Heat Induced depression in flies compared to Control. **(C)** Effect of MP-Water on Cold and Heat Induced depression in flies compared to Control. **(D)** Effect of MP-Meth on Cold and Heat Induced depression in flies compared to Control





Bars with different letters (a, b, c) indicate statistically significant differences ($p < 0.05$). 'a' represents the lowest activity level, with higher letters indicating progressively greater increases

Fig. 2 Effect of different antidepressant on the Catalase activity of CMS Induced Depression in *D. melanogaster* in comparison to control ($p < 0.05$). Baseline catalase activity in the control group was **0.5087 µmol of H_2O_2/min/mg** protein under both stress conditions. After CMS induction:

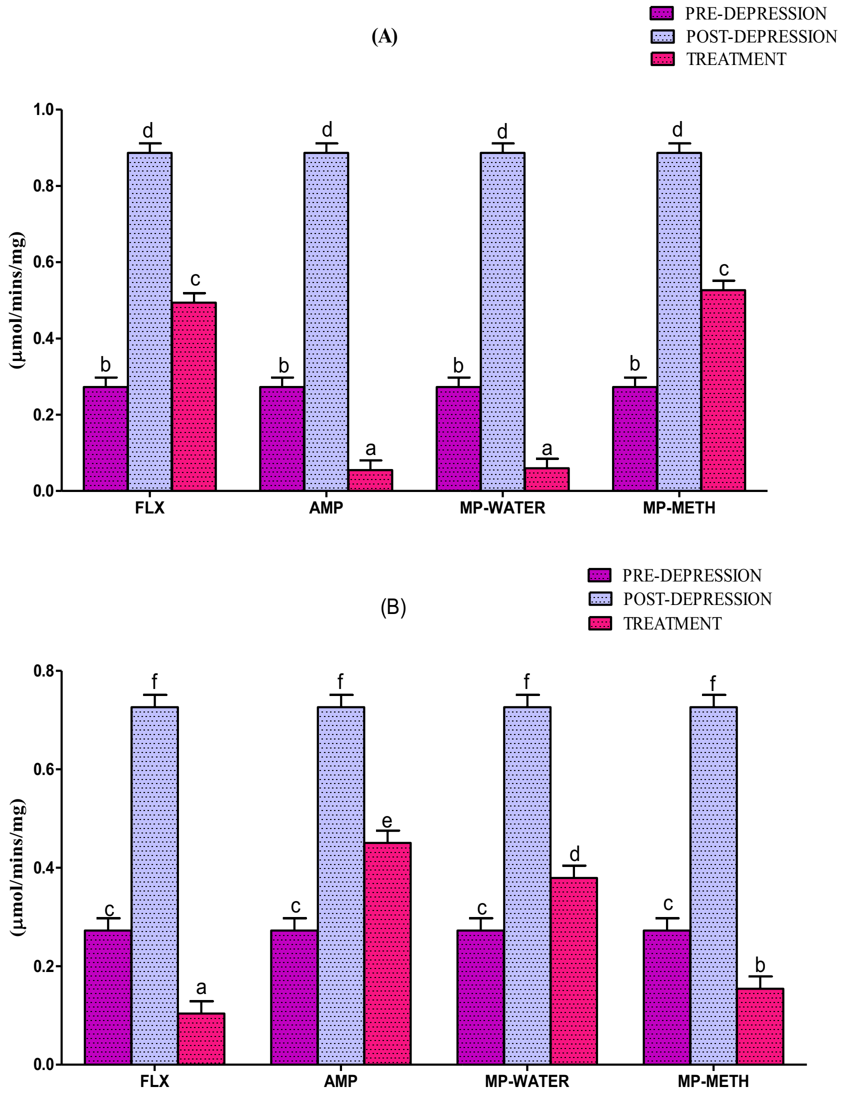
- **Under cold stress (B)**, lipid peroxidation was **4.46×10^{-9} µmol/ml** at baseline. MP-Water reduced lipid peroxidation to **3.60×10^{-9} µmol/ml**. AMP slightly increased lipid peroxidation to **4.57×10^{-9} µmol/ml**, FLX increased to **5.29×10^{-9} µmol/ml**, while MP-Meth showed the highest increase to **5.90×10^{-9} µmol/ml** Fig. 5.

Discussion

Phytochemical analysis

Mucuna pruriens is a medicinal plant known for its rich secondary metabolites, particularly alkaloids, vital to its neuroprotective properties. Salim et al. [28] reported that *M. pruriens* seed extract contains several bioactive compounds, including alkaloids, phenols, flavonoids, terpenoids, steroids, and tannins. Similarly, the qualitative analysis confirmed the presence of these compounds in both methanolic and aqueous extracts, except for saponins [29]. These phytochemicals have significant antioxidant and neuroprotective effects, contributing to the therapeutic potential [30].

Fig. 3 Effect of different anti-depressant on the G-S-T activity of CMS Induced Depression in *D. melanogaster* in comparison to control with ($p < 0.05$). Baseline G-S-T activity in the control group was **0.2475** µmol/min/mg protein**.** After CMS induction:



Bars with different letters (a, b, c, d, e, f) indicate statistically significant differences ($p < 0.05$). 'a' represents the lowest G-S-T activity, with higher letters indicating progressively greater increases

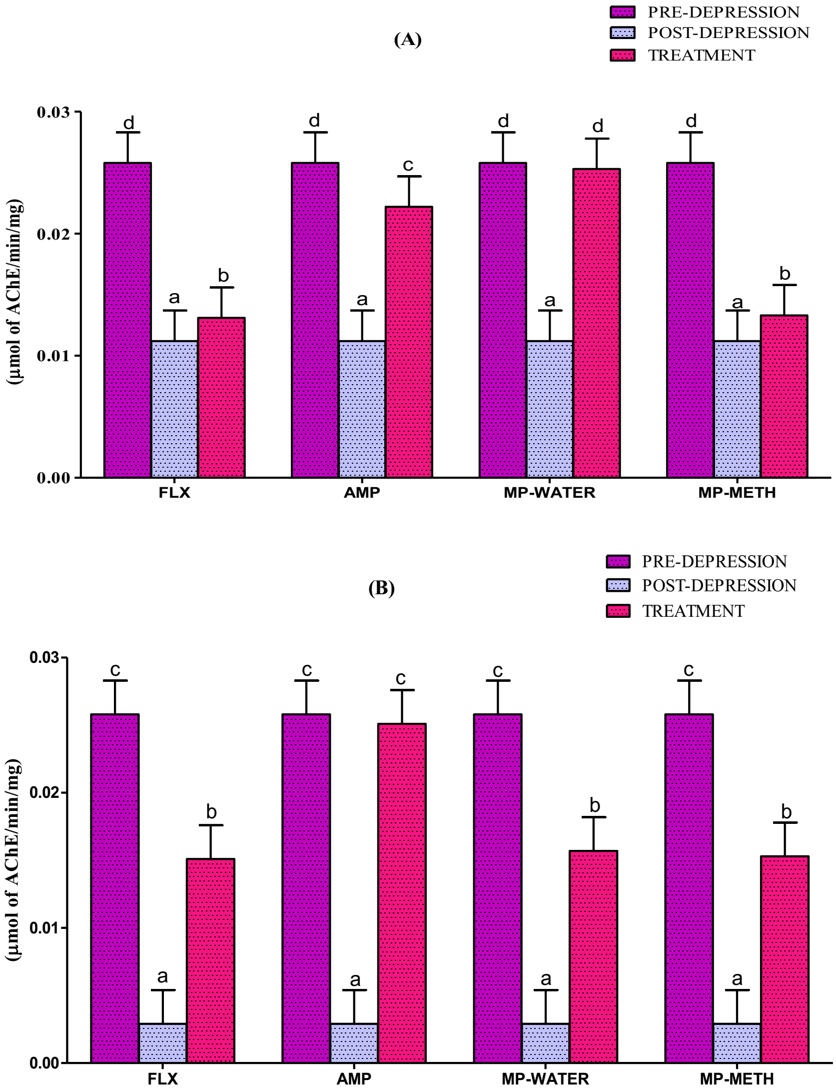
Survival rate

The survival rate analysis under cold and heat stress indicated that the treatments had different effects. Fluoxetine (FLX) increased survival under cold stress but negatively affected control flies, while Amitriptyline (AMP) improved survival in both cold and heat stress. MP-Water was effective under heat stress, and MP-Meth under cold stress, suggesting that the neuroprotective effects of *M. pruriens* are stressor-specific.

Catalase activity

Catalase is an essential antioxidant enzyme that mitigates oxidative stress by converting hydrogen peroxide to water and oxygen [31]. Depression is often associated with oxidative stress, leading to altered catalase activity [32]. In this study, AMP and MP-Meth significantly reduced catalase activity under heat and cold stress, respectively, suggesting that these treatments effectively modulate oxidative stress. The presence of phenolic compounds in M. pruriens is responsible for this antioxidant properties [33].

Fig. 4 Effect of different antidepressant on the AChE activity on CMS Induced Depression in *D. melanogaster* in comparison to control. All the treatment increased the AChE activity with ($p < 0.05$). The baseline AChE activity in the control group was **0.0233 µmol/min/mg protein**. After CMS induction:



Bars with different letters (a, b, c, d) indicate statistically significant differences ($p < 0.05$). 'a' represents the lowest AChE activity, with higher letters indicating progressively greater increases

Glutatihione-S-Transferase activity

Glutathione (GSH) plays a crucial role in detoxifying reactive oxygen species [8]. Depression is linked to reduced GSH levels, contributing to oxidative stress [34]. In this study, all treatments reduced G-S-T activity in both stress conditions, with AMP showing the highest reduction in heat stress and MP-Meth in cold stress. This reduction suggests that *M. pruriens* extracts, particularly MP-Meth, effectively ameliorates oxidative stress, due to high alkaloid and flavonoid content [35].

Acetylcholinesterase activity

Acetylcholinesterase (AChE) is involved in the breakdown of acetylcholine, a neurotransmitter crucial for cognitive function and mood regulation [36]. Increased AChE activity has been observed in depression, as a compensatory mechanism (Dulawa et al. 2002). In this study, all treatments increased AChE activity under stress conditions, with AMP

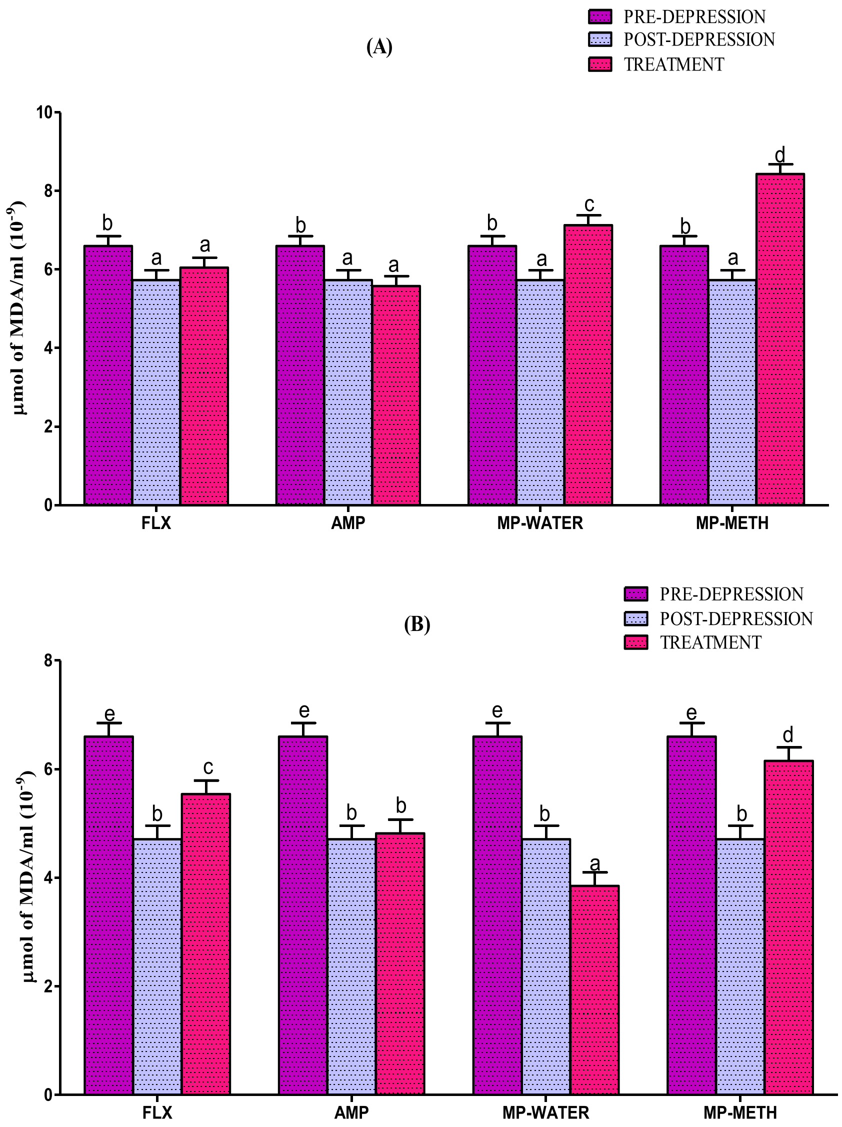


Fig. 5 Effect of different antidepressant on the Lipid Peroxidation of CMS Induced Depression in *D. melanogaster* in comparison to control with ($p < 0.05$). The baseline lipid peroxidation level in the control group was **6.35×10^{-9} µmol of malondialdehyde (MDA) formed/ml**. After CMS induction:

Bars with different letters (a, b, c, d, e) indicate statistically significant differences ($p < 0.05$). 'a' represents the lowest level of lipid peroxidation, with higher letters representing greater peroxidation levels

restoring AChE activity to near pre-depression levels under cold stress and MP-Water showing the highest increase under heat stress. These findings suggest that *M. pruriens* extract, particularly MP-Water, modulate cholinergic pathways and may protect against stress-induced neurodegeneration [37].

Lipid peroxidation

Lipid peroxidation, a process where ROS damage cell membranes, is indicated in the pathophysiology of depression [38]. Although lipid peroxidation levels did not increase under the stress conditions in this study, AMP and MP-Water reduced lipid peroxidation under heat and cold stress, respectively. These findings align with the antioxidant properties of *M. pruriens*, particularly its phenolic and flavonoid content, which are known to inhibit lipid peroxidation [39].

Conclusions

The findings suggest that the phytochemical constituents of *Mucuna pruriens* contribute to its antidepressant effects, potentially through modulation of oxidative stress and cholinergic pathways. Both *M. pruriens* extracts demonstrated unique efficacy across various biochemical markers and stress conditions, emphasizing their potential as innovative treatments for depression. There should be further exploration of the aqueous and methanol extracts from *Mucuna pruriens* as promising therapeutic agents for enhancing depression management.

Recommendation

Further research is needed to elucidate the specific molecular mechanisms underlying the observed effects of standard antidepressant drugs and plant extracts on *Drosophila melanogaster* under stress-induced depression conditions. Clinical studies should also assess whether the effects observed in *Drosophila* translate to humans, with a particular focus on the potential therapeutic benefits and risks of *Mucuna pruriens* extracts compared to standard antidepressant drugs. Also, a combination therapy between both extracts is recommended for better treatment as each has its peculiarity.

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Authors' contributions MES wrote the manuscript and, together with MAA, conducted the study. NBAB principally supervised the project and edited the manuscript. ROY and SIA co-supervised the project. KFN performed the phytochemical screening. ROO, KTGW and WS assisted with biochemical assay evaluation.

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Availability of data and materials All the data for this study are included in the manuscript and are available for the readers.

Declarations

Ethics approval and consent to participate Not applicable.

Consent for publication Not applicable.

Competing interests The authors declare that they have no competing interests.

REFERENCES

1. Hanson ND, Owens MJ, Nemeroff CB. Depression, Antidepressants, and Neurogenesis: A Critical Reappraisal. Neuropsychopharmacology. 2011;36(13):2589-2602. https://doi.org/10.1038/npp.2011.220
2. Willner P. The chronic mild stress (CMS) model of depression: History, evaluation and usage. Neurobiology of Stress. 2017;6:78-93. https://doi.org/10.1016/j.ynstr.2016.08.002
3. Zhang J, Zhou H, Yang J, et al. Low-intensity pulsed ultrasound ameliorates depression-like behaviors in a rat model of chronic unpredictable stress. CNS Neuroscience and Therapeutics. 2020;27(2):233-243. https://doi.org/10.1111/cns.13463
4. McGorry PD, Purcell R, Hickie IB, Jorm AF. Investing in youth mental health is a best buy. Medical Journal of Australia. 2007;187(S7). https://doi.org/10.5694/j.1326-5377.2007.tb01326.x
5. Setorki M. Medicinal herbs with anti-depressant effects. Journal of Herbmed Pharmacology. 2020;9(4):309–317. https://doi.org/10.34172/jhp.2020.39
6. World Health Organisation. Depression and Other Common Mental Disorders Global Health Estimates.; 2017. https://apps.who.int/iris/bitstream/handle/10665/254610/WHO-MSD-MER-2017.2-eng.pdf
7. Nduna M, Jewkes RK, Dunkle KL, Jama Shai NP, Colman I. Prevalence and factors associated with depressive symptoms among young women and men in the Eastern Cape Province, South Africa. Journal of Child and Adolescent Mental Health. 2013;25(1):43-54. https://doi.org/10.2989/17280583.2012.731410
8. Bhatt S, Nagappa AN, Patil CR. Role of oxidative stress in depression. Drug Discovery Today. 2020;25(7):1270-1276. https://doi.org/10.1016/j.drudis.2020.05.001

9. Correia AS, Cardoso A, Vale N. Oxidative Stress in Depression: The Link with the Stress Response, Neuroinflammation, Serotonin, Neurogenesis and Synaptic Plasticity. Antioxidants. 2023;12(2):470. https://doi.org/10.3390/antiox12020470
10. Dulawa SC, Janowsky DS. Cholinergic regulation of mood: from basic and clinical studies to emerging therapeutics. Molecular Psychiatry. 2019;24(5):694-709. https://doi.org/10.1038/s41380-018-0219-x
11. Chakrabarty M, Bhat P, Kumari S, et al. Cortico-hippocampal salvage in chronic aluminium induced neurodegeneration by Celastrus paniculatus seed oil: Neurobehavioural, biochemical, histological study. PubMed. Published online April 1, 2012. https://doi.org/10.4103/0976-500x.95520
12. Cuijpers P, Karyotaki E, Weitz E, Andersson G, Hollon SD, van Straten A. The effects of psychotherapies for major depression in adults on remission, recovery and improvement: A meta-analysis. Journal of Affective Disorders. 2014;159(159):118-126. https://doi.org/10.1016/j.jad.2014.02.026
13. Geddes JR, Carney SM, Davies C, et al. Relapse prevention with antidepressant drug treatment in depressive disorders: a systematic review. The Lancet. 2003;361(9358):653-661. https://doi.org/10.1016/s0140-6736(03)12599-8
14. Manyam BV, Dhanasekaran M, Hare TA. Neuroprotective effects of the antiparkinson drug Mucuna pruriens. Phytotherapy Research. 2004;18(9):706-712. https://doi.org/10.1002/ptr.1514
15. Patel J, Galani VJ. Investigation of noradrenaline and serotonin mediated antidepressant action of Mucuna pruriens (L) D.C seeds using various experimental models. Oriental Pharmacy and Experimental Medicine. 2012;13(2):143–148. https://doi.org/10.1007/s13596-012-0089-8
16. Karege F, Perret G, Bondolfi G, Schwald M, Bertschy G, Aubry JM. Decreased serum brain-derived neurotrophic factor levels in major depressed patients. Psychiatry Research. 2002;109(2):143-148. https://doi.org/10.1016/s0165-1781(02)00005-7
17. Ugur B, Chen K, Bellen HJ. Drosophila tools and assays for the study of human diseases. Disease models and mechanisms. 2016;9(3):235-244. https://doi.org/10.1242/dmm.023762
18. Dietzl G, Chen D, Schnorrer F, et al. A genome-wide transgenic RNAi library for conditional gene inactivation in Drosophila. Nature. 2007;448(7150):151-156. https://doi.org/10.1038/nature05954
19. Rubin GM, Lewis EB. A brief history of Drosophila's contributions to genome research. Science. 2000;287(5461):2216-2218. https://doi.org/10.1126/science.287.5461.2216
20. Aquadro CF, Bauer DuMont V, Reed FA. Genome-wide variation in the human and fruitfly: a comparison. Current Opinion in Genetics and Development. 2001;11(6):627-634. https://doi.org/10.1016/S0959-437X(00)00245-8
21. Folarin R, Kayode A, Adenowo TK, Adeyanju MM, Olugbode J, Obadeyin E. GEOTACTICAL AND NEUROCHEMICAL PHENOTYPES OF DROSOPHILA MELANOGASTER FOLLOWING NIGELLA SATIVA EXPOSURE. AfricArXiv (OSF Preprints). Published online May 28, 2019. https://doi.org/10.31730/osf.io/xfuqg
22. Jimoh MA, Idris OA, Jimoh MO. Cytotoxicity, Phytochemical, Antiparasitic Screening, and Antioxidant Activities of Mucuna pruriens (Fabaceae). Plants. 2020;9(9):1249. https://doi.org/10.3390/plants9091249
23. Shah MD, Hossain MA. Total flavonoids content and biochemical screening of the leaves of tropical endemic medicinal plant Merremia borneensis. Arabian Journal of Chemistry. 2014;7(6):1034-1038. https://doi.org/10.1016/j.arabjc.2010.12.033
24. Phuyal A, Ojha PK, Guragain B, Chaudhary NK. Phytochemical screening, metal concentration determination, antioxidant activity, and antibacterial evaluation of Drymaria diandra plant. Beni-Suef University Journal of Basic and Applied Sciences. 2019;8(1). https://doi.org/10.1186/s43088-019-0020-1
25. Santhi K, Sengottuvel R. Qualitative and Quantitative Phytochemical analysis of Moringa concanensis Nimmo. International Journal of Current Microbiology and Applied Sciences. 2016;5(1):633–640. https://doi.org/10.20546/ijcmas.2016.501.064
26. Araujo SM, Poetini MR, Bortolotto VC, et al. Chronic unpredictable mild stress-induced depressive-like behavior and dysregulation of brain levels of biogenic amines in Drosophila melanogaster. Behavioural Brain Research. 2018;351:104-113. https://doi.org/10.1016/j.bbr.2018.05.016
27. Adedara AO, Babalola AD, Stephano F, et al. An assessment of the rescue action of resveratrol in parkin loss of function-induced oxidative stress in Drosophila melanogaster. Scientific Reports. 2022;12(1):3922. https://doi.org/10.1038/s41598-022-07909-7
28. Salim M, Prajapati IP, Singh M. Investigations into the Phytochemical Composition of Mucuna pruriens (L.) Seeds. Published online November 16, 2023:54–61. https://doi.org/10.9734/bpi/mono/978-81-967669-6-2/ch8
29. Hadimani, Gavishiddappa A, Desai SD, et al. Evaluation of Acute Oral Toxicity and Phytoconstituents of Methanolic Extract of Mucuna pruriens. Journal of Pharmaceutical Sciences and Research. Journal of Pharmaceutical Sciences and Research. 2015;Vol. 7(1)(0975–1459):33–36.
30. Yadav SK, Prakash J, Chouhan S, et al. Comparison of the neuroprotective potential of Mucuna pruriens seed extract with estrogen in 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-induced PD mice model. Neurochemistry International Journal. Published online January 2014:65:1–13. https://doi.org/10.1016/j.neuint.2013.12.001
31. Chelikani P, Fita I, Loewen PC. Diversity of structures and properties among catalases. Cellular and Molecular Life Sciences (CMLS). 2004;61(2):192-208. https://doi.org/10.1007/s00018-003-3206-5
32. Gałecki P, Szemraj J, BieńkiewiczM, Zboralski K, GałeckaE. Oxidative stress parameters after combined fluoxetine and acetylsalicylic acid therapy in depressive patients. Human Psychopharmacology: Clinical and Experimental. 2009;24(4):277–286. https://doi.org/10.1002/hup.1014
33. Tavares R, Vasconcelos M, Dutra M, et al. Mucuna pruriens Administration Minimizes Neuroinflammation and Shows Anxiolytic, Antidepressant and Slimming Effects in Obese Rats. Molecules. Molecules. 2020;25(23). https://doi.org/10.3390/molecules25235559
34. Maes M, Galecki P, Chang YS, Berk M. A review on the oxidative and nitrosative stress (OandNS) pathways in major depression and their possible contribution to the (neuro)degenerative processes in that illness. Progress in Neuro-Psychopharmacology and Biological Psychiatry. 2011;35(3):676-692. https://doi.org/10.1016/j.pnpbp.2010.05.004

35. Madhyastha S, Chauhan R, Rao G, Umesh H. Neuroprotective effects of Mucuna pruriens against stress-induced oxidative damage. Journal of Physiological and Biomedical Sciences. 2011;24(2):28-33.
36. Ullas KS, Chaturvedi A, Bhaskar YD, Kundapura N, Amin N, Devaramane V. Increased Levels of Acetylcholinesterase, Paraoxonase 1, and Copper in Patients with Moderate Depression- a Preliminary Study. Reports of Biochemistry and Molecular Biology. 2019;7(2):174-180.
37. Walczak-Nowicka ŁJ, Herbet M. Acetylcholinesterase Inhibitors in the Treatment of Neurodegenerative Diseases and the Role of Acetylcholinesterase in their Pathogenesis. International Journal of Molecular Sciences . 2021;22(17). https://doi.org/10.3390/ijms22179290
38. Mao C, Lei G, Horbath A, Gan B. Assessment of lipid peroxidation in irradiated cells. Radiation Oncology and Radiotherapy Part A. Published online 2022:37–50. https://doi.org/10.1016/bs.mcb.2022.05.003
39. Joshi YB, Domenico Praticò. Lipid Peroxidation in Psychiatric Illness: Overview of Clinical Evidence. Oxidative Medicine and Cellular Longevity. 2014;2014:1–5. https://doi.org/10.1155/2014/828702

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