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Evaluation of anti-depressant properties of ethanol extract of *Zingiber officinale* rhizome in mice

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ABSTRACT: Depression, a common psychiatric disease, is associated with moodiness, disinterest, and anhedonia. *Zingiber officinale* is a traditional herb used to treat various disorders. This study evaluated the effect of ethanol extract of *Zingiber officinale* (EEZO) rhizome on depression in mice. Forty-eight male mice (28±2 g) were used and divided into six groups of 8 mice each. Depression was induced using the chronic mild stress model and then treated for three days afterwards. Group 1: control (normal saline), group 2: depressed, group 3: standard drug (diazepam; 1 mg/kg), groups 4, 5 and 6: treatment (50 mg/kg, 100 mg/kg and 200 mg/kg) body weight of EEZO respectively. Behavioural tests (open field, tail suspension, sucrose preference, dark and light box, hole maze and object exploration) were carried out on the mice before and after treatment. Concentration of inflammatory cytokines such as prostaglandins E_2, interleukin-1, tumour necrosis factor-α, interferon gamma, cyclooxygenase and nitric oxide was determined. The extract significantly ($p < 0.05$) improved behavioural pattern of mice and reduced the level of the inflammatory biomarkers, relative to the depressed mice. The results implied that EEZO reduced stress-induced depression in mice and could be a potential alternative for anti-depressant drug formulation.

Keyword: Depression, Chronic mild stress model, *Zingiber officinale*, cytokines, behaviour tests

Introduction

Depression is a chronic multifactorial disorder that affects mood, thought, behaviour and physical health. It has assumed a global health concern status as it affects up to 20% of the world's population [1]. Anxiety and depression have a long, close relationship in psychiatry investigation [2]. The role of physical stress as a common anxiogenic method in the development of depression has been reported [3]. Additionally, many inflammatory markers such as prostaglandins, interleukins, interferons, cyclooxygenase, and nitric oxide have been suggested as contributors to the development of depression. Clinical studies have reported higher levels of circulating pro-inflammatory cytokines, such as interleukin-1β, interleukin-6 (IL-6), and tumour necrosis factor-α (TNF-α), in depressed individuals [4]. Irregular distortion of the concentrations of these active proteins has been associated with depression [5]. Synthetic antidepressant drugs available to manage the disorder are not only mentally addictive but also not affordable

and laden with serious side effects [6]. Phytoconstituents gotten from herbs are gaining increased attention to manage disorders, including depression and anxiety owing to the myriads of bioactive constituents found in them. Additionally, the relative safety, affordability, and multi-therapeutic efficacy of phytotherapy have endeared it to more drug-discovery research in recent times [7]. Previous studies had described some anti-depressant plant extracts such as *Kaempferia parviflora* [8], *Rosmarinus officinalis* [9], *Hemerocallis Citrina* [10], among others. The plant of interest in this study is *Zingiber officinale*, a plant native to South-eastern Asia [11]. *Z. officinale* is commonly called ginger and has different names in Nigerian local dialects: 'Ata ile', 'chita' and 'jinga' in Yoruba, Hausa and Igbo languages respectively [12].

In folklore medicine, the plant has been used to treat many diseases such as nausea, gastrointestinal disorders, respiratory disorders, atherosclerosis, migraine, depression, gastric ulcer, cholesterol, inflammation, rheumatoid arthritis etc. [13]. The effect of *Zingiber officinale* on central nervous system had been previously described, as well as its *in silico* antidepressant study. The current study aims to investigate the effect of ethanol extract of *Zingiber officinale* on stressed-induced depressive mice by determining its effect on pro-inflammatory cytokines such as interleukin-1, interferon and tumour necrosis factor-α (TNF-α), as well as prostaglandins, COX and nitric oxide in the brain and serum of mice. Additionally, mice behavioural pattern was also assessed after inducing stress through the chronic mild stress (CMS) model.

Materials and Methods

Plant material

Fresh *Zingiber officinale* rhizome used were purchased from a local market in Ipata, Kwara State, North-west Nigeria. The plant rhizome parts were authenticated (UILH/001/2019/1168) at the Herbarium unit of the Department of Plant Biology, University of Ilorin, Kwara State, and a voucher specimen was deposited at the University Herbarium. The *Zingiber officinale* rhizome were cleaned to remove dust and dirt, they were then chopped into tiny pieces and oven-dried for 2 to 3 days till completely dried.

Extract preparation

The dried *Z. officinale* were pulverized using a blender (Binatone model, Lagos, Nigeria). The powder was packed into an airtight container and labelled accordingly. The extraction protocols described by Fatope *et al.* (1993) were adopted [14]. Briefly, 500 g of the *Zingiber officinale* rhizome powder were soaked in 1500 mL of 95% ethanol in a flask and was allowed to stand for 24 hours with vigorous shaking to ensure full extraction of the active ingredients. The suspension was then filtered using Whatman filter paper and the filtrate was kept separately from the residue. The residue obtained was re-soaked with 1500 mL of 95 % ethanol for 24 hr and filtered as well. These steps were repeated until the colour of the filtrate gotten was faint to ensure complete extraction. The resulting filtrate were pooled together and concentrated in a rotary evaporator. The resulting ethanol extract was then weighed, and the percentage yield was 8.34%. The ethanol extract of *Z. officinale* (EEZO) was used to prepare desired doses (50, 100 and 200 mg/kg body weight of mice). Diazepam was also prepared as 1 mg/kg body weight of mice.

Chemicals and reagents

Diazepam (Valium, LOT D0513005, EXP 08/2020) used was obtained from Masjad Pharmacy, Ilorin, Kwara State, Nigeria. The pro-inflammatory cytokine kits were products of ElabScience Inc., USA. The water used was glass-distilled and obtained from Medical Biochemistry and Pharmacology Laboratory, Kwara State University, Malete, Nigeria. Except otherwise stated, all other reagents and chemicals used are of high analytical grades.

Experimental animals

Forty-eight (48) male mice with weight within 28.20 ± 0.02 g were used for the study which was purchased from the Kwara State University animal house. The animals were maintained under hygienic conditions, and they were kept in clean ventilated plastic cages with free access to food and water, for five days to acclimatize to the environment.

Ethical approval

Animal handling strictly followed the principles of laboratory animal care (NIH Publication No. 85-23, 1996) and ethical clearance (KWERC/2019/12) was obtained from the Institutional Animal ethics committee before commencement of the study.

Experimental design

The forty-eight (48) male mice were used for the study out of which 40 were subjected to stress and were divided into six groups with 8 mice in each group:

Group 1: Control mice
Group 2: Stressed mice
Group 3: Mice treated with 1 mg/kg diazepam
Group 4: Mice treated with 50 mg/kg EEZO rhizome
Group 5: Mice treated with 100 mg/kg EEZO rhizome
Group 6: Mice treated with 200 mg/kg EEZO rhizome

Phase 1 study: Induction of depression

Forty (40) mice (involving groups 2, 3, 4, 5 and 6) were subjected to stress to induce depression as described in Table 1.

Table 1: Experimental procedure carried out to induce and check for depression in mice

Days	Treatment	Description
Days 1-3	Exposure to rat	Mice were subjected to stress by exposing them to rat to induce depression
Day 4	Restraint	Rats were separated from the mice to avoid overstressing and perhaps mortality
Days 5-7	Exposure to rat	Mice were further subjected to stress by exposing them to rat to further induce depression
Day 8	Behavioural tests	Behavioural tests were carried out on mice pre- and post-treatment to check for depression Tests such as: Open field test Tail suspension test Sucrose preference test Dark and light box test Object exploration test Hole maze test were conducted

Behavioural tests

Open Field Test (OFT)

In OFT, plywood was cut into the size of the container and placed in it. The plank was divided and marked into sixteen 18 x 18 cm squares (Figure 1). Mice were put in the center and their behavior was

recorded on camera for 2 min. The number of squares visited by each mouse was calculated as previously described by Holmes [15].

Tail Suspension Test (TST)

In TST, each mouse was individually suspended at a height of 30 cm from the floor, by adhesive tape placed approximately 1 cm from the tip of the tail (Figure 2). The immobility period was recorded for 6 min. Mouse was immobile when it did not show any body movement, hung passively and completely immobile [16].

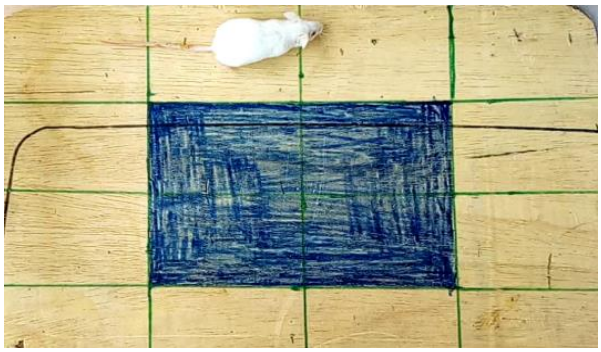

Figure 1: Open field test

Figure 2: Tail suspension test

Sucrose preference test

Animals were given hours of free choice between two bottles of either 1% sucrose or distilled water, as described elsewhere (2018) as they were left overnight [17]. At the beginning and end of the period, the bottles were weighed, and consumption was calculated.

Dark and light box test

Mice were placed into the dark compartment, from where they could visit the lit box, illuminated by ray of sunlight. The latency of the first exit to the light compartment, the total duration of time spent in the lit area, and the number of visits to this depression-related compartment (dark area) were scored by visual observation over 2 min. The dark/light box test is also based on the rodents' innate aversion to brightly illuminated areas and on the spontaneous exploratory behaviour of the animals [18].

Object exploration test

Test is done to check for mice innate desire to explore novel environment by placing them in different cages with different objects, which measures the latency and consumption of food in a novel unfamiliar environment denoting whether they are active or not [19].

Hole maze test

Mice attitude to open spaces and heights is checked by placing them in the intersection of the maze (Fig. 3) and allowing them to move freely, there activeness is recorded relative to their exploration around the maze in 2 min testing session [15].

Figure 3: Hole maze test

Phase 2 study: Treatment phase (post-depression)

The mice were grouped and treated with EEZO rhizome or diazepam post-depression (as described below):

Group 1: control mice (received 2 mL normal saline intraperitoneally for three days).
Group 2: stressed mice (non-treated received 2 mL distilled water intraperitoneally for three days).
Group 3: standard drug mice (treated with 2 mL of 1 mg/kg diazepam intraperitoneally for three days).
Group 4: 50 mg/kg EEZO mice (treated with 2 mL of 50 mg/kg EEZO rhizome intraperitoneally for three days).
Group 5: 100 mg/kg EEZO mice (treated with 2 mL of 100 mg/kg EEZO rhizome intraperitoneally for three days).
Group 6: 200 mg/kg EEZO mice (treated with 2 mL of 200 mg/kg EEZO rhizome intraperitoneally for three days).

**Behavioural tests were carried out after three (3) days of EEZO rhizome/diazepam treatment to evaluate effects on depression*

Biochemical indices

Measurements of inflammatory cytokines

The level of the proinflammatory cytokines IFN-γ, IL-1β, TNF-α. was determined based on the manufacturer's instruction. PGE_2 and COX were determined according to the methods hitherto reported by Stachowska [20]. The concentration of nitric oxide concentration followed the procedures of Kumar and Chanana [21].

Statistical analysis

Statistical analysis was performed using Graphpad Prism 5 program (GraphPad Software Inc., La Jolla, Ca, USA). Analysis of variance (ANOVA) followed by post-hoc Tukey's test was used for the analysis. Data were expressed as mean ± a SEM value, which was performed using Microsoft Excel software 2007. $p < 0.05$ (probability level) was considered as statistically significant.

Results

Behavioural tests

The results of the behavioural tests are presented in Figures 4-7 and Table 2. In the OFT, the distance travelled by the EEZO-treated (200 mg/kg) mice is comparable to the control and the diazepam group after three days of treatment (Figure 4). There was a significant increase ($p < 0.05$) in locomotor activity, measured as the distance covered by mice treated with EEZO rhizome relative to the non-treated group. The effect of EEZO rhizome on the time spent in immobile state in TST is presented in Figure 5. Before treatment with EEZO Rhizome, mice spent longer time in the immobile state in comparison with control group. However, the immobility time significantly ($p < 0.05$) decreased within days 1 and 3 of treatment with EEZO rhizome.

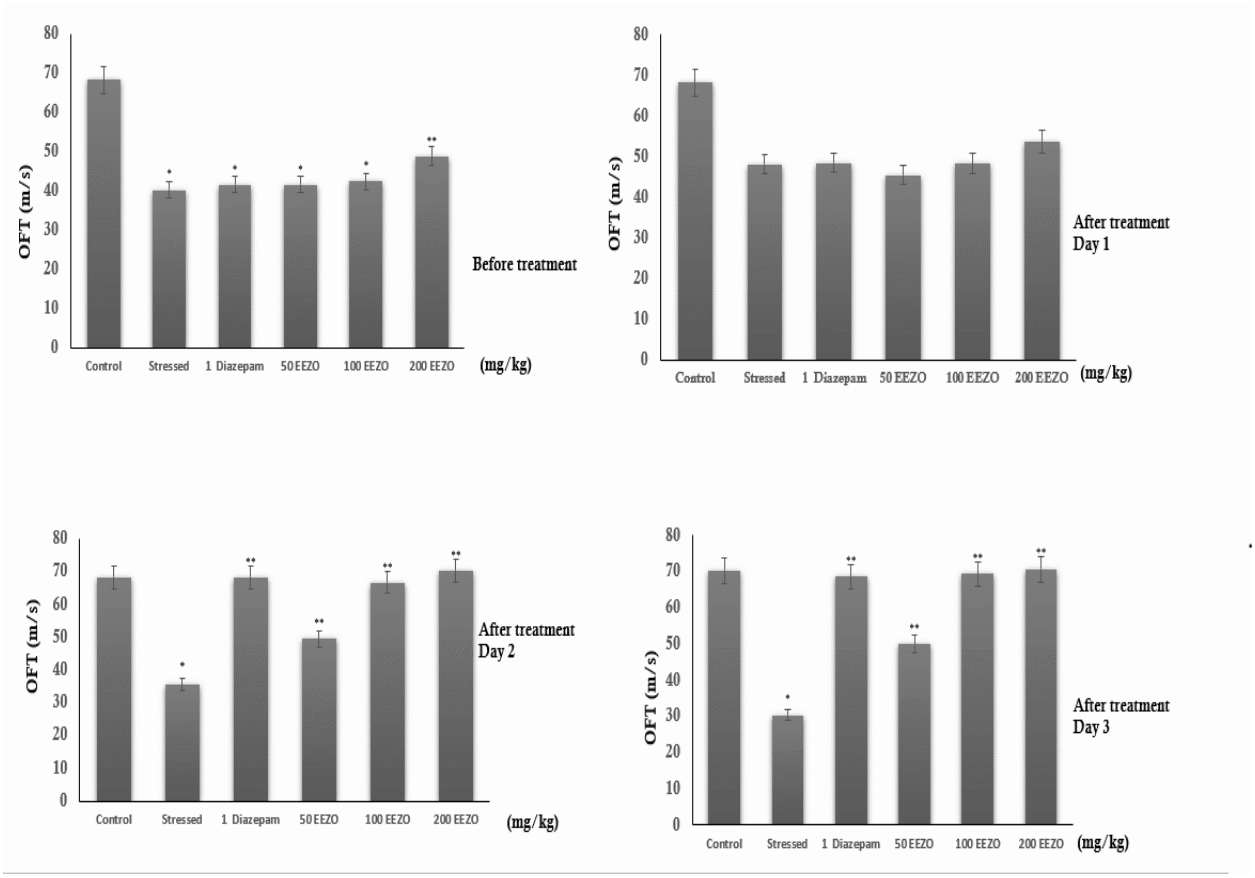

Figure 4: Effect of EEZO rhizome on the distance covered by CMS-induce depressed mice in OFT

The reduction in immobility time was dose-dependent, as the mice treated with 200 mg/kg of the extract exhibited increased mobility comparable to those in the diazepam and the control group. As shown in Fig. 6, exposure of mice to CMS significantly reduced the volume of sucrose consumption in the stressed mice which remained to be significantly lower ($p < 0.05$) in comparison with the control group. Mice treated with 100 and 200 mg/kg of EEZO rhizome consumed more sucrose solution and therefore significantly ameliorated depression behaviour induce by chronic mild stress procedure ($p < 0.05$) (Figure 6). Figure 7 shows the effect of EEZO rhizome on the time spent by mice in dark and light transition test chamber.

Treatment with EEZO rhizome over the course of three days significantly improved the mice affinity for light. Depressed mice spent more time in the dark box than the treated mice. Comparing the mice behaviour before and after three (3) days of treatment with EEZO rhizome, there was significant ($p < 0.05$) increase in mice aversion towards the light space dose dependently. Mice treated with 200 mg/kg of EEZO rhizome spent more time in the light section than the diazepam and the control groups.

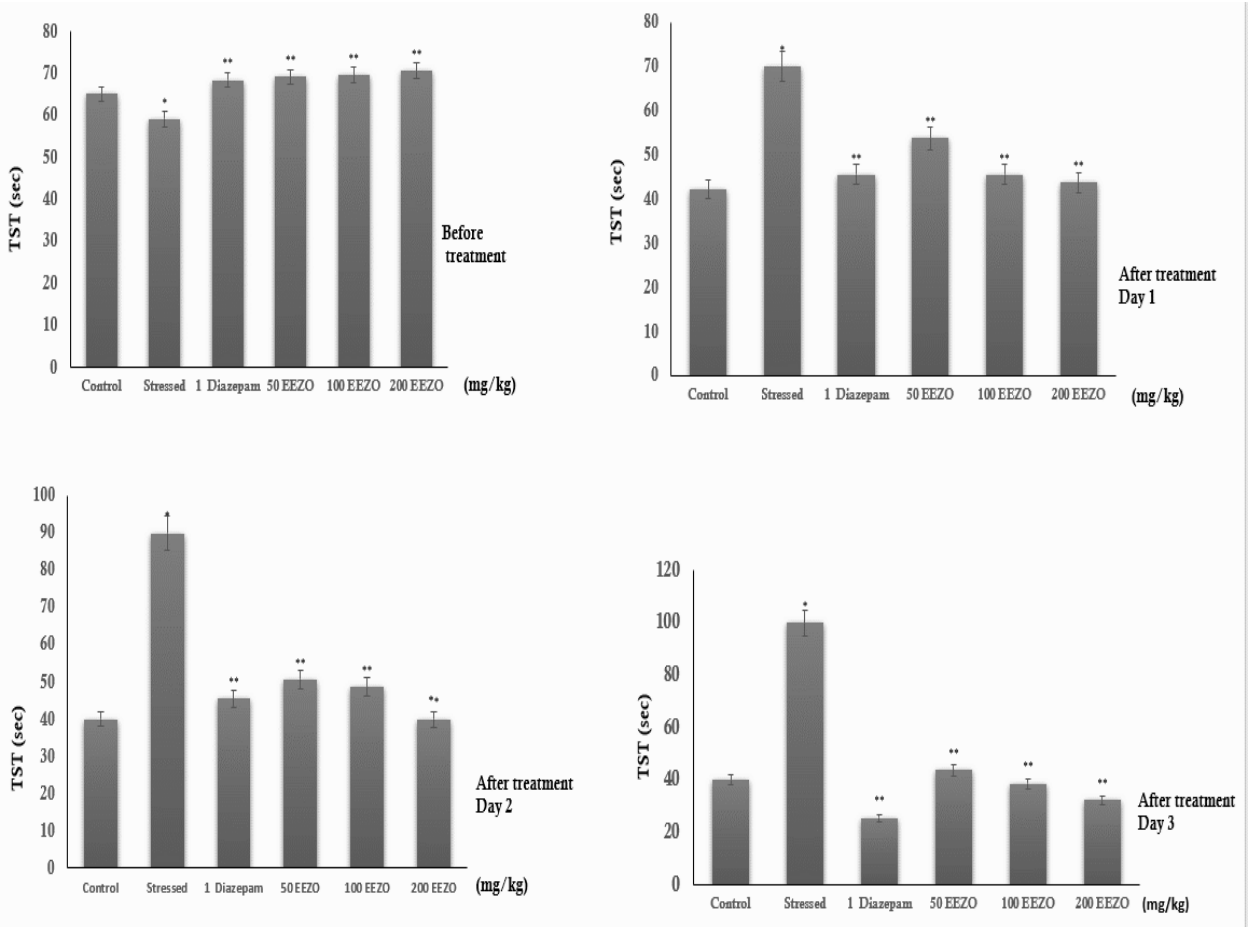

Figure 5: Effect of EEZO rhizome on the immobility state of CMS-induce depressed mice in the TST.

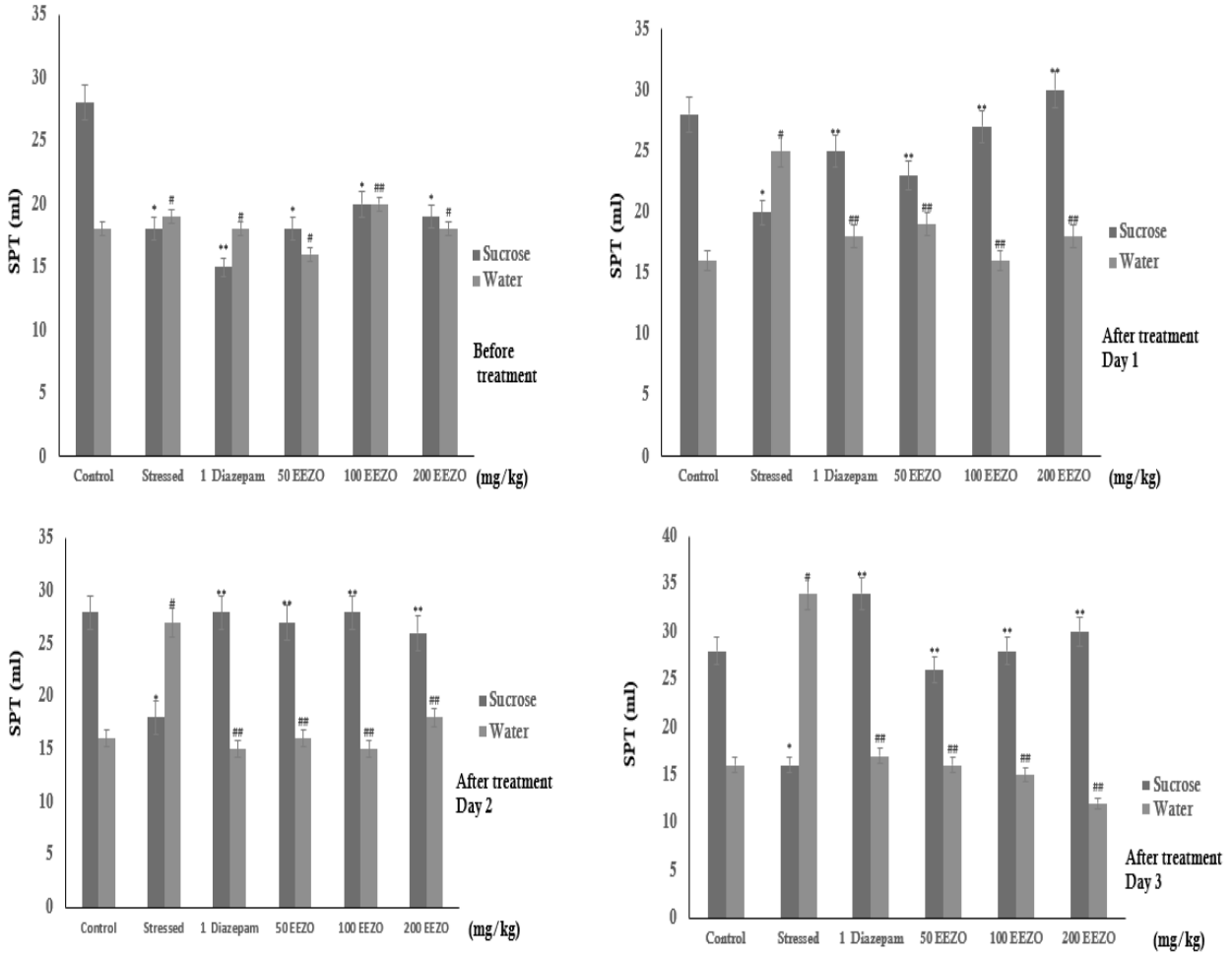

Figure 6: Effect of EEZO rhizome on sucrose solution consumption by CMS-induce depressed mice in SPT.

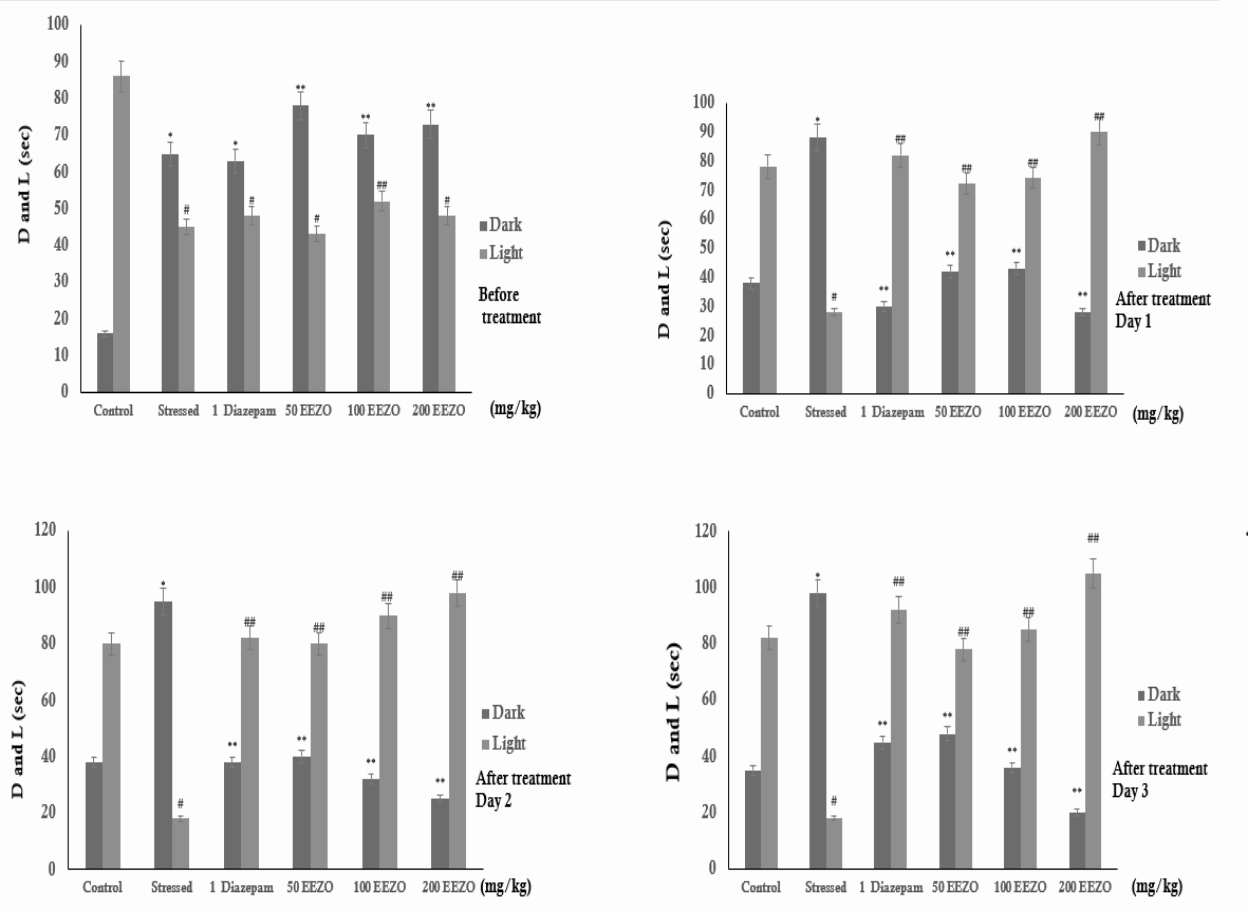

Figure 7: Effect of EEZO rhizome on the time spent in dark and light transition chamber by CMS-induce depressed mice.

Table 2 shows the effect of EEZO rhizome on movement activity of mice in hole maze and object exploration test. It was noted that the control group explored fully while the depressed group explored partially which then declined significantly in three days. Mice treated with EEZO rhizome did not also explore fully after day 1 but explored fully after three days of treatment. It was also observed that mice treated with 200 mg/kg dose of EEZO rhizome were more exploratory with objects and moved around new spaces checking for entries and exits in the hole maze compared to the control mice, whereas the depressed mice had lower tendency to stay in the space.

Inflammatory biomarkers

The concentration of prostaglandin E_2 in the brain and serum of mice is presented in Table 3. The level of PGE_2 is significantly higher in the brain and serum (51.46±0.56 and 122.89±2.60 respectively pg/ml) of depressed mice than in other groups. The mice treated ethanol extract of EEZO rhizome showed significantly ($p < 0.05$) higher concentration of PGE_2 in the brain and serum than the control group, except for the 100 mg/kg-treated mice which had significantly ($p<0.05$) lower concentration of PGE_2 in the serum (21.41±0.62 pg/mL).

Table 2: Effect of EEZO rhizome on exploratory behaviour in CMS-induce depressed mice

	HOLE MAZE TEST				NOVEL CAGE EXPLORATION TEST			
GROUPS	**Before treatment**	**After treatment day 1**	**After treatment day 2**	**After treatment day 3**	**Before treatment**	**After treatment day 1**	**After treatment day 2**	**After treatment day 3**
Control	explored fully	explored fully	moderate exploration	explored fully	explored completely	explored fully	explored fully	explored fully
Stressed	explored partially	mild movements	did not explore	did not explore	mild exploration	mild exploration	did not explore	did not explore
Diazepam (1 mg/kg)	did not explore	moderate exploration	explored fully	explored fully	did not explore	explored fully	explored fully	Explored fully
EEZO (50 mg/kg)	did not explore	explored partially	explored partially	moderate exploration	did not explore	explored partially	moderate exploration	moderate exploration
EEZO (100 mg/kg)	did not explore	explore moderately	explore moderately	explored fully	partial exploration (+)	partial exploration (++)	explored fully	explored fully
EEZO (200 mg/kg)	explored partially	fully explored	fully explored	fully explored	partial exploration (+)	partial exploration (++)	explored fully	explored fully

Table 3: Effect of EEZO rhizome on the level of inflammatory biomarkers in the serum and brain of CMS-induce depressed mice

Groups	Prostagladin E2 (ρg/mL)		Interleukin 1 (ρg/mL)		Tumor Necrosis Factor Alpha (ρg/mL)		Interferon Gamma (ρg/mL)		Nitric Oxide (mg/dL)		Cyclooxygenase (Cox) (U/L)	
	Brain	**Serum**	**Brain**	**Serum**	**Brain**	**Serum**	**Brain**	**Serum**	**Brain**	**Serum**	**Brain**	**Serum**
Control	40.42±1.48	24.25± 1.98	0.68±0.00	0.56± 0.03	114.41± 1.76	100.96± 0.42	53.9±2.45	27.14± 2.19	19.10±1.21	28.51±1.47	0.39±0.06	7.73±0.86
Stressed	51.46±0.56	122.89± 2.60	5.866± 0.04	0.66± 0.00	179.88± 0.67	144.81± 1.60	63.45± 1.89	42.62± 0.83	31.38±0.43	38.07±0.10	7.24±0.48	22.36±0.46
Diazepam (1 mg/kg)	53.00±2.00	37.95± 0.66	0.83± 0.012	0.57± 0.07	127.25± 1.42	120.89± 0.25	54.40± 1.54	26.18± 0.34	21.01±2.77	33.43±1.41	1.22±0.12	11.13±0.57
EEZO (50 mg/kg)	44.26±0.37	31.91± 0.26	1.78±0.77	0.63± 0.06	153.19± 1.68	125.29± 0.81	57.37± 1.41	37.69± 1.03	23.12±1.62	36.00±4.40	3.36±0.02	12.86±0.38
EEZO (100 mg/kg)	43.22±0.67	21.41± 0.62	1.13±0.50	0.62± 0.01	148.26± 1.65	122.99± 2.10	52.62± 0.82	32.66± 2.29	21.10±1.25	35.51±0.71	2.06±0.74	12.66±2.47
EEZO (200 mg/kg)	54.10±1.04	56.65± 1.71	3.71±0.41	0.65± 0.00	154.86± 1.29	134.00± 0.09	57.70± 0.45	42.23± 1.87	28.03±1.19	38.72±0.57	4.62±0.01	13.46±0.56

Mice treated with 200 mg/kg dose of EEZO rhizome had significantly ($p < 0.05$) higher level of PGE_2 in the brain and lower amount in the serum (54.10±1.04 and 56.65±1.71 pg/mL respectively) relative to the mice treated with the 1 mg/kg dose of Diazepam (53.00±2.00 and 37.95±0.66 pg/mL, respectively). Table 3 also shows the concentration of IL-1 in the brain and serum of mice. The mice treated with the highest dose (200 mg/kg) of EEZO rhizome had significantly ($p < 0.05$) higher level of IL-1 in the brain and serum than both control and standard groups. The depressed mice however had the highest amount of IL-1 in the brain (5.866±0.04 pg/mL) and its serum concentration (0.66±0.00 pg/mL) is comparable, though significantly different, with those of mice treated with 200 mg/kg dose of EEZO rhizome (0.65±0.00 pg/ml). Other treatment groups also showed significantly higher concentration of IL-1 in the brain relative to the serum.

This observation is however not dose dependent. Similarly, concentrations of TNFα in the brain and serum of mice of the treated mice are significantly ($p < 0.05$) higher than the control group. It was however observed that depressed mice had the highest concentration of TNFα in both the brain and serum (179.88±0.67 and 144.81±1.60 pg/mL) which are significantly ($p < 0.05$) different from other treatment groups and the control group (Table 3). For IFNγ, the depressed mice had the highest concentration of IFNγ in the brain and serum which are significantly different from all other groups.

The EEZO-treated groups had significantly ($p < 0.05$) higher level of IFNγ in the brain and serum than the standard group, except for 100 mg/kg group which had significantly lower level of IFNγ in the brain (52.62±0.82 pg/mL) compared with the standard group (54.40±1.54 pg/mL) and the control group (53.99±2.45 pg/mL). The level of NO in the brain and serum (28.03±1.19 and 38.72±0.57 mg/dL, respectively) of 200 mg/kg-treated mice is significantly ($p < 0.05$) higher than the standard (21.01±2.77 and 33.43±1.41 mg/dL, respectively) and control (19.10±1.21 and 28.51±1.47 mg/dL, respectively) groups. Other treatment groups (50 and 100 mg/mL) had significantly ($p < 0.05$) higher level of NO in the serum than the standard group which has a comparable level of NO in the brain with the 100 mg/kg-treated mice. The depressed mice however had the highest concentration of NO in the brain and serum (31.38±0.43 and 38.07±0.10 mg/dL, respectively), which are significantly different from the treatment and control groups. As shown in Table 3, the levels of COX in the EEZO-treated mice are significantly ($p < 0.05$) elevated in both serum and brain in comparison with the mice treated with 1 mg/kg diazepam, with the depressed mice having the highest in both the serum and the brain. The control group had the lowest concentrations of COX in both the brain and the serum.

Discussion

Phytotherapy has remained a viable option in the management of different diseases and disorders due to its cost effectiveness, availability, and reduced toxicity [22]. One of such predominantly used and sought-after herbal plants is *Zingiber officinale* which has been previously reported for its antioxidant [22], antidiabetic [23], antiulcer [24], anticancer properties [25], among others. In the current study, depression was induced using the chronic mild stress (CMS) method as previously described by Willner [26], and the behavioural pattern of depressed mice was investigated after treatment with EEZO rhizome. The results of the behavioural tests suggest that the administration of EEZO rhizome significantly increases the distance covered by the mice in the OFT and improves sucrose consumption in the SPT, comparable with the standard and control groups. These could suggest that EEZO rhizome is capable of attenuating depressive behaviours in mice.

This finding is in line with previous reports of Wang (2019) and Li (2018) where plant extracts have been shown to possess antidepressant activities [27, 28]. The TST has been noted as a reliable behavioural test model in rodents and a potent tool for psychiatry investigation [29]. The observed immobility in depressed rodents in the TST is a similitude of lowered mood or depressive behaviour in humans [30]. In this study, the 200 mg/kg of EEZO rhizome significantly reduced the immobility time in mice. This marked

display of antidepressant potential by EEZO rhizome in the TST is attributable to its inhibitory effect on monoamine oxidase A, the enzyme responsible for serotonin degradation.

The role of serotonin in depression and anxiety have been previously reported by Mazarati [31]. Anxiety is directly related to depression and remains an important parameter in investigating depressive behaviours [32]. Consequently, the light and dark test and the hole maze test are often used to establish the anxiolytic potentials of plants, in addition to their antidepressant potentials [33, 34]. Thus, the antidepressant activity of EEZO rhizome was investigated with these two behaviour tests. The treated mice spent significant time in the light section in contrast with the depressed mice that were more drawn to the dark portion of the box, suggestive of the antidepressant property of the extract. Buttressing this, there was a significant increase in the number of open arm crossings by the treated mice and the exploratory behaviour displayed in contrast with the depressed mice in the hole maze test.

This finding agrees with reports of Mendonça Netto [35] and Barua [36] where plant extracts reversed depressive behaviour occasioned by stress and anxiety. Inflammation is gradually emerging as a common mechanism for various diseases, including neuropsychiatric disorders such as depression [37]. Increased peripheral concentration of proinflammatory cytokines has been shown to contribute to the pathophysiology of major depression [38]. Similarly, elevated concentration of prostaglandins (PGs), cyclooxygenase (COX) and nitric oxide (NO) have also been used as biomarkers to established depressive conditions [39,40]. Consequently, the current study evaluated the concentration of these important biomarkers such as PGE_2, IL-1, TNFα, IFNγ, COX and NO in the brain and serum of mice to determine their impacts on depressive behaviour.

EEZO rhizome at the highest dose (200 mg/kg) significantly reduced the concentration of PGE_2 in the brain and the serum of the depressed mice which is comparable to those treated with diazepam. This may be due to the ability of ZO to inhibit prostaglandin synthesis pathway by inhibiting the essential enzyme(s) of the pathway. Expectedly, the elevated activity of COX in the brain and serum of the depressed mice was reversed after treatment with ZO, buttressing our earlier position on PGE_2 since COX is chiefly involved in PGs biosynthesis. This also agrees with previous reports of Lopresti [41] and Xia [42].

The results of this study suggest that the CMS model triggered the production of pro-inflammatory cytokines, in agreement with the previous report of You [43]. Accordingly, the concentrations of all the three pro-inflammatory biomarkers measured (IL-1, TNF and IFNγ) are elevated in the depressed mice. Treatment with EEZO rhizome potentiated the reversal of these elevated concentrations in both the brain and serum of the erstwhile depressed mice. Surprisingly, this observation is not dose dependent as the 100 mg/kg of *Z. Officinale* reduce the brain concentration of TNFα more than the 200 mg/kg and is comparable with the standard and control groups. Since EEZO rhizome reduced the concentration of the measured cytokines, it could be inferred that it has the ability to alter the synthesis of the implicated cytokines at the hippocampus and specific hypothalamic regions in the brain of the treated mice. This further supports the antidepressant efficacy of EEZO rhizome.

Previous reports have also reported other plant extracts as lowering the concentration of pro-inflammatory cytokines [44, 45]. NO plays vital roles in neurogenesis, neurotransmission, synaptic plasticity [39]. It also regulates emotional and cognitive functions, suggesting its culpability in anxiety and depression [21]. Elevated plasma NO concentration has been linked to major depression and rectifying aberrant NO signalling could play a role in combating depression [46]. As observed in this study, the mice treated with EEZO rhizome significantly reduced NO concentration in both the serum and brain relative to the depressed non-treated mice. This could imply that the extract has an inhibitory influence on NO synthesis. It has been hitherto reported that NO is increased in the hippocampus and cerebral cortex regions of the brain in mice following stress [47], and NO inhibition, as displayed by ZO in this study, results in antidepressant-like effects in rodents [48].

Conclusion

Judging by the results of this study, EEZO rhizome displayed remarkable antidepressant properties. The CMS model employed distorted the behavioural patterns of the mice and caused the induction of pro-inflammatory cytokines and other depression-related biomarkers. The intraperitoneal administration of

mice with EEZO rhizome for three days restored normalcy in behaviour and reduced the concentration of depression-related biomarkers. Further study is however suggested to establish the specific compounds responsible for the antidepressant displayed by EEZO rhizome.

Data Availability

The corresponding author may be contacted (asiat.naallah@kwasu.edu.ng) for any requests regarding data for this study.

Conflict of Interest

The authors declare that there is no conflict of interest regarding the publication of this article.

Funding Statement

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