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ANTIDEPRESSANT EFFECT OF METHANOL EXTRACT OF *TAPINANTHUS DODONEIFOLIUS* (DC) DANSER INVOLVES NEUTROTROPHIC AND NEUROENDOCRINE SYSTEMS FOLLOWING CHRONIC STRESS IN MICE

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ABSTRACT

The antidepressant effect of *Tapinanthus dodoneifolius* in mouse despair model has been reported. This study investigates mechanism(s) of its activity using the chronic mild stress model. Chronic unpredictable mild stress (CUMS) was used to induce depression in mice for five weeks. Forty-eight animals were grouped into six groups of eight animals each involving five stress groups and one unstressed group. Weekly weighing was carried out for all the groups. Behavioural tests including tail suspension (TST), open field test (OFT) and sucrose consumption test (SCT) were carried out at base line, 2 weeks and at completion. The stressed groups were treated orally for three weeks starting from second week with graded doses (250, 500 and 1000 mg//kg) of the extract, fluoxetine and distilled water respectively. At the end of the fifth week, all the animals were euthanized and their brains were isolated. The brains were subjected to biochemical assay using ELISA kits for cortisol, brain derived neurotrophic factor (BDNF) and cytokines (IL-1 and IL-6) respectively. The extract produced a significant ($p < 0.05$) reduction in immobility time, ambulation, and anhedonia. Also, the extract significantly ($p < 0.05$) ameliorates CUMS-induced reduction in brain BDNF level and increased brain cortisol level. However, the brain cytokines level was not significantly affected. The study revealed that the extract relieves depressive-like state through the mitigation of despair and anhedonia likely through the neuroendocrine and neurotrophic mechanisms.

KEYWORDS: **Depression; Despair; Anhedonia; brain derived neurotrophic factor (BDNF); Cortisol; Cytokines.**

INTRODUCTION

Depression is a common psychiatric disorder usually characterized by a state of low mood, aversion to activity or apathy which usually affects thoughts, feelings, behavior and sense of well-being [1]. Its worst case results to suicide which is the second leading cause of death among people aged 10-34 or attempt from unattended physical issues making it to be considered as the most serious psychiatric problem [2]. Depression sets in insidiously, though it can be abrupt sometimes, and the course varies considerably based on individual difference, nature of trigger, and treatment. While it presents in an episodic manner in a lot of individuals, it is naturally unforeseeable in terms of number, duration and pattern among individuals [3]. In practice currently, treatment of depression involves non-pharmacological and orthodox drugs. the commonly used orthodox drugs enhance the activity of monoamines [4]. However, the currently available treatments do not produce effective and complete remission and also lead to unwanted adverse effects. Hence, recovery from depression after treatment is about 60% within two years, 40% within four years and 20% within six years and the percentage reduces with age of onset and about 80% experience recurrent episode within their lifetime after recovery which is also affected by

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comorbidities like anxiety [5]. Chronic depressive illness develops when individuals fail to recover after treatment depending on their characteristics at onset and nature of treatment [6]. Meanwhile, the detection, diagnosis, and management of depression often pose great challenges due to its heterogeneous manifestation, unforeseeable course and prognosis, as well as variable response to treatment [7]. Therefore, there is need to develop a safer and more effective pharmacological interventions for the management of depression. There are many animal models developed to evaluate the activity of new antidepressant agents among which the chronic unpredictable mild stress is often described as a prototype and most common model that produce deluge of behavioural modulations typical to depression such as decreased reward response, anhedonia, a behavioural marker of the clinical core feature of depression, in which, rodents are subjected chronically to unpredictable micro-stressors, which can be managed by chronic treatment with antidepressants [8]. Moreover, the chronic unpredictable mild stress produces cellular and molecular changes resulting from stress response such as hyperactivity of the hypothalamic– pituitary–adrenal (HPA) axis leading to increased level of cortisol; the major stress biomarker, impaired neurogenesis and neuroplasticity (measured by determining the level of neurotrophins like the brain-derived neurotrophic factor, BDNF and others) which are important translational biomarkers that enhance understanding about the pathophysiology of depression [9]. Recent evidence shows the role played by cytokines, such as interleukin (IL)-1β; IL-6 and tumor necrosis factor alpha (TNF-α) in the brain to affect neurotransmission, neuroendocrine function, and behaviors associated with depression [10]. The levels of the inflammatory cytokines are up-regulated in individuals with depression, and treatment with anti-inflammatory agents in yields antidepressant effects especially in patients with autoimmune and inflammatory disorders suggesting the involvement of inflammatory processes and brain-systemic immune interactions in the pathophysiology of depression [11]. Medicinal plants play a crucial role in the development of safe alternatives for the management of various diseases presenting in a chronic course and prove resistant to available treatment options. *Tapinanthus dodoneifolius*, besides being used locally for the management of depression, has been reported to possess antidepressant effect in the acute despair model of depression in mice [12]. This study is aimed at evaluating the antidepressant effect of the extract in chronic unpredictable mild stress model of depression by evaluating behavioral changes such as anhedonia using sucrose preference test; despair using tail suspension test and open field test as well as investigate possible involvement of neuroendocrine, neurotrophic and neuro-inflammatory mechanisms.

MATERIALS AND METHODS

Drugs and Chemicals

Fluoxetine capsule 20mg (V.S International pvt. Ltd. Dabhel, India): The drug was prepared during each administration by dissolving the powder from a capsule in 10mL distilled water with 1% to obtain 2mg/mL stock solution which was administered to the animals according to their body weights at the dose 20mg/Kg.

Sucrose (Sigma Aldrich): It was prepared as a 2% solution in distilled water and used for sucrose consumption test.

Distilled water (prepared in the department of pharmacology and therapeutics, Ahmadu Bello University Zaria): it was used for preparation of sucrose solution, suspension of the extract and drugs as well as administration as negative control to the animals.

Animals

Swiss mice (23-28g) of both sex obtained from the Animal House Facility, Department of Pharmacology and Therapeutics, Ahmadu Bello University Zaria were used for the experiment. The animals were maintained in cages under natural day and light cycle, normal environmental hygiene and fed with molded laboratory rodent feed and water *ad libitum*. Relevant ethical approval for the use of animals was sought from the Ahmadu Bello University Committee on Animal Use and Care (ABUCAUC/2020/010).

Plant material

The plant together with part from its host, *Perkia biglobosa* was collected from a forest area in Sabon-Gari Local Government, Kaduna State, Nigeria in March, 2019. It was identified and authenticated at the herbariums section, Department of Botany, Faculty of Life Sciences, Ahmadu Bello University, Zaria (Voucher number: 0350/02846 for the plant and the host). The plant was subjected to soxhlet extraction using 3 L of 70% methanol as a solvent, concentrated to dryness and subjected to phytochemical screening and was confirmed on a thin layer chromatography (TLC) [13]. The extract

was labelled as the methanol extract of *Tapinanthus dodoneifolius.*

Chronic unpredictable mild stress (CUMS)

Animals grouping for CUMS

Forty-eight mice were randomly divided into six groups (treatment groups), based on individual sucrose consumption. The groups are as follows: Group 1 which received stress + distilled water (10mL/kg); group 2 which received fluoxetine (20mg/Kg); group 3 which received stress + TD extract (1000 mg/kg); group 4 which received stress + TD extract (500 mg/kg); group 5 which received stress + TD extract (250 mg/kg); group 6 which received no stress and no treatment. The animals in the treatment groups were further divided into another eight groups (CUMS groups) with one animal from all the treatment groups in each CUMS group.

The CUMS procedure

The CUMS procedure was carried out according to the method described by Shehu *et al.,* [14] with modifications. The stressed mice were singly housed and exposed to the following stressors: 2-h immobilization, 24-h food deprivation, 24-h water deprivation, 5-min cold swim at 4 °C, wet bedding, 24-h cage tilt at 45°, 24-h empty cage exposure, and 1-min tail pinch with a needle placed at 1 cm from the base of the tail. The same stress was not applied successively so that the mice did not anticipate the occurrence of stress. At the end of each stress session, the mice were returned to their home cages with free access to food and water except for the 21-h period of food and water deprivation before the sucrose consumption test until the next stress session. However, for the non-stressor group, all the mice were kept in one cage (male and female separated) with free access to water and food except for 21-h deprivation prior to the sucrose consumption test. They animals were not disturbed except for the purpose of weekly weighing and cage-cleaning. The stress procedure was carried out for 5 weeks. Drug was administered for 3 weeks, starting 2 weeks after CUMS exposure up to the end of the procedure. OFT, TST, and SPT were carried out on three occasions (i.e., before stress, before drug administration, and at the end of drug administration). The experiment took 45 days to complete in the following temporal arrangement: adaptation period: 1st – 3rd day; OFT: 4th, 18th and 39th day; sucrose consumption test: 5th – 8th, 22nd and 43rd day; TST: 9th, 23rd and 44th day; sacrifice: 45th day; stress administration: 10th - 45th day; drug administration: 24th – 45th day.

The sucrose consumption test (SCT)

The test was carried out at 0, 2nd, and 5th weeks. All the mice were housed singly in cages and provided with a bottle of sucrose solution for training to consume 2% w/v sucrose solution for 24 h prior to testing. Subsequently, the bottle of sucrose solution was replaced with tap water for 24 h. At the end of adaptation, mice were deprived of food and water for 24 h. a bottle containing 20mL of 2% sucrose solution was introduced over a 24-h period, and Sucrose consumption was measured by measuring the volume of sucrose solution remaining in the test bottle [15].

The open field test (OFT)

The test was carried out at weeks 0, 2 and 5. Each mouse was placed in a white wooden open field apparatus (70 × 70 × 35 cm, length × breadth × height) of which one wall and the floor were made of Plexiglas. The Plexiglas floor was divided into 16 visible squares (15 × 15 cm) with a large central square. The behavior of mice as peripheral line crossing was recorded for 5 min. The arena was cleaned with 10% ethanol between each tests [16].

The tail suspension test (TST)

This test was carried out at weeks 0, 2, and 5. The total duration of immobility following tail suspension was measured according to the method described by [17]. One hour after treatment, mice were suspended on the edge of a table, 50 cm above the floor using an adhesive tape placed at approximately 1 cm from the tip of the tail. Immobility time was recorded within the last 4 mins period in each animal suspended for 6 minutes. An animal was considered to be immobile when it did not show any movement of the body indicating escape struggle and hanged passively.

Brain tissue harvesting

At the completion of all the behavioral tests, four mice were randomly selected from each group and euthanized by decapitation. The brain samples were harvested and stored in 0.1 M sodium phosphate buffer, pH 7.4, at –20 °C till needed for further investigations. Each brain was minced and homogenized in phosphate buffered saline (PBS; 0.01 M, pH 7.4) using a glass homogenizer on ice. The homogenates were centrifuged at 5000g to obtain the supernatant. The brain homogenates were subjected to analysis using ELISA to determine cortisol, brain derived neurotrophic factor (BDNF) and inflammatory markers (IL - 1 and IL - 6) levels in the brain.

Determination of brain cortisol level

The brain cortisol level was measured using commercially available ELISA kit (Wuhan Fine Biotech Co. Ltd.) following the manufacturer's instruction thus, the plates were washed twice before adding the samples. 50 µL of the sample and standard solutions were added to a precoated antibody plate provided with the kit, and incubated (Incubator model DHP-9035A) for 45 min at 37 °C. The plates were removed, aspirated, and then washed three times, followed by the addition of 100 µL of SABC working solution into each well and incubation for further 30 min at 37 °C. The plates were removed, aspirated, and washed five times, followed by the addition of 90 µL of TMB substrate. The plates were further incubated for 15 min at 37 °C. The reaction was stopped by adding 50 µL of stop solution, and the absorbance was read at 450 nm using a micro plate reader (Rayto-RT-2100C). The sensitivity of the assay was <0.234 ng/mL of cortisol.

Determination of brain-derived neurotrophic factor (BDNF) level

The BDNF level was determined using commercially available ELISA kit (Wuhan Fine Biotech Co., Ltd.), by following the manufacturer's instruction thus, the plate was washed twice before adding the standard, sample, and zero wells. Then, 100 µL of the standard and samples were added to each well and incubated for 90 min at 37 °C, after which the plates were aspirated and washed three times. 100 µL of the biotin-labeled antibody working solution was added to each well and incubated for 60 min at 37 °C, after which the plate was removed, aspirated, and washed three times. Subsequently, 100 µL of SABC working solution was added to each well and incubated for 30 min at 37 °C. The plates were removed, aspirated, and washed five times, 90 µL TMB substrate was added, and finally incubated for 15 min at 37 °C. The plates were removed and 50 µL of stop solution was added into each well. The absorbance was measured immediately at 450 nm using a micro plate reader (Rayto-RT-2100C). The sensitivity of the assay was <2.0 pg/mL of BDNF.

Determination of inflammatory cytokines (IL-1 and IL-6) levels

The brain cytokines level was measured using commercially available ELISA kit (Wuhan Fine Biotech Co. Ltd.) following the manufacturer's instruction thus, standard curve was prepared for a total of 8 points. 100 µL/well of samples were added to the appropriate wells. The precoated plate was sealed and incubated at room temperature for 2 hours. Wells were aspirated and washed 3 times with >250 µL/well Wash Buffer. 100 µL/well of detection antibody (diluted in 1X Assay Diluent) was added, sealed and incubated at room temperature for 1 hour. 100 µL/well of Avidin-HRP diluted in 1X Assay Diluent was added to the plate, sealed and incubated at room temperature for 30 minutes. 100 µL/well of Substrate Solution was added to each well. The plate was incubated at room temperature for 15 minutes. 50 µL of Stop Solution was added to each well. The plate was read at 450 nm using the micro plate reader.

Statistical analysis

All data were presented as mean ± SEM. The data were analyzed by either One Way Analysis of Variance (ANOVA) or Repeated Measure Analysis of Variance (ANOVA) followed by Bonferroni *post hoc* test for multiple comparison to determine the source of significant main effect using statistical package for social science (SPSS)® software version 20. Differences were considered significant at $p < 0.05$.

RESULTS

Effect of methanol extract of *Tapinanthus dodoneifolius* on body weight in mice

At the beginning of the stress, there were no significant ($p > 0.05$) difference between the body weights of the mice in the six groups up to the third week when a significant decrease ($p < 0.05$) was observed with the negative control (distilled water treated) compared to unstressed group which proceeded up to the fourth week. Beginning from the third week, there was a significant ($p < 0.05$) increase in fluoxetine treated group compared to the negative control. However, at the fifth week following treatment with TD extract, there was also a significant increase ($p < 0.05$) in the unstressed, and the TD extract at 250mg/kg compared to the negative control (Figure 1).

Effect of methanol extract of *Tapinanthus dodoneifolius* on sucrose consumption in mice

Before beginning the stress (week 0), there was no significant difference in sucrose consumption across the groups. At the second week of the stress (week 2), there was a significant ($p < 0.05$) reduction in sucrose consumption by all the groups compared to the unstressed group. Following a 3-week treatment with TD extract (250, 500, and 1000 mg/kg) and fluoxetine (20 mg/kg), there was a significant ($p \leq 0.05$) increase between the unstressed, fluoxetine treated and the extract at 1000 mg/kg compared to

the negative control group. Moreover, for groups treated with 1000 and 500 mg/Kg, there was a significant increase in sucrose consumption from week 2 to week 3 (Figure 2).

Effect of methanol extract of *Tapinanthus dodoneifolius* on ambulation of mice in open Field Test (OFT)

Before commencing the stress, at base line (week 0), there was no difference in the number of lines crossed in the Open Field across all the groups. At the second week (week 2), there was reduction in the number of lines crossed by animals in the stressed groups but was insignificant compared to the unstressed group. However, following treatment with TD and fluoxetine (week 5) the fluoxetine treated group and the unstressed showed a significant ($p < 0.05$) improvement in the number of lines crossed while the extract treated groups (1000, 500 and 250 mg/kg) showed a more significant ($p < 0.005$) improvement in the number of lines crossed compared to the negative control (Distilled Water treated) group. Moreover, there was a significant increase in the number of lines crossed from week 2 to week 5 in all treatment groups (1000, 500 and 250 mg/kg) and also a significant increase from baseline (week 0) in group treated with 1000 mg/Kg (Figure 3).

Effect of methanol extract of *Tapinanthus dodoneifolius* on immobility of mice in tail suspension test (TST)

Before commencing the stress at base line (week 0), there was no difference in immobility duration across the groups. Two weeks into the stress (week 2), all the stressed groups showed a significant ($p < 0.05$) increase in immobility duration compared with the unstressed group. Following treatment for three weeks (week 5), all the treatment groups (TD 1000, 500, 250 mg/kg and fluoxetine 20 mg/kg) produced a significantly ($p < 0.05$) reduced immobility time compared to the negative control group (Figure 4).

Effect of methanol extract of *Tapinanthus dodoneifolius* on cortisol level

The level of cortisol in the mice brain was significantly ($p < 0.005$) increased in the stressed and untreated group compared to the unstressed group. Also, the stress induced increase in cortisol level was significantly ($p < 0.005$) reduced in all the TD treated (TD 1000, 500, 250 mg/kg) groups in dose dependent manner and fluoxetine 20 mg/kg compared to the Negative control group (Figure 5).

Effect of methanol extract of *Tapinanthus dodoneifolius* on BDNF level

The brain level of BDNF in the mice was increased insignificantly in the stressed and untreated group compared to the unstressed group. Also, the stress induced decrease in BDNF level was significantly ($p < 0.05$) enhanced in the groups treated with fluoxetine, 1000 and 500 mg/kg of TD respectively compared to the negative control group (Figure 6).

Effect of methanol extract of *Tapinanthus dodoneifolius* on IL-1 level

The Inflammatory/proinflammatory (IL-1) level was not significantly affected by the stress and the treatment though the level after treatment was highest in the negative control (distilled water treated) group and lowest in the fluoxetine treated group followed by the lowest dose of the extract (250 mg/kg) and then the higher doses (500 and 1000 mg/kg) and the unstressed remained at the same level (Figure 7).

Effect of methanol extract of *Tapinanthus dodoneifolius* on IL–6 level

The Inflammatory/proinflammatory mediator (IL-6) level was also not significantly affected in the study though highest level was produced in the negative control (distilled water treated) group while the lowest level was produced in the highest dose (1000 mg/kg) of TD followed by the fluoxetine treated group and then the unstressed and the lower doses (500 and 250 mg/kg) respectively (Figure 8).

DISCUSSION

The chronic unpredictable mild stress is a well-established model used to induce depression in rodents and is used to screen potential antidepressant agents by mimicking the various adversities in human lives in the form of stressors resulting to development of a deluge of behavioral changes, such as decreased response to rewards, a behavioral correlate of the clinical core symptom of depression, anhedonia [18]. The mice subjected to the stressors daily for the 5 consecutive weeks exhibited obvious symptoms of depression as evident by significant weight loss; likely due to depressed appetite, impaired sucrose consumption, and elevated immobility durations in the tail suspension test (TST) as well as some specific biochemical changes. Anhedonia, which is prominent in human depressive state, is widely applied to assess depression-like behaviors in preclinical research [19]. Sucrose preference is

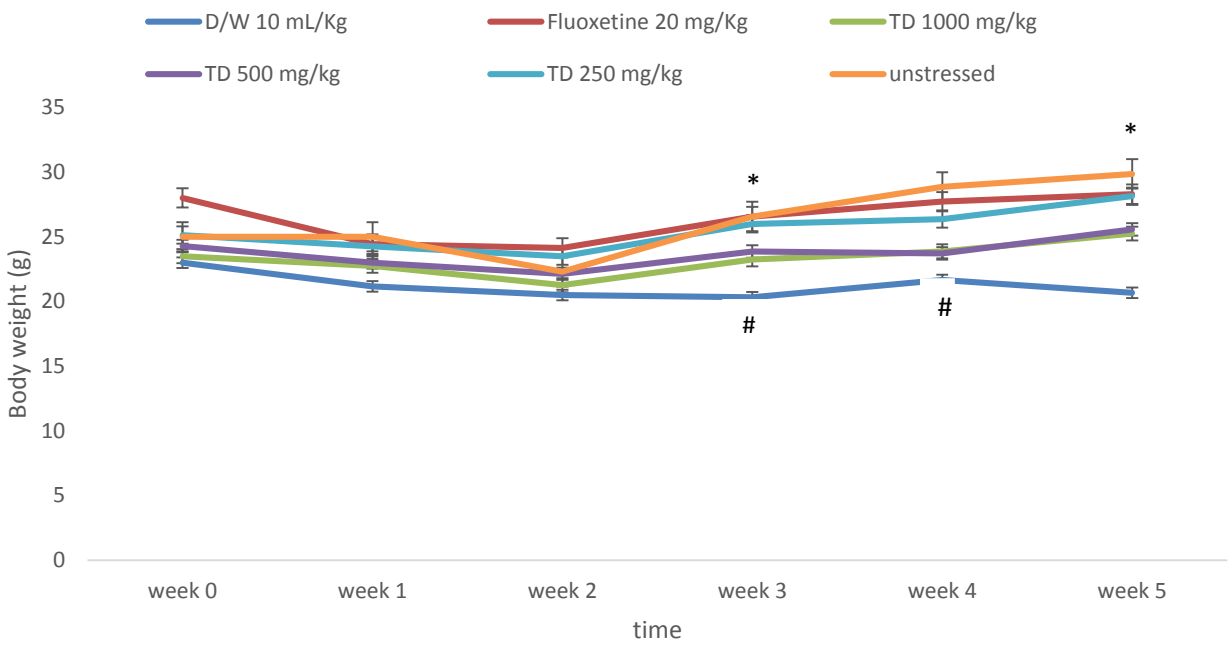

Figure 1: Effect of Methanol Extract of *Tapinanthus dodoneifolius* on Stress-Induced Change in Body Weight in Mice.

Weight presented as Mean ± SEM. (n= 8). Data was analyzed using One-way repeated measure ANOVA followed by Bonferroni *post hoc* test. # = p< 0.05 vs. Unstressed group. * = $p < 0.05$ vs. Negative control (Distilled water treated) group. DW = Distilled Water; TD = Methanol Extract of *Tapinanthus dodoneifolius*. CUMS = Chronic Unpredictable mild stress.

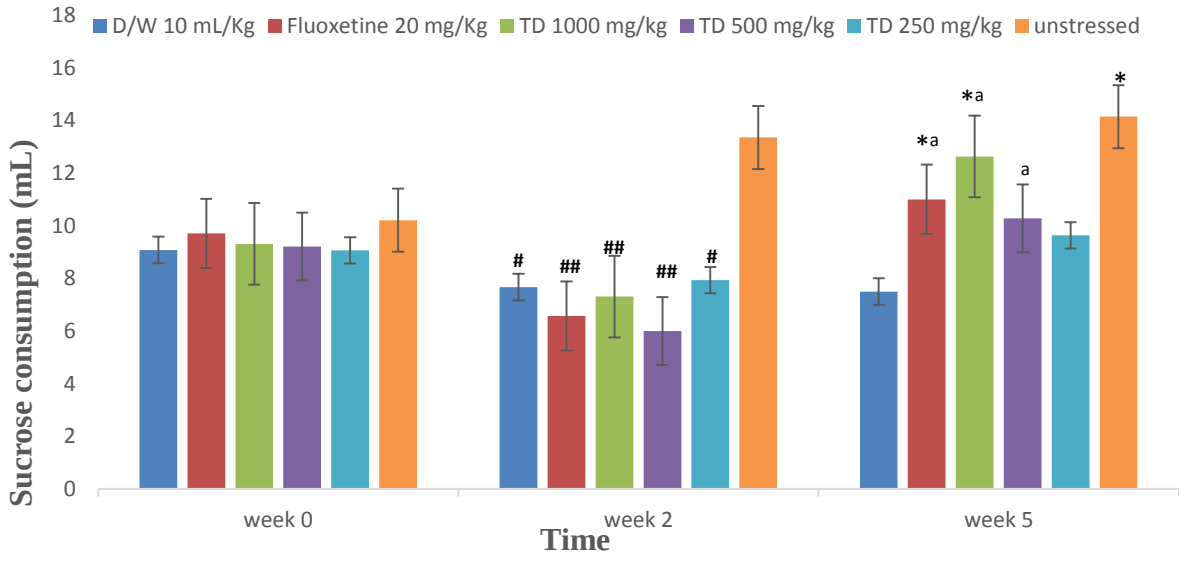

Figure 2: Effect of methanol extract of *Tapinanthus dodoneifolius* on stress-induced change in sucrose consumption of mice.

Amount of sucrose consumed presented as Mean ± SEM. (n= 8). Data was analyzed using One-way repeated measure ANOVA followed by Bonferroni *post hoc* test. # = $p < 0.05$ vs. Unstressed group. ## = $p < 0.005$ vs. unstressed group. * = $p < 0.05$ vs. Negative control (Distilled water treated) group. [a] = $p < 0.05$ vs. week 2
DW = Distilled Water; TD = Methanol Extract of *T. dodoneifolius*. CUMS = Chronic Unpredictable Mild Stress.

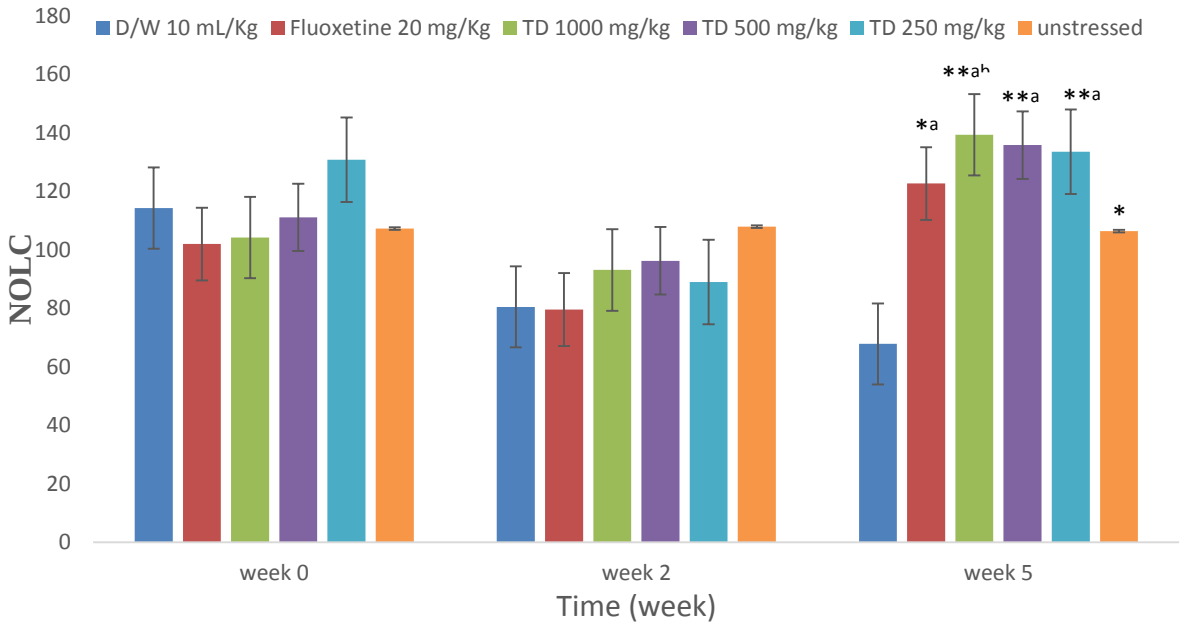

Figure 3: Effect of methanol Extract of *Tapinanthus dodoneifolius* on stress-induced ambulation of mice in open field test (OFT).

NOLC presented as Mean ± SEM. (n= 8). Data was analyzed using One-way repeated measure ANOVA followed by Bonferroni *post hoc* test. * = $p < 0.05$ vs. Negative control (Distilled Water treated) group. ** = $p < 0.005$ vs. Negative control (Distilled Water treated) group. [a] = $p < 0.05$ vs. week 2. [b] = $p < 0.05$ vs. week 0

DW = Distilled Water; TD = Methanol Extract of *T. dodoneifolius*; NOLC = Number of lines Crossed.

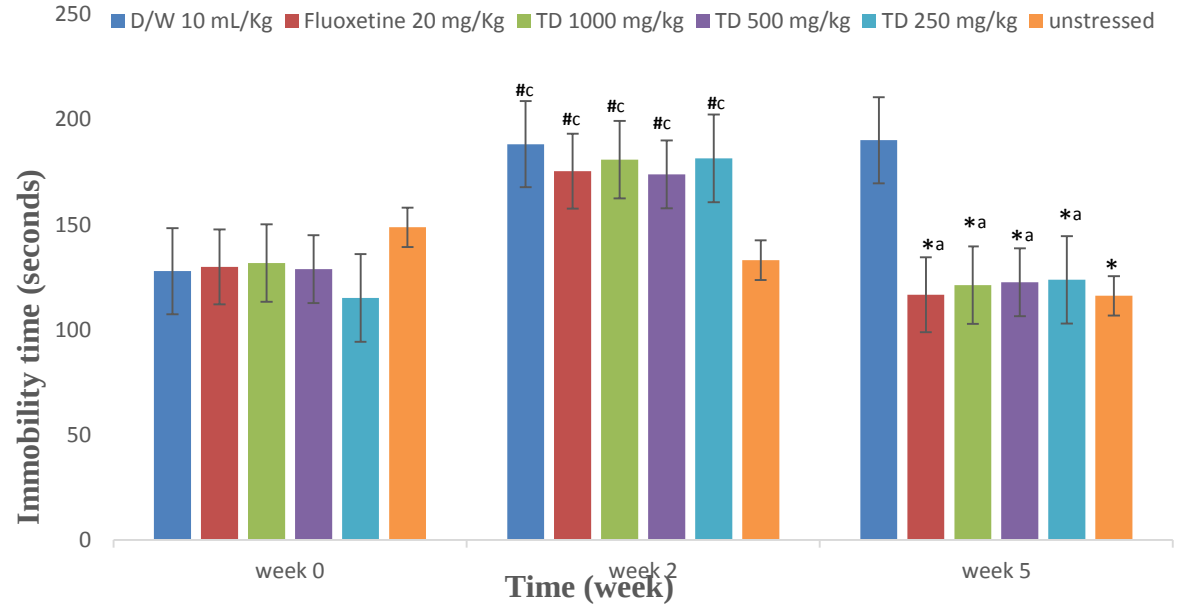

Figure 4: Effect of methanol extract of *Tapinanthus dodoneifolius* on stress-induced change in immobility duration of mice in tail suspension test (TST).

Immobility time presented as Mean ± SEM. (n= 8). Data was analyzed using One-way repeated measure ANOVA followed by Bonferroni *post hoc* test. [#] = $p < 0.05$ vs. Unstressed group. * = $p < 0.05$ vs. Negative control (Distilled water treated) group. [c] = $p < 0.05$ vs. week 0. [a] = $p < 0.05$ vs. week 5.

DW = Distilled Water; TD = Methanol Extract of *T. dodoneifolius*.

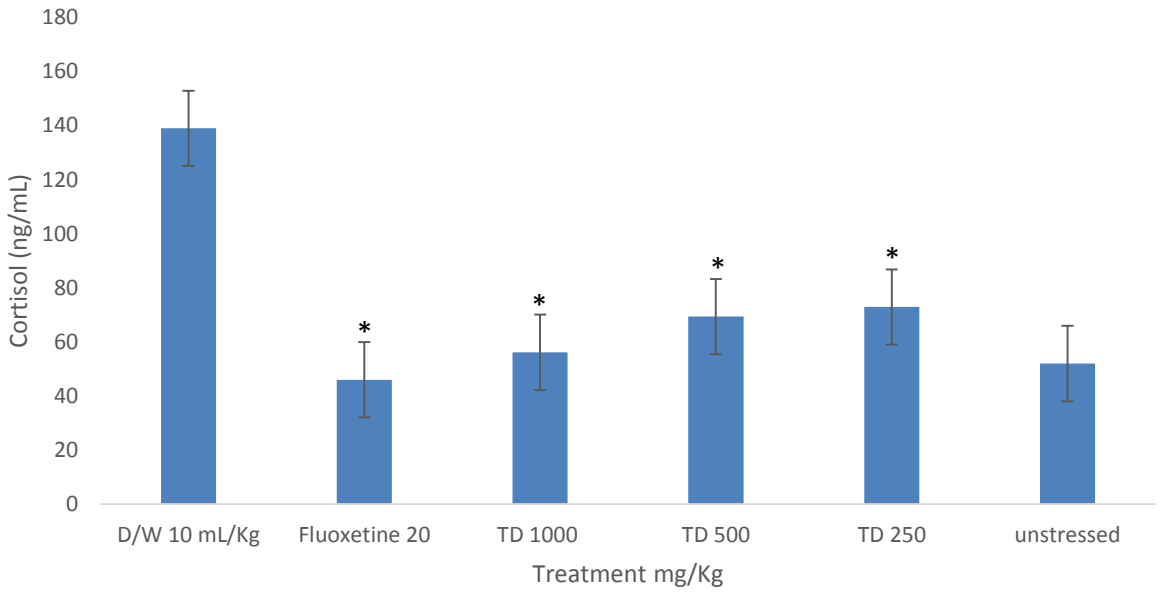

Figure 5: Effect of methanol extract of *Tapinanthus dodoneifolius* on stress-induced change in cortisol level.

Cortisol level presented as Mean ± SEM. (n= 4). Data was analyzed using One-way ANOVA followed by Bonferroni *post hoc* test. * = $p < 0.005$ vs. Negative control (Distilled Water treated) group.
DW = Distilled Water; TD = Methanol Extract of *Tapinanthus dodoneifolius*: CUMS = Chronic Unpredictable Mild Stress.

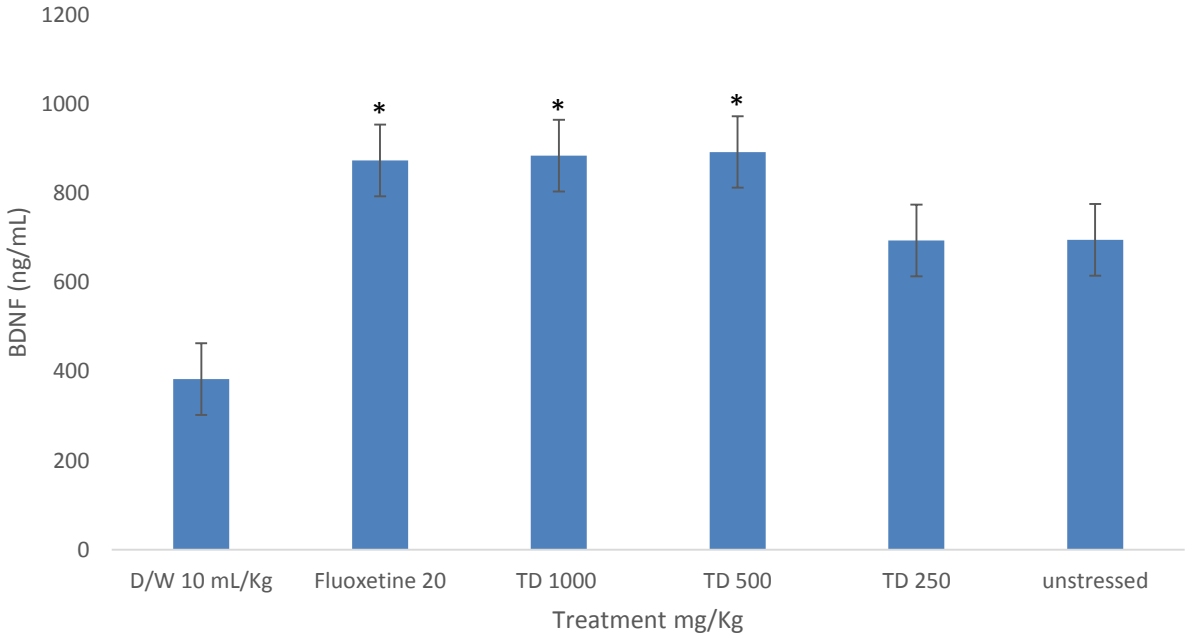

Figure 6: Effect of methanol extract of *Tapinanthus dodoneifolius* on stress-induced change in BDNF level in mice.

BDNF level presented as Mean ± SEM. (n= 4). Data was analyzed using One-way ANOVA followed by Bonferroni *post hoc* test. * = $p < 0.05$ vs. Negative control (Distilled Water treated) group.
DW = Distilled Water; TD = Methanol Extract of *Tapinanthus dodoneifolius*: CUMS = Chronic Unpredictable Mild Stress. BDNF = Brain Derived Neurotrophic Factor.

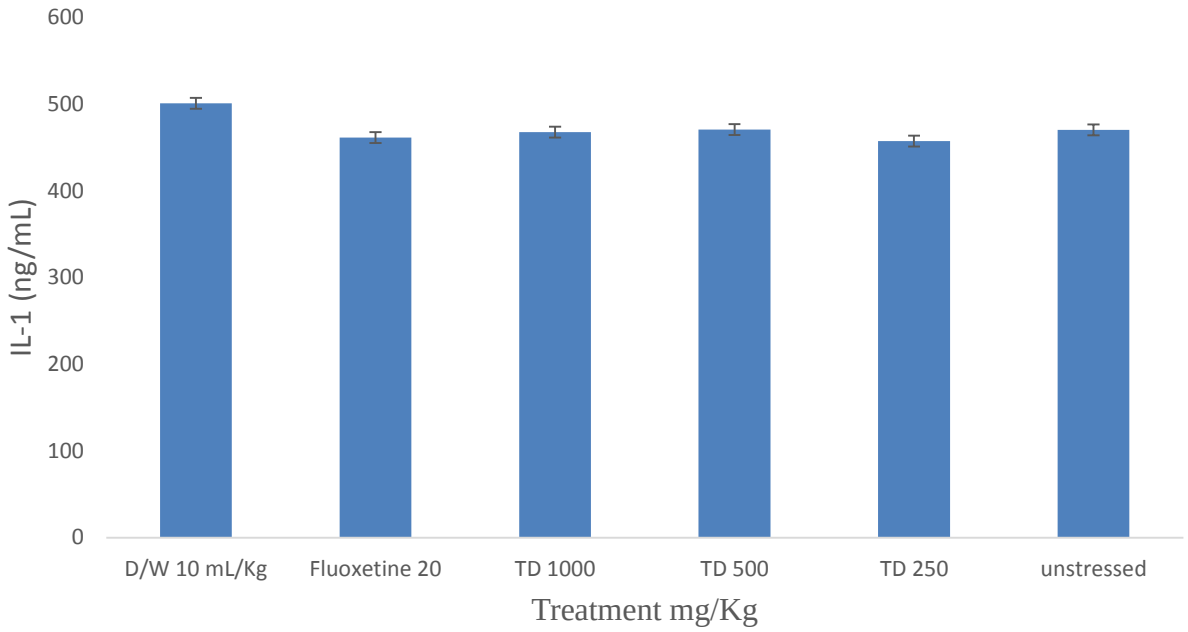

Figure 7: Effect of methanol extract of *Tapinanthus dodoneifolius* on stress-induced change in the level ofiInterleukin-1β.

IL-1 level presented as Mean ± SEM. (n= 4). Data was analyzed using One-way ANOVA

DW = Distilled Water; TD = Methanol Extract of *Tapinanthus dodoneifolius*; CUMS = Chronic Unpredictable Mild Stress; IL-1 = Interleukin-1.

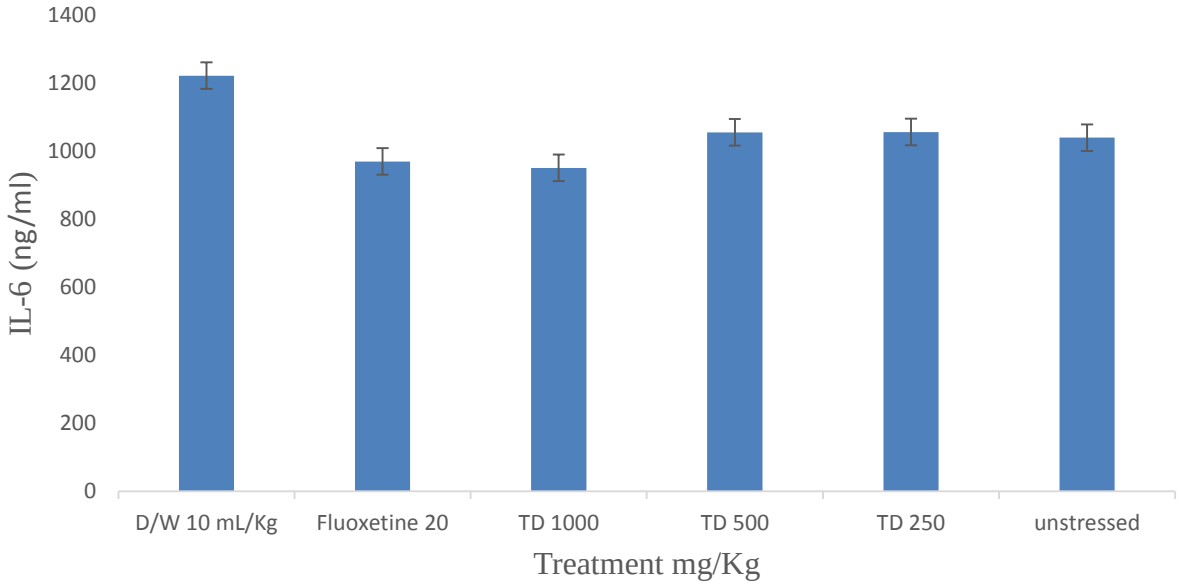

Figure 8: Effect of methanol extract of *Tapinanthus dodoneifolius* on stress-induced change in the level of interleukin-6 level.

IL-6 level presented as Mean ± SEM. (n= 4). Data was analyzed using One-way ANOVA

DW = Distilled Water; TD = Methanol Extract of *Tapinanthus dodoneifolius*: CUMS = Chronic Unpredictable Mild Stress; IL–6 = Interleukin-6.

widely utilized to represent anhedonia behavior in CUMS depression model, indicating a loss of interest or pleasure [20]. The animals displayed a reduction of sucrose consumption compared to the control animals, which was congruent to previous findings [14]. Chronic administration of the extract significantly ameliorated the behavioral change, indicating its antidepressant-like activity in CUMS induced depression in mice. The TST which is to be used in depicting despair paradigm, a common indicator of depression in animals is the most widely applied model for evaluating potential antidepressant agents and determining underlying mechanisms involved in antidepressant activity as it is easy to use in addition to having enhanced predictive validity and reliability [21]. Various studies indicated that mice subjected to repeated stress demonstrate obvious elongation of immobility duration in the TST and reduced locomotor activity in the open field test (OFT) [22]. Agreeably, in this experiment, an increased total time of immobility, reduced locomotor activity in the TST and OFT respectively was also observed in mice subjected to CUMS compared to the unstressed mice. The result also showed that the extract administration for three (3) weeks significantly shorten the immobility duration of CUMS treated mice and ameliorate reduced locomotor activity similar to the established antidepressant effect of fluoxetine. This is an indication of likely presence of a quercetin glycoside in the extract which was found to decrease mobility in tail suspension after chronic administration [23]. Additionally, the hypothalamic–pituitary–adrenocortical axis (HPA) system has an important role to play in the neuroendocrine system by contributing in the control of stress responses and regulation of many physical activities. Hyperactivity of the HPA axis is one of the key pathophysiological changes associated with depression [24]. In depression, the HPA axis is hyperactive, and a cascade of hormonal reactions is activated where corticotrophin-releasing-hormone (CRH) is released from neurons in the hypothalamus due to activation by multiple stressors thereby induces the synthesis and secretion of Adrenocorticotrophic hormone (ACTH) from the adenohypophysis which further activates the cortex of the adrenal glands to synthesize and release glucocorticoids such as cortisol, especially in animals [25]. The activity of the HPA axis is evaluated by measuring the level of corticosterone in the brain [26]. Interestingly, the mice subjected to CUMS and treated with distilled water (vehicle) demonstrated higher plasma corticosterone concentrations when compared with the unstressed mice, and all the treatment groups had the abnormal alterations restored, especially at higher doses suggesting that the antidepressant-like effect of the extract may be associated with inhibition of the HPA axis activity in CUMS mice. This may also be associated with phytochemicals such as the flavonoid, garnistin that may be present in the extract which was known to exert antidepressant effect through the endocrine mechanism [27]. Further mechanistic investigation into the pathogenesis of depression suggested that impairment of neurogenesis and neuronal plasticity sequel to stress resulting into stress-induced neuronal atrophy and inhibition of development in the brain tissues, particularly the hippocampus, amygdala and prefrontal cortex areas generally involved in the regulation of mood [28]. Furthermore, chronic stress was shown to adversely affect function of the hippocampus through down regulating the expression of neurotrophic factors and this change could be reversed by antidepressant treatment [29]. The BDNF signaling pathway is thought to be the major trophic factor associated with the development of depressive disease and associated to antidepressants activity as evident from various preclinical and clinical studies [30]. CUMS has been confirmed to cause reduction of BDNF expressions (mRNA and protein) in the hippocampus and prefrontal cortex, while chronic treatment with antidepressants obviously elevated BDNF levels in those region [31]. In the current study, CUMS was observed to reduce levels of BDNF in the homogenizes brain tissues of CUMS vehicle (distilled water treated) group and the extract averted the decrease induced by CUMS which provided evidence that elevation of BDNF expression might be a contributor to the antidepressant property of the extract. This might as well be an indication for the presence of flavonoids such as chrysin or hisperidine which exert antidepressant activity by causing elevation in BDNF or, other similar or different compounds in the extract [32]. Furthermore, Inflammatory processes have also been shown to be involved in the pathophysiology of depression as it is now well established how dysregulation of the innate as well as adaptive immune systems occur in depressed patients and hinder favorable prognosis, including antidepressant responses [33]. Cytokines produced by microglia and other CNS cells in the brain are essential in the modulation of various functions in the CNS, such as maintenance of neuroplasticity [34]. However, prolonged activity of inflammatory cytokine attenuates several nervous functions such as, alteration in neurotransmitters synthesis, release, signaling and reuptake [33]. The activity of cytokines in the brain can be elevated to

detrimental level by various mechanisms such as physical and psychological stressors, albeit, it is unclear how they affect neuronal function and therefore, it has been proposed that the combination and sources of the cytokines determine the effect on brain function [35]. Correspondingly, in this study, the mice in the CUMS vehicle (distilled water treated) group had elevated levels of these cytokines; Interleukin-1 (IL-1) and Interleukin-6 (IL-6), however, the levels in the extract treated group were not significantly lower than those in the CUMS vehicle (distilled water treated) group and were similar to the levels in mice given the antidepressant Fluoxetine. Hence, the antidepressant effect the extract may not significantly involve amelioration of inflammatory response due to stress or the stress induced in the animals did not trigger a significant inflammatory response. A monoterpene phenol, carvacrol, sourced from plants such as oregano and thyme commonly known for anti-inflammatory effect produced antidepressant effect [36]. This further buttresses the antidepressant effect of plant secondary metabolites with anti-inflammatory activity.

CONCLUSION

The methanol extract of *Tapinanthus dodoneifolius* ameliorates CUMS-induced depression in mice likely through neurobehavioral, neuroendocrine and neurotrophic mechanisms as evidenced by reduction in despair, reduced anhedonia, improved level of neurotrophic factor and reduction in the level of stress hormone, cortisol. However, further phytochemical and mechanistic investigations, especially using more specific solvent and receptors involvement may also be of significance for better understanding and guide to isolation of a novel active principle from the plant.

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