N-butanol fraction of Olax subscorpioidea Oliv. (Olacaceae) attenuates lipopolysaccharide-induced depressive-like symptoms in mice.

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Abstract

Background: The leaves of *Olax subscorpioidea* have become a mainstay in the management of inflammatory diseases and mental illness in folkloric medicine in Nigeria. Previous studies have shown its antidepressant and anti-inflammatory properties in experimental animals. Recently its antidepressant action was linked to the involvement of monoaminergic transmission. However, with accumulating evidences suggesting link between immuno-inflammatory signaling pathways and depression, there is dearth of information about its beneficial effect on inflammation associated depression. We thus evaluated the effect of n-butanol fraction of *O. subscorpioidea* leaves on lipopolysaccharide (LPS)-induced depressive-like behaviours and investigated its antidepressant effect with respect to its action on inflammatory and oxidative pathways.

Methods: Fifty Swiss male mice were randomly assigned into five groups (n= 10): group 1 (vehicle only), group 2 (nBFOS 5mg/kg), group 3 (nBFOS 10 mg/kg), group 4 (imipramine 10 mg/kg), group 5 (vehicle). Mice were treated with vehicle or nBFOS (5 & 10 mg/kg) or imipramine intraperitoneal for seven days. Thirty minutes after treatment on day seven, animals were injected with LPS (0.83 mg/kg, i.p.) except group 1 (vehicle only). Twenty-four hours following LPS injection, animals were assessed for depressive symptoms using sucrose preference test and immobility using tail suspension test (TST). Brain levels of pro-inflammatory mediators interleukin-1β (IL-1β) and tumor necrosis factor (TNF), oxidative stress biomarkers (malondialdehyde and reduced glutathione) and plasma level of corticosterone were measured by Enzyme Linked immunosorbent assay.

Results: LPS significantly ($p < 0.05$) increased immobility of mice in TST and decreased sucrose preference which is indicative of depressive-like behaviours. The depressive behaviours were significantly ($p < 0.05$) attenuated by nBFOS and imipramine compared to control. Furthermore, LPS-induced increase in malondialdehyde, corticosterone, TNF, IL-1β, and decrease in reduced glutathione, in the brain were significantly reversed by treatment with nBFOS and imipramine.

Conclusion: The findings suggest that attenuation of LPS-induced depressive-like behaviours by the fraction of *O. subscorpioidea* leaves may be related to suppression of oxidative stress and inhibitory effect on inflammatory mediators in the central nervous system.

Highlights

- Lipopolysaccharide causes depressive-like behaviours in rodents
- N-butanol fraction of *Olax subscorpioidea* abrogates LPS-induced depressive like syndrome via inhibition of oxidative stress and neuroinflammation.

Introduction

Major depressive disorder (MDD) is a chronic psychiatric disease, which ranks high as one of the main causes of loss of performance and disability worldwide. The vast majority of antidepressants available

for the treatment of this disorder largely modulate monoaminergic neurotransmission and their therapeutic benefits in the management of depression are ridiculously marginal with lower remission rate, and delayed onset of action. This thus suggests that dysfunction of monoaminergic systems may not only account for the disease pathophysiology. It is therefore pertinent to understand other underlying factors of depression pathophysiology in order to identify new neurobiological targets for the development of novel drug candidates (Machado-Vieira et al., 2009).

There have been several reports of activation of immuno-inflammatory pathways and proinflammatory cytokines as contributing factors in the pathogenesis of major depressive disorder (Maes 1995; Berk et al., 1997; Khairova et al., 2009). Consistently, studies have shown depressed patients presenting with elevated proinflammatory cytokine (Felger and Lotrich, 2013), while increased incidences of depression among patients with infection have been reported (Felger and Lotrich, 2013). Interestingly, treatment with conventional anti-inflammatory drugs, such as aspirin (Mendlewicz et al., 2006), celecoxib (Muller et al., 2006) and etanercept (Tyring et al., 2006) have reportedly improved behaviours in adults with MDD. In another preclinical study, doxycycline a known anti-inflammatory tetracycline also demonstrated a comparable antidepressant-like effect with imipramine in mice (Mello et al., 2013). All these put together thus suggest an overlapping link between depression and inflammatory responses. Furthermore, activation of oxidative and nitrosative pathways, and imbalance antioxidant defense system have been implicated as contributing factors in depressive disorder (Maes et al., 2011). Altered levels of antioxidant defenses, in the postmortem of MDD brain has been reported (Gawryluk et al., 2011). Similarly, evidences of elevated reactive oxygen species (ROS) and reactive nitrogen species have been reported in depressed patients (Suzuki et al., 2001; Maes et al., 2010; Dhir and Kulkarni, 2011).

Lipopolysaccharide (LPS) is a bacterial endotoxin and strong immune cells inducer. Its use as a pharmacological tool in investigating neuroinflammation and depressive-like symptoms in rodents has been widely accepted (Jiang et al., 2017). As a robust inflammatory model of depression, a single acute LPS injection has been used to trigger sickness behaviour, anhedonia and hypoactivity in despair tests with evidence of elevated pro-inflammatory cytokines (O'Connor et al., 2009; Mello et al., 2013; Jiang et al., 2017). Furthermore, reduction in exploratory behaviour, increasing slow wave sleep, food consumption and decreased social interaction in rodents were demonstrated following LPS, IL-1β and TNF administration (Bluthé et al., 2000). The inflammatory theory of depression and discovery of LPS as a microglial stimulators have given an insight and formed the basis for identifying new neurobiological targets and a potent etiological therapeutics for depression symptomatology (Kreisel et al., 2014). In this regard, it can be hypothesized that agents with potent anti-inflammatory property may abrogate depressive syndromes and thus serve as a good alternative for treatment of depression. Therefore, we employed a single acute LPS administration as a model to evaluate antidepressant-like effect of n-butanol fraction of *Olax subscorpioidea* Oliv. (Olacaceae) in inflammation associated depression.

Olax subscorpioidea is a popular herb among various cultures in Nigeria. It is referred to as Ifon in Yoruba traditional medicine and belongs to Olacacea family (Ibrahim et al., 2006, Adeoluwa et al., 2014, Adeoluwa et al., 2016). It is a major recipe in the concoctions traditionally prepared to treat pain,

inflammatory related diseases and mental disorders (Ibrahim et al., 2006). The main chemical constituents of the plant are alkaloids, steroids, glycosides, saponins, flavonoids and terpenoids (Ayandele and Adebiyi, 2007; Victoria et al., 2010). High performance liquid chromatograph (HPLC) analysis of the butanol fraction has revealed the presence of caffeic acid, rutin, morin and quercetin following mapping of peaks with their respective commercial standards (Adeoluwa et al., 2019). In our previous studies, we validated the traditional claim of its analgesic effect (Adeoluwa et al., 2014) and reported an antidepressant property of the crude extract in rodents using despair tests (Adeoluwa et al., 2015). Ishola et al. (2015) reported anti-inflammatory property of the crude extract of *O. subscorpioidea.* Furthermore, anti-immobility action and antidepressant effect of the butanol fraction of the leaves in the predictive models of depression were reported and it was concluded that the effect might be mediated via monoaminergic neurotransmission (Adeoluwa et al., 2019). However, with a growing body of evidence linking neuroinflammation and depressive symptoms, there is a need to investigate the potential therapeutic benefit of this plant in inflammatory-related depression. The current study thus aimed to evaluate the modulatory effect of n-butanol fraction of *O. subscorpioidea* (nBFOS) in LPS-induced depressive-like behaviour in mice.

Materials And Methods

2.1 Plant materials

The fresh leaves of the plant (*O. subscorpioidea*) were collected at Gambari Forest Reserve area of Ibadan, located between latitude 7° 261 N and longitude 3°541 E, Oyo state, Nigeria. The plant was identified and authenticated by O.A. Ugbogu and O.S. Shasanya at the Forestry Research Institute of Nigeria (FRIN), Ibadan herbarium section and preserved with voucher specimen identification number 109924.

2.2 Preparation of plant materials

N-butanol fraction of *O. subscorpioidea* was prepared from the hydroethanolic extract of *O. subscorpioidea* as reported previously by Adeoluwa et al. (2019). Briefly, the dried and powdered leaves (500g) of *O. subscorpioidea* Oliv. were placed in a glass container with 50% ethanol and allowed to stand at room temperature for 48 hours and filtered, this extraction procedure was repeated two times and the combined filtrate was concentrated to dryness using a rotary evaporator. The dry crude extract (50.46 g) was then partitioned between ethyl acetate, n-butanol and water [EAF (2.00 g), nBFOS (9.79 g) and AF (25.45 g) respectively].

2.3 Animals

Adult male Swiss mice between 3–4 months old (20–25 g) raised in the Laboratory Animal Centre of University of Ibadan were used for the study. They were kept in groups (4–5 animals per cage) in a room

temperature controlled environment with 12hr/12hr (light/dark) cycle, 40–70% relative humidity, with water and standard rodent chow ad libitum. University of Ibadan Animal Care and Use Research Ethics Committee (UI-ACUREC/App/2015/064) approved the experimental procedure and it was carried out in line with care and animal use guideline by NIH.

2.4 Drugs and Chemicals

Lipopolysaccharide – LPS (Sigma, Germany), Thiobabituric acid-TBA (Guanghua Chemical Factory Co. Ltd., China), 5,5'-dithio-bis(2-nitrobenzoicacid) –DTNB (Aldrich, Germany),), Acetic acid (Sigma-Aldrich, Inc., St Louis, USA), Trichloroacetic acid-TCA (Burgoyne Burbidges & Co., Mumbai, India), Tris (hydroxymethyl) – amino-methane (Tris-buffer) (Hopkin & Williams Company, USA NaOH (J.T Baker Chemicals Co., Phillipsburg, N.J., USA), Sodium Carbonate (Fisons, Loughborough Leics, England), $NaHCO_3$, $Na_2HPO_4.H_2O$, $NaH_2PO_4.H_2O$, K_2HPO_4, $K_2Cr_2O_7$, KCl (BDH Chemical Ltd, Poole, England) were used in the study.

2.5 Experimental procedure

Fifty male Swiss mice were allocated randomly into five groups (n = 10): the first group (vehicle only), second group (nBFOS 5 mg/kg), third group (nBFOS 10 mg/kg), forth group (Imipramine 10 mg/kg), and fifth group (vehicle). Vehicle or nBFOS or imipramine was administered to mice for seven days. On day 7, thirty minutes following treatments, animals were injected with LPS (0.83 mg/kg, i.p.) except group 1 (vehicle only). The doses of the n-butanol fraction and imipramine used in this study were based on the previous work (Adeoluwa et al., 2019). The dose of LPS used is due to its already established ability to reliably induce acute sickness behaviour as well as increase brain indolemine 2, 3-dioxyegenase (IDO) activity, a putative mechanism in LPS-induced depressive-like phenotypes (O'Connor et al., 2009). Twenty-four hours after the LPS injection, depressive-like symptoms were determined in each animal. Animals were sacrificed immediately after behavioural studies. The brain tissues and blood samples were collected for bioassay of pro-inflammatory mediators (IL-1β and TNF), oxidative stress biomarkers (malondialdehyde and reduced glutathione) and plasma level of corticosterone were assayed with the enzyme-linked immunosorbent assay (ELISA) kits. In this study, animals were assigned into different groups using simple random sampling technique and during behavioural studies single blind fold method was adopted in order to eliminate bias.

2.5.1 Sample size determination

Sample size for the study was determined using the following formula:

E = Total number of animals – Total number of groups. Attrition of 10% was put into consideration. However, the authors did not perform a priori sample size calculation to obtain sufficient power for the

study

2.6 Behavioural studies

All behavioural studies were performed 24 hours after LPS administration.

2.6.1 Sucrose preference test

Sixty hours before the LPS-induced depression procedure, sucrose preference training was carried out. This involves adaptation to 1% sucrose solution (w/v) in which two bottles containing this solution were placed in each cage. At exactly 24 hours, one of the bottles was replaced with distilled water and left for 24 hours. After adaptation period of 48 hours, food and water were withdrawn for 12 hours. Thereafter, sucrose test was performed by re-introducing two bottles containing water and sucrose to the animals at the same time, for one hour. The volume consumed from each bottle was recorded and animal's preference for sucrose was determined from the following expression:

$$\frac{\text{Sucrose consumption}}{\text{Sucrose consumption + water consumption}} \times 100$$

2.6.2 Tail Suspension Test

The tail suspension test (TST) was conducted following the anhedonic test. This test is often employed in screening antidepressant. Mice were suspended for six minutes at the tip of its tail not more than 1cm, using adhesive tape on a platform that is 50 cm above the floor. Immobility i.e. the time during which animals were immobile was measured. A mouse is regarded immobile in the absence of any visible body movements and hangs passively (Porsolt et al., 1977). An extended immobile duration in this test is adjudged state of despair or hopelessness.

2.6.3 Collection and preservation of brain tissues

Mice were sacrificed immediately after behavioural tests by cervical dislocation. The brain tissues were excised from the skull, were homogenized and centrifuged with the speed of 10,000 rpm at 4^{O}C for 15 minutes. The supernatants were separated and stored at -80 ^{O}C until biochemical assays.

2.6.4 Measurement of reduced glutathione (GSH) level

The reduced glutathione in the brain was estimated with the method of Moron et al., (1979). After centrifuging the brain homogenate, 0.4 mL of the brain samples and 0.4 mL of 20% TCA were added and

centrifuged at 10,000 rpm for 20 minutes at 4^{o}C. Thereafter, 2 mL of 0.6mM DTNB and 0.25mL supernatant were mixed with addition of 0.75 mL of phosphate buffer to make final volume of 3 mL. The absorbance was quickly read against blank at 412 nm, using spectrophotometer. The values of GSH were expressed in micromoles per gram tissue (µmol/g tissue).

2.6.5 Measurement of malondialdehyde (MDA) level

Malondialdehyde was quantified using the method described by Adam-vizi and Seregi (1982). This involves centrifugation of the brain samples at 10,000 rpm. The supernantaant (0.4 mL) was mixed with Tris-KCL buffer (1.6 mL) and 0.5 mL of 30% TCA was added. Then 0.5 mL of 0.75% Thiobabituric acid (TBA) was added. The mixture was heated at 80 ^{o}C in a water bath for 45 minutes. It was allowed to cool before centrifuging at 3000 revolution per minute for fifteen minutes. The optical density of the supernatant against a reference blank at 532 nm was read. Molar extinction coefficient of 1.56×10^{5} M^{-1} cm^{-1} was used to calculate MDA.

2.6.6 Measurement of Interlukin-1β and Tumor Necrosis Factor with ELISA techniques

The concentrations of IL-1β and TNF in the supernatants of the brain samples were measured by specific mouse IL-1β ELISA kit (Assaypro, USA, Cat No. ERT2010-1) and TNF ELISA kit (Ray Biotech, USA, Cat No. ELR-IL1b) respectively. All assays were carried out under standard conditions in accordance with the respective manufacturers' instructions and absorbance read at 450 nm wavelength. The concentrations of cytokines from the tissues were extrapolated from the standard curve obtained from the optical densities of the known standard concentrations included in the assay kits. The levels of the cytokines in the brain were expressed as pg/mL.

2.6.7 Estimation of corticosterone concentration

Blood samples were collected through puncture of the retrobulbar plexus under anesthesia into plain tubes. The samples were allowed to stand at room temperature and later centrifuged for 15 minutes at 3000 rpm. The serum was kept frozen at − 80 ◦C freezer until assayed. Concentration of serum corticosterone was determined using a commercially available ELISA kits (corticosterone ELISA, IBL International GMBH, Hamburgh Germany) according the manufacturer's instructions. Data were expressed as ng/mL.

2.7 Statistical analysis

All data are expressed as mean ± SD. Normality of distribution and variance homogeneity were examined with **Shapiro-Wilk test and Levene's test respectively.** One-way analysis of variance (ANOVA) followed by multiple comparison post hoc tests (Tukey) was used to analyse the data, while Kruskwalis test was performed for non-parametric data with Dunn's multiple comparison post hoc tests. The level of significance for all tests was set at $p < 0.05$.

Results

3.1 N-butanol fraction of *O. subscorpioidea* attenuates depressive like behaviours in LPS mice

Tail suspension test and sucrose preference test were performed to investigate antiimmobility and anti-anhedonic effect of the fraction. The results show that nBFOS has antidepressant like property in TST and SPT. The data for immobility time in the TST test passed Shapiro-Wilk normality test ($W = 0.8828$, $p = 0.3220$; $W = 0.8804$, $p = 0.3112$; $W = 0.9297$, $p = 0.5946$; $W = 0.9377$, $p = 0.6495$; $W = 0.9668$, $p = 0.8544$) and variance homogeneity test [$F_{(4, 20)} = 1.731$, $p = 0.183$], hence parametric test was performed using One-Way ANOVA. There is a significant difference among the treatment groups [$F_{(4, 20)} = 50.06$, $p < 0.0001$]. Post hoc analysis with Turkey, further revealed that LPS increased significantly immobility in TST relative to control (Fig. 1A), however, treatment with nBFOS (5 and 10 mg/kg) significantly, ($p < 0.05$) attenuated LPS-induced immobility (Fig. 1A). Furthermore, data for sucrose preference having passed Shapiro-Wilk normality test ($W = 0.9789$, $p = 0.9287$; $W = 0.9286$, $p = 0.5869$; $W = 0.8098$, $p = 0.0972$; $W = 0.8714$, $p = 0.2722$; $W = 0.9621$, $p = 0.8223$), violated variance homogeneity [$F_{(4, 20)} = 2.89$, $p = 0.049$]. Kruskal Wallis H test revealed a significant difference among the group ($X^2 = 12.904$, $df = 4$, $p = 0.012$. Following Dunn test, LPS significantly reduced sucrose consumption behaviour of mice treated with LPS relative to control (i.e. vehicle-treated mice) (Fig. 1B). Treatment with nBFOS (5 and 10 mg/kg) significantly ($p < 0.05$) reversed the LPS-induced decrease in sucrose consumption (Fig. 1B).

3.2 N-butanol fraction of *O. subscorpioidea* mitigates LPS-induced oxidative stress

In this study, we assayed for oxidative stress biomarkers and evaluate the mitigating action of n-butanol fraction of *O. subscorpioidea* by measuring reduced glutathione (GSH) and lipid periodixation.

Data from both assays passed Shapiro-Wilk normality test GSH ($W = 0.7775$, $p = 0.0525$; $W = 0.7989$, $p = 0.0794$; $W = 0.8817$, $p = 0.3171$; $W = 0.8327$, $p = 0.1457$; $W = 0.9882$, $p = 0.9730$); MDA ($W = 0.8327$, $p = 0.1458$; $W = 0.7098$, $p = 0.0121$; $W = 0.8236$, $p = 0.1243$; $W = 0.8896$, $p = 0.3552$; $W = 0.8649$, $p = 0.2463$) and Levene's test GSH [$F_{(4, 20)} = 1.521$, $p = 0.234$], MDA [$F_{(4, 20)} = 0.898$, $p = 0.483$].

One-way ANOVA shows significant difference among the groups in GSH [$F_{(4, 20)} = 19.18$, $p < 0.0001$] and MDA [$F_{(4, 20)} = 9.573$, $P = 0.0002$]. Post hoc analysis shows that LPS (0.83 mg/kg, i.p.) caused significant ($p < 0.05$) depletion of GSH (Fig. 2A) and significant increase in MDA (Fig. 2B) which suggests increase in

oxidative stress) compared to control (VEH-treated mice). Treatment with nBFOS (5 and 10 mg/kg) or imipramine (10 mg/kg, i.p.) significantly reduced GSH depletion and concentration of MDA caused by LPS administration.

3.3 N-butanol fraction of *O. subscorpioidea* inhibits LPS-induced pro-inflammatory cytokines in the brain

Proinflammatory cytokines (IL1-β and TNF) were measured in order to evaluate anti-neuroinflammatory effect of nBFOS in mice exposed to LPS. The results show that nBFOS has antineuroinflammatory effect. The data for TNF assay were normally distributed (W = 0.8227, p = 0.1224; W = 0.6757, p = 0.0052; W = 0.8108, p = 0.0990; W = 0.5847, p = 0.0004; W = 0.8119, p = 0.1010) and met homogeneity of variance [$F_{(4, 20)} = 2.802$, p = 0.054], hence parametric test was performed using One-Way ANOVA. One-way ANOVA revealed a significant difference among the groups in [$F_{(4, 20)} = 43.82$, $p < 0.0001$]. Post hoc analysis, further revealed that LPS (0.83 mg/kg i.p.) induced a significant increase in the central TNF (Fig. 3A) compared to control (VEH-treated mice), however, treatment with nBFOS (5 and 10 mg/kg) significantly, ($p < 0.05$) reduced LPS-induced TNF (Fig. 3A). Furthermore, data for IL-1β having passed Shapiro-Wilk normality test (W = 0.9647, p = 0.8400; W = 0.9749, p = 0.9057; W = 0.7971, p = 0.0768; W = 0.9012, p = 0.4164; W = 0.9741, p = 0.9010), violated variance homogeneity [$F_{(4, 20)} = 5.830$, p = 0.003]. Kruskal Wallis H test revealed a significant difference among the group ($X^2 = 16.61$, df = 5, p = 0.0023. Following Dunn test, treatment with LPS significantly increase brain IL-1β compared to control (i.e. vehicle-treated mice) (Fig. 3B). However, only nBFOS (10 mg/kg) or imipramine (10 mg/kg, i.p.) significantly ($p < 0.05$) reduced LPS-induced. Dose of the fraction at 5 mg/kg showed no significant reduction ($P > 0.9999$) in the level of IL-1β.

3.4 N-butanol fraction of *O. subscorpioidea* inhibits LPS-induced increase in corticosterone

To evaluate the effect of n-butanol fraction of *O. subscorpioidea* on HPA axis, blood level of corticosterone was measured. There was a normal distribution of data (W = 0.8835, p = 0.3254; W = 0.9020, p = 0.4211; W = 0.6840, p = 0.0065; W = 0.8835, p = 0.3254; W = 0.8208, p = 0.1185) and homogeneity of variance was met [$F_{(4, 20)} = 1.752$, p = 0.178]. One-way ANOVA revealed a significant difference among the groups [$F_{(4, 20)} = 15.675$, $p < 0.0001$]. Post hoc test revealed that administration of LPS (0.83 mg/kg i.p.) significantly ($p < 0.05$) increased the level of blood corticosterone compared to control (VEH-treated mice), whereas treatment with nBFOS (5 and 10 mg/kg) or imipramine (10 mg/kg, i.p.) significantly attenuated (*$P < 0.05$*) LPS-induced increased corticosterone (Fig. 4).

Discussion

Findings from this study present a potential for n-butanol fraction of *Olax subscorpioidea* to inhibit inflammatory and oxidative stress markers, regulate HPA-axis and reverse depressive symptoms in LPS treated mice. Our data showed a pattern of neuroinflammation (elevated TNF and IL-1β) and antioxidant

imbalance (elevated malondialdehyde and depletion of reduced glutathione) in the brain and dysregulated HPA-axis following administration of lipopolysaccharide as a model of depression. However, treatment with the n-butanol fraction of *Olax subscorpioidea* showed a significant reversal of the LPS-induced depressive-like symptoms.

In the present study, depressive-like symptom was modeled in rodents with lipopolysaccharide, a bacterial endotoxin that is potent enough to cause immune and inflammatory responses. It is an important pharmacological tool to model neurodegeneration and psychiatric disorders in rodents. Several studies have demonstrated depressive-like behaviours in rodents following systemic administration of LPS (Szot et al., 2017, Wickens et al., 2017, Yamawaki et al., 2018), and accompanying these behavioural dysfunctions are elevated brain inflammatory madiators and evidence of oxidative stress biomarkers (Mello et al., 2013; Jiang et al., 2017). In this study, compared with the normal control animals, we observed that animals treated with LPS alone displayed depressive-like symptoms line decrease sucrose consumption and increased immobility in the TST paradigm. This finding is consistent with the observations of Henry et al. (2008)d Connor et al. (2009) that systemic administration of LPS caused a marked increase in immobility in the TST and induction of anhedonia with significant decrease in sucrose preference. However, we observed that the groups pretreated with nBFOS and later injected with LPS showed antidepressive like effects with increased mobility and sucrose consumption compared to LPS group. Crude extract and butanol fraction of *Olax subscorpioidea* have previously been demonstrated to possess antidepressant effect in forced swim and tail suspension tests (Adeoluwa et al., 2015; Adeoluwa et al., 2019).

Oxidative stress is an important factor in the pathophysiology of depression (Maes et al., 2009). Evidence of elevated reactive species has been reported in patients living with psychiatric and mood disorders (Maes et al., 2011). Malondialdehyde, a biomarker of oxidative stress was reportedly high in depressed patients (Sarandol et al., 2007; Rybka et al., 2014), while reduced glutathione, an antioxidant defense protein has been reportedly low in depressive disorder (Kodydková et al., 2009). High level of lipid peroxidation has been observed in some depressive disorders like auditory-verbal working memory, and short-term and delayed declarative memory (Maes et al., 2011; Talarowska, 2012). Similarly, some preclinical studies have demonstrated evidence of oxidative stress in LPS model of depression in mice (Mello et al., 2013 and Jiang et al., 2017). In this study, injection of LPS significantly caused rise in lipid peroxidation and depletion of reduced glutathione in animals challenged with LPS. We however, observed that the group pretreated with-butanol fraction of *O. subscorpioidea* had improved depressive behaviours with significant reduction in lipid peroxidation and increase in reduced glutathione, thus demonstrating its capacity to inhibit oxidative stress and correct antioxidant system imbalance in depressive disorder.

Over the past decades, inflammation has become the cornerstone in the study of neurodegenerative and neuropsychiatric disorders. There are reports of proinflammatory cytokines and/or chemokines such as TNF, IL-1β, IL-6 and CC chemokine ligand family playing important roles in the pathogenesis of major depressive disorder (Khairova et al., 2009). Consistently, high incidence of depression has been frequently reported in patients with infection, while depressed patients present with increased levels of

proinflammatory cytokine (Felger and Lotrich, 2013). Pre-clinically, proinflammatory cytokines or immune inducers have been used to activate microglial cells and trigger sickness behaviours, and depressive-like syndromes in mice (O'Connor et al., 2009; Mello et al., 2013). In this study, a single acute dose of lipopolysaccharide was used as a robust inflammatory model of depression in mice. Behavioural abnormalities were noticed in the group of mice treated with LPS with characteristic elevated pro-inflammatory cytokines, and dysregulated HPA-axis (i.e. increase in corticosterone). Previous preclinical evidences have shown cytokine inducers (LPS and M-CSF) causing behavioural deficits (sickness behaviour, anhedonia, immobility and reduced exploratory activity) in mice (O'Connor et al., 2009; Mello et al., 2013; Jiang et al., 2017) and dysregulation of hypothalamic-pituitary-adrenal axis (Tilders et al., 1994).

Our data in this study revealed that animals in groups treated with n-butanol fraction of *O. subscorpioidea* showed reduced cytokines concentration (TNF and IL-1β) and normalized HPA-axis evident by reduction in the plasma corticosterone. Alleviation of LPS-induced depressive-like symptoms with marked reduction in corticosterone, oxidative stress and inflammatory biomarkers thus demonstrates the anti-neuroinflammation and HPA-axis modulatory actions of n-butanol fraction of *O. subscorpioidea*. It can thus be said that the antidepressant effect this fraction may be due to its inhibitory actions on oxidative stress inflammatory mediators.

In conclusion, acute injection of LPS in mice has provoked release of inflammatory and oxidative stress mediators and produced depressive-like behaviours. Treatment with nBFOS reversed depressive behaviours in mice, suggesting that its antidepressant-like effect may be due to inhibition of oxidative stress and inflammatory mediators. However, further studies are required to establish the inhibitory action of the extract against neuroinflammatory and oxidative pathways.

Abbreviations

nBFOS - n-butanol fraction of *O. subscorpioidea*

LPS – lipopolysaccharide

Tail suspension test (TST).

IL-1β- interleukin-1β

TNF- tumor necrosis factor

IL-6- interlukin-6

HPLC- High performance liquid chromatograph

FRIN- Forestry Research Institute of Nigeria

UI-ACUREC - University of Ibadan Animal Care and Use Research Ethics Committee

MDA- malondialdehyde

GSH- reduced glutathione

TBA- Thiobabituric acid

DTNB- 5,5'-dithio-bis- 2-nitrobenzoicacid

TCA- Trichloroacetic acid

Declarations

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Code availability: Not applicable

Consent to participate: Not applicable

Consent to publish: All authors agreed to publish data obtained from this research

Ethics approval: University of Ibadan Animal Care and Use Research Ethics Committee (UI-ACUREC/App/2015/064) approved the experimental procedure and it was carried out in line with care and animal use guideline by NIH.

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Figures

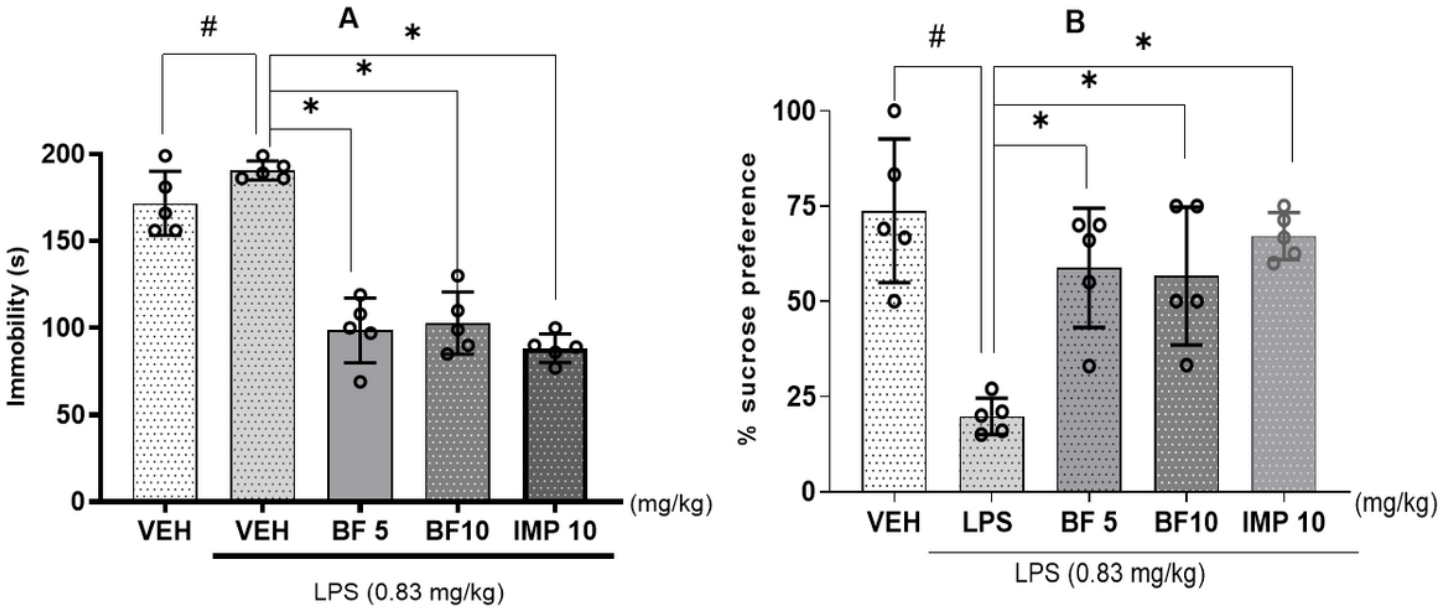

Figure 1

N-butanol fraction of *O. subscorpioidea* attenuates depressive like behaviours in LPS mice. Animals were repeatedly treated with nBFOS (5 and 10 mg/kg) or VEH (10 mL/kg) for seven days before LPS treatment. Twenty-four hours post LPS administration; immobility (**A**) and sucrose preference (**B**) were measured. **The d**ata are presented asthe mean ± SD.

shows statistical significance relative to VEH, $p < 0.05$;

* shows statistical significance relative to VEH/LPS-treatment, $p < 0.05$

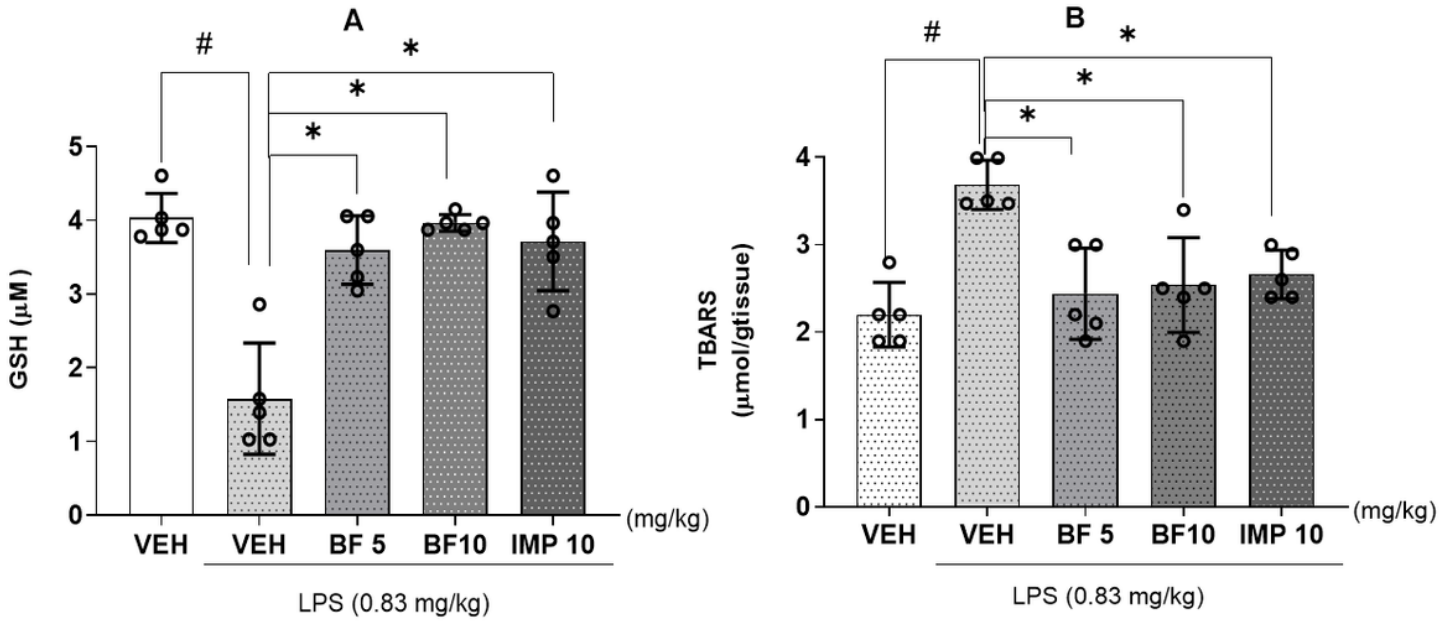

Figure 2

N-butanol fraction of *O. subscorpioidea* mitigates LPS-induced oxidative stress. Mice were repeatedly treated with nBFOS (5 and 10 mg/kg) or VEH (10 mL/kg) for seven days before LPS treatment. Twenty-four hours post LPS administration, concentrations of GSH (A) and MDA (B) were determined. Data are presented as the mean ± SD.

shows statistical significance relative to VEH $p < 0.05$;

* shows statistical significance relative to VEH/LPS-treatment, $p < 0.05$

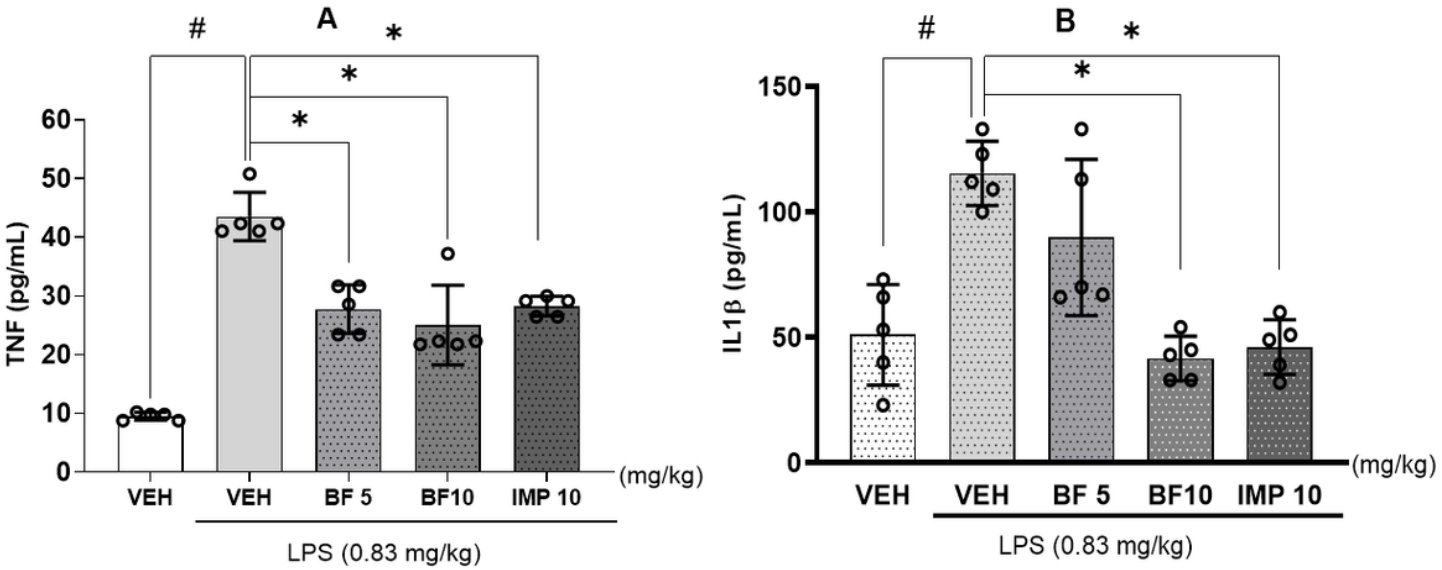

Figure 3

N-butanol fraction of *O. subscorpioidea* inhibits LPS-induced pro-inflammatory cytokines in the brain. Mice were repeatedly treated with nBFOS (5 and 10 mg/kg) or VEH (10 mL/kg) for seven days before LPS treatment. Twenty-four hours post LPS administration, concentrations of TNF (A) and IL-1β (B) were determined. Data are presented asthe mean ± SD

shows statistical significance relative to VEH p < 0.05;

* shows statistical significance relative to VEH/LPS-treatment, p < 0.05

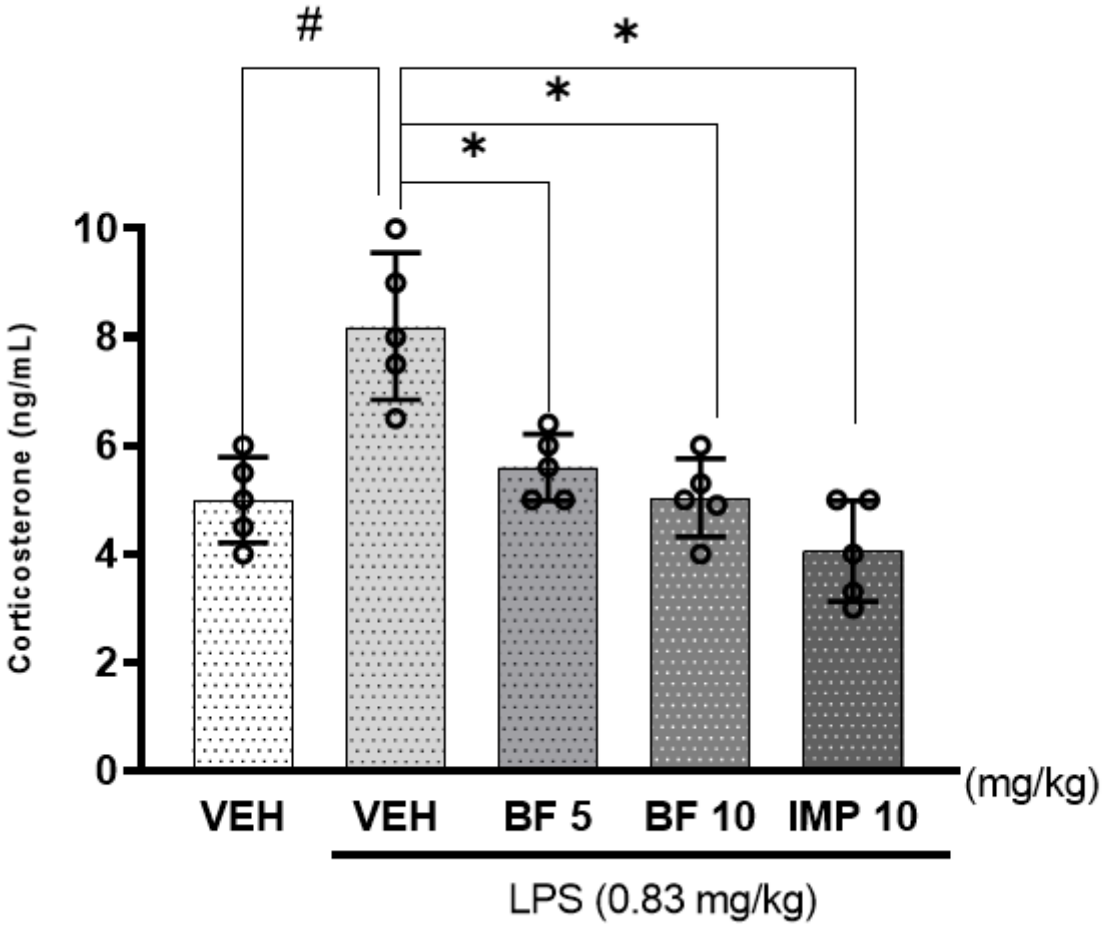

Figure 4

N-butanol fraction of *O. subscorpioidea* inhibits LPS-induced increase in corticosterone. Mice were repeatedly treated with nBFOS (5 and 10 mg/kg) or VEH (10 mL/kg) for seven days before LPS treatment. Twenty-four hours post LPS administration, concentrations of corticosterone was determined. Data are presented as the mean ± SD

shows statistical significance relative to VEH p < 0.05;

* shows statistical significance relative to VEH/LPS-treatment, p < 0.05