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Antidepressant-Like Effect of Ethanol Extract of *Blighia Unijugata* Bak. (Sapindaceae) Leaves in Acute and Chronic Models of Depression in Mice

[1]Bakre A.G., [2]Olayemi J. O., [1]Ojo O. R., [1]Odusanya S. T., [1]Agu G. A. and [1]Aderibigbe A.O*

[1]*Department of Pharmacology and Therapeutics, College of Medicine, University of Ibadan, Nigeria.*
[2]*Department of Pharmacognosy, Faculty of Pharmacy, University of Ibadan, Nigeria.*

Summary: *Blighia unijugata* (Sapindaceae) is an indigenous tree belonging to the tropical forests of West Africa. It is called "Ako Isin" by the Yoruba people of Southern-Western part of Nigeria, where it is among plants used traditionally in the management of depressive psychosis. The aim of this present study was to evaluate the anti-depressant activity of ethanol extract of *Blighia unijugata* leaves *in-vivo* using acute and chronic experimental models of depression. The antidepressant activity of ethanol extract of *B. unijugata* leaves was investigated using acute and chronic unpredictable mild stress. Depression tests used included forced swimming, tail suspension, yohimbine induced lethality and reserpine induced depression tests. Oxidative stress markers were also assessed in the brain homogenates after chronic unpredictable mild stress. The LD_{50} via oral route of administration was 1414 mg/kg. The results showed that, *B. unijugata* produced significant reduction in immobility time in forced swimming and tail suspension tests without stimulating in locomotor activity in open field test. It was also found that *B. unijugata* significantly reversed diarrhea, ptosis and hypothermia in reserpine model of depression. 2.5 mg/kg *B. unijugata* potentiated yohimbine induced lethality in mice and also reduced the oxidative stress markers. The ethanol extract of *B. unijugata* leaves possessed antidepressant action, thus justifying its use in the management of mental illness.

Keywords*:* Flavonoids, Blighia unijugata*; antidepressant; immobility; yohimbine; reserpine; ptosis.*

**Address for correspondence*: *bakreeyitemi@live.com*

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Introduction

Depression is a common psychiatric disorder, affecting approximately 20% of the worldwide population (Gao *et al.,* 2013). The World Health Organization (2009) predicts that it will become the second leading cause of premature death or disability worldwide by the year 2020 (Wager-Smith and Markou, 2011). Chronic psychosocial stress majorly predisposes most susceptible individuals to depression (De Kloet et al., 2005; Siegrist, 2008). Sadness, loss of interest or pleasure, feelings of low self-worth, disturbed sleep and poor concentration are some of the characteristics of depression (Chowdhury and Juvekar, 2014). The primary mechanism of this disorder include depletion of monoamines, oxidative stress and hyperactivity of HPA-axis (Dhingra and Bhankher, 2014). Although, the pathophysiology of this mental illness is not very clear, increasing evidence support the hypothesis that neurotransmitters deficiencies are extraordinarily important to its etiology; this has made the prescription of monoamines oxidase inhibitors and selective serotonin reuptake inhibitors (SSRIs) more frequent for patients suffering depression (Shen et al., 2016). Most of the drugs in use currently, target this deficit, which is empirical, symptom oriented and not disease specific. Also, these drugs have numerous limitations including unpleasant side effects (Nash and Nut, 2005). This gave rise to the use of plants as a therapy for this disorder; especially those with little or no side effects.

Reports by World Health Organization (1985), reveal that about 80% of the people living in developing countries almost exclusively use herbal medicine for their primary health care needs. The screening of these herbal medicines which are mostly of plant origin is a potential source of novel drug prototypes (Rabe and van Staden, 1997; Afolayan, 2003). Plants are the most natural and accessible sources of therapeutically active biological compounds. *Blighia Unijgata* belongs to Sapindaceae plant family; it is widely distributed with 136 genera and 2000 species (Urdampilleta et al., 2005). Many species in this family have been reported to possess a number of biological and pharmacological

activities (Basile et al., 2005). *Blighia unijugata*, (BU) has been reportedly used traditionally in the management of psychosis in the Southern-Western part of Nigeria (Sofidiya et al., 2011).

Significantly, there is paucity of studies investigating BU leaf extract's antidepressant activity and its probable extended pharmacological effect (side effects) which is a bane of drugs currently used for the treatment of depression. We therefore undertook this study considering the profile of side effects of currently used antidepressants and the paucity of antidepressant studies on BU. The aim of the study was to investigate the antidepressant-like effect of the ethanol extract of *Blighia Unijgata* leaves in acute and chronic models of depression.

Materials and Methods

Plant materials

Fresh leaves of *Blighia Unijugata* were collected at the Forestry Research Institute of Nigeria (FRIN), Ibadan, Nigeria. The leaves was also authenticated at the FRIN, where a voucher specimen with the number (FHI 110119) was deposited.

Preparation of plant material and drugs

The leaves were air-dried, pulverized and 100g was macerated for 48 hours in 1.75 L of 50% ethanol. The ethanol extract was decanted, filtered and concentrated under a rotary evaporator at the pharmaceutical chemistry laboratory of the University of Ibadan. The concentrated extract of *Blighia Unijugata* (BU) was dried and stored in a desiccator. On each day of the experiment, the extract obtained was freshly dissolved in distilled water which served as vehicle.

Experimental Animals

Male Swiss mice (20-25 g) were obtained from the Animal Centre, College of Medicine, University of Ibadan, Nigeria, and were housed in plastic cages at room temperature. They were fed with balanced rodent pellet diet and water *ad libitum*. The animals were acclimatized for at least 1 week before being used for the experiments. The experimental procedures were in compliance with the National Institutes of Health Guide for Care and Use of Laboratory Animals (Publication No. 85-23, revised 1985).

Drug and Chemicals

Imipramine (Sigma-Aldrich, St. Louis, USA), Thiobuturic acid (TBA), Trichoroacetic acid (TCA), DTNB reagent, phosphate buffer, ethanol and distilled water.

Acute toxicity test

The method described by Lorke (1983) was used to determine the LD_{50} which is the index of acute toxicity. Male Swiss mice (20 - 25 g) were used for the test. Briefly, this method involved an initial dose finding procedure, in which the mice were divided into three groups of three mice each. Doses of BU (10, 100 and 1000 mg/Kg) were administered intraperitoneally, one dose for each group. The treated animals were monitored for mortality and general behavior for 24 hours. From the result of the dose finding step, four higher doses of BU (2000, 3000, 4000, 5000 mg/Kg) were chosen and administered (i.p.) respectively to four groups of one mouse per group. The treated animals were monitored for 24 hours. The LD_{50} was then calculated as the geometric mean of the highest dose showing no death and the lowest dose showing death.

Tail Suspension Test (TST)

Tail Suspension Test is a commonly employed behavioral model for screening antidepressant activity in mice (Steru *et al*., 1985). The total duration of immobility following tail suspension was measured using the model for evaluating potential antidepressants postulated by Rodrigues *et al.*, (2002). Briefly, each mouse was individually suspended 50 cm above the floor by the tip of the tail (approximately 1 cm) adhered to a lever. The mouse under testing was quarantined during the test duration. A total testing period of 6 minutes was allowed. After the first 2 minutes following suspension of the mouse by the tail; the duration of immobility was manually recorded using a stopwatch during the next 4 minutes of test. The mouse was considered to be immobile when it did not show any body movement, hung passively and was completely motionless. The test was conducted between 8 am -12 pm in a quiet room to avoid change in biological rhythm and disturbance. The protocol was repeated for each mouse in the experimental groups consisting of 5 mice. The groups are as follows: group 1 received distilled water (0.2 mL/20 g), groups 2-4 received BU (1.25, 2.5 and 5 mg/Kg) and group 5 received imipramine (10 mg/Kg). All treatments were administered 30 minutes before the test.

Forced Swimming test (FST)

Forced Swim Test is a behavioral test for assessment of antidepressant activity of compounds. The test was performed according to the procedure described by Porsolt *et al*., (1997). The rodents were placed individually in Plexiglas cylinders (40 cm in height, 18 cm in diameter) filled with water (25 ◦C) up to 15 cm. Two minutes pre-swimming period was followed later by 4 minutes test period during which the total immobility time was measured. The mice were considered immobile when they made no further attempts to escape for necessary movements to keep their heads above the water. The absence of hind leg movement was recorded as immobility by stopwatch during the exposures. The water in the cylinder was changed before every trial and the mice were towel dried before being returned into their home cage after the swimming sessions.

Locomotor activity in the Open Field

Motor activity was measured in the open field apparatus (white Plexiglass box measuring 28 cm × 28cm × 25cm, with the floor equally divided into 16 equal squares marked with painted black grid). Thirty minutes after the administration of an extract or standard drug, each mouse was placed separately in the centre of the box, and the number of squares crossed by all four paws were counted for 5 minutes. The floor of the open field apparatus was cleaned with 70% ethanol and allowed a 5 minutes interval before the next animal was assessed (Akanmu *et al.,* 2011).

Yohimbine Induced Lethality Test

The involvement of noradrenergic system in the antidepressant-like effect of the extract was evaluated using yohimbine-induced lethality test. The test was performed as described by Vogel & Vogel (1997). Fifty (50) mice were assigned into five groups (n=10). Group 1 received distilled water (10 mL/Kg); groups 2-4 received different doses (1.25, 2.5 and 5.0 mg/Kg; i.p.) of leaf extract of BU; group 5 received imipramine (10 mg/Kg; i.p.). All the treatments were done thirty minutes prior to administration of Yohimbine (35 mg/kg; i.p.). The number of death and percentage lethality was calculated 24 hours after the injection of Yohimbine.

Reserpine-induced hypothermia, ptosis and diarrhea in mice

The test of reserpine-induced hypothermia, ptosis and diarrhea were in accordance with those of Bourin *et al.,* (1983). The mice were administered reserpine (2.5 mg/kg) 30 minutes after treatment with either distilled water, BU or imipiramine. The treatment was performed in five groups of male mice (n=5). Group 1 was given distilled water (0.2 mL/20 g), while groups (2-4) were given different doses of BU (1.25, 2.5, 5 mg/Kg); and group 5 was give imipramine (10 mg/Kg). The rectal temperature were recorded at 0, 1, 2, 3 and 4 hours, respectively, after the administration of reserpine. The degree ptosis was evaluated after 4 hours according to the following rating scale: eyes open = 0, quarter closed =1, eyes half closed = 2, eyes three-quarters closed = 3 and eyes completely closed = 4. Body temperature was measured using a rectal thermometer. The probe of the thermometer was inserted 1.5cm into the rectum. The pre-drug recording served as the reference point for the determination of temperature changes (Parimaladevi *et al.,* 2003). Diarrhea was evaluated as number of droppings at time points.

Chronic Unpredictable Mild Stress

Animals were subjected to various stress paradigms once a day, thirty minutes after treatment for a period of 2 weeks as described by (Kumar et al., 2011). Mice were randomly distributed to 6 groups (n=5). Groups 1-2 were administered 10 mL/Kg distilled water (vehicle), groups 3 – 5 were administered BU (1.25, 2.5, and 5.0 mg/Kg) and group 6 was administered imipramine (10 mg/Kg). The mice in group 1 were not stressed, while those in groups 2 – 6 were exposed to various stress conditions over a period of two weeks. Typical stressors included overnight illumination, periods of food or water restriction, cage tilt, and isolation or crowded housing. All administration was done by intraperitoneal route. Behavioral testing was done in independent groups of mice on the 15th day. Tail Suspension Test (TST) and Open Field Test (OFT) were the models employed for evaluation of the presence of antidepressant activity.

Biochemical assay

Determination of brain glutathione (GSH) concentration

The animals were sacrificed under ether anesthesia and the brains were rapidly removed. Thereafter, half of the whole brain were weighed and homogenized with 10% w/v phosphate buffer (0.1M, pH 7.4). Each brain tissue homogenates was separated into two portions for the different biochemical assays. Aliquots of brain homogenates of individual mouse in the respective treatment groups were taken and GSH concentration was determined using the method of Moron *et al.,* (1979). Equal volume (0.4 ml) of brain supernatant and 20% trichloroacetic acid (TCA) (0.4 ml) were mixed and then centrifuged using centrifuge at 2,000 rpm for 10 min. The supernatant (0.25 ml) was added to 2 ml of 0.6 mM 5,5′-Dithiobis-(2-nitrobenzoic acid) (DTNB) and the final volume was made up to 3 ml with phosphate buffer (0.2 M, pH 8.0). The absorbance was read at 412 nm against blank reagent using a spectrophotometer. The concentrations of GSH in the brain tissues are expressed as micromoles per gram tissue (µmol/g tissue).

Estimation of lipid peroxidation

Lipid peroxidation (LPO) was determined by estimating malodialdeyde (MDA) levels as described by Ohkawa et al., (1979). MDA and other aldehydes have been identified as products of lipids that react with TBA to give a pink coloured species that absorbs visible light spectrum at 532 nm. The method involved heating of biological samples with TBA reagent for 20 mins in a boiling water bath. TBA reagent contain 20% TCA, 0.5% TBA and 2.5 N HCl. After cooling, the solution was centrifuged at 2,000 rpm for 10 mins and the precipitate obtained was removed. The absorbance of the supernatant was determined at 532 nm against a blank that contained all the reagents minus the biological sample. The MDA equivalents of the sample were calculated using an extinction coefficient of 1.56×10^{5} M^{-1} cm^{-1}.

Statistical Analysis

Data were analysed using Graph Pad Prism version 5.0 and expressed as mean ± S.E.M. Statistical analysis

was done using one-way ANOVA followed by Newman-keuls post- hoc test. P values < 0.05 were considered statistically significant.

RESULT

Acute toxicity test: No lethality/mortality was recorded when doses as high as 1000 mg/kg was given to the mice. The mouse administered the dose 2000 mg/Kg died, and this was used along with the 1000 mg/Kg to calculate the LD_{50}. The LD_{50} of crude extract of *Blighia Unijugata* in mice was found to be 1414 mg/Kg; i. p. body weight.

BU reduced the locomotor activity of mice in OFT: BU at 5.0 mg/kg significantly reduced [F (4, 29) = 4.942, p< 0.05] line crossing activity in the OFT compared to vehicle (Figure 1).

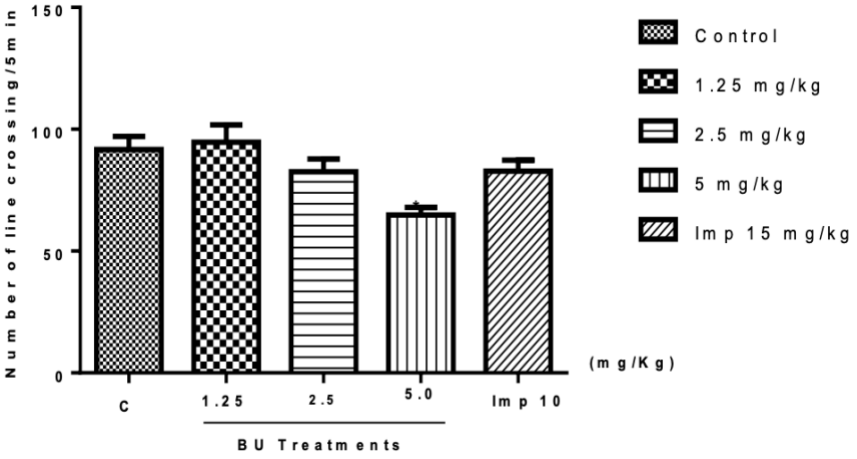

Figure 1:
Effect of BU the locomotor activity of mice in OFT *p < 0.05 in comparison with control. C=Control 10 ml/kg, Imp=imipiramine

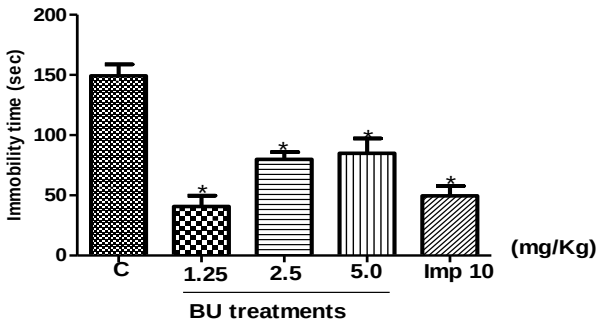

Figure 2:
Effect of BU on immobility time of mice in FST *p < 0.05 in comparison with control. C=Control 10 ml/kg, Imp=imipiramine

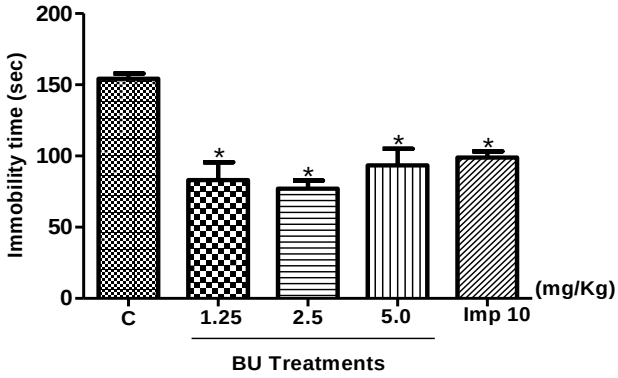

Figure 3:
BU reduced the immobility time of mice in TST *p < 0.05 in comparison with control. C=Control 10 ml/kg, Imp=imipiramine

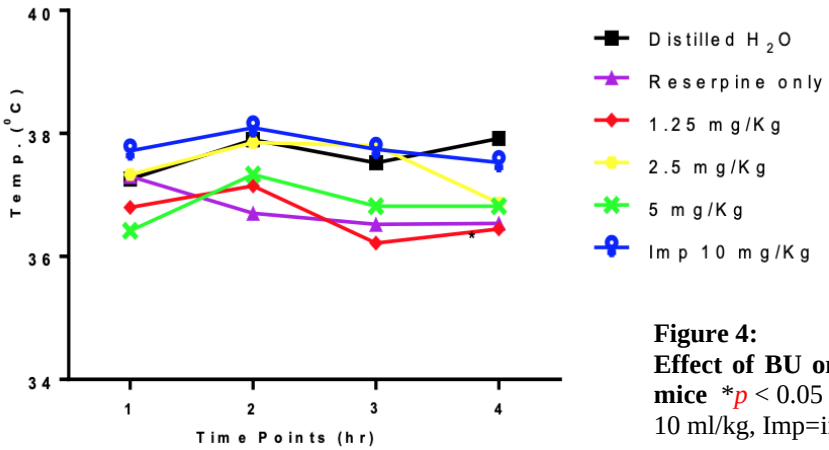

Figure 4:
Effect of BU on reserpine induced hypothermia in mice *p < 0.05 in comparison with control. C=Control 10 ml/kg, Imp=imipiramine

BU reduced the immobility time of mice in FST: The administration of BU (1.25, 2.5 and 5.0 mg/Kg, p.o.) and imipiramine (10 mg/Kg) significantly reduced [F (4, 29) = 21.25, p< 0.05) the immobility time in the forced swim test (Figure 2)

BU reduced the immobility time of mice in TST: The administration of BU (1.25, 2.5 and 5.0. mg/kg, p.o.) and imipiramine (10 mg/Kg; i.p.) significantly reduced [F (4, 29) = 13.28, p< 0.05) the immobility time in the tail suspension test (Figure 3).

BU reverses reserpine induced hypothermia in mice

The administration of BU (2.5 and 5.0 mg/Kg) prevent reserpine induced hypothermia in mice when compared to reserpine only. BU 2.5 mg/Kg and imipiramine 10mg/Kg and significantly [F (5, 29) = 3.992, p< 0.05) reversed reserpine induced hypothermia (Figure 4)

BU reverses the degree of ptosis in reserpine-induced depression

The administration of BU (1.25, 2.5 and 5.0 mg/Kg) and imipramine (10 mg/Kg) significantly reversed the degree of ptosis during reserpine-induced depression (Table 1).

Table 1:
Effect of ethanol extract of *B. Unijugata* leaves on ptosis and diarrhea in reserpine-induced depression
*P<0.05

Treatment	Score of Ptosis	Diarrhea
Vehicle (10 ml/kg)	1.75±0.23	3.43±0.83
BU (1.25 mg/kg)	0.67±0.17*	1.63±0.40*
BU (2.5 mg/kg)	0.79±0.23*	1.96±0.57*
BU (5.0 mg/kg)	0.167±0.07*	1.67±0.49*
Imipramine (10 mg/kg)	0.67±0.33*	1.33±0.33*

BU reverses score of diarrhea in reserpine-induced depression

The administration of BU (1.25, 2.5 and 5.0 mg/Kg, i.p.) and imipramine (10 mg/Kg) significantly reversed the degree of diarrhea during reserpine-induced depression (Table 1).

Table 2. Effect of ethanol extract of *Blighia Unijugata* leaves on Yohimbine Lethality Test

Treatment	Number of death (n)	% Mortality
Vehicle (10 ml/kg)	2/10	20
BU (1.25 mg/kg)	0/10	0
BU (2.5 mg/kg)	6/10	60*
BU (5.0 mg/kg)	2/10	20
Imipramine (10 mg/kg)	7/10	70*

*P<0.05

Effect of ethanol extract of BU leaves on Yohimbine lethality

2.5 mg/Kg BU and 10 mg/Kg imipiramine produced a significant increase in the number of deaths (P < 0.05) as compared with control (Table 2).

Effect of BU on locomotor activity of mice subjected to chronic unpredictable mild stress

Chronic unpredictable mild stress significantly decreased the locomotor activity in stressed mice as compared to vehicle-treated unstressed control. Imipiramine (10 mg/Kg) significantly (p < 0.001) decreased the immobility period as compared to stressed mice. The administration of BU (1.25- 5 mg/Kg) did not significantly decrease the locomotor activity when compared with stressed control mice (Figure 5).

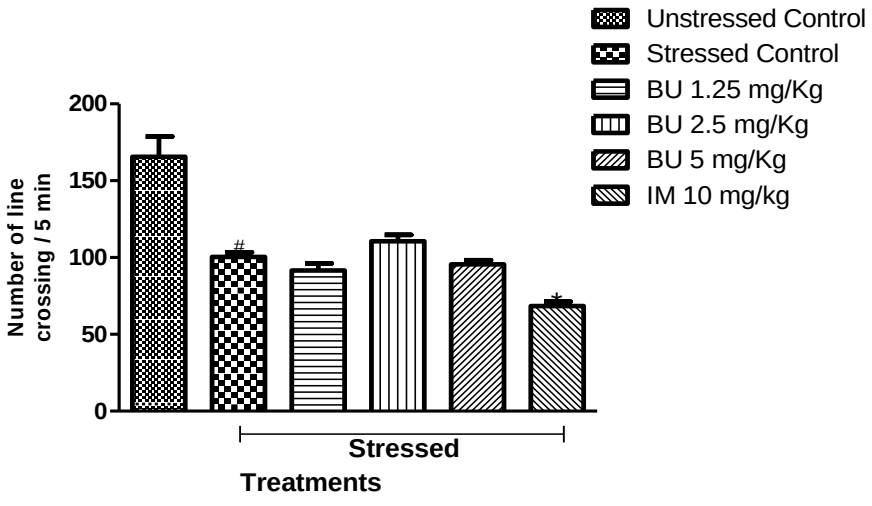

Figure 5:
Effect of BU on locomotor activity in mice subjected to CUMS * indicates significant difference from the stressed control $p < 0.05$ # indicates significant difference from the unstressed control treated $p < 0.05$ Control 10 ml/kg, Imp=imipiramine

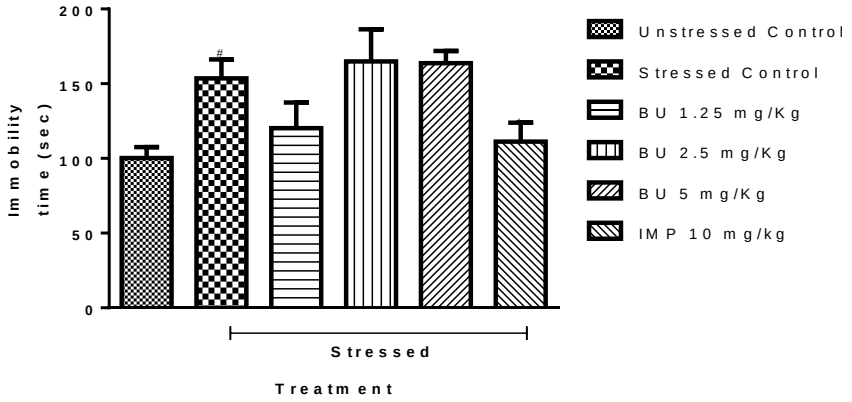

Figure 6:
Effect of BU on immobility time in mice subjected to CUMS * indicates significant difference from the stressed control $p < 0.05$ # indicates significant difference from the unstressed control treated $p < 0.05$

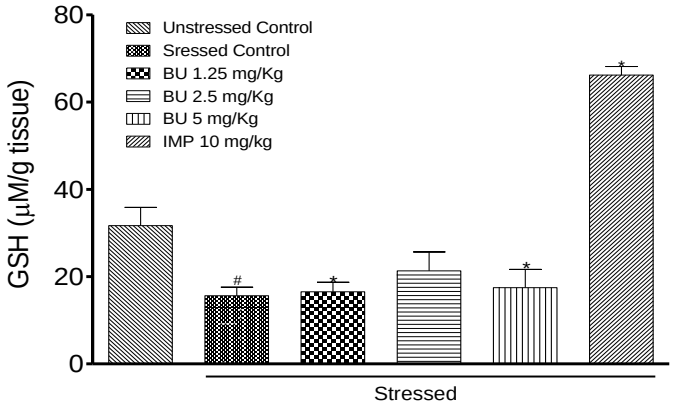

Figure 7:
Effect of BU on brain Glutathione Levels in CUMS * indicates significant difference from the unstressed control $p < 0.05$ # indicates significant difference from the unstressed control treated $p < 0.05$

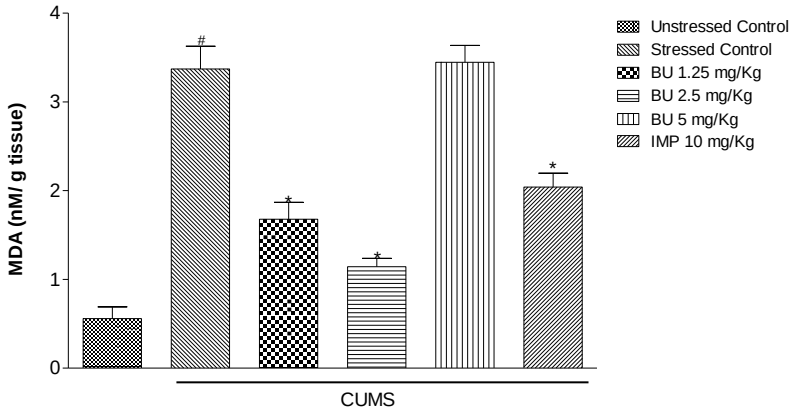

Figure 8:
Effect of BU on brain Malondialdehyde Levels * indicates significant difference from the unstressed control $p < 0.05$ # indicates significant difference from the unstressed control treated $p < 0.05$

Effect of BU on immobility time in TST of mice subjected to CUMS

Chronic unpredictable mild stress significantly increased the immobility time in stressed mice as compared to vehicle-treated unstressed control. The administration of BU (1.25- 5 mg/Kg) and imipiramine (10 mg/Kg) did not significantly decrease the immobility time as compared to stressed control mice (Figure 6).

Effect of BU on brain Glutathione Levels

GSH levels were significantly ($p < 0.001$) decreased in brains of vehicle-treated stressed mice when compared with unstressed control (Figure 7). BU (1.25, 2.5 and 5mg/Kg) and imipramine (10 mg/kg) produced a significant ($p < 0.001$) increase in GSH levels in respective treated mice in comparison with stressed control mice.

Effect of BU on brain Malondialdehyde Levels

Malondialdehyde levels were significantly ($p < 0.001$) increased in brains of vehicle-treated stressed mice compared with unstressed control (Figure 8). BU (1.25, 2.5 and 5mg/Kg) and imipramine (10 mg/Kg) produced a significant ($p < 0.001$) decrease in MDA levels in respective treated mice in comparison with stressed control mice.

DISCUSSION

Behavioural studies have been shown to play an important part in the evaluation and development of antidepressant drugs (Xu, et al., 2008). The behavioral paradigms used in the present study include Forced Swim Test (FST), Tail Suspension Test (TST) and open field test. Yohimbine lethality test and reserpine induced depression were also used to investigate the probable mechanism of action. Behavioural and biochemical changes were also studied after inducing chronic depression using Chronic Unpredictable Mild Stress (CUMS). The CUMS was used to determine the underlying factors of depression which may involve the generation of free radicals such as reactive oxygen species. The study established that the acute lethal dose (LD50) of the ethanol extract of *Blighia Unijugata* (intraperitoneal route) is 1414 mg/kg using the method described by Lorke (1983). It could therefore be assumed to have a wide safety margin especially in use as a potential candidate for antidepressant drug discovery. Jebunnessa et al. (2009) extracted alkaloids, triterpenoids, phenolic compounds, carotenoids, steroids and ketones from the leaves of *Blighia Unijugata*.

The immobility displayed by rodents subjected to unavoidable and inescapable stress (in FST and TST) is postulated as reflecting behavioural despair which may reflect depressive disorder in humans (Porsolt *et al.*, 1977). This study revealed that ethanol extract of *Blighia Unijugata* (BU) produces a statistically significant reduction in immobility time in FST and TST. Imipramine, an antidepressant, expectedly produced significant reduction in the immobility time of the tested rodents. It has been established that immobility is reduced by a variety of therapeutically active antidepressants as tricyclics, monoamine oxidase inhibitors, and other new antidepressants (Cryan and Lucki, 2000). The capacity of BU to also trigger a reduction in immobility time suggests that it may possess antidepressant activity.

The possible attribution of the antidepressant effects of test substances to stimulant effect in behavioural models of depression are usually characterized by locomotor activity test (open field test) (Bourin *et al*., 2001). Amphetamines, convulsants and anticholinergic are some compounds which can enhance locomotor activity or cause hyperkinesias in OFT and still produce false positive results in FST and TST (Butterweck *et al*., 2003). Antidepressants and psycho-stimulants are usually discriminated by increased locomotor activity in OFT (Borsini and Meli, 1988).

The possible mechanism underlying the antidepressant activity of BU, was evaluated using the reserpine induced depression reversal test. Existing studies have revealed that reserpine can cause the depletion of amine stores and irreversibly inhibit the vesicular uptake of monoamines. The resulting reduction level of monoamines in the brain is an underlying factor in pathophysiology of depression; and it leads to physiological effects such as diarrhoea, ptosis and hypothermia which have all been associated with reserpine induced depression (Bourin *et al*., 1983). The major classes of antidepressant drugs have reversed or inhibited these syndromes associated with reserpine induced depression. BU (2.5 and 5.0 mg/kg) and imipramine (10 mg/kg) have been observed to significantly reverse hypothermia induced by reserpine. The degree of ptosis and diarrhea were also significantly reversed by BU and imipramine when compared with the control. BU significantly antagonized the clinical observations induced by reserpine. BU might be mediated via the monoamine pathway to trigger the mechanism of its antidepressant-like effect.

To identify neurotransmitter systems that may be involved in the mechanism of actions of antidepressant drugs, the yohimbine induced lethality test is recommended (Leonard *et al*, 1986). Yohimbine is an α_2 adrenergic antagonist which is responsible for increased sympathetic discharge in the peripheral and in the central nervous system. As an antagonist, it also increases the level of serotonin and other biogenic amines when it inhibits the negative feedback regulation of their release (Blier and Montigny, 1994). All currently approved antidepressants (ADs) increase the synaptic availability of one or more of the biogenic amine transmitters: noradrenaline, dopamine, and serotonin. The proposed mechanism of antidepressant drugs in potentiation of yohimbine induced lethality is via involvement of noradrenaline. BU at 2.5 mg/kg potentiated the effect of yohimbine, which resulted in 60% mortality as compared with the control (20% mortality). This effect might either be via inhibition of reuptake or inactivation of monoamine oxidase.

Chronic Unpredictable Mild Stress (CUMS) induced depression is considered the most valid animal model of depressive behavior observed in humans after a long-term exposure to multiple stressors (Willner, 2005). Cortisol (stress hormone) is released in excess via the activation of the hypothalamic pituitary adrenal axis inducing a damage to the dopaminergic, serotoninergic or glutamatergic neurons (Vyas et al, 2016). The consequence of all these changes is reduction in the size of the hippocampus and the frontal cortex, which is characteristic for patients with

severe depression (De Andrade et al., 2013). Animal studies in CUMS model have shown that long-acting stressors cause atrophy of hippocampal pyramidal cells and impair the neurogenesis resulting in a reduction in size (Drevets et al., 2001). The administration of BU (1.25, 2.5, 5 mg/kg) (14-day) reverses the structural and functional changes in the hippocampus and the frontal cortex induced by different chronic stressors. BU might increase the size of the hippocampus via enhancement of neurogenesis in the hippocampus and frontal cortex. Imipramine was responsible for the reversal of the hippocampal atrophy and the inhibition of neurogenesis in the hippocampus and in the cortex.

Also, CUMS impairs the antioxidant status of brain tissue due to excessive production of reactive oxygen species (Bilici *et al.*, 2001). Reactive oxygen species (ROS) plays a vital role in the pathogenesis of neuropsychiatric disorders that cause oxidative damage to macromolecules (lipids, proteins and DNA) and result in neuronal dysfunction and depression (Esch et al., 2002). Lipid peroxidation and other antioxidant enzymes may be biomarkers of major depression due to the fact that they return to normal levels after treatment with antidepressants (Bilici et al., 2001). In this study, 14 days of successive exposure to unpredictable mild stress using different stressors resulted in the reduction in GSH and increased in the amount of MDA in stress exposed mice. BU (2.5 mg/kg) and imipramine significantly increased GSH level in the brain. BU (1.25 and 2.5 mg/kg) and imipramine also significantly reduced the brain level of MDA. Thus, BU might possess a neuroprotective effect against oxidative stress in CUMS-induced depression. Some species of plants such as *Bacopa monneira, Withania somnifera* and *Asparagus racemosus*, have been reported to have antidepressant-like properties attributable to their antioxidant activity (Sairam et al., 2002; Bhattacharya et al., 2000). Therefore, it is possible that the antioxidant activity of the BU may contribute to its antidepressant-like effect.

Finally, the results of this study showed that the leaves of *Blighia unijugata* possess antidepressant properties. This neuropharmacological property is possibly mediated through the facilitation of noradrenergic pathways and antioxidant activity. However, considering the limitations of the models used, clinical studies involving humans may be need to be carried out to further confirm the results of our study.

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