Original Research

ETHANOL STEM BARK EXTRACT OF *BLIGHIA SAPIDA* (*SAPINDACEAE*) REVERSE KETAMINE INDUCED SCHIZOPHRENIA LIKE PHENOTYPES AND OXIDATIVE STRESS IN MICE

ABSTRACTS

This study investigated the effects of ethanol extract of *Blighia sapida* (EEBS) stem bark on ketamine-induced schizophrenia-like behaviour and oxidative stress in mice. Thirty-five mice were divided into groups and treated with ketamine (20 mg/kg/day) or saline for 14 days, followed by EEBS (10, 20, 40, or 80 mg/kg) or risperidone (0.5 mg/kg) from days 8 to 14. Behaviour was assessed 24 hours after the final treatment using the open field and Y-maze tests. Oxidative stress markers, including nitrite levels, glutathione synthase (GSH), and malondialdehyde (MDA) concentrations, were measured. EEBS and risperidone significantly reversed ketamine-induced behavioural changes, with EEBS showing dose-dependent effects. EEBS at 80 mg/kg (i.p) notably reduced locomotor activity (mean value 373.8 ± 14.38) compared to ketamine alone (584.2 ± 16.23) and 40 and 80 mg/kg improved cognitive performance in the Y-maze test (75.68 ± 1.563% and 79.70 ± 2.381% alternation respectively), compare to ketamine alone (56.53 ± 1.248%). Oxidative stress parameters showed that EEBS and risperidone decreased nitrite levels, increased GSH activity, and reduced MDA levels compared to ketamine alone. These findings suggest that *Blighia sapida* has neuroprotective properties, likely through its antioxidant effects.

Keywords: *Blighia sapida*, Stem bark, Schizophrenia, Oxidative stress, Mice.

INTRODUCTION

Schizophrenia is a cluster of disorders characterized by fundamental disturbances of thinking, perception and emotions. Behavioural manifestations of schizophrenia are categorized as positive or negative and, a sufferer usually exhibits a mixture of the two symptoms (Reena et al. 2011, Ben-Azu et al. 2018a, 2022). The negative symptoms of schizophrenia may include: lethargy, apathy, reduction in speech, social withdrawal whereas, positive symptoms usually manifest as hallucinations, delusions, tangentiality, pressure of speech, inappropriate dressing. However, the cognitive symptoms are known to manifest as learning and memory impairment (McCutcheon et al. 2023), however significantly associated with extra-pyramidal side effects such as tardive dyskinesia and akathisia. The atypical class notably, clozapine and risperidone were originally believed to be effective in ameliorating the positive and negative symptoms and, are better tolerated with respect to extra-pyramidal adverse effects but they exhibit significant cardiovascular risk (Chatterjee, 2012). While the disease is associated with several underlying pathological factors consistently linked to disruption of neurochemical imbalances such as dopamine, glutamate, serotonin, and gamma aminobutyric acid (GABA), the role of glutamatergic receptor such as N-methyl-D-aspartate (NMDA) receptor notably linked to oxidative stress has been strongly implicated in the pathogenesis of the disease (Monte et al. 2013; Ben-Azu et al. 2018a, 2018b, 2023).

Ketamine, a short acting dissociative anaesthetic agent, and non-competitive NMDA receptor antagonist, is known to induce the positive, negative symptoms, and cognitive deficits in humans (Hallak et al. 2011) and in preclinical animal models (Monte et al. 2013; Ben-Azu et al. 2018a, 2018b, Usman et al. 2019) at sub anaesthetic dose (20mg/kg). Single or repeated ketamine administration induces changes in systems involving glutamate, dopamine, serotonin and gamma

aminobutyric acid (GABA), neurotransmitters implicated in schizophrenia pathophysiology (Lindefors et al. 1997), eliciting oxidative stress and subsequent altered endogeneous antioxidant systems (Ben-Azu et al. 2018a, 2018b, 2023) . Oxidative stress which is a state of an imbalance in the generation of free and antioxidant systems might overwhelm neurotransmitter metabolism, leading to dopamine auto-oxidation and impaired glutaminergic neurotransmission, as evident in hyperdopaminergic state in schizophrenia, whereas excess dopamine activity result in increased oxidative damage (Ben-Azu et al. 2018a, 2018b, 2023). Glutamate causes excitotoxic damage by binding to non-NMDA receptors, increasing calcium input, enhancing the neuronal nitric oxide species (NOS) activity and increase nitric oxide NO production (Nakao et al. 2003). Sustained oxidative stress decreased glutathione synthase (GSH) activity and increased levels of malondialdehyde MDA, which correlates with clinical symptoms and cognitive deficits in schizophrenia (Dadheech et al. 2008).This suggests that oxidative mechanism may be involved in the pathophysiology of schizophrenia (Akyol et al. 2004)

Blighia Sapida, commonly known as Ackee is a soap berry plant of the family *Sapindaceae.* It is a perennial herbaceous plant that is prominently found in Western Tropical Africa. It is a medicinal plant used in traditional medicine among many ethnic nationalities in Africa (Olusegun and Olutomi, 2013). Various parts of *Blighia Sapida* plants, alone or in combination, have been reportedly used for the treatment of psychosis, cancer, gonorrhea, stomach ache, hernia, backache, diarrhoea and constipation (Owonubi, 2006). *Blighia sapida* stem bark contains phytochemicals such as flavonoids, polyphenols, and carotenoids (Sofowora, 2006, Bachtela and Israni-Wingerb, 2020). These compounds possess antioxidant properties that may contribute to neuroprotective effects in the brain (Olusegun and Olutomi, 2013). Ethanol extract of the plant stem bark had

previously been reported to possess antipsychotic properties in apomorphine induced stereotypy behaviour in mice (Usman et al. 2019).

MATERIALS AND METHODS

Plant material

The bark of the stem of *Blighia sapida (sapindaecae)* was collected during the raining season from Igboora farm settlement in Afijio, Oyo state, and identified at the Forestry Research Institute of Nigeria (FRIN), Ibadan with voucher number 110254.

P**reparations of *Blighia sapida* stem bark extract**

The ethanol extract of *Blighia sapida* (EEBS) was prepared using cold extraction. The stem bark was air – dried for 4 weeks, and was pulverized with an electric crusher. A 200 g of pulverized stem bark was soaked in 70% ethanol and left for 48 hours. After which, it was filtered on absorbent cotton and on Whatman 3 mm paper. The volume of the filtrate was concentrated using Rotary evaporator at 40℃ and the paste, of dark brown colour obtained was dried to a constant weight in a dessicator before it was kept in a sterilized glass vial for use.

Animals

Male and female Swiss mice (*Mus musculus)* weighing between 18-24 g were obtained from the Central Animal House, University of Ibadan. The animals were initially (for a period of one week) housed in plastic cages in a controlled environment at an ambient room temperature and approximately light:dark cycle of 12:12 with unrestricted access to standard pellet food and water.

Drugs and chemicals

Ketamine hydrochloride (Swiss pharma), risperidone (RISP; Ranbaxy) were obtained in powder form and the drug solutions were prepared fresh in normal saline and administered intraperitoneally (i.p) in a constant volume of 1 ml/body weight of the animal, phosphate buffer (0.1M, p^H 7.4), An aliquot of 0.2ml, 0.05M carbonate buffer (pH 10.2), 0.3mM adrenaline (epinephrine), Griess reagent , Sodium nitrite, 5′, 5′–dithiobis-(2-nitrobenzoic acid), Sulfhydryl compounds, 0.4ml of 20% trichloroacetic acid (TCA), Tris-potassium chloride (Tris-KCl) buffer, Thiobarbituric acid (TBA).

Safety profile and Dose determination.

Acute toxicity test of ethanol stem bark extract of *Blighia sapida* was previously carried out by Usman et al. of which median lethal dose (LD_{50}) was determined as the index of acute toxicity using Lorke's method. Doses from the previously conducted study of the extract by Usman et al. was adopted for this study.

Experimental procedure

Drug treatment

The effect of the ethanol extract of stem bark of *Blighia sapida (sapindaecae)* in the reversal of ketamine induced oxidative stress in mice model of schizophrenia, as described by Aline et al. (Ben-Azu et al. 2016). Thirty-five animals were randomly divided into 7 groups (n=5). One of the animal grouping (Vehicle) received distilled water (10 mL/kg), while the remaining 6 groups were given Ketamine (20 mg/kg) intraperitoneally for 14 days. However, from days 8-14, four (4) of the ketamine treated groups were additionally given graded doses of the extract (10 mg/kg, 20 mg/kg, 40 mg/kg, and 80 mg/kg) intraperitoneally (KET+EEBS groups), the doses of EEBS was selected base on previous study by Usman et al. 2019. Another of the ketamine treated group

received risperidone (0.5 mg/kg i.p) daily along with the ketamine treatment as the positive control group (KET+RISP group), the last ketamine treated group received ketamine alone intraperitoneally for the 14 days as the negative control group (KET). Twenty-Four (24) hours after the last treatment (on the 15th day), behavioural test, locomotor activity and oxidative stress parameters were assessed.

Locomotor activity

Open field test.

Spontaneous locomotor activity was measured in an activity cage (Ugo Basile, Verase, Italy) having dimensions of 39 × 28 × 26 cm. The data are presented as the number of horizontal and vertical motor activity measured by the apparatus in response to animal movements. The activity of each mouse was automatically recorded for 5 min. Each animal was placed in the activity cage and allowed to explore the apparatus for 5 min. The testing apparatus was thoroughly cleaned with 70% ethanol before introducing subsequent mouse (Becker and Grecksch, 2004).

Cognitive Assessment

Y-maze test

Alternation performance was assessed by placing each animal in a Y-maze apparatus, measuring: 33 × 11 × 12 cm with each arm at an angle 120° to one another. Respective mice were gently placed at the end of one arm, and were allowed to freely explore the Y-maze for 5 min while taking the following parameters: the number of arm visits, and sequence of arm visits (Chindo et al. 2012, Ben-Azu et al. 2016).

Determination of oxidative stress parameters: Each mouse was sacrificed through cervical dislocation after the behavioural analysis, following which the whole brain of each mouse was collected and homogenized with 5ml of phosphate buffer (0.1M, p^H 7.4). Homogenates were centrifuged at 10,000g for 10 min at 4 ^{o} C, the pellet were discarded and the supernatants were immediately separated into portions for the different antioxidant biochemical assays, namely: Reduced glutathione (GSH), Lipid peroxidation (Malondialdehyde, MDA), and nitric oxide (NO) as described below (Dadheech et al. 2008, Ben-Azu et al. 2016)

Nitrite determination: To determine nitric oxide (NO) production, nitrite contents was measured in all groups. 10% (w/v) homogenates brain were centrifuged (800 ×g; 10 min), supernatants were collected and nitric oxide production was determined based on Griess reaction (Ben-Azu et al. 2016). Briefly, 100 µL of the supernatant was incubated with 100 µL of Griess reagent at room temperature for 10 min. Absorbance was measured using a microplate reader at 550 nm. Nitrite concentration was determined from a standard nitrite curve produced using $NaNO_2$ and was expressed as nmol/mg protein (Dadheech et al. 2008, Ben-Azu et al. 2016).

Determination of GSH: Reduced glutathione concentration was measured by calculating the content of non-protein sulfhydryl groups by technique previously described by Sedlak and Lindsay 1968. 400 µL of the homogenate was added to 320 µL of distilled water and 80 µL of trichloroacetic acid at 50%, the mixture was centrifuged for 15 min at 3000 rpm and then 400 µL of the supernatant was taken and added to 800 µL of Tris–HCl buffer (0.4 M, pH 8.9) and 20 µL of 5, 5′-dithiobis (2-nitrobenzoic acid) (DTNB) 0,1M and after 1 min the reading was made at 412

nm. The concentration of GSH was expressed in nanogram per gram of tissue (Ben Azu et al. 2016).

Determination of lipid peroxidation: Lipid peroxidation levels in all groups were analyzed by measuring the thiobarbituric acid reactive substances (TBARSs) in 10% (w/v) homogenates brain, as previously described by Okhawa et al. (Ben-Azu et al.. 2016, Dadheech et al. 2008). Briefly, the samples were mixed with 1 mL 10% trichloroacetic acid and 1 mL 0.67% thiobarbituric acid. They were then heated in a boiling water bath for 15 min and butanol (2:1, v/v) was added to the solution. After centrifugation (800 ×g; 5 min), thiobarbituric-acid-reacting substances were determined from the absorbance at 535 nm. Results were expressed as nmol of malondialdehyde (MDA)/g wet tissue (Ben Azu et al. 2016).

$$\text{MDA (units/mg protein)} = \frac{\text{Absorbance x volume of mixture}}{E_{532nm} \text{ x volume of sample x mg protein}}$$

Statistical analysis

Data were expressed as Mean ± S.E.M and were analyzed using one–way analysis of variance (ANOVA) and post hoc tests (Student's Newman-Keuls) for the multiple comparisons where appropriate using GraphPad InStat® Biostatistics software. The level of significant was set at $p < 0.05$.

RESULTS

Effect of ethanol extract of *Blighia sapida* in the reversal of ketamine-induced hyper-locomotion

Animals that received varied doses of EEBS (10, 20, 40, 80 mg/kg i.p) and risperidone (0.5mg/kg *i.p*) showed significant (P<0.05) decrease in activity counts/5minutes respectively, compare with the negative control (KET) that received ketamine (20mg/kg i.p) alone. (Table .1).

Table 1: Effect of EEBS on reversal treatment of chronic ketamine-induced hyperlocomotion

S/N	Treatment and Dose	Locomotor Activity (count/5minute)
1	VEHICLE 10 mL/kg	382.4 ±14.21
2	KET 20 mg/kg i.p	584.2 ±16.21$^{\#}$
3	KET + EEBS 10mg/kg i.p	461.4 ± 26.13*
4	KET + EEBS 20mg/kg i.p	496.6 ±13.99*
5	KET + EEBS 40mg/kg i.p	422.0 ± 30.78*
6	KET + EEBS 80mg/kg i.p	373. 8 ± 14.38*
7	KET + RISP 0.5 mg/kg i.p	401.8 ± 40.93*

Values represents the Mean ± SEM (n=5). One way ANOVA, significant [F (6, 28) = 8.228, P< 0.0001] differences between various treatment groups for number of activity counts for 5 minutes. $^{\#}p$< 0.05 as compared to vehicle group, * p< 0.05 as compared with ketamine group.

KET = Ketamine, **RISP** = Risperidone, **EEBS** = Ethanol extract of *Blighia Sapida* Stem Bark

Effect of ethanol extract of *Blighia sapida* in the reversal of ketamine-induced cognitive dysfunction

EEBS (40 and 80 mg/kg i.p) and Risperidone (0.5 mg/kg, i.p) significantly (P<0.05) showed increased percentage alternation compared with ketamine (20mg/kg i.p) alone. EEBS at dose 10 and 20mg/kg showed no significant increase in percentage (%) alternations compared with the negative control group (Table. 2).

Table.2: Effect of EEBS on reversal treatment of ketamine-induced cognitive dysfunction

S/N	Treatment and Dose	Percentage Alternation (%)
1	VEHICLE 10 mL/kg	77.75 ± 2.861
2	KET 20 mg/kg i.p	56.53 ±1.248$^{\#}$
3	KET + EEBS 10mg/kg i.p	60.79 ±3.250
4	KET + EEBS 20mg/kg i.p	64.75 ±2.615
5	KET + EEBS 40mg/kg i.p	75.68 ± 1.563*
6	KET + EEBS 80mg/kg i.p	79.70 ±2.381*
7	KET + RISP 0.5 mg/kg i.p	80.33 ± 3.223*

Value represents the Mean ± S.E.M (n=5). One way ANOVA revealed that there is significant [F (6, 29) = 7.961, P< 0.0001] difference between various treatment groups.

$^{\#}p$< 0.05 as compared to vehicle group, $^{*}p$< 0.05 as compared with ketamine group.

KET = Ketamine, **RISP** = Risperidone, **EEBS** = Ethanol extract of *Blighia sapida* Stem Bark

Effects of ethanol extract of *Blighia sapida* on Nitrite level in the reversal of ketamine induced oxidative stress

Animals in extract treated groups KET + EEBS that received doses of EEBS (10, 20, 40, 80 mg/kg i.p) and KET + RISP (positive control) animals that received risperidone (0.5mg/kg i.p) showed significantly (P<0.05) decreased nitrite level compared with negative control animals (Fig.1).

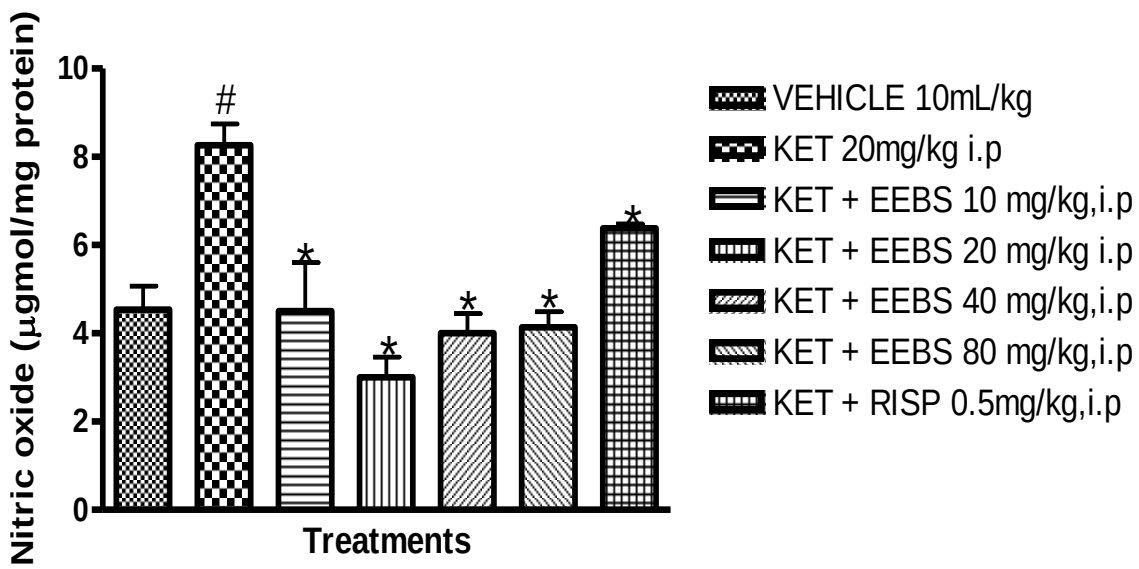

Fig. 1: Effects of EEBS on Nitrite level in mice brain.

Value represents the Mean± SEM (n=5).One way ANOVA shows that there is significant [F (6, 28) = 9.587, P <0.0001] difference between various treatments groups when compared with negative control group that received ketamine (20mg/kg i.p) alone.

$^{\#}p<0.05$ when ketamine group was compare to vehicle.* $p<0.05$ as compare with ketamine group.

KET= Ketamine, **RISP**=Risperidone, **EEBS**= Ethanol extract of *Blighia sapida* Stem Bark

Effects of ethanol extract of *Blighia sapida* on glutathione synthase (GSH) activity in the reversal of ketamine induced oxidative stress

KET + EEBS animals that received EEBS (20, 40, 80 mg/kg i.p respectively) and positive control animals that were given risperidone (0.5mg/kg i.p) significantly (P<0.05) showed increased GSH level compare with the negative control (KET 20mg/kg i.p) that received ketamine (20mg/kg i.p) (Fig.2).

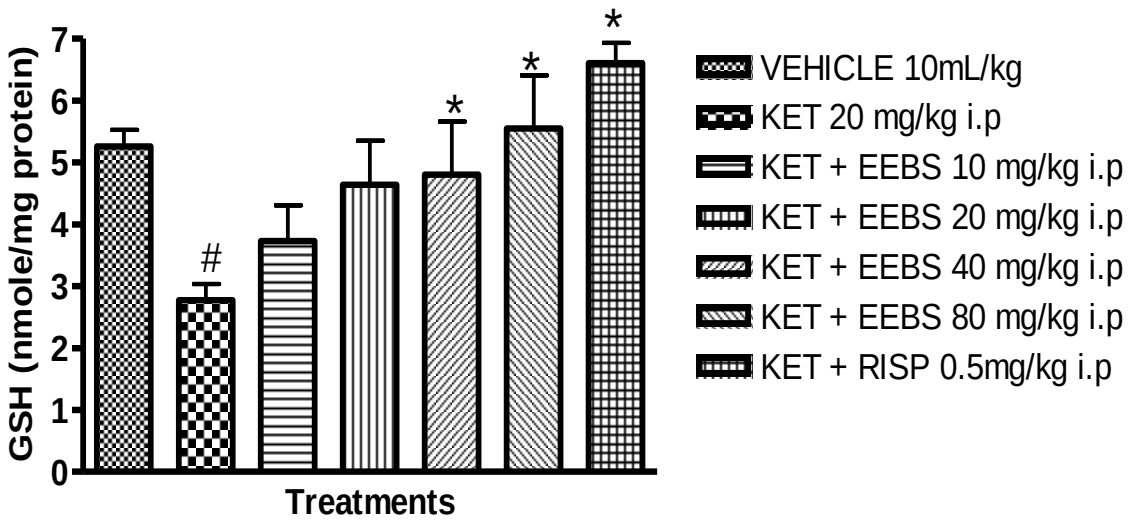

Fig.2: Effects of EEBS on glutathione synthase (GSH) activity in mice brain.

Value represents the Mean± SEM (n=5).One way ANOVA shows that there is significant [F (6, 41) =8.275, P < 0.0001] difference between various treatments groups when compared with negative control group that received ketamine (20 mg/kg) alone.

$^{\#}p< 0.05$ as compared with vehicle.* $p< 0.05$ as compare with ketamine group.

KET= Ketamine, **RISP**=Risperidone, **EEBS=** Ethanol extract of *Blighia sapida* Stem Bark

Effects of ethanol extract of *Blighia sapida* on Malondialdehyde (MDA) concentration in the reversal of ketamine induced oxidative stress.

EEBS at doses 10,20,40,80 mg/kg (i.p) and risperidone (0.5mg/kg i.p) significantly (P<0.05), decreased MDA level when compared with result obtained in negative control group. (Fig.3).

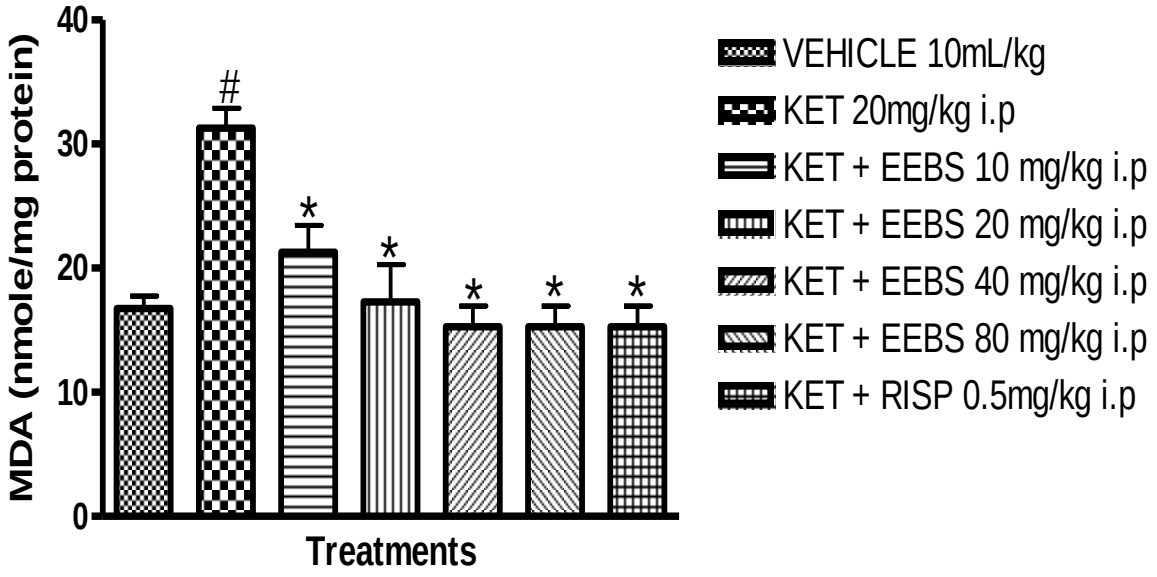

Fig.3: Effects of EEBS on Malondialdehyde (MDA) conentration in mice brain**.**

Value represents the Mean± SEM (n=5).One way ANOVA shows that there is significant [F (6, 41) =8.275, P < 0.0001] difference between various treatments groups when compared with negative control group that received ketamine (20 mg/kg) alone.

$^{\#}p< 0.05$ as compared with vehicle. * $p< 0.05$ as compare with ketamine group.

KET= Ketamine, **RISP**=Risperidone, **EEBS**= Ethanol extract of *Blighia sapida* Stem Bark

DISCUSSION

Pharmacological experiments have demonstrated that sub anaesthetic doses of ketamine induce schizophrenia-like symptoms in humans (hyperactivity) as well as behavioural activation in

experimental animals i.e hyper locomotion and alterations in working memory and cognition that resemble those observed in schizophrenia patients (Becker and Grecksch, 2004). Stimulation of schizophrenic-like behaviour in mice by ketamine has been partially associated with its direct antagonistic effect on NMDA receptors and indirect dopamine agonist activity. Open field and Y-maze tests have been used previously to evaluate the effects of antipsychotic drugs (e.g., haloperidol, olanzapine and clozapine) on locomotor activity and cognitive function in rodents (Ben Azu et al. 2016). Present study showed that risperidone and EEBS (10,20,40,80 mg/kg) significantly reduced activity counts / 5minute compared with negative control in a spontaneous activity cage (open field test), a measure of locomotor activity in mice, suggesting EEBS may have indirect effect on dopaminergic transmission via glutamatergic NMDA receptor, which is same for most psychotropic drugs. Phytochemicals present in *Blighia sapida* exhibit anti-oxidant, anti-inflammatory and neuroprotective properties that may modulate neurotransmitter levels, particularly dopamine and glutamate, which are implicated in schizophrenia (Phani Kumar et al. 2015, Anupama and Sunilkumar, 2019, Bachtela and Israni-Wingerb, 2020). EEBS (80mg/kg i.p) and risperidone (0.5mg/kg i.p) treated mice showed reduced activity counts compare with untreated mice (vehicles), this confirmed the sedative effect of EEBS and probable anti-psychotic like effect (Usman et al. 2019), also EEBS (40, 80 mg/kg i.p) treated mice significantly showed increased percentage alternation in ketamine induced cognitive dysfunction compare with the negative control mice. This may be attributed to the activities of phytochemicals like flavonoids, phenolics, and saponins compounds in EEBS that may influence synaptic plasticity and regulate neutrophic factors, contributing to improved cognitive function, as seen in effects of atypical antipsychotic drugs.

Oxidative stress plays a key component in schizophrenia pathophysiology, constituting a central point where other factors of vulnerability meet and their interactions could play a decisive role in the severity of symptoms of the disease (Padurariu et al. 2010, Ben-Azu et al. 2022). Moreover, increased plasma levels of MDA, a product of lipid peroxidation, decreased glutathione peroxidase, an enzyme that reduces lipid hydroperoxides and free hydrogen peroxides, and other markers of oxidative stress (Sarandol et al. 2007).

In context of this study, animals in negative control group showed increased nitrite level, high MDA levels and decreased GSH compared with the vehicle group. Extract-treated animals (EBSS 80 mg/kg i.p) showed significantly decreased nitrite levels compared to negative control animals. The effect of the highest dose of EEBS (80mg/kg i.p) administered to KET + EEBS 80mg/kg i.p animals is similar to that of risperidone (0.5mg/kg) treated animals (positive control). Ability of phytochemicals contained in EEBS such as flavonoids, polyphenols, and carotenoids to chelate or bind to biometals, preventing them from participating in reactions that produce reactive oxygen species could be a significant factor responsible for reduced Nitrite level in schizophrenia.

Specifically, EEBS treatments significantly increased GSH levels in the animals similarly with risperidone which is consistent with previous studies that demonstrated that risperidone treatment increased GSH levels (Monte et al. 2013, Ben-Azu et al. 2018b, 2018c, 2023). GSH is a water soluble tripeptide antioxidant enzyme consisting of cysteine, glycine, and glutamic acid. It is known to serve as a neuroprotective antioxidant agent to that reduces lipid hydroperoxides and to a great extent its enhancement reverses oxidative damage in the brain of rats treated with ketamine (Stojkovic et al. 2012, Aoyama, 2021). Complementarily to this, like risperidone, EEBS treated animals showed reduced MDA level compared with ketamine control animals, suggesting reduced lipid peroxidation in neuronal cells. Previous studies have shown that phytochemicals contained

in *Blighia sapida*, possesses anti-oxidant effects that may help to neutralize free radicals generation and consequently reduce oxidative stress, which is particularly implicated in the pathogenesis of schizophrenia. Some of these phytochemicals have been documented to elicit beneficial effects (Phani-Kumar et al. 2015) in protecting neurons, by mitigating oxidative stress, inflammation and other disease exacerbating mechanisms.

CONCLUSION

In conclusion, the findings from this study showed that ethanol extract of *Blighia sapida* stem bark reverses ketamine-induced schizophrenic-like phenotypes such as hyperlocomotion and cognitive impairment, through mechanisms associated with oxidative stress inhibition in mice brains.

CONFLICT OF INTEREST

The authors declared no conflict of interest.

ETHICAL STATEMENT

The institutional Animal Care and Use Research Ethics Committee, ACUREC, (University of Ibadan, Ibadan, Nigeria) approved this animal study and its experimental procedures, which was performed in accordance with the university, Animal Care and Use Research Ethics Committee guidelines 2014.The animals were handled according to the National Institute of Health (NIH 2011) guidelines for care and use of laboratory animals.

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