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Neurobehavioral Effect of Methanol Extract of *Cassia singueana* Del. (Fabaceae) Against Ketamine-Induced Behavioral Deficit in Mice

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Abstract

Background: Schizophrenia is a chronic neurodevelopmental disorder that affect about 1% of the global population. Studies have shown that the plant possesses numerous pharmacological activities and could be beneficial in managing schizophrenia. **Aim:** This study was aimed at investigating the actions of *C. singueana* extract on the ketamine-induced behavioral deficit in mice. **Method:** Acute toxicity profiling was performed as stated by the Organization for Economic Co-operation and Development (OECD 425) at the dose of 2000 and 5000 mg/kg. Oral doses of 100, 200 and 400 mg/kg of *C. singueana* extract were used for efficacy studies in the ketamine-induced behavioral deficit studies. Cognitive function was evaluated using open field (OFT), Y-maze (Y-M), novel object recognition tests (NORT) and forced swim test (FST). The brain tissues were evaluated for the concentrations of malondialdehyde (MDA), reduced glutathione (GSH) and superoxide dismutase (SOD) using standard laboratory techniques. **Results:** The oral median lethal dose of *C. singueana* extract was determined to be greater or equal to 5000 mg/kg in mice. The extract significantly (p<0.05) increased the recognition index and spontaneous alternation in NORT and Y-M respectively. The extract considerably shortened the duration of immobility and hyperlocomotion in FST and OFT respectively. The plant also improved the level of antioxidant enzymes in the brain tissue. **Conclusion:** *Cassia singueana* extract is relatively safe after acute administration and possesses an antischizophrenic effect in ketamine-induced schizophrenic mice.

Keywords: *Schizophrenia, Cognitive deficit, Ketamine, Cassia singueana, Oxidative stress*

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1. Introduction

Neuropsychiatric disorders include: Schizophrenia, the manic phase of bipolar disorder, Acute idiopathic illness and other illnesses marked with severe agitation (Baldessarini and Farazi, 2006). Schizophrenia is a chronic debilitating neuropsychiatric disorder affecting approximately 1% of the population worldwide (Erskine *et al.*, 2017). Schizophrenia is a major neuropsychiatric illness manifesting in complex phenotypes including positive, negative, and cognitive symptoms. This mental illness affects nearly 1% of the population (Perala *et al.*, 2007), leading to significant societal, economic, and personal costs. Despite its societal threat, the pathological mechanisms illustrating the real cause of schizophrenia remained poorly understood. Several series of evidence implicate disturbed neuroplasticity in the pathophysiology of schizophrenia, incorporating alterations in neurotransmitter systems, receptor dysfunction and cortical connectivity with clinical observations of cognitive deficits and negative symptoms (Paz *et al.*, 2008; Benes *et al.*, 2007; Coyle, 2006; Stephan *et al.*, 2009; Schmitt *et al.*, 2011; Hasan *et al.*, 2013).

Strengthening neuronal connections in consistently and highly activated pathways is termed long-term potentiation (LTP). The weakening of insufficiently activated neuronal pathways is termed long-term depression (LTD). LTP and LTD are the neural mechanisms underlying learning, memory and cognition (Hebb *et al.*, 2013). Glutamatergic N-methyl-d-aspartate (NMDA) receptors play a vital role in LTP and LTD molecular processes (Hasan *et al.*, 2013). Many researchers have reported a link between NMDA receptor hypofunction to aberrant LTP and LTD in schizophrenia. Precisely, the administration of NMDA receptor antagonists, such as ketamine and phencyclidine, provides a favorable support for the theory of NMDA receptor hypofunction and impaired plasticity in people with schizophrenia (Coyle, 2006; Schmidt *et al.*, 2012; Corlett *et al.*, 2011), and these eventually lead to anterograde amnesia which further translates to poor learning ability and avolition behavior in mice. It has also been recently reported that; dysfunction of glutamatergic NMDA receptors with both a hypoglutamatergic and a periodic hyperglutamatergic state in schizophrenic patients has been evident (Paz *et al.*, 2008; Konradi and Heckers, 2003; Paz *et al.*, 2006).

The current treatments for schizophrenia have severe limitation as they are efficient for only about half of patients. Negative symptoms (flat affect and social withdrawal) and cognitive (learning and attention disorders) symptoms remain untreated (Carbon and Correll, 2014). Also, they invoke severe neurological and metabolic side effects and may lead to sexual dysfunction or agranulocytosis as seen in clozapine (De Berardis*et al.*, 2018). Therefore, a need to explore medicinal plant archives to explore novel treatments for this disease is of imminent importance (Vyas *et al.*, 2019). Scientific exploration of medicinal plants has led to the breakthrough of many pharmacologically active constituents which act as lead compounds for developing novel drugs against many therapeutic targets (Uddin *et al.*, 2018). Constant exploration of bioactive compounds from medicinal plants that will be an asset in managing of cognitive dysfunction is also increasing (Tewari *et al.*, 2018; Vyas *et al.*, 2019). *Cassia singueana* is commonly known as winter. Cassia, a shrub or tree with great medicinal importance across Africa (Hiben *et al.*, 2016) including Nigeria which is found abundantly in the northern part of the country. The leaf juice is traditionally used to treat syphilis, ulcer, malaria, pneumonia, snake bite and eye infection (Schmelzer *et al.*, 2008), antimalarial (Hiben *et al.*, 2016) and antioxidant (Ibrahim and Islam, 2013). The plant has also been reported to enhance blood circulation in lactating mothers (Ifeanyi and Ode, 2012). The aim of this study is to evaluating neurobehavioral effect of *Cassia singueana* methanol extract on ketamine-induced behavioral deficits in mice.

2. Methods

2.1. Drugs and Chemicals

Ketamine, Thiobarbituric acid, Tetrachloroacetic acid, Epinephrine (Ranbaxy Laboratories Limited, USA); risperidone (Roche, Italy); methanol (Sigma-Aldrich, USA).

2.2. Experimental Animals

Swiss Albino male mice (18-22 g) were obtained from the animal house of the Department of Pharmacology and Therapeutics, Ahmadu Bello University, Zaria. The mice were maintained in regular cages with access to water and food and kept under a natural day and light cycle. Before commencing the experiment, the animals

were taken to the behavioral room (25±3 °C, relative humidity 30-70%) for 1 week to acclimatize to the laboratory environment. The experimental protocols were authorized by Ahmadu Bello University Committee on Animal Use and Care. Animal maintenance and treatment were also performed according to the guidelines set by Animal Research: Reporting of In-Vivo Experiments (ARRIVE).

2.3. Plant Material

The plant of *Cassia singueana* was obtained from Marabar Safana, Safana Local Government area of Katsina State, Nigeria, in November, 2019. It was confirmed by Mr Musa in the Department of Botany, Usmanu Danfodiyo University, Sokoto. A voucher number (0027A) was issued by matching with a reference voucher specimen formerly kept in the herbarium.

2.4. Plant Extraction

The plant leaf was air-dried under shade and then milled using mortar and pestle. The pulverized plant material (1600 g) was subjected to cold maceration with 50 L of 80% v /v methanol for 12 days. The filtrate obtained was concentrated with the aid of a rotary evaporator and the extractive yield was determined. The extract was labelled and then stored in a desiccator until needed for further studies.

2.5. Preparation of Stock Solutions

Stock solutions of the extract and risperidone were prepared by dissolving a specified quantity in distilled water followed by serial dilution to acquire the desired concentrations for the studies. The drug solutions were prepared fresh for each day's experiment to preserve their stability.

2.6. Acute Toxicity Study

The up-and-down method as described by the Organization for Economic Co-operation and Development (OECD) was adopted to establish the acute oral toxicity profile of CSE in mice (OECD 425). Two groups of 5 mice each were used ($n = 5$); the first group was assigned as control (administered distilled water, 10 mL/kg), while the second group (test group) of mice were sequentially administered a single dose of CSE (5000 mg/kg) by oral gavage (p.o). The mice were deprived of food (but not water for 3-4 h) before dosing and afterwards. They were carefully observed during the first 24 h and then daily for two weeks, after which the median lethal dose (LD_{50}) was estimated. The animals were sedated under soft chloroform and then euthanised. Blood samples were collected for haematology and brain organ for histopathology.

2.7. Experimental Design for Neurobehavioral Studies

Neurobehavioral studies to evaluate the effect of CSE were done using ketamine-induced behavioral deficits in mice. A total of 42 mice were randomly divided into 6 groups of seven each ($n = 7$). The mice were subjected sequentially to an Open Field Test (OFT), Forced Swim Test (FST), Y-maze (Y-M) and Novel Object Recognition Tests (NORT). For each study, the mice were pretreated with graded doses of CSE (100, 200 and 400 mg/kg, p.o) an hour before each trial, and Ketamine (25 mg/kg, i.p.) was given 30 min before each test.

2.8. Novel Object Recognition Test

Novel object recognition is a widely used test for assessing the efficacy of therapeutic approaches to schizophrenia in animal models (Amann *et al.*, 2011; Grayson *et al.*, 2007).

Mice were placed individually in a 32x30 cm box with beige walls for 5 min habituation followed by injection with saline or extract (before or after acquisition) and returned to the chamber. Twenty minutes later mice were injected (i.p.) with saline or 100 mg/kg ketamine and 30 min later, it was placed in a chamber with two identical objects for 10 min (acquisition session). After the acquisition, mice were returned to home cages and 1.5 h later they were placed back into the testing chamber in the presence of one of the original objects and one novel object of about the same size but a different shape and color (recognition session). The acquisition and recognition sessions were video recorded and the time spent exploring the objects was scored by an observer blinded to the drug treatments. Exploratory behavior was defined as sniffing, touching and directing attention to the object. For the recognition session, the recognition index was calculated as (time exploring the novel object/time exploring both the familiar and the novel object)/100.

2.9. Y-Maze

The Y-maze can be used for short-term working memory and locomotive activity. Spontaneous alternation is the measure of spatial working memory. To alternate among spatial locations a mouse must remember its previous location (Akanmu *et al.*, 2007). The effects of antipsychotic drugs on cognitive function as an index for the cognitive dysfunction of schizophrenia are assessed using the method of Monte *et al.* (2013). Six groups of mice (n = 6) were randomly selected and treated orally or by intraperitoneal injection; each mouse was only tested once. Group 1 was given distilled water (10 mL/kg) once daily for 14 days. Groups 2 to 6 were pretreated with sub-anesthetic doses of ketamine (20 mg/kg) once daily for 14 days. From 8th to 14th day of treatment, group 2 was treated with vehicle (10 mL/kg) once daily as negative control; group 6 (positive control) received risperidone 1 mg/kg. Group 3 to 5 were given three graded dose of the extract till day 14th. Twenty-four hours after the last treatment (15th day), animals were assessed for behavioral activity on Y-maze. The apparatus consists of three identical arms (33 x 11 x 12 cm) in which arms are symmetrically separated at 120^{o}. Specifically each mouse is place at the end of arm A and allowed to explore all the three arms (A, B, C freely for 5 min taking record of the number of arms visited and the sequence (alternation) of arms visits visually. An arms entries is body of mouse except its tail into an apartment. Alternation is defined as the entry into all three arms on consecutive devices. The percentage alternation was determined as the ratio of actual alternations to visible alternations (defined as total number of arm entries minus two) multiply by 100 (Akanmu *et al.*, 2007). After each mouse observation the chamber were cleaned with 70% ethanol.

2.10. Forced Swimming Test

Forced swimming test, as described previously by Chatterjee *et al.* (2011) in mice is a measure of despair behavior. In brief, mice were placed individually in glass cylinders (20 cm height, 10 cm diameter) containing 10 cm water depth at 25 ^{o}C. After 5 min, the animals were removed from the water, dried and returned back to their home cages. They were again placed in the cylinder 24 h later and after the initial 1 min acclimatization period, the total duration of immobility was measured for 5 min. the swimming was recorded by a camera mounted above the cylinders for the duration of 6 min.

2.11. Open Field Test (Locomotor Activity)

Gross open field activity (Blesa *et al.*, 2012) was studied using plexiglass arena, ûtted with a video camera containing horizontal square lines on the floor of the arena. The number of interruptions of the central and peripheral square cross by the animals was interpreted as horizontal activity and locomotive behavior. Group 1 received only distilled water, while group 2-6 received ketamine with 3, 4, 5 receiving graded doses of extract and group 6 received risperidone 1 mg/kg. Prior to the experiment, the control and the treated animals were habituated in the experimental cage for 15 min. After the initial habituation process, the activities of the animals were studied for 5 min. All enclosures were connected to the video camera.

2.12. Evaluation of Antioxidant Activity

2.12.1. Estimation of Malondialdehyde (MDA)

A portion of the brain sample (1 mL) was added to 3ml of trichloroacetic acid-Thiobarbituric acid- hydrochloric acid reagent (TCA-TBA-HCl reagent) and mixed thoroughly. The solution was then heated for 15 min in boiling water bath. Thereafter, the reaction mixture was allowed to cool and it was centrifuged for 10 min at 1000 g to remove the flocculent precipitate. The absorbance of the clear supernatant was then read at 535 nm against the reference tube and the concentration of the MDA was calculated using a Molar extinction coefficient of 1.56 x 10^{4} $M^{-1}cm^{-1}$

$$MDA\ (Units/g\ tissue) = \frac{A \times V \times I}{The\ Molar\ extinction\ coefficient \times v \times X}$$

where, A is OD at 535 nm, V is the total volume of reaction mixture, v is the volume of sample, X is the weight of tissue in reaction medium (g).

The value of *X* was calculated from the weight of tissue homogenized in a given volume of solution. 1 g of tissue was homogenized in 5 mL of PSB buffer, the weight of the tissue in the volume of sample used for the assay was calculated using proportion.

2.13. Determination of Superoxide Dismutase (SOD)

Two sets up are used for this assay. The first is the reference tube prepared by mixing 0.2 mL of distilled water and 2.5 mL of 0.05 M carbonate buffer (pH 10.2). This is followed by adding 0.3 mL of freshly prepared, cold epinephrine solution. This was very rapidly mixed and absorbance was taken at 420 nm. Absorbance readings are taken after 120 s at 30 s intervals and the change in absorbance per minute was determined. The sample tubes are prepared in the same way as the reference tubes except, the respective samples replaced the distilled water. The percentage inhibition is then calculated using the following expression:

$$\%\ Inhibition = \frac{DA_{ref} - DA_{test}}{DA_{ref}} \times 100$$

$$SOD\ activity\ (Units/g\ of\ wet\ tissue) = \frac{\%\ Inhibition}{50y}$$

y is the amount (mg) of tissue in the volume sample used. This was deduced from the weight of tissue homogenized in a given buffer volume.

2.14. Determination of GSH

The GSH was assayed using following the method described by Jallow *et al.* (1974). This method based upon production of relatively stable yellow colour when 5′-5′ Dithiobis (2-nitrobenzoic acid) (DTNB) is added to sulfhydryl compound. The chromophoric product resulting from reaction of DTNB with reduced glutathione, 2-nitro, 5-thiobenzoic acid is maximally absorbed at 412 nm and the amount of reduced glutathione in sample was proportional to the absorbance at the wave length.

Briefly 0.4 mL of each sample was added to 0.4 mL of 20% trichloroacetic acid (TCA) and mixed by gentle swirling motion and centrifuge at 10,000 rpm for 10 min at 4 °C (in cooled centrifuge). 0.25 mL of the supernatant was withdrawn and added to 2 mL of 0.6 mM DTNB and final volume of the solution was made up to 3 mL with (0.75 mL) phosphate buffer (0.2 M, pH 8.0). Absorbance was read at 412 nm against black reagent (2 mL of 0.6 mM DTNB+ 1 mL phosphate buffer (0.2 M, pH 8.0) using spectrophotometer. The concentration of reduced glutathione in the brain tissue is expressed as micromole per gram of protein (umole/g).

2.15. Histology

Histopathological examination of the hippocampus was done by fixing the brains in 10% formalin solution. The organs were prepared using ethanol and paraffin wax for embedding. Thin sections (5 µm) of the organs were made with the aid of a microtome and dewaxed using xylene. Afterwards, they were placed on a microscope slide and stained with haematoxylin–eosin. The sections were observed for histopathological changes such as tissue integrity, degeneration, necrosis and leukocyte infiltration (Rolls, 2011).

2.16. Statistical Analysis

Data obtained were analysed using Statistical Package for Social Sciences (SPSS) software (Version 23). Difference between means of groups was analyze usig one-way analysis of variance (ANOVA) followed by Bonferroni's post-hoc test. Values of $p<0.005$ was considered significant in all the statistical tests. Data obtained were expressed as mean ± standard error of the mean (SEM)

3. Results

3.1. Acute Toxicity Profile of Cassia singueana Extract in Mice

The single oral administration of CSE 5000 mg/kg body weight in mice did not produce signs of toxicity or death during the 14 days observation period. The oral LD_{50} of CSE was therefore determined to be greater than

5000 mg/kg in mice. Also, there were no substantial changes in skin, behavior and body weights due to extract treatment on mice when compared to control.

3.1.1. Effect of Cassia singueana *Extract on Subchronic Ketamine Induced Hyperactivity in Open Field Test*

The extract has significantly ($p< 0.05$) decreased the number of central and peripheral square cross at the dose of 200 and 400 mg/kg when compared with the distilled water treated group as stated in Figure 1.

3.1.2. Effect of Cassia singueana *Methanol Leaf Extract on Subchronic Ketamine Induced Immobility in Forced Swim Test*

The extract has significantly ($p< 0.05$) decreased the duration of immobility at the dose of 400 mg/kg when compared with the distilled water treated group as stated in Figure 2.

3.1.3. Effect of Cassia singueana *Extract on Subchronic Ketamine Induced Memory Impairment in Novel Object Recognition Test*

The extract has significantly ($p< 0.05$) and dose dependently increased duration of novel object exploration and the percentage recognition index when compared with the distilled water treated group as stated in Figure 3.

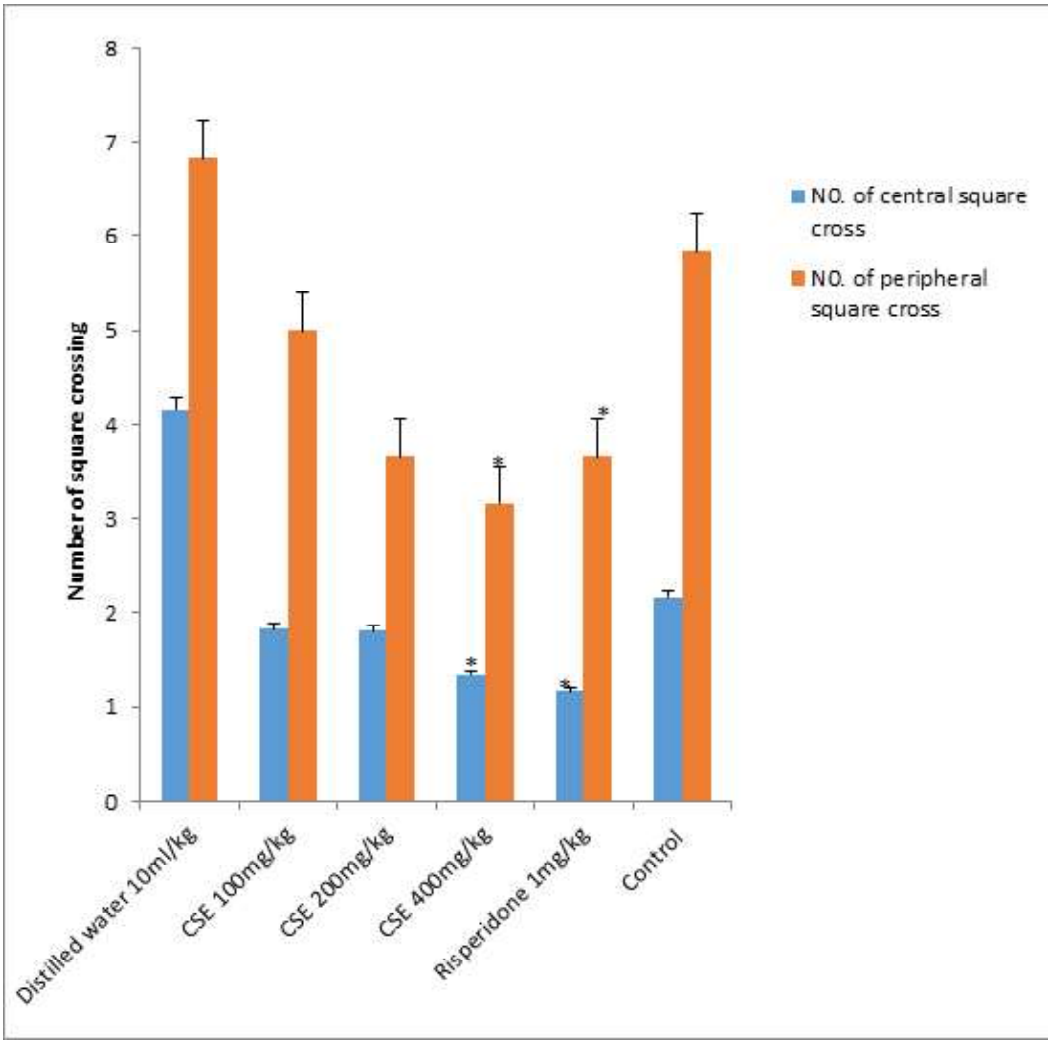

Figure 1: Effect of *Cassia singueana* Extract on Subchronic Ketamine Induced Hyperactivity in Open Field Test

Note: Values are Mean ± S.E.M; *= $p< 0.05$ as compared to Distilled water group – One way ANOVA followed by Dunnet post hoc test, CSE = *Cassia singueana* Extract.

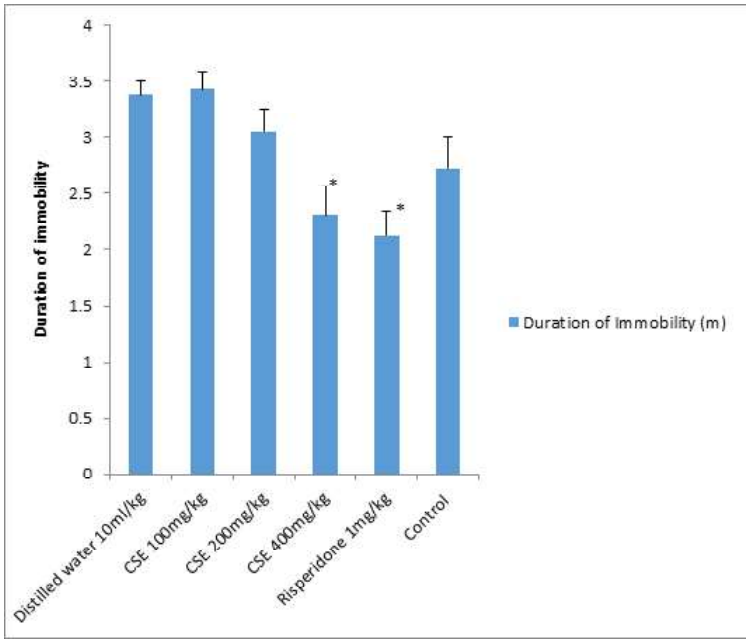

Figure 2: Effect of *Cassia singueana* Methanol Leaf Extract on Subchronic Ketamine Induced Immobility in Forced Swim Test

Note: Values are Mean ± S.E.M; *= $p< 0.05$ as compared to Distilled water group – One way ANOVA followed by Bonferroni's post hoc test, CSE = *Cassia singueana.*

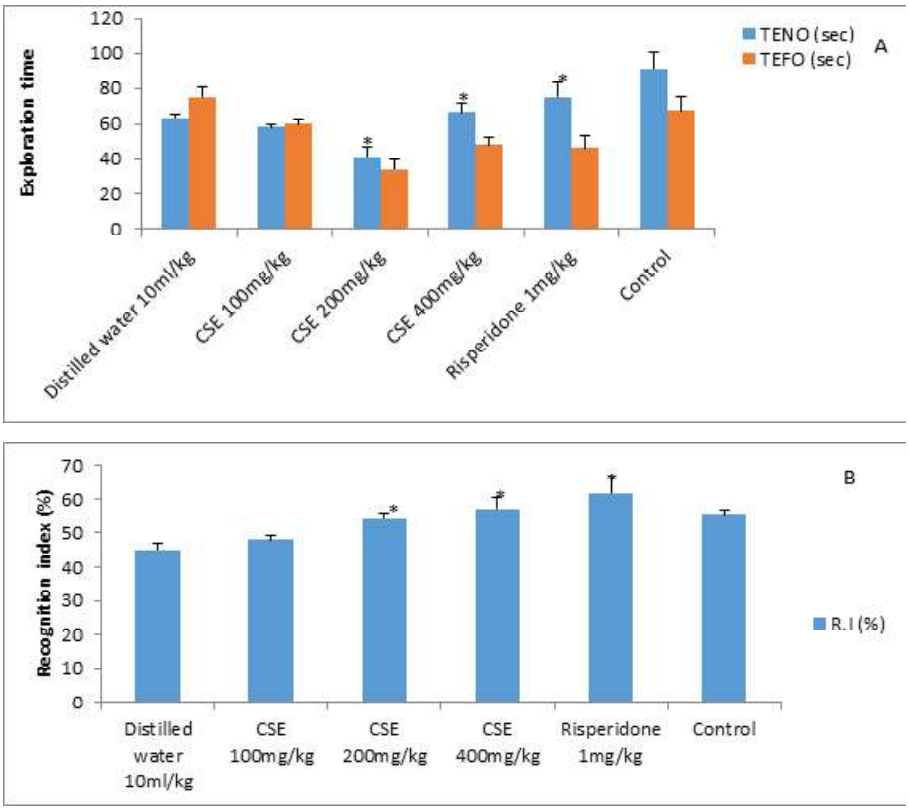

Figure 3: Effect of *Cassia singueana* Extract on Subchronic Ketamine Induced Memory Impairment in Novel Object Recognition Test

Note: A = Exploration time, B = Recognition index. Values are Mean ± S.E.M; *= $p< 0.05$ as compared to Distilled water group - One way ANOVA followed by Bonferroni post hoc test, CSE = *Cassia singueana* extract, TENO = Time exploring novel object, TEFO = Time exploring familiar object, R.I = Recognition index.

3.1.4. Effect of Cassia singueana *Methanol Leaf Extract on Subchronic Ketamine Induced Spatial Memory Impairment in Y- maze*

The extract has significantly ($p< 0.05$) and dose dependently increased the percentage spontaneous alternation when compared with the distilled water treated group as stated in Figure 4.

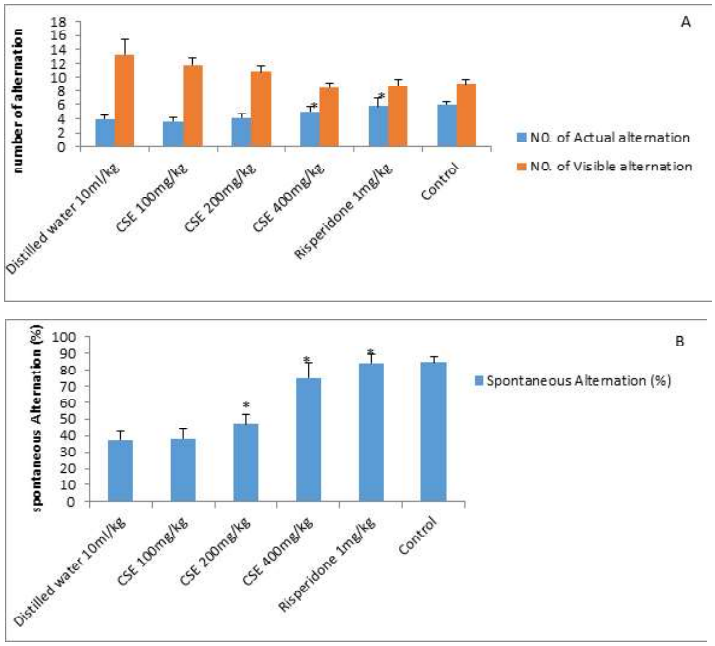

Figure 4: Effect of *Cassia singueana* Extract on Subchronic Ketamine Induced Memory Impairment in Y-maze

Note: A = Number of alternation, B = Percentage spontaneous alternation Values are Mean ± S.E.M; *= $p<0.05$, as compared to Distilled water group - One way ANOVA followed by Dunnet post hoc test, CSE = *Cassia singueana* Extract.

3.1.5. Effect of Cassia singueana *Extract on Oxidative Stress Biomarkers in Hippocampus of Rats*

The extract has significantly ($p< 0.05$) and dose dependently decreased the level of MDA, increased the level

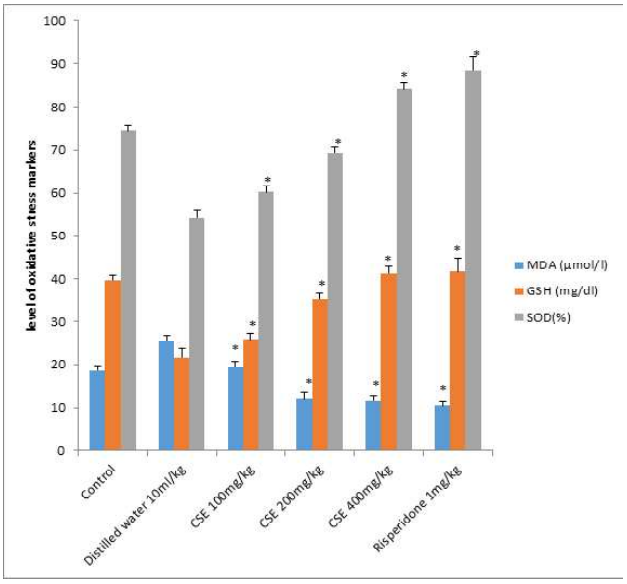

Figure 5: Effect of *Cassia singueana* Extract on Oxidative Stress Biomarkers in Brain of Rats

Note: Values are Mean ± S.E.M; *$p<0.05$ significance as compared to Distilled water group - One way ANOVA followed by Bonferonni post hoc test, CSE = *Cassia singueana* Extract.

of GSH and SOD in the brain homogenate of isolation reared rats compared with the distilled water treated group as stated in Figure 5.

3.1.6. Effect of Methanol Leaf Extract of Cassia singueana *on Haematological Indices Following 28 Days Subchronic Oral Treatment In Wistar Rats*

After twenty eight days oral administration of methanol leaf extract of *Cassia singueana*, there was no significant (p<0.05) increase or decrease in white blood cell count in all the extract treated groups (100, 200 and 400 mg/kg). However there was significant increase in red blood cell count and no significant increase or decrease in other hematological parameters such as, haemoglobin, platlets, packed cell volume, mean cell volume and haematocrit (Table 1).

Table 1: Effect of Methanol Leaf Extract of *Cassia singueana* on Haematological Indices Following 28 Days Subchronic Oral Treatment in Wistar Rats

Treatment/Dose	WBC	RBC	HGB	HCT	PLT	MCV
(mg/kg)	($x10^3\mu L$)	($x10^3\mu L$)	(g/dl)	(%)	($\mu Lx10^3$)	(fl)
Distilled water	20.71±1.60	7.98±0.43	13.92±0.74	39.25±1.45	635.5±2.10	65.68±0.42
100CSE	18.85±0.62	7.27±0.41	12.37±0.41	31.80±1.31	520.0±4.14	63.22±0.71
200CSE	17.92±0.51	8.6±0.24*	9.55±2.24	29.37±0.16	568.0±5.9	49.90±1.03
400CSE	19.00±0.11	9.9±0.28*	13.92±0.51	34.10±1.91	657.5±4.27	48.07±1.71

Note: Data expressed as Mean±SEM, SEM = Standard Error of Mean n= 6, * p<0.05, WBC = White Blood Cells, RBC = Red Blood Cells, HCT = Haematocrit, HGB =Haemoglobin, PCV = Packed Cell Volume, PLT = Platelet, MCV = Mean Cells Volume, CSE = *Cassia singueana* methanol leaf extract. Dunnet post-hoc test.

3.1.7. Effect of Methanol Leaf Extract of Cassia singueana *on Histology of Brain following 28 days Oral Administration in Albino Rats*

The histology of the brain sections of rats following 28 days oral administration of methanol leaf extract of *Cassia singueana* reveals well preserved pyramidal neuronal cells in both the control and extract treated groups (Plate I).

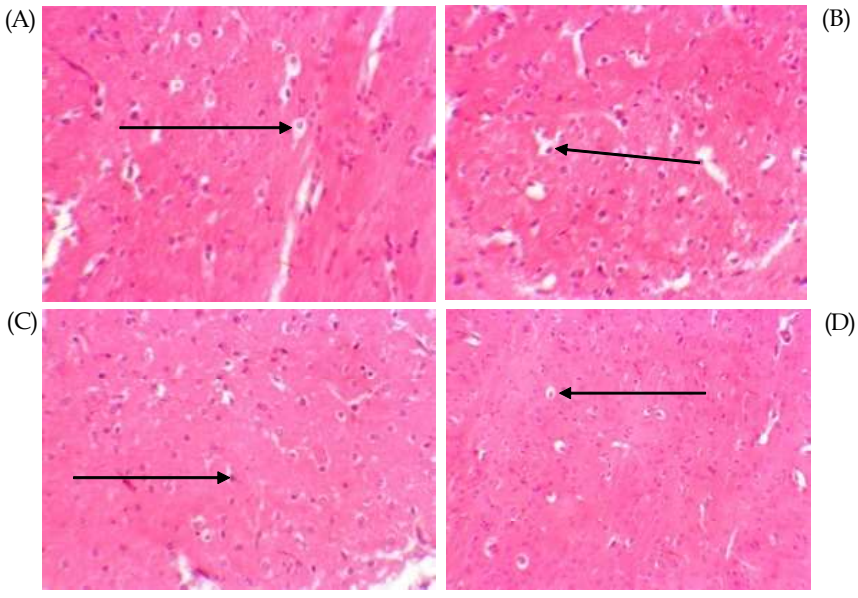

Plate I: Photomicrograph of a Section of Rat Brain Treated with Methanol Leaf Extract of *Cassia singueana* (MLECS) Following 28 Days Oral Administration (H&EX 100)

Note: (A) Distilled water treated showing pyramidal neuronal cells neutrophils. B, C, and D are 100, 200 and 400 mg/kg extract treated groups respectively, showing also pyramidal neuronal cells with black arrow.

4. Discussion

Cassia singueana leaf decoction is used in management of insanity and lactation enhancer by traditional medicine practitioners. Numbers of phytochemical compounds were isolated from the plant and they could be responsible for the observed neurobehavioral effects (Jibril *et al.,* 2019). There is paucity of data on whether *Cassia singueana* leaf extract could ameliorate symptoms of schizophrenia in laboratory animals' models; hence we explored the behavioral effect of the plant on ketamine-induced behavioral deficit in mice using suitable rodent schizophrenia models. Our results revealed that Cassia singueana extract significantly neutralized the ketamine-induced behavioral deficit in forced swim test, novel object recognition, Y-maze and open field tests. The extract also found to elicits some antioxidant effect and indicates some safety profile in respect to brain tissue and haematological indices.

Acute toxicity investigation is of paramount important in preclinical studies as it assesses the adverse effects that could possibly occur due to intentional or unintentional short-term exposure to chemicals (Erhirhie *et al.,* 2018). It also serves as a guide in dose selection for efficacy studies. The oral LD_{50} of CSE was estimated to be > 5000 mg/kg, and according to the classification of LD_{50} values of xenobiotics (Loomis and Hayes, 1996), the CSE was considered to be relatively safe in mice after oral administration.

Cognitive impairments such as deficits in attention, executive function, working short term memory, and learning, are core symptoms in patients with schizophrenia (Harvey *et al.,* 2004). Among these, learning and memory impairments are known to be particularly severe and they are suggested to be major determinants of the amount of disability in patients with schizophrenia and they experience difficulty in social and occupational functioning and in independent living (Green *et al.,* 2000). Ketamine, disrupts cognitive function in both humans and animals, producing deficits paralleling those present in schizophrenic patients (Trivedi and Jarbe, 2011). Therefore, in our study we have used the ketamine model, subchronic administration to evaluate the efficacy of *Cassia singueana* as potential antischizophrenic agent. Different brain regions contribute to recognition memory processing, including the perihirnal cortex, the medial prefrontal cortex and the hippocampus (Barker *et al.,* 2017). Lesions in these areas have been shown to produce deficits in recognition memory (Warburton, 2018). Our findings revealed *Cassia singueana* methanol leaf extract reverse the cognition impairment induced by subchronic ketamine administration in mice by significantly increasing the percentage recognition index and percentage spontaneous alternation in novel objection recognition test and Y-maze test respectively. The N-methyl-D-aspartate-antagonists induced rodent model of schizophrenia shows increased oxidative stress specifically in the prefrontal cortex (Ozyurt *et al.,* 2014). Higher levels of brain mitochondrial Reactive Oxygen Specie (ROS) have been found in a ketamine-induced rat model of schizophrenia (Faizi *et al.,* 2014). It has also been reported that glutathione (GSH) depletion in rat's brain leads to schizophrenic symptoms (Cruz-Aguado *et al.,* 2001). In addition, knockout mice that lack a crucial subunit of the GCL enzyme show significant reduction of glutathione levels in the anterior cortex (Das *et al.,* 2012) and schizophrenic symptoms in the hippocampus (HIP) of the adult knockout mice, including an increase in oxidative stress, this inferred the likelihood that our extract elicits its pharmacological effects via antioxidant mechanism.

Also in this research, our findings revealed that *Cassia singueana* extract significantly reverse the ketamine induced hyperactivity at doses 200 and 400 mg/kg. The stimulation of locomotor activity has been mainly attributed to the dopaminergic hyperactivation in the striatal areas of mice brain (Irifune *et al.,* 1991). It has been also reported that dopamine neurotransmission is involved in the motor activating effects of NMDA, since the systemic administration of dopamine antagonists counteracts the motor activation induced by the systemic administration of NMDA (Gimenez-Llort *et al.,* 1997). Moreover, dopamine release in the nigrostriatal neurons remains in direct presynaptic control of glutamate via both AMPA and NMDA receptors located in nerve terminals of dopaminergic neurons (Cheramy *et al.,* 1998). An indirect inhibitory regulation of DA release was also demonstrated due to the combined stimulatory effect of NMDA on the medium sized GABAergic efferent neurons (Krebs *et al.,* 1994). Also, a possible mechanism by which ketamine might produce this adverse behavioral effects, have been related to the blockade of NMDA receptors located on inhibitory GABAergic neurons in the limbic and subcortical brain regions (Nakao *et al.,* 2003), our finding indicates the likelihood that *Cassia singueana* phytochemical (s) elicits its activity via antagonism of dopamine receptors and modulation of NMDA receptor.

The prolonged duration of immobility after subchronic administration of ketamine has been used previously as a model for the negative symptoms of schizophrenia, such as flattening of affect and avolition (Chatterjee *et al.,* 2011). Subchronic administration of *Cassia singueana* extract reduced the duration of immobility in the forced swim test, similar to risperidone. The efficacy of atypical antipsychotic agents is believed to be through their 5HT2a receptor antagonism (Kawaura *et al.,* 2015), our extract as well likely interacts negatively with this serotonin receptor. A similar finding was also reported by Marsden *et al. (*2011).

Oxidative stress occurs as a result of imbalance between cellular generations of Reactive Oxygen Species (ROS) and the power of cells to eliminate them through activation of endogenous antioxidant defense mechanisms (Pizzino *et al.,* 2017). Certainly, brain damage as a result of radicals accumulation can lead to cognitive impairment (Cheignon *et al.,* 2018). Malonedialdehyde (MDA) is a known biomarker of lipid peroxidation and a measure of free radical generation and membrane dysfunction (Shichiri, 2014). It has also been reported that lipid peroxidation may increase because of reduced GSH stores in the brain (Lee *et al.,* 2020). The extract of *Cassia singueana* significantly decreased the level of malonedialdehyde (MDA) and increased the level of reduced glutathione (GSH) and superoxide dismutase (SOD) in the brain homogenate treated rats, On the other hand, SOD is an important antioxidant enzyme that performs a vital function in clearing superoxide anions, which otherwise injures the cell membranes and macromolecules (Kurutas, 2016). However during continuous exposure of rats to ketamine could lead to the decrease in level of antioxidants which exposed the oligodentrocyte to reactive oxygen species radicals and eventually lead to prefrontal cortex hypomylination, which correlates with behavioral and cognitive dysfunction (Liu *et al.,* 2012), our extract increased the level of endogenous antioxidants such as GSH and SOD which might enhance the protection of OPC and eventually improved cognition.

The physiological system responsible for haematopoiesis represents an eminent target for toxic xenobiotics, specifically the bone marrow which is the site for the production of Red Blood Cells (RBCs) (Kifayatullah *et al.,* 2015). As such, haematological indices are generally used as biomarkers of toxicity because of their sensitivity to toxins or their metabolites (Abubakar *et al.,* 2019). Increase in white blood cell count specifically, the granulocyte (neutrophils) and lymphocyte counts occur under many conditions, including xenobiotics exposure, inflammatory disease, stress or secondary to treatment-induced tissue anomaly in other organs (Greaves, 2012). Granulocytes are the prominent WBCs that increase during non viral infections and lymphocytes got increased in viral infections (Merriman, 2014). Our studies disclosed significant increase red blood cell count and slight decrease in white blood cell count after subchronic administration of CSE which translate to less likelihood of the extract to cause agronolocytosis like the convention atypical antipsychotic agents.

Histological examination of hippocampus tissue of the ketamine treated group displayed slight irregular neutrophils aggregation but, such changes were not evident with the extract treated groups, indicating possible neuroprotective effect of *Cassia singueana* extract on brain tissue.

5. Conclusion

The methanol leaf extract of *Cassia singueana* ameliorated ketamine-induced behavioral deficit and the observed elicited activity by the extract could be mediated through its antioxidant property or its possible ability to modulate NMDA receptor function. This also strengthened its ethno-medical use in the management of insanity.

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Author Contributions

IYA designed the research, conducted the experiment, data analysis and drafted the manuscript. MGM and JY participated in the research design and supervised the experiments. IYA, MGM, and JY revised and approved the submission of the final manuscript. All authors read and approved the final manuscript.

Conflicts of Interest

The authors have no conflicts of interests to disclose.

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Availability of Data and Materials

All data related to this study are included herein, otherwise available on request.

Ethical Approval and Consent to Participate

The experimental protocols were authorized by Ahmadu Bello University Committee on Animal Use and Care (Approval number: ABUCAU) and performed according to the guidelines set by Animal Research Reporting of In Vivo Experiments (ARRIVE).

Consent for Publication

Not applicable.

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Appendix

Abbreviations

AA	:	Actual alternation;
ANOVA	:	Analysis of variance;
CSE	:	Cassia singueana extract;
GABA	:	Gamma amino butyric acid;
GSH	:	Glutathione reductase;
HCT	:	Haematocrit;
H&E	:	Haematoxylin and eosin;
HGB	:	Haemoglobin;
LD50	:	Median lethal dose;
LYMP	:	Lymphocytes;
MDA	:	Malondialdehyde;
NORT	:	Novel object recognition test;
OECD	:	Organization for Economic Co-operation and Development;
PLT	:	Platelets;
RBC	:	Red blood cells;
RI	:	Recognition index;
ROS	:	Reactive oxygen species;
SA	:	Spontaneous alternation
SEM	:	Standard error of mean;
SOD	:	Superoxide dismutase;
SPSS	:	Statistical package for social sciences;
TENO	:	Time exploring novel object;
TEFO	:	Time exploring familiar object;
VA	:	Visible alternation:
WBC	:	White blood cells;
Y-M	:	Y-maze;

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