(RESEARCH ARTICLE)

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Ameliorative effect of *Cassia singueana* extract against acute ketamine induced cognitive impairment in murine models of schizophrenia

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Abstract

Introduction: In the quest for search of novel pharmacotherapy from medicinal plants for psychiatric disorders, it has progressed appreciably for the past few decades and their therapeutic potential has been evaluated in a variety of murine models. *Cassia singueana* Del. is a commonly used medicinal plant in Nigeria for management of insanity and as lactation enhancer.

Aim: The main aim of this study was to evaluate the cognitive potential of *Cassia singueana* extract against ketamine-induced cognitive impairment in mice.

Methods: The phytochemicals and elemental minerals present in the extract were determined using standard protocol and antioxidant potential of the extract was evaluated using TBA-TCAA protocols, while its oral median lethal dose (LD_{50}) in mice was estimated by OECD protocol. The effect of *Cassia singueana* extract (100, 200 and 400 mg/kg) on neurobehavioral deficit was evaluated in mice using behavioural paradigms: Y- maze, novel object recognition, forced swim test, open field test and catalepsy test.

Results: The phytochemical constituents of *Cassia singueana* were alkaloids, cardiac glycosides, tannins, flavonoids, saponins, steroids and triterpenes. The extract also significantly decreased the level of MDA and increased the level of SOD and GSH. Oral LD_{50} was estimated to be >5000 mg/kg. *Cassia singueana* extract significantly (P< 0.05) increased the exploration time of novel object and percentage recognition index during the retention phase of the NORT. In Y-maze test, the extract significantly (p<0.05) increased the number of actual alternation and the percentage spontaneous alternation. It also significantly (p<0.05) decreased the duration of immobility in forced swim test. The extract also significantly (p<0.05) increased the number of central and peripheral square cross in open field. The extract also did not prolong the duration of catalepsy induced by haloperidol in glass bar test.

Conclusion: *Cassia singueana* extract contained phytochemicals that could ameliorate cognitive impairment and negative symptoms of schizophrenics.

Keywords: Ketamine; Cognitive impairment; Schizophrenia; *Cassia singueana*; Antioxidant

1. Introduction

Schizophrenia is a chronic developmental psychiatric disorder affecting greater than 1% of the world population (Bhugra, 2005 and Fatemi, 2008). Drugs prescribed for the treatment of psychosis can be grouped as first and second generation antipsychotics. The first generation or typical class of antipsychotics are effective against the positive

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symptoms, i.e. hallucinations, delusions, and euphoria and also have the highest tendency to cause extra-pyramidal side effects. Whereas the atypical class are effective in ameliorating the positive, negative and the cognitive symptoms of schizophrenia and possess lesser tendency to cause extra-pyramidal side effects but however, they hold the greater risk of cardiovascular diseases, diabetes, weight gain and agranuloctosis. Long term usage of these agents may also increase oxidative load and thereby further enhance the progression of the disease (Meltzer, 2010; Meyer, 2011 and Reus, 2008). In this very circumstance, there is need for some drugs with lesser adverse effects. Long ago, research interest and activities to validate, invalidate and document medicinal plants have increased greatly due to lesser side effect. Since psychiatric disorders such as depression, anxiety, mania and schizophrenia are on rise, researchers are looking for alternative remedies and also trying to exploit herbal medications for the treatment of neurobehavioural disorders. At the same time, this revival of interest in herbal medicines is supported by the explosion of the ethno-medicine literature and by an increased awareness of people and the recognition of natural herbal medicines globally (Verma *et al.,* 2010). Apart from possessing lesser adverse effects, natural herbal products structurally possess broad spectrum chemical diversity, biochemical specificity and other medicinal properties that also make them friendly as lead compounds for the management of a number neuropsychiatric disorders including schizophrenia (Carlini, 2003 and Chatterjee *et al.,* 2011). Henceforth, we have directed our attention towards establishing the scientific rational in governing the efficacy of one of the medicinal plant, *Cassia singueana* which have been reported to be used in ethno-medicine for management of insanity and lactation enhencer. *Cassia singueana* Del. (Fabaceae) native plant of Northern Nigeria, is now also cultivated and used in many regions of the country and other countries. It has been shown that number of phytochemical compounds have been isolated from the plant namely; luteolin has been reported from the leaf extract of C. singueana (Ode and Asuzu, 2011), stigmasterol (1), stigmast4-en-3-one (2a), stigmasta-4, 22-dien-3-one (2b), 1-heneicosanol (3) and hexyl heneicosanoate (Jibril *et al.,* 2019). The leaf juice is traditionally used to treat syphilis, ulcer, malaria, pneumonia, snake bite and eye infection (Schmelzer*et. al*., 2008), leaf extract is also reported to possess antimalarial activity (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). We therefore hypothesized that it may be beneficial for schizophrenic and allied diseases wherein the mood symptoms are affected. Therefore, to establish the scientific prove for the role of *Cassia singueana* in schizophrenia, a number of parameters in animal models were assessed, specifically those involving elucidation of their effect against positive, negative and cognitive symptoms imparted by the compound ketamine in acute animal models of psychosis. Moreover, the extrapyramidal side effects commonly found in antipsychotics were also assessed using catalepsy test. Furthermore, an effort was also dissipated to find the possible underlying mechanism through antioxidant effect of the plant extract.

2. Material and methods

2.1. Animals

Adult male Swiss albino mice weighing 25–30 g were employed in the study. They were housed five to seven per cage at room temperature (25 ± 20 ℃) and 12/12 h light/ dark (7:00 a.m. to 7:00 p.m.). They were allowed to acclimatize for at least 2 week prior to experiments and were allowed free access to regular food pellets and water ad libitum. 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).

2.2. Drugs and Treatment Schedule

Ketamine (as injectable vials) was purchased from Ranbaxy, India. All other compounds were procured from Sigma–Aldrich, UK, unless otherwise specified. For these acute studies, mice were pretreated with single injection of vehicle or ketamine (100 mg/kg, i.p.)

2.3. 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, 10ml/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 afterward. 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 euthanized. Blood samples were collected for biochemical test and brain organ for antioxidant studies.

2.4. Behavioural Observations

Behaviour assays were performed according to the method described previously (Chatterjee *et al.,* 2011). Briefly, mice, acclimatized previously were randomly selected and distributed into various groups of 7 animals (n = 7), i.e.: (a) hyperlocomotor activity (representing positive symptoms, evaluated using Open field test), (b) Forced Swim Test (representing negative symptoms) (c) Novel Object recognition test and Y-maze (representing cognitive symptoms). Drug administration was generally done one hour prior to ketamine treatment.

2.5. Novel object recognition test

Novel object recognition is widely used test for assessing the efficacy of therapeutic approaches to schizophrenia in animal models. (Amann *et al.,* 2010 and Grayson *et al.,* 2007)

Mice were place 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 100mg/kg ketamine and 30 min later, it was placed in a chamber with two identical objects for 10 min (acquisition session). After acquisition, mice was returned to home cages and 1.5 h later they were place 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 who was blinded to the drug treatments. Exploratory behavior was defined as sniffing, touching and direct attention to the object. Exploration times were expressed as the means ± s.e.m.

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

2.6. 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 location a mouse must remember its previous location (Akanmu*et al.,* 2007). The effects of antipsychotic drug 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 is only tested once. Group 1 was given distilled water (10ml/kg) once daily for 14 days. Group 2 to 6 were pretreated with sub-anesthetic dose 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 1mg/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 one twenty degree specifically each mouse is place at the end of arm A and allow to explore all the three arms (A, B, C freely for 5 minutes taking record of the number of arms visited and the sequence (alternation) of arm visits visually. An arm entry is defined as the entry of the body of mouse except its tail into an apartment. Alternation is defined as the entry into all three arms on consecutive device. The percentage alternation was determined as the ratio of actual alternation to visible alternation (defined as total number of arm entry minus two) multiply by 100 (Akanmu*et al.,* 2007). After each mouse observation the chamber will be clean with 70% ethanol.

2.7. Forced Swimming Test

Force 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 depth of water at 25 ℃. After 5 min, the animals were removed from 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 duration of swimming during the 6 min test period was recorded by a camera mounted above the cylinders.

2.8. Open field test (Locomotor Activity)

Gross open field activity (Blesa*et al* 2012) was studied using plexiglass arena, fitted 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 1mg/kg. Prior to the experiment, both 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 cages were connected with video camera.

2.8.1. Catalepsy Test

The catalepsy procedure has been described previously (Prinssen*et al.,* 2002). Animals will first be examined in the crossed-leg position test, and immediately thereafter in the bar test. In the crossed-leg position test, the hind limbs are placed over the ipsilateral forelimbs and the time during which an animal remained in this position was determined up to a maximum of 30 seconds. In the bar test, the forelimbs were placed on a horizontal, cylindrical metal bar (diameter 1.25 cm; height 10 cm) and the time during which both forelimbs remained on the bar was determined up to a maximum of 30 seconds. The bartest was repeated 3 and 6 min later and the mean of three trials was used for data analysis. Animals were returned to their cage between tests. Six animals were tested in each group. Positive control: Haloperidol, negative control: distilled water and test groups received 100, 200 and 400 mg/kg of the extract.

2.9. Evaluation of Antioxidant Activity

2.9.1. Estimation of Malonedialdehyde (MDA)

A portion of the sample (1ml) 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 minutes in boiling water bath. Thereafter, the reaction mixture was allowed to cool and it was centrifuged for 10minutes at 1000g in order to remove the flocculent precipitate. The absorbance of the clear supernatant was then read at 535nm against reference tube and concentration of the MDA is calculated using Molar extinction coefficient of $1.56 \times 10^4 M^{-1} cm^{-1}$

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

Where A is OD at 535 nm, V is total volume of reaction mixture, v is volume of sample, X is 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. 1g of tissue was homogenized in 5ml of PSB buffer, the weight of the tissue in the volume of sample used for the assay was calculated using proportion.

2.9.2. Determination of superoxide dismutase (SOD)

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

$$\% \text{ Inhibition} = \frac{DAref - DAtest}{DAref} \times 100$$

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

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

2.9.3. 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 412nm and the amount of reduced glutathione in sample was proportional to the absorbance at the wave length.

Briefly 0.4ml of each sample was added to 0.4ml of 20% trichloroacetic acid (TCA) and mixed by gentle swirling motion and centrifuge at 10,000rpm for 10minutes at 4^0C (in cooled centrifuge). 0.25ml of the supernatant was withdrawn and added to 2ml of 0.6mM DTNB and final volume of the solution was made up to 3ml with (0.75ml) phosphate buffer (0.2M, pH 8.0). Absorbance was read at 412nm against black reagent (2ml of 0.6mM DTNB+ 1ml phosphate buffer(0.2M, pH 8.0) using spectrophotometer. The concentration of reduced glutathione in the brain tissue is expressed as micromole per gram of protein (umole/g

3. Results

3.1. Acute toxicity profile of *Cassia singueana* extract in mice

The single oral administration of CSE 5000mg/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 5000mg/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.

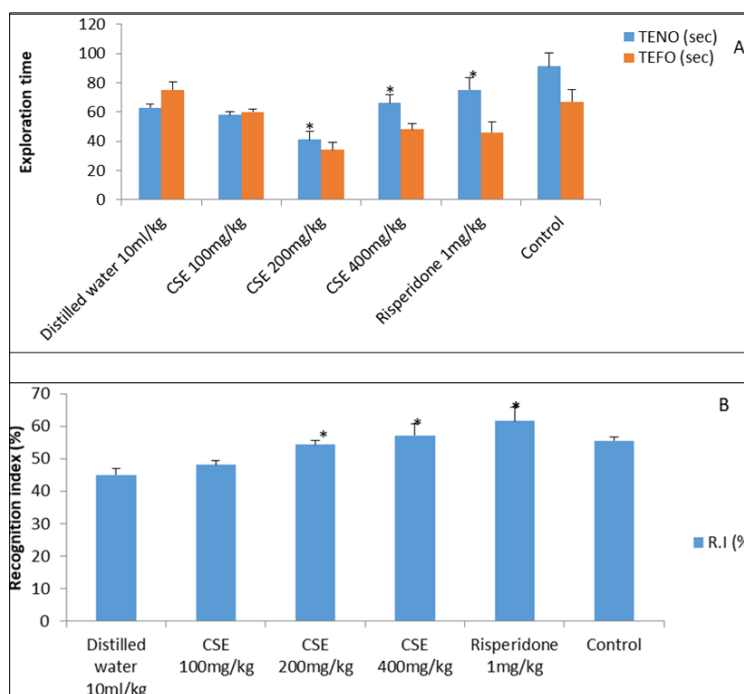**Table 1** Phytochemical Constituents of Methanol Leaf Extract of *Cassia singueana*

Test	Result
Saponins	+
Carbohydrates	+
Phenols	+
Flavonoids	+
Tannins	+
Cardiac glycosides	+
Steroids	+
Alkaloids	+
Anthraquinones	+
Proteins	+

Key: + = Present

The elemental content of *Cassia singueana* reveals the presence of Potassium, calcium, magnesium, manganess and zinc

3.2. Effect of *Cassia singueana* extract on acute ketamine induced memory impairment in Novel object recognition test.
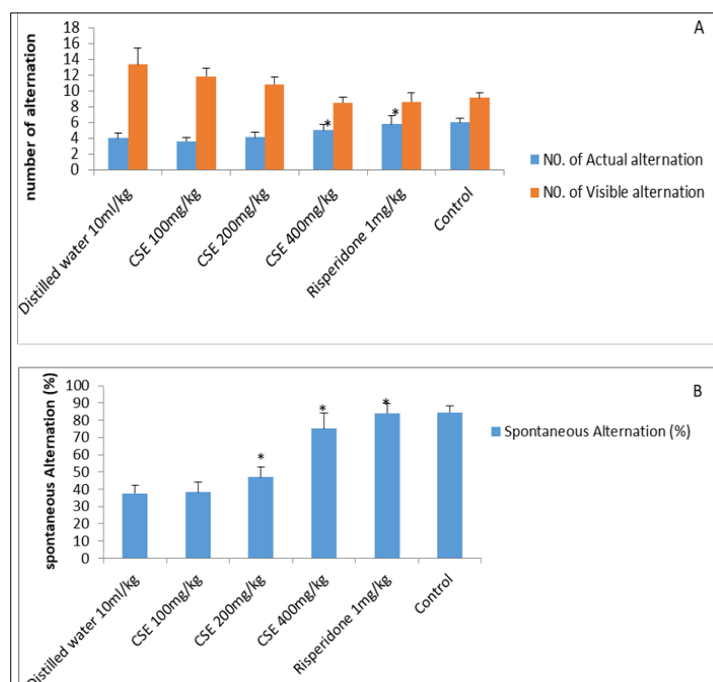
The extract has significantly (p< 0.05) and dose dependently increased duration of novel object exploration and the percentage recognition index when compared with the distilled water treated group as stated below in Figure 1

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

Figure 1 Effect of *Cassia singueana* extract on subchronic ketamine induced memory impairment in Novel object recognition test

3.3. Effect of *Cassia singueana* methanol leaf extract on acute ketamine induced spatial memory impairment in Y- maze

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.

Figure 2 Effect of *Cassia singueana* extract on subchronic ketamine-induced 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 below in table 2.

3.4. Effect of *Cassia singueana* extract on acute ketamine induced hyperactivity in open field test.

The extract has significantly (p< 0.05) decreased the number of central and peripheral square cross indicating decreased hyper locomotor activity as stated below in table 2.

Table 2 Effect of *Cassia singueana* extract on acute ketamine induced hyperactivity in open field test

Treatments	N0. of central square cross	N0. of peripheral square cross
Distilled water 10ml/kg	7.83 ± 0.90	15.83 ± 0.79
CSE 100mg/kg	6.33 ± .093	13.50 ± 1.20
CSE 200mg/kg	5.50 ± 1.05	7.83 ± 1.70*
CSE 400mg/kg	2.66 ± 0.60*	5.66 ± 1.17*
Risperidone 1mg/kg	0.66 ± 0.22*	0.83 ± 0.16*
Control	5.33 ± 0.80	9.16 ± 1.07

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.

3.5. Effect of *Cassia singueana*methanol leaf extract on acute ketamine induced immobility in forced swim test

The extract has significantly (p< 0.05) decreased the duration of immobility at the dose of 400mg/kg as stated below in table 4.9.

Table 3 Effect of *Cassia singueana*extract on acute ketamine induced immobility in forced swim test

Treatments	Duration of Immobility (m)
Distilled water 10ml/kg	3.43 ± 0.13
CSE 100mg/kg	3.31 ± 0.33
CSE 200mg/kg	3.06 ± 0.13
CSE 400mg/kg	2.48 ± 0.21*
Risperidone 1mg/kg	2.10 ± 0.21*
Control	2.81 ± 0.25

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*

3.6. Effect of *Cassia singueana* extract on catalepsy duration in glass bar test.

There was no significantly (p< 0.05) increase in the duration of catalepsy induced by haloperidol when compared with the distilled water treated group as stated below in table 4.

Table 4 Effect of *Cassia singueana* extract on catalepsy duration

Treatments	Catalepsy (sec)
Distilled water 10ml/kg	29.50 ± 5.41
CSE 100mg/kg	25.00 ± 5.16
CSE 200mg/kg	16.33 ± 2.33
CSE 400mg/kg	17.50 ± 2.71
Risperidone 1mg/kg	17.89 ± 1.21

Values are Mean ± S.E.M; – One way ANOVA followed by Bonferroni post hoc test, CSE = *Cassia singueana* Extract.

3.7. Effect of *Cassia singueana* extract on oxidative stress biomarkers in prefrontal cortex of rats

The extract has significantly (p< 0.05) and dose dependently decreased the level of MDA, increased the level of GSH and SOD in the brain homogenate of isolation reared rats compared with the distilled water treated group as stated below in table 5.

Table 5 Effect of *Cassia singueana*extract on oxidative stress biomarkers in prefrontal cortex of rats

Treatments	MDA (μmol/l)	GSH (mg/dl)	SOD(%)
Distilled water 10ml/kg	20.40 ± 1.23	14.62 ± 3.02	45.10 ± 2.18
CSE 100mg/kg	15.40 ± 2.20	17.10 ± 1.20*	49.21 ± 1.10*
CSE 200mg/kg	8.01 ± 0.12*	28.10 ± 1.20*	56.10 ± 1.10*
CSE 400mg/kg	6.16 ± 1.08*	32.21 ±1.11*	71.00 ± 1.12*
Risperidone 1mg/kg	5.40 ± 0.14*	32.64 ± 1.01*	76.21 ± 2.11*

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

3.8. Effect of Extract of *Cassia singueana* on renal Function Indices following 28 days subchronic Oral Treatment in mice

There was no significant (p< 0.05) increase or decrease in serum urea and sodium at dose of 100 and 200mg/kg, while in 400mg/kg extract treated group we noted significant (p< 0.05) decrease in serum urea only as stated in table 6 below.

Table 6 Effect of *Cassia singueana* on renal function indices following 28 days subchronic oral treatment in mice

Treatment/Dose (mg/kg)	Urea (mmol)	Creatinine(mmol)	Na^+(mmol)	K^+ (mmol)	Cl^- (mmol)	HCO_3 (mmol)
Distilled water	4.82±0.21	0.80±0.13	98.25±1.88	2.32±0.19	80.50±2.11	20.50±1.31
100CSE	3.10±0.20	0.60±0.08	88.50±2.53	3.10±0.11	84.75±2.01	18.75±1.30
200CSE	3.27±0.17	0.63±0.06	97.10±2.90	2.30±0.13	83.50±2.18	21.11±1.12
400CSE	5.42±0.22*	1.01±0.85	90.11±1.97	2.45±0.30	79.25±4.11	19.10±1.62

Data expressed as Mean±SEM, SEM = Standard Error of Mean n= 6, * P <0.05, Na^+ = Sodium ion, K^+ = Potassium ion, Cl^- = Chloride ion, HCO_3 = Bicarbonate, CSE = *Cassia singueana* methanol leaf extract. Bonferroni Post-hoc test.

3.9. Effect of *Cassia singueana* on liver function indices following 28 days subchronic oral treatment in mice

There was no significant decrease in all the liver function parameters following 28 days oral administration of (100, 200 and 400mg/kg) methanol leaf extract of *Cassia singueana* (Table 7).

Table 7 Effect of *Cassia singueana* on liver function indices following 28 days subchronic oral treatment in mice

Treatment/Dose (mg/kg)	T.B (Mg %)	D.B (Mg %)	ALK (m/l)	AST (m/l)	ALT (m/l)	T.P (g/l)	Albumin (g/l)
Distilled water	0.57±0.01	0.20±0.01	67.75±2.11	48.00±3.01	65.50±2.11	51.25±1.56	30.50±1.04
200CSE	0.55±0.04	0.10±0.01	66.25±2.21	52.00±1.51	63.50±2.31	58.50±3.12	29.75±2.41
400CSE	0.67±0.12	0.21±0.04	64.15±2.42	47.25±2.12	57.75±3.71	51.25±1.10	33.25±2.00
800CSE	0.75±0.01	0.35±0.01	61.05±6.31	52.40±2.17	61.15±2.11	56.75±2.71	34.11±1.21

Data expressed as Mean±SEM, SEM = Standard Error of Mean n= 6, T.B= Total Bilirubin, D.B = Direct Biluribin, ALK = Alkaline Phosphatase, AST = Aspartase Transaminase, ALT= Alanine Transaminase, T.P = Total Protein, CSE = *Cassia singueana*methanol leaf extract. Dunnet Post-hoc test.

4. Discussion

Medicinal plants due to their antioxidant properties and lesser side-effects remains the preferred choice in comparison to synthetic chemical compounds. Hence we select *Cassia singueana* plant for its vast spectrum of medicinal value starting from antiulcer to memory enhancing property. The study has evaluated the effects of CSE against the ketamine induced experimental psychosis model in mice. In this study, we evaluated the effects of CSE which is known to possess neuroactive properties for management of insanity and lactation enhancing effects. Pharmacological, post-mortem, neuroimaging and clinical studies have implicated the glutamatergic N-methyl-D-aspartate (NMDA) receptor in the pathology of schizophrenia (Olney and Farber, 1995). NMDA receptor antagonists such as MK 801, phencyclidine and ketamine induce schizophrenia-like symptoms, in healthy humans (Adler *et al.,* 1999), and augment psychotic symptoms in patients with schizophrenia (Charttajee*et al.,* 2011). Most importantly, phencyclidine, as well as its derivatives, MK-801 and ketamine, also impaired cognitive function in both humans and animals (Jentsch *et al.,* 1999), producing anomaly matching those present in schizophrenia (Trivedi, 2011). Therefore, in this present study, we have used the ketamine model to investigate the efficacy of CSE as possible antischizophrenic agent. In our findings, CSE was able to reverse the ketamine induced hyperactivity at doses 200 and 400mg/kg, however, showed a delayed response in blocking ketamine effects in 100mg/kg dose. The stimulation of high locomotor activity has been mainly administration of dopamine agonist and, the dopamine antagonists counteracts the motor activation induced by the systemic administration of NMDA (Gimenez-Llort*et al.,* 1997). Furthermore, dopamine release in the nigrostriatal neurons remains in direct presynaptic control of glutamate via both AMPA and NMDA receptors located in neuronal terminals of dopamine neurons. An indirect inhibitory regulation of dopamine release was also demonstrated due to the combined stimulatory effect of N-methyl D-aspartate on the mediumsized GABAergic efferent neurons. Also, a possible mechanism by which ketamine might produce this adverse behavioural effects, at least partially, have been related to the blockade of NMDA receptors located on inhibitory GABAergic neurons in the mesolimbic and subcortical brain regions (Nakao *et al.,* 2003). This disinhibitory action has been reported to facilitate the neuronal activity and excessive DA release in the limbic striatal regions (Lorrain *et al.,* 2003). Our finding that CSE is able to attenuate dopamine levels in the striatal areas and could not normalize D2 receptor gene expression, indicates that CSE may be able to modulate dopamine and hence it was able to show efficacy against positive symptoms of schizophrenia. Cognitive impairments such as deficits in attention, executive function, working short memory, and long-term memory, are core symptoms in patients with schizophrenia (Harvey *et al.,* 2004). Among these, learning and memory impairments are known to be particularly severe and resistant to treatment by convention antipsychotics (Saykin*et al.,* 1991). Novel object recognition test The NORT is a widely accepted method for assessing recognition memory in mice based on their natural innate character for exploring novelty (Lueptow 2017). In this study, the groups treated with CSE displayed a clear preference for novelty whereas the ketamine along treated group were unable to pass the novelty preference test indicating memory impairment. Similar outcomes were observed with the percentage recognition index; the extract and risperidone treated groups signifcantly improved the recognition index indicating the ability of the animals to retain preference for the novel object. This reveals that the CSE ameliorated object recognition memory that was impaired by ketamine. Based on this behavioural tests, it can be inferred that CSE improved the retention and recognition memory and can augment the effect of antipsychotic drugs on memory. Our findings also depict the memory impairing properties of ketamine (Chatterjee *et al.,* 2011), which was reverted by CSE treatment and also by standard atypical drug, Risperidone. Also the Y- maze can be used for short term working memory. Spontaneous alternation is the measure of spatial working memory. To alternate among spatial location a mouse must remember its previous location (Akanmu*et al.,* 2007). The effect of antipsychotic drug on cognitive function as an index for the cognitive dysfunction in schizophrenics is assessed using the paradigm described by Monte et al., (2013), our findings revealed that CSE was able to increase the number of actual alternation and improve spontaneous alternation in Y maze which indicate a favorable effect toward cognition as glutamate via NMDA receptors mediates long term potentiation, memory formation and ketamine administration could decrease in glutamate content and reversal by CSE could correlate its memory improving aspects. Oxidative stress results due to dysregulation of cellular generation of reactive oxygen species (ROS) and the ability of the cells to eliminate the ROS using endogenous physiological antioxidant defense system (Pizzino*et al.* 2017). The brain is very vulnerable to damage by oxidative stress radicals owing to its high oxygen content (Ma *et al.,* 2018). Certainly, brain damage as a result of free radical accumulation can cause impairment in learning and memory abilities (Fukui et al. 2002; Cheignonet al. 2018). In the same vein, ketamine-induced memory impairment in mice is associated with increased brain lipid peroxides (such as MDA) and reduced brain repository of antioxidants like reduced glutathione (GSH) and superoxide dismutase (SOD) (Ferdousy et al. 2016; Sevastre-Berghian et al. 2017). MDA is an intermediate of lipid peroxidation process and served as a biomarker of free radical generation. Lipid peroxidation is a vital marker of brain neurodegeneration which would eventually affects the structure and function of neuronal membrane (Shichiri 2014). GSH is a natural antioxidant found in all mamalian cells in varying concentrations and an indicator of oxidative stress (Birket al. 2013). Neuronal protection against hydrogen peroxide radicals that affect brain tissues, is largely mediated by the glutathione anoxidant system. Furthermore, lipid peroxidation may increase because of reduced GSH stores in the brain (Ansari and Schef 2010; Lee et al. 2020). It has been reported also that, SOD is an

antioxidant enzyme that clears away superoxide anions, which usually damage the cell membranes and other useful macromolecules (Kurutas 2016). In the present study, ketamine appreciably increased the MDA levels, while the GSH and SOD levels were reduced. However reduction in MDA level and increased GHS and SOD by CSE in the prefrontal cortex of mice brain indicates its antioxidant potential, which may be beneficial for memory formation and can confer neuroprotection against free radicals mediated cell injury.

The enhancement of immobility after 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). Administration of CSE reduced the immobility duration in the forced swim test, similar to risperidone. The efficacy of atypical agents in the negative symptom of schizophrenia has been attributed to their 5HT-2 receptor blocking ability; hence our CSE possibly contained phytochemical(s) that antagonize the 5HT2a receptors in the brain.

Urea and creatinine are the major biomarkers of renal toxicity. Serum urea accumulation is used as the acute marker, while serum creatinine accumulation is used in detecting chronic renal toxicity (Borges *et al.,* 2005). In this study, the oral administration of CSE for 28 days did not produce significant alteration in the renal function indices. The histological section of the rats indicates normal glumeruli and regular renal tubules. A similar finding was also reported by (Ezedinju*et al.,* 2014) who evaluated the renoprotective effects of *Moringa oleifera* leaf extract on the kidneys of adult mice and observed no alteration in the renal function indices and no tissue lesion in the histopathology study.

The degree of liver damage induced by a chemical substance can be evaluated by determining the level of biochemical markers of the liver function such as Aspertate transaminase (AST), Alanine transaminase (ALT), Alkaline phosphatase (ALP) (Udenze*et al.,* 2012). ALP is located in the cytoplasm and is release into circulation after liver cells damage. ALT and AST are also enzymes released as a result of hepatic injury, especially damage to mitochondria of liver cells. (Udenze*et al.,* 2012). Elevation of level of these enzymes can be an indication of cellular damage, leakage and loss of functional integrity of liver cell membrane. The result of this study dispalyed no significant differences in the level of liver enzyme when CSE treated groups are compared with the distilled water group.

5. Conclusion

Therefore, our present findings show that CSE would be effective against positive, negative and cognitive impairment induced by ketamine. The extract displayed no extrapyramidal side effects which are usually observed in antipsychotic medications. Hence, CSE and its bioactive phytochemicals may provide us an important lead in the discovery of a novel antipsychotic drug which can be further exploited clinically for patient with predominant negative and cognitive dysfunction which are resistant to treatment by conventional agents in majority of schizophrenics or the CSE can be used as an adjunct with the current clinical drug for treatment of schizophrenic patients. Moreover, its antipsychotic properties may be related to its modulatory effect on NMDAR, normalization of dopamine and serotonergic neurotransmission and reduction of reactive oxygen species accumulation activities.

Compliance with ethical standards

Disclosure of conflict of interest

The authors have declared that, there is no conflict of interest.

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