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Behavioral effect of *Cassia singueana* Del. (Fabaceae) extract against isolation rearing-induced cognitive impairment in albino rats

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Abstract

Early life stress negatively alters brain development and couldlead to the development of psychiatricdisorder. Exposing animals to early-life adverse events, including maternal separation or social isolation, greatly affects brain development and adult behaviour and may contribute to the occurrence of psychiatric manifestations, such as positive, negative and cognitive symptoms of schizophrenia. These symptoms can be depicted in different animal models.

Aim: This study is aimed at evaluating the effect of *Cassia singueana* on isolation rearing-induced cognitive impairment in rats.

Materials and Methods: Pharmacological intervention (Risperidone 1mg/kg/day, graded doses of *Cassia singueana* extract or Distilled water 10ml/kg) was introduced 4 weeks(adolescence) and one group 8 weeks(young adulthood) after IR (i.e., rats were 7- or 11-week-old). The intervention lasted for 8 weeks, after which the rats in all the various groups were subjected toopen field test, forced swim test, novel object recognition test, y-maze and post isolation grooming test. Rats' hippocampus immediately after sacrifice was removed to measure the level of MDA, SOD and GSH.

Results: *Cassia singueana* extract significantly decrease the hyper locomotion in open field, it also significantly decrease the duration of immobility in forced swim test. CSE also increase the exploration time of novel object and improved the recognition index. The extract also significantly increases the number of actual alternation and spontaneous alternation in y-maze. However the extract did not improve the duration of grooming in isolation reared rats. CSE also significantly improved the level of antioxidant enzymes.

Conclusion: The study results revalidate the utility of the isolation rearing paradigm in exploring mental disorders with neurodevelopmental origin, especially schizophrenia and *Cassia singueana* extract contained phytochemicals with tendency to ameliorate all the three symptoms of schizophrenia possibly through their antioxidant properties.

Keywords: *Cassia singueana*; Cognitive impairment; Schizophrenia; Isolation rearing; Oxidative stress

1 Introduction

Long term adversity is a major environmental risk factor for the development of neuropsychiatric disorders. Specifically, early life stress, such as social isolation, compromises brain development, and can contribute to mental illness (Fone and Porkess, 2008; Varese*et al.,* 2012). Abnormal social environment during the early life neurodevelopment are relevant in disorders such as autism spectrum disorder, attention-deficit/ hyperactivity disorder, and schizophrenia (Meyer-Lindenberg and Tost, 2012; Meyer-Lindenberg, 2014). It has been proposed that

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social experiences and relationships may influence dopaminergic neurotransmission in the CNS (Seltenet *al.,* 2017; Howeset *al.,* 2017). It has been reported that, greater dopaminergic and cortisol responses to a psychological stressors were observed in young adults that reported poor parental care when compared to controls (Pruessneret *al.,* 2004), this suggests that early-life experiences may have a long impact on systems implied in stress response. Similar to this notion, exposure of children to abuse, adversityand unstable family situations is positively correlated with elevated dopaminergic functionality in the striatum at adulthood (Egertonet *al.,* 2016), as well as dysregulation in excitatory/inhibitory neurotransmitters, which may lead tocertain cognitive dysfunctions (Allenet *al.,* 2019). Isolation reared ratsare commonly used method in which weanling rats are reared in social separation. They are allowed to see, smell, and hear other rats but not to have social contact with them. The IR rats exhibit majorsocial, behavioral, and neurochemical changes when they grow up, similar to the full manifestationof schizophrenic symptoms in thepatients'. Isolation rearing (IR) rats are therefore suitable for modeling mental disorders with a pathology based on neurodevelopmental hypothesis (Foneet *al.,* 2008),such as schizophrenia. The cardinal core features of IR-induced behavioral changes are locomotor hyperactivity and a defect in learning ability and are is particularly useful in examining the pathogenic mechanism of schizophrenia (Liuet *al.,* 2011; Yangt *al.,* 2015). In our present research, if the neurodevelopmental process, i.e., the isolation rearing maneuver has a crucial impact on the oxidative stress-related abnormalities in schizophrenia, it is expected that these abnormalities may be revealed in rats reared in social isolation (i.e., IR rats) and not socially reared rats (SOC), and pharmacological interventions at an early stage of IR might reverse the dysfunctions. For the pharmacological interventions, the treatment of schizophrenia has been greatly improved with the extensive use of second-generation antipsychotics as they have greater tendancy to relieve negative symptoms in some schizophrenics, specifically risperidone is employed in this study (Tandon, 2011). In the present study, we also examined the IR effects on oxidative stress-relevant dysfunctions by assessing the level of endogenous antioxidant biomarkers and to see if *Cassia singueana* extractmay reverse these dysfunctions. Nowadays, social isolation represents a significant mental health issue. Certainly, children and adolescents are more sensitive to the negative effects of isolation, the COVID-19 quarantine measures might result in increased anxiety and depression rates among those vulnerable subjects (Misiakt *al.,* 2017), and therefore the period could bean interesting event that required further insights on the consequences of social deprivation. A common animal model used to study different facets of mental disorders, such as schizophrenia is early-life social isolation in rats (Fone and Porkess, 2008). In rats, this isolation can induce long-lasting alterations in functional connectivity, behavior, and molecular expression (Fone and Porkess, 2008;Reinwaldt *al.,* 2018 and Cattaneoand Riva, 2015). The exposure to social deprivation is considered one of the most reliable preclinical models of schizophrenia, as it simulates the core features of mental disorders, e.g. cognitive deficits, alterations in social behavior which translate as negative symptoms of schizophrenia, hyperactivity which translate as positive symptom, and sensory gating deficits (Fone and Porkess, 2008).

Cassia species are used widely around the globe as traditional medicines and in pharmaceutical companies (Lim, 2012; Ayo, 2010). They are found across tropical and sub-tropical regions (Bhalerao and Kelkar, 2012). *Cassia singueana* Del. (Fabaceae), commonly known as winter Cassia is a shrub or tree with many medicinal values across Africa (Hiben, *et al*., 2016).The negative and cognitive impairments in schizophrenia are resistant to treatment with current antipsychotics, even after remission of the psychotic symptoms, which limits their therapeutic efficacy.The leaf extract of CS has been reported to be used in enhancing circulation in nursing mothers (Ifeanyi and Ode, 2012) and in management of insanity in traditional usage, hence our present research is aimed at exploring the effect of *Cassia singueana* extract against behavioral deficits induced by isolation rearing of rats.

2 Material and methods

2.1 Animals

Male albino rats (reared in ABU zaria, animal house of pharmacology and therapeutics department) were used in all experiments. The rats were 21-day-old and had been weaned on that day at the animal house of the pharmacology and therapeutics department A.B.U. Zaria, Kaduna state, Nigeria. Rats in the IR group were housed singly per cage. They could see, hear, and smell others but were kept separate and denied physical contact. The control group for IR was a social rearing (SOC) group. These rats were from the same batch as the IR rats but were reared socially (n = 4 for each cage). All rats were housed in a temperature (25°C–30°C) and humidity (30%–60%) controlled holding facility with 12 h light/dark cycles (light on from 700 to 1900) and received food and water ad libitum. After weaning (3 weeks postnatal age), rats were randomly and equally assigned to the IR or SOC group. Pharmacological intervention were give as follows; Grouping, The isolation reared rats were randomly distributed into six groups of 6 rats each while one group is socially reared rat which serve as control (SOC), making total seven groups. Group 1 received 10ml/kg/day vehicle, group 5 received risperidone 1mg/kg/day and group 2, 3 and 4 received graded doses of extract, the late intervention group 6 start receiving 400mg extract at their 11th week. The regimens of Risperidone, extract or vehicle have continued for 8 weeks. Locomotor activity was employed to validate the IR effect, after which the rats

weresubjected to neurobehavioral tests, such as force swim test, open field, Y-maze test for spontaneous alternation and novel object recognition. Immediately after completion of the final test, the rats were sacrificed, and their brains were removed to measure brain tissue level of oxidative stress markers (SOD, GSH, MDA) using TBA-TCA chemical protocol. All experimental procedures were approved by the 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 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 profle 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 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.

2.3 Neurobehavioral studies

Neurobehavioral studies to evaluate the effect of CSEon behavioral deficits induced by isolation rearing were performed as follows. A total of 36isolation reared rats were randomly divided into 6 groups of seven mice each (n=6). The rats were subjected to open field test (OFT), Forced swim test (FST), Y-maze (Y-M) and novel object recognition tests (NORT). For each study, the rats were pretreated with graded doses of CSE (100, 200 and 400 mg/kg, p.o) for 8weeks and on the day for the behavioral test, an hour before each trialbefore each test.

2.4 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. After acquisition, ratswas 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 familiarand the novel object)/100

2.5 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). Isolation reared rats were assessed for behavioral activity on Y- maze. The apparatus consists of three identical arms (33 x 11 x 12cm)in which arms are symmetrically separated at 120^{o}specifically each rat 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 rat 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 rat observation the chamber will be clean with 70% ethanol.

2.6 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^{0}C. 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.

2.7 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 extracts 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 Evaluation of Antioxidant Activity

2.8.1 Estimation of Malonedialdehyde (MDA) in rats hippocampus

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 $1.56 \times 10^{4} M^{-1} cm^{-1}$

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

Where A is OD at 535nm, 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.

2.8.2 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:

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

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

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.

2.8.3 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^{0}C (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).

2.9 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 (S.E.M.)

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.

3.2 Effect of *Cassia singueana* extract on hyper locomotion of isolation reared rats in open field test

The extract at the highest dose (400mg/kg) significantly ($p< 0.05$) decrease the number of central and peripheral square crossing in the open field arena when compared with the distilled water treated group, while no significant decrease in squares cross is observed in the late intervention group. The early intervention indicates a positive effect of the extract on isolation-induced hyperactivity in isolation reared rats as stated below in table 1.

Table 1 Effect of *Cassia singueana*methanol leaf extract on isolation reared rats in open field test

Treatments	N0. of central square cross	N0. of peripheral square cross
Distilled water 10 ml/kg	11.60±1.60	12.60±1.60
CSE 100 mg/kg	10.20±1.80	13.40±1.63
CSE 200 mg/kg	9.40±1.40	10.60±1.36
CSE 400 mg/kg	5.40±1.70*	10.80±1.24
Risperidone 1 mg/kg	3.80±1.10*	5.40±0.87*
LI CSE 400 mg/kg	10.40±1.30	12.00±2.30
SOCR	8.00±0.80	9.60±1.20

Values are Mean ± S.E.M; *= $p< 0.05$ as compared to Distilled water group – One way ANOVA followed by Bonferonni post hoc test, CSE = *Cassia singueana* Extract, LI = Late intervention, SOCR = socially reared rats

3.3 Effect of *Cassia singueana* extract on immobility of isolation reared rats in forced swim test

Table 2 Effect of *Cassia singueana*methanol leaf extract on isolation reared rats in forced swim test

Treatments	Duration of Immobility (m)
Distilled water 10 ml/kg	3.86 ± 0.19
CSE 100 mg/kg	3.45 ± 0.24
CSE 200 mg/kg	2.78 ±0.29*
CSE 400 mg/kg	2.45 ± 0.19*
Risperidone 1 mg/kg	1.91 ± 0.30*
LI CSE 400 mg/kg	3.73 ±0.19
SOCR	2.13 ± 0.29

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, LI = Late intervention, SOCR = socially reared rats

The extract has significantly (p< 0.05) decrease the duration of immobility at the dose of 200 and 400mg when compared with the distilled water treated group. However no effect is observed on the group that received late intervention of the extract as stated below in table 2.

3.4 Effect of *Cassia singueana* extract on spontaneous alternation of isolation reared rats in Y- maze

The extract has significantly (p< 0.05) increased the percentage spontaneous alternation at the dose of 200 and 400mg when compared with the distilled water treated group. However no effect is observed on the group that received late intervention of the extract as stated below in table 3.

Table 3 Effect of *Cassia singueana*methanol leaf extract on spontaneous alternation of isolation reared rats in Y- maze

Treatments	N0. of Actual alternation	N0. of Visible alternation	Spontaneous Alternation (%)
Distilled water 10 ml/kg	2.33 ± 0.40	10.83 ± 1.77	28.66 ± 4.71
CSE 100 mg/kg	1.66 ± 0.33	6.83 ± 0.62	35.66 ± 7.12
CSE 200 mg/kg	2.16 ± 0.47	7.50 ± 1.50	51.00 ± 1.07*
CSE 400 mg/kg	2.50 ± 0.42	5.83 ± 0.73	69.50 ± 6.90*
Risperidone 1 mg/kg	2.50 ± 0.50	6.00 ± 1.34	74.16 ± 1.17**
LI CSE 400 mg/kg	2.50 ± 0.42	10.33 ± 1.63	33.33 ± 5.77
SOCR	4.00 ± 0.57	6.83 ± 0.87	86.33 ± 4.44

Values are Mean ± S.E.M; *= p< 0.05, **= p<0.01 as compared to Distilled water group – One way ANOVA followed by Dunnet post hoc test, CSE = *Cassia singueana* Extract, LI = Late intervention, SOCR = socially reared rats

3.5 Effect of *Cassia singueana* extract on object recognition of isolation reared rats in Novel object recognition test

The extract has significantly (p< 0.05) increased the percentage recognition index at the dose of 400mg/kg early intervention when compared with the distilled water treated group. However no effect is observed on the group that received late intervention of the same dose of the extract as stated below in table 4.

Table 4 Effect of *Cassia singueana* extract on object recognition of isolation reared rats

Treatments	TENO (sec)	TEFO (sec)	R.I (%)
Distilled water 10ml/kg	85.25 ± 10.63	109.2 ± 6.25	43.20 ± 2.08
CSE 100 mg/kg	116.4 ± 8.43	120.0 ± 5.10	48.8 ± 1.80
CSE 200 mg/kg	105.6 ± 5.84	112.8 ± 8.42	48.0 ± 2.80
CSE 400 mg/kg	75.60 ± 11.63	56.40 ± 5.91	61.2 ± 6.21*
Risperidone 1 mg/kg	115.2 ± 11.90	60.00 ± 10.4	69.4 ± 6.50*
LI CSE 400 mg/kg	115.2 ± 6.43	122.4 ± 10.8	48.20 ± 1.24
SOCR	67.20 ± 3.94	49.20 ± 5.21	58.00 ± 1.44

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, LI = Late intervention, SOCR = socially reared rats, TENO = Time exploring novel object, TEFO = Time exploring familiar object, R.I = Recognition index

3.6 Effect of *Cassia singueana* extract on swim induced-grooming in isolation reared rats.

The extract has significantly (p< 0.05) decreased the duration of grooming at the dose of 100mg/kg early intervention when compared with the distilled water treated group. However less effect was observed on the subsequent groups treated with the extract as stated below in table 5.

Table 5 Effect of *Cassia singueana* extract on grooming of isolation reared rats in swim induced grooming

Treatments	Duration of Grooming (m)
Distilled water 10 ml/kg	1.64 ± 0.22
CSE 100 mg/kg	3.76 ± 0.25*
CSE 200 mg/kg	2.40 ± 0.28
CSE 400 mg/kg	1.86 ± 0.22
Risperidone 1 mg/kg	3.06 ± 0.13*
SOCR	3.36 ± 1.10

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, SOCR = socially reared rats

3.7 Effect of *Cassia singueana* extract on oxidative stress biomarkers in hippocampus of Isolation reared 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 6.

Table 6 Effect of *Cassia singueana*extract on oxidative stress biomarkers in brain of isolation reared rats

Treatments	MDA (µmol/l)	GSH (mg/dl)	SOD(%)
SOC	18.60 ± 1.10	39.70 ± 0.93	74.50 ± 1.20
Distilled water 10 ml/kg	25.40 ± 1.20	21.60 ± 1.12	54.00 ± 2.11
CSE 100 mg/kg	19.40 ± 1.30	25.80 ± 1.23*	60.20 ± 1.50*
CSE 200 mg/kg	12.00 ± 0.77*	35.20 ± 1.21*	69.40 ± 1.40*
CSE 400 mg/kg	11.60 ± 1.02*	41.20 ±1.10*	84.00 ± 1.50*
Risperidone 1 mg/kg	10.40 ± 0.92*	41.60 ± 0.92*	88.40 ± 3.17*

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.

4 Discussion

In the oral acute toxicity study of the plant extract we calculate the median lethal dose to be greater or equal to 5000mg/kg. According to Dietrich Lorke, 1mg/kg is considered highly toxic, 10mg/kg is considered toxic, 100mg/kg is moderately toxic, 1000 mg/kg is slightly toxic and 5000mg/kg is considered not toxic (Lorke, 1983). Our result indicates that large dose of the extract can be relatively safe. Mortality was not observed in all the phasesof the experiment employed. According to the American Society for testing safety of Materials, any chemical substance with LD_{50} less than 2000mg/kg/oral route but greater than 1000mg/kg/oral could be considered to be slightly toxic.

The environmental stress that likely induces oxidative stress which eventually lead to dysfunctions of numerous CNS neurotransmitters and their receptors are crucial in the pathoetiology of schizophrenia, and as the latter is a psychiatric disorder with the neurodevelopmental origin, an animal model of developmental impact on brain function appears suitable paradigm to examine the hypothesis that the neurodevelopmental process has a crucial impact on neurobehavioral abnormalities. Social isolation of rat pups from the age of weaning (postnatal day 21) alters brain development and causes behavioural deficits at adulthood, including hyper locomotion (Lapiz*et al*., 2003). Isolation rearing in rats leads to hyperfunctional mesolimbic dopaminergic systems, hypofunctional mesocortical dopaminergic systems, enhanced serotonergic function in the nucleus accumbens, and attenuated serotonergic function in the frontal cortex and hippocampus (Fone&Porkess, 2008). It been reported that, greater dopaminergic and cortisol responses to a psychosocial stressor were observed in young adults that reported low parental care when compared to controls (Pruessner*et al.,* 2004), suggesting that early-life experiences may have a deep impact on systems implied in stress response. Corroborating this notion, exposure of children to abuse or unstable family situations is linked with elevated

dopaminergic functionality in the striatum at adulthood (Egerton*et al*., 2016) as well as with imbalance in excitatory/inhibitory neurotransmitters, which may underlie some cognitive dysfunctions (Allen*et al.,* 2019).

Isolation reared rats when exposed to open field tend to display hyperactivity by repetitive locomotion in an open field arena, this hyperactivity reflect an increased in mesolombic dopamine activity which is usually translated as positive symptom of schizophrenia (Del Arco *et al*., 2004). The conventional antipsychotics haloperidol, olanzapine and risperidone also reverse isolation-induced hyper-activity (Fabricius*et al*., 2010; Jones *et al*., 2011) also the NMDA receptor modulator, L-serine helps in treating isolation induced hyperactivity (Bendikov*et al*., 2007). *Cassia singueana* methanol leaf extract significantly decreased the number of square crossing of isolation reared rats in open field arena indicating decrease hyper locomotion activity, these revealed that the extract phytochemicals likely interact with dopamine and N-methyl D-aspartate receptors in eliciting their pharmacological effects. A similar finding of decreased locomotor activity was reported by (Gaurav*et al.,* 2012).

Social isolation rearing induced immobility in forced swim test translates to decreased volition and despair behavior, a negative symptoms of schizophrenia (Minae*et al.,* 2011). The extract significantly and dose dependently decreased the duration of immobility of isolation reared rats in forced swim test, this finding is correlated with the ability to attenuate the negative symptoms of schizophrenia as reported by (Minae*et al.,* 2011).

The *Cassia singueana* extract also significantly increased the percentage spontaneous alternation in Y- maze, inferring positive neurocognitive effect, the extract enhanced short term spatial memory and attenuate the anterograde amnesia induced by social isolation (Minae*et al.,* 2011). While spatial learning and acquisition are highly dependent on hippocampal–neocortical pathways, performing an attentional shift to learn a new rule depends primarily on prefrontal cortex–striatal pathways, which would appear to be preferentially affected by social isolation from weaning (Quan et al., 2010), the phytochemicals of this extract likely interact with the receptors in these brain regions and reverse the cognitive deficit induced by isolation rearing.

Humans and rodents alike have an innate curiosity to preferentially explore novel over familiar objects, and assessment of this differential exploration forms the basis of the novel object recognition task, which is thought to assess visual episodic memory (Dere*et al*., 2007; Winters *et al*., 2008) and to map in a translational manner to the visual learning and memory domain affected in schizophrenia (Young *et al*., 2009). Isolation reared rats displayed inability to discriminate between novel and familiar objects in the retention test of Novel object recognition test (McLean *et al*., 2010 and Marsden *et al*., 2011). Seven to ten days intervention with some antipsychotics, or specifically the 5-HT6 receptor antagonist, PRX-07037 (Porkess et al., 2006; King et al., 2007) both restored novel object recognition to levels seen in sociallyreared control rats, suggesting that this behavioural deficit may be a valuable tool with which to examine novel therapeutic treatments for cognitive impairments relevant to those seen in schizophrenia. Our extract has significantly increased the percentage recognition index in isolation reared rats; a similar result was also reported by (Nazifi*et al.,* 2022). The impairment in object recognition is likely to reflect deficits in recognition memory, which is one of the core cognitive symptoms of schizophrenia(McLean *et al*., 2010), so by this gesture the extract is likely to manage the cognitive symptoms of schizophrenia. Recently, some studies have shown the ability of 5-HT6 receptor antagonists (King *et al*., 2007), dopamine D3 receptor antagonists (Watson*et al*., 2011) and mGluR2/3 agonists (Jones et al., 2011) to reverse isolation-induced deficits in object recognition, and these also inferred the likelihood of our extract to interact with these receptors in eliciting its pharmacological effects.

Oxidative stress occurs as a result of disparity 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*etal,.* 2018).MDA is aknownbiomarker 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 of isolation reared 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). There has been report on important association between cortico-striatal oxidative stress and schizophrenia-related behaviours in rats subjected to isolation rearing. The early life period is very sensitive and critical stage, it is characterized by intense brain development, during which external stimuli may influence or interfere with ongoing structural and functional changes (Andersen, 2003;Andersen, 2015). Therefore, it is highly likely that challenges that occur during this period may have long-term consequences on brain functions, potentially leading to the outbreak of pathological conditions later in life (Inta*et al.,* 2014). Environmental insults later in life, such as social isolation, are associated with oxidative stress and represent great risk factors for development of schizophrenia (Zugno*et al.,* 2015).

Myelin is produced by oligodendrocytes (OLs) that are derived from OL precursor cells (OPCs) in the developing as well as the adult brain (River*set al.,* 2008). Plasticity in the formation and retraction of myelin sheaths by OLs also occurs from early childhood to adulthood (River*set al.,* 2008). Neuronal activity can instruct OPCs to divide and mature, and can stimulate myelin sheath production by OLs (Mensch*et al.,* 2015), leading to increased myelination and improved behavioural performance (Gibson*et al.,* 2014).However during the period of isolation in rats there is decrease in level of antioxidants which exposed the oligodentrocyte to reactive oxygen species radicals and eventually lead to prefrontal cortex hypomylination, which correlates with behavioural 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 improved cognition.

5 Conclusion

The methanol leaf extract of *Cassia singueana* possesses antioxidant and memory-enhancing activities. This could served as scientific justification for its ethnomedicinal use in management of insanity and as lactation enhencer

Acknowledgement Compliance with ethical standards

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Disclosure of conflict of interest

The authors have no conflict of interest to declare.

Statement of ethical approval

The research committee on animals research of Ahmadu Bello University, Zaria has approved all the experimental protocols that involved used of animals and the ethical approval was received.

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