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Contextual Fear, Anxiety and Motor Impairments Induced by Ketamine Neurotoxicity and the Roles of Curcuma Longa in Female Wistar Rats

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Abstract

Objective: *To investigate the roles of Curcuma longa in ketamine-induced motor impairments, anxiety, and contextual fear caused by alterations in the hippocampus, prefrontal cortex and cerebellum.*

Methods: *The thirty-five female Wistar rats were assigned into five groups of seven rats. Group A served as a control; Group B received ketamine only. Groups C, D, and E were treated with 200mg/kg, 400mg/kg, and 600mg/kg of extract, respectively. The Elevated Plus Maze and open field test (OFP) assessed locomotion, anxiety, and fear, while GPx, GOT, GST, GR, GSH, and GPO were evaluated using the serum.*

Results: *After each induction, behaviours like hallucinations, long sleeping time, staggering, heavy breathing, and restlessness upon waking up were noted. There was a significant reduction in voracious locomotion, anxiety, and fear in the treated group, while serum concentrations of GPx, GOT, and GST significantly decreased (Fig. 6). The concentrations of GPO, GSH, and GR significantly increased at $P<0.05$ (Fig. 5) in treated groups. The tissue sections showed mild healing after treatment with Curcuma longa.*

Conclusion: *Curcuma longa can ameliorate ketamine toxicity and should be part of daily meals capable of improving motor activities.*

Keywords: Anxiety, Contextual Fear, Ketamine, Impairment, Motor Activity

Introduction

Ketamine was initially created as an antagonist and used as a dissociative anesthetic, and it is still often used for this clinical reason today, according to Kim et al. [1]. Lately, there have been reports of people using ketamine recreationally, especially in nightclubs and following the "rave culture" properties [2]. Its quick onsets, brief action time, and unusual "K-hole" psychoactive side effects, such as bewilderment, dissociation, and depersonalization, are often blamed for the recorded abuse [3]. Neurobehavioral abnormalities such as hallucinations, anxiety, despair, cognitive deficits, and delusion are the most common effects of ketamine use for recreational purposes, as abuse and dependence are reported properties [4]. In addition, there have been gastrointestinal toxicity, urological issues, particularly hemorrhagic cystitis, and neuropsychiatric disturbances (a state resembling schizophrenia) in long-term usage. Ketamine has attracted much attention as a novel therapy in the past few years in the scientific and lay/media worlds due to its effectiveness as a quick-acting antidepressant [5]. Esketamine nasal spray was registered and approved by the US Food and Drug Administration in March 2019 as an additional therapy for depression that has not responded to other treatments [6]. However, the adverse effects and misuse of ketamine have prevented its more frequent and routine clinical usage [7].

Nonetheless, researchers who have extensively investigated its antidepressant characteristics have suggested that subanesthetic doses may provide a unique therapeutic pathway for acute neuronal damage and other neuropsychiatric disorders [8]. In model systems, ketamine appears to display context-dependent neurotoxic and neuroprotective properties [9]. At anesthetic doses applied during neurodevelopmental windows, ketamine contributes to inflammation, autophagy, and apoptosis and enhances levels of reactive oxygen species [10]. Different neuronal cell

types are well organized into layers in the Hippocampus and are frequently used as a model system for studying neurophysiology [1]. The neural plasticity known as long-term potential (LTP) was first discovered in the Hippocampus and has been studied in this structure [12]. LTP is widely understood to be one of the effective neural mechanisms for storing memory in the brain [13]. Researchers have recently picked interest in hippocampal olfactory responses, especially the role hippocampus plays in odor memory, but few people believe that olfaction is its primary function today [14].

Recent studies have shown turmeric as a medicinal spice with anti-inflammatory and antioxidant properties [15, 16]. Moreover, an ointment base on the sauce is used as an antiseptic in India, decreases Kapha, and removes mucus in the throat, watery discharges like leucorrhea, and pus in the eyes, ears, or wounds; used as a tooth powder or paste [17]. Hence, the need to check in this research the therapeutic role of Curcuma longa against spatial memory, learning, and cognitive impairments induced by ketamine neurotoxicity on rodents' Hippocampus and prefrontal cortex.

Methodology

Ethical Clearance

This research considered and obeyed all OECD guidelines for testing chemical usage in experimental animals [18]. The Animal and Biological Science Ethical Committee on Research of Alex Ekwueme Federal University Ndufu-Alike, Ikwo, Ebonyi State, gave the ethical certificate to conduct this research. The ethical reference number is AE-FUNAI-2021/003565.

Purchase, Identification, and Preparation of Extract

Curcuma longa belongs to the family zingerbecea, as identified by a taxonomist in the Department of plant science and Biotechnology at the University of Nigeria Nsukka, with herbarium number 998a. The turmeric cloves were obtained from a local market in Abakaliki town, peeled, and dried for fourteen days before grinding into powder. The powder was soaked in water for 48 hours to extract active ingredients and sieved with cheesecloth and then with Whiteman filter paper. The filtrate was evaporated at 400c until a sticky paste was obtained.

Ketamine Induction

Ketamine has been in use over several decades by physicians as anesthesia, but recently, some outlaws have converted it to use as a social drug. The ketamine used in this study was purchased from a pharmaceutical unit of Alex Ekwueme Federal University Teaching Hospital, Abakaliki (AE-FUTHA). It has batch number 5C80115, manufactured on Oct-18-2021 and expires on Sept-2022, and NAFDAC Reg. NO: A4-8208. The animals were given ketamine intraperitoneal injections six times at two days interval per induction.

Experimental Design

The thirty-five (35) female albino Wistar rats weighing between 150g and 300g were obtained from the animal house of Alex Ekwueme Federal University Ndufu-Alike. After 7 days of acclimatization, the rats were shared into five groups of seven rats each group. Group A served as negative control and received normal saline. Group B was given 10mg/kg of ketamine and served as an untreated group. Groups C, D, and E received 10mg/kg of ketamine and were later treated with 200mg/kg, 400mg/kg, and 600mg/kg of Curcuma longa, respectively. Groups B, C, D, and E received six intra-peritoneal injections of ketamine at two days intervals while C, D, and E served as low, middle, and high doses of Curcuma longa treated orally, respectively. Ketamine induction lasted twelve days, the treatment with the extract lasted seven days, and the experiment took 27 days, including acclimatization.

Elevated Plus Maze (EPM)

The elevated plus maze (EPM) is an exploratory model of anxiety that measures animals' response to a novel approach and their relative exploration of two distinct environments. EPM could be used to assess anxiety, emotionality, and reactivity based on the animals' aversion to open and elevated surfaces, according to Munekazu et al. [19]. The apparatus comprises two open arms (25 x 5 x 0.5 cm) and two perpendicular closed arms (25 x 5 x 16 cm) with a center platform (5 x 5 x 0.5 cm). The open arms have a minimal (0.5 cm) wall to decrease the number of falls, whereas the closed arms have high (16 cm) walls to enclose the components [20]. The experiment was recorded with a camera attached to a computer and scored at convenience, and the following parameters were collected; the number of open arm entries, the number of close arm entries, the number of Rearing, head dip, risk assessment, time spent in Rearing, time spent freezing, time spent grooming, time spent in open arm, time spent in the close associate [21]. This test was performed on the 25th day of the experiment.

Assessment of Open Field Test (OFT)

A square Open Field Apparatus constructed with plywood to measure 40×40cm and a high wall of 40cm with a detachable floor. The floor was divided into 16 small boxes of 18×18cm squares and smoothly polished white and grey paint [21]. The Open Field Test was conducted according to previous studies by Lee et al. Rats were placed at the center facing the walls, allowed to explore the apparatus for 10 mins, and monitored by video tracking [22-24]. Rats were returned to home cages and apparatus cleaned with ethanol between tests. The parameters collected include line crossing, center square entries, time spent in the center square, and Rearing; according to Sturman et al., OFT was carried out on the 26th of the experiment after the last treatment [25, 26].

Estimation of Glutathione Peroxidase (GPX) and Glutathione-S-Transferase (GST.)

The technique applied in determining Glutathione peroxidase (GPx) was, according to Rotruck et al. due to the ability of GPx to oxidize reduced glutathione (GSH) to produce H2O in the presence of H2O2. The amount of GSH consumed is directly proportional to the activity of GPx and expressed as U/ml (µmol of GSH consumed/minute). GST activity was estimated based on the procedure described by Habig et al. [27] The absorbance change was monitored at 340 nm for 3 min at 30s intervals spectrophotometrically, and specific activity was expressed as µmoles ml-1 min-1 mg-1 protein.

Estimation of GSH, GPO, and GR.

The GSH remaining reacted with 5'-5' dithiobis-2-nitrobenzoic acid (DTNB) to form a yellow complex that absorbs maximally at 412 nm. The reaction mixture contained 0.4 ml of phosphate buffer (pH 7.0), 0.1 ml sodium hydroxide and 0.2 ml of the plasma or standard or blank, 0.2 ml of glutathione, and 0.1

ml of H2O2. The solution was mixed thoroughly and incubated at 37°C for 10 minutes. The reaction was arrested by adding 0.4 ml of 10% trichloroacetic acid (TCA). The content was centrifuged at a speed of 4000 rpm for 5 minutes, and 0.5 ml of the supernatant was added to a cleaned test tube, followed by 2 ml of phosphate buffer (PH=7.0) and 0.5 ml of 40 mM DTNB. The solution was thoroughly mixed, and the yellow color was read at 412 nm. A blank was treated the same way except that it contained 0.2 ml of distilled water instead of the sample 20 mg/100 ml of GSH standard (0.651µmol/ml) was also used [27]. The glutathione peroxidase activity was expressed as U/mL of plasma (µmoles of GSH utilized/minute).

Estimation of Glutamate Oxaloacetate Transaminase (GOT).

GOT was spectrophotometrically determined, according to the principle of Zhong et al. and Kim et al., with some modifications [28-30]. First, 0.1ml of serum was put in a test tube and mixed with 0.5ml buffer. The incubation period of the mixture was 30 minutes at 370C. Next, 0.5ml of 2, 4- dinitrophenylhydrazine was added and maintained at a temperature of 250C for 20 minutes, and the reaction ended immediately after 5.0ml of 0.4Mol/L NaOH was added to the mixture. Finally, the reading of the absorbance was taken at 546nm.

Animal Sacrifice

The animals were euthanized to reduce pain and subsequently sacrificed using cervical decapitation at the end of the experiment, and the brain was removed and fixed in 10% formal saline. The tissue was then processed, and a thin section was obtained for histological examination of turmeric's role in drug intoxication.

Statistical Analysis

Statistical Package for Social Sciences (SPSS) was applied in analyzing the data from this experiment, and the results were tabulated and presented as Mean± SEM. In addition, the inferential statistics of Analysis of Variance (ANOVA) were adopted to check the significance level, and a significant group was established at $p < 0.05$.

Results

Effects of Curcuma Longa Anxiogenic and Anxiolytic Behaviors

The animals in the ketamine group spent a significant amount of time in an elevated plus maze closed arm with less entry at $P<0.05$ compared to the control group (Fig. 1). Low dose group spent an increased time in the closed arm. In contrast, medium and high doses spent less time in the close associate compared to the ketamine group. Furthermore, the time spent in the open arms by the rats significantly reduced in the ketamine group compared to the control at $P<0.05$, but among the treated groups, only the medium dose showed a significant increase in both entry frequency and time in the open arms while low and high dose reduced significantly compared ketamine group (Fig. 1b and c).

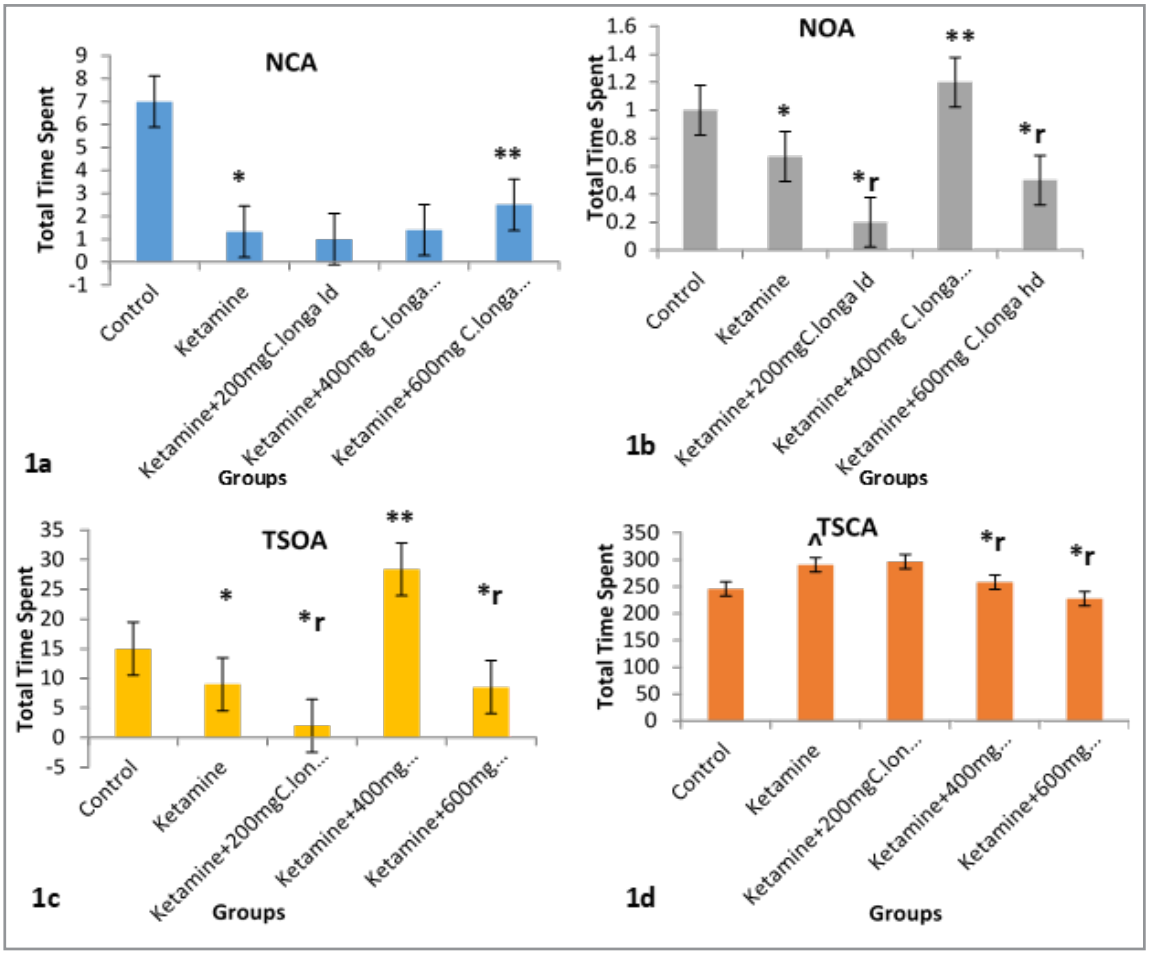


Fig 1: The effects of C. longa on ketamine induce anxiety behaviors. Note NOA-Number of open arm entries; TSOA-Time spent in the open arm; NCA-Number of close arm entries, TSCA-Time spent in the closed arm. * Significant decrease compared to control at P≤0.05; **Significant increase compared to ketamine at P≤0.05; *r Significant decrease compared to ketamine at P≤0.05; ^Significant increase compared to control at P≤0.05

Effects of Curcuma Longa Verticality Behaviors

Abnormal Verticality behaviors were measured in this research using an elevated plus maze, and rearing and grooming behaviors were recorded, as presented in Figure 2 below. The ketamine group was observed to spend a significantly reduced rearing time and did not even groom during the period under consideration compared to the control, as shown in Figure 2 ($P<0.05$). The extract significantly increased the time spent by the animals in Rearing and grooming compared to the ketamine group (Fig. 2). The number of grooming increased in low doses in contrast with the medium and high doses recorded with a reduced grooming frequency but more time spent during the same period at $P<0.05$.

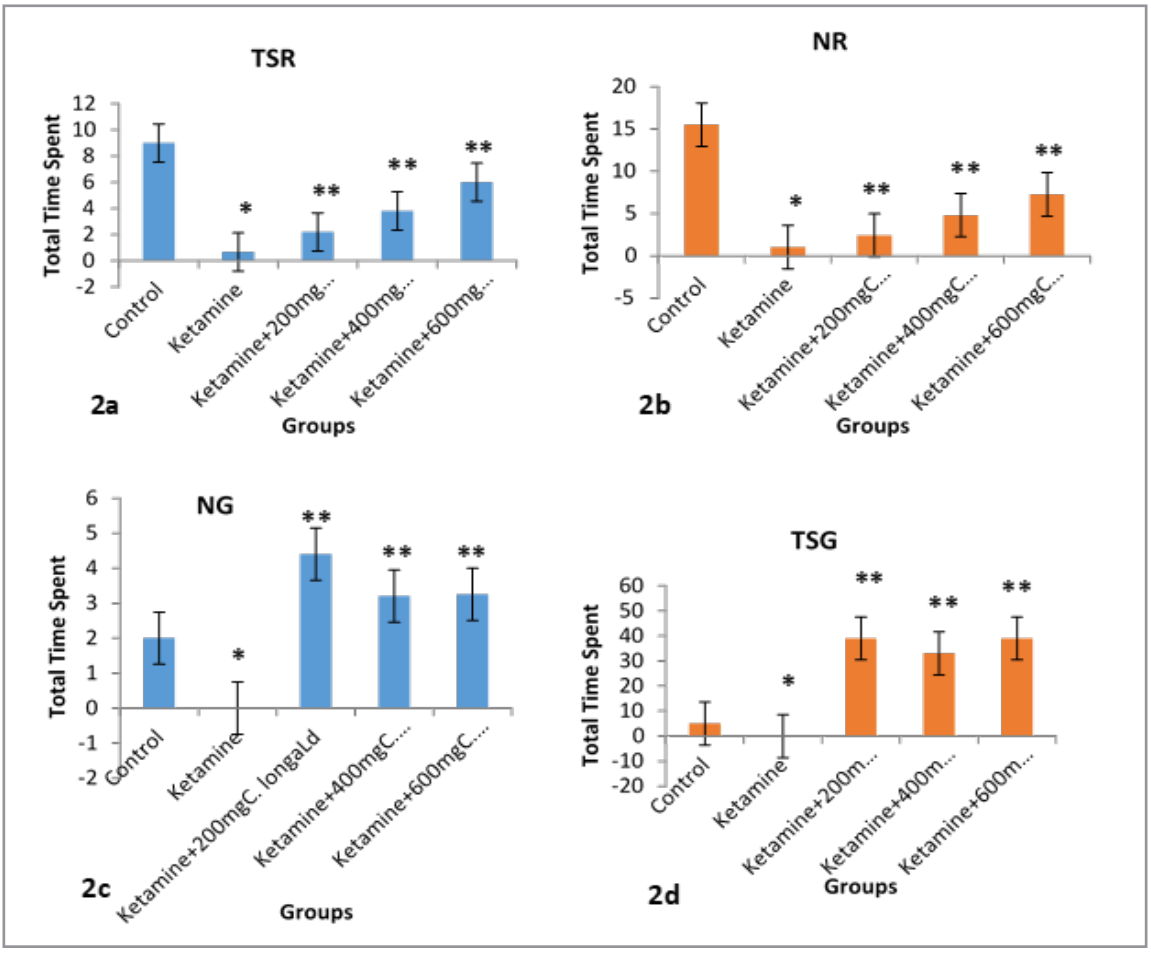
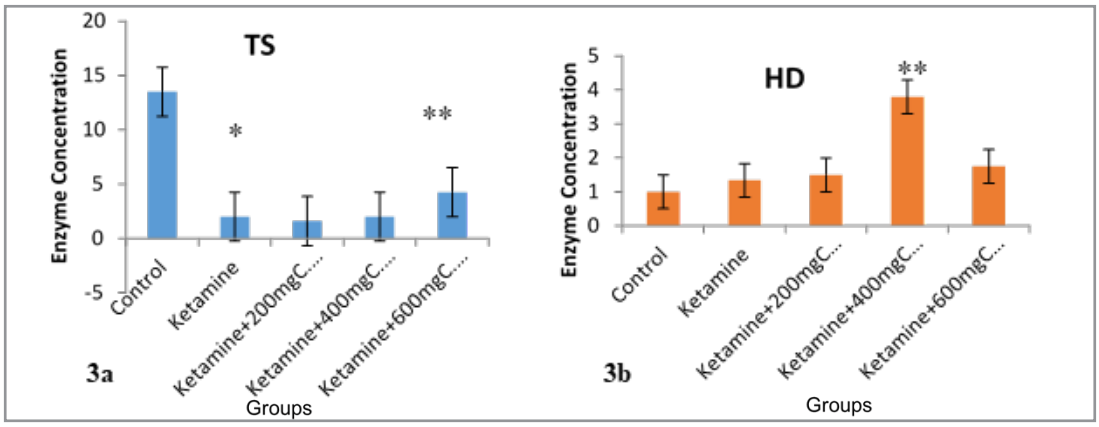


Fig 2: Effects of Curcuma longa on abnormal verticality behaviors induced by ketamine using elevated plus maze. NR-Number of rearing, TSIR-Time spent rearing, NG-Number of grooming TSG-Time spent grooming *Significant decrease compared to A at $P\leq0.05$; **Significant increase compared to B at $P\leq0.05$.

Effects of Ketamine and Curcuma Longa on Contextual Fear

At a height, the animals are bound to exhibit some characteristics of fear. The elevated plus maze is one of a height, so the animals tend to assess their environments before making any move. In this research, head dip, stretching, and freezing were used to measure the animals' ability to determine their environment and the level of fear at such a point. In the ketamine group, the animals' stretching reduced significantly at $P<0.05$ (Fig. 3a). The high extract dose caused a significant increase in the total stretching recorded by the animals compared to the ketamine group at $P\leq0.05$ (Fig. 3a). The animals spent a substantial increase in time in ketamine group to freeze compared to control. All the treated groups spent lesser time freezing, with the low dose being the highest and the high dose the least at $P<0.05$ (Fig. 3c). The high dose group accessed its environment more often with a frequent head dip, as seen in Figure 3b.



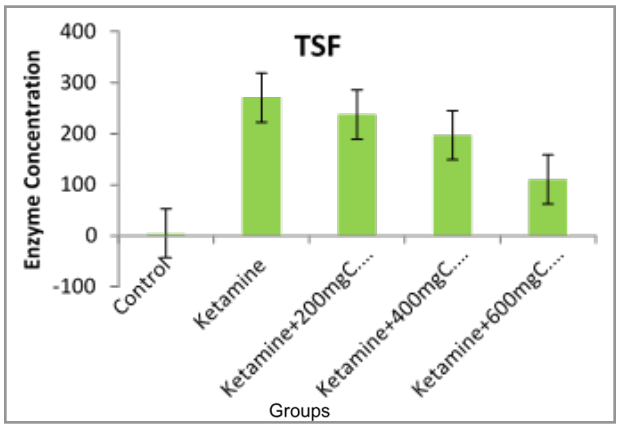


Fig 3: Effect of Curcuma longa on rats' risk assessment and freezing behavior from contextual fear conditioning testing following ketamine induction using elevated plus maze. HD-Head dip, TS-Total Stretch, TSF-Time spent freezing. *Significant decrease compared to A at P≤0.05; **Significant increase compared to B at P≤0.05; *r Significant decrease compared to B at P≤0.05

Effects of Ketamine and Curcuma Longa on Locomotive

Figure 4 shows the animal's locomotive behaviors in an open field. The untreated group spent a substantial amount of time at the center and as well crossed more lines at P<0.05 compared to the control (Fig. 4). Since it spent significant time at the center, the time spent close to the wall of the open field decreased significantly compared to the control group at P<0.05. After receiving the extract's low, medium, and high doses, the animals' voracious movement in crossing lines was reduced significantly at P<0.05 compared to the ketamine group. The significant increase in time spent at the center by the medium may have informed the substantial reduction in time spent close to the walls, as shown in Figure 4, while time spent at the center significantly reduced in the high dose group at P<0.05.

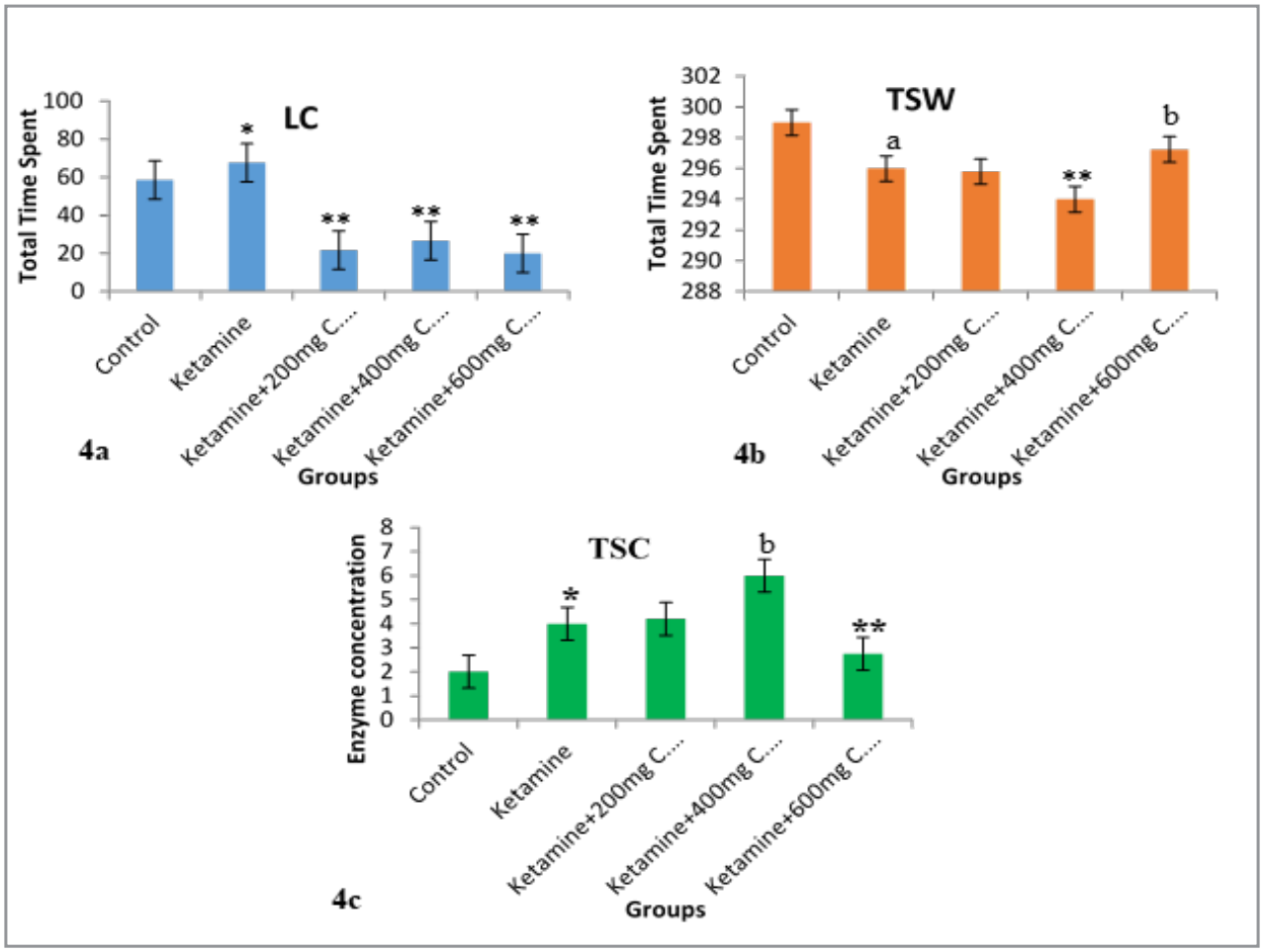


Fig 4: Effect of C. longa on the ketamine-induced locomotive and exploratory behaviors on adult female Wistar rats using open field test. TSC= Time spent at the Centre, TSW=Time spent closer to the walls; LC=Line crossing, *Significant increase compared to A at P≤0.01; **Significant decrease compared to B at P≤0.05, a Significant decrease compared to A at P≤0.05; b Significant increase compared to B at P≤0.05,

The Serum Concentration of GPO, GSH, and GR

Ketamine reduced the serum concentration of GR, GSH significantly, and GPO in this experiment at P<0.05 compared to the control group (Fig. 5). On treatment with Curcuma longa, the serum concentration significantly increased at P<0.05 compared to the ketamine group, see figure 4 below. In addition, the concentration of GSH enzyme was reduced considerably by ketamine compared to the control and increased significantly in the high-dose group compared to the ketamine group at P<0.05 (Fig. 4).

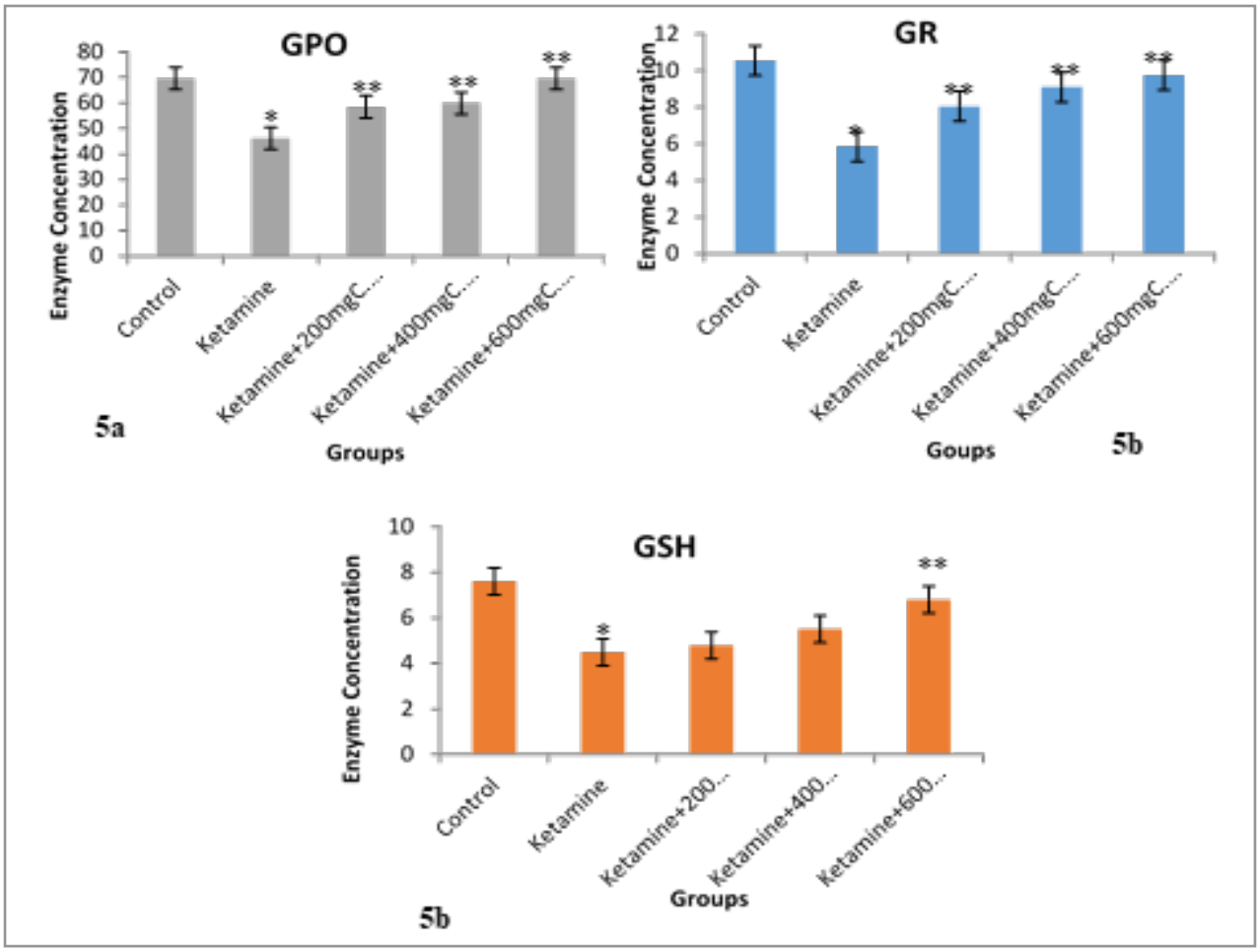
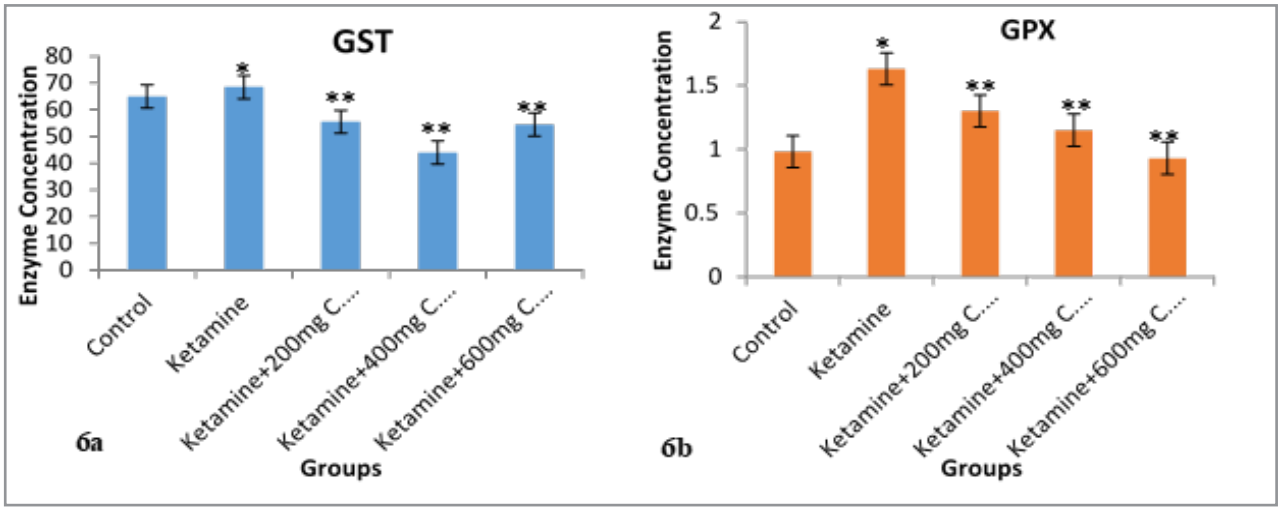


Fig 5: Effect of C. longa on ketamine-induced some brain biomarkers; GPO-Glutathione Peroxidase; GR- Glutathione Reductase, and GSH- Glutathione-S-Transferase. **Significant increase compared to B at P≤0.05; *Significant decrease compared to A at P≤0.05

The Concentration of GST, GOT, and GPX

Table 6 showed that GST, GOT, and GPX concentration in group B significantly increased compared to the control group at P<0.05. On the contrary, on the administration of the extract of C. longa, GST and GOT decreased significantly in low, medium, and high dose groups compared to the ketamine group at P<0.05 (Fig. 6).



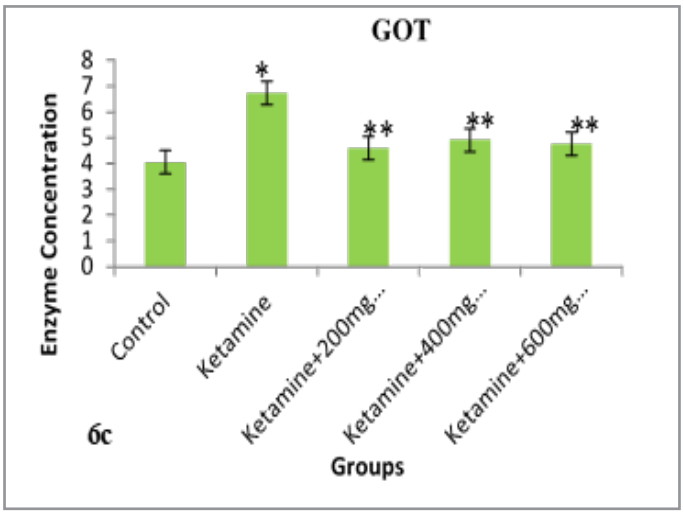


Fig 6: Effect of Curcuma longa the concentration of some enzyme markers on ketamine-induced toxicity in adult female Wistar rats. *Significant increase compared to A at P≤0.05; **Significant decrease compared to B at P≤0.05

Microscopical Examination

On examination of sections of the Hippocampus from the control group, a normal histoarchitecture with distinct layers having prominent nuclei and all the CA1, CA2, CA3, CA4, and dentate gyrus were present (Figure 7A) compared to the ketamine group with severe necrosis, focal area of hemorrhage, empty optical spaces and loss of nuclei (Figure 7B). The low and medium doses displayed moderate healing with vessel widening, few lost nuclei, and large nuclei, as shown in Figures 7C and D. The high dose group showed better recovery with distinct dentate gyrus cells and normal blood vessels (Figure 7E).

Microscopic Examinations of the Hippocampus

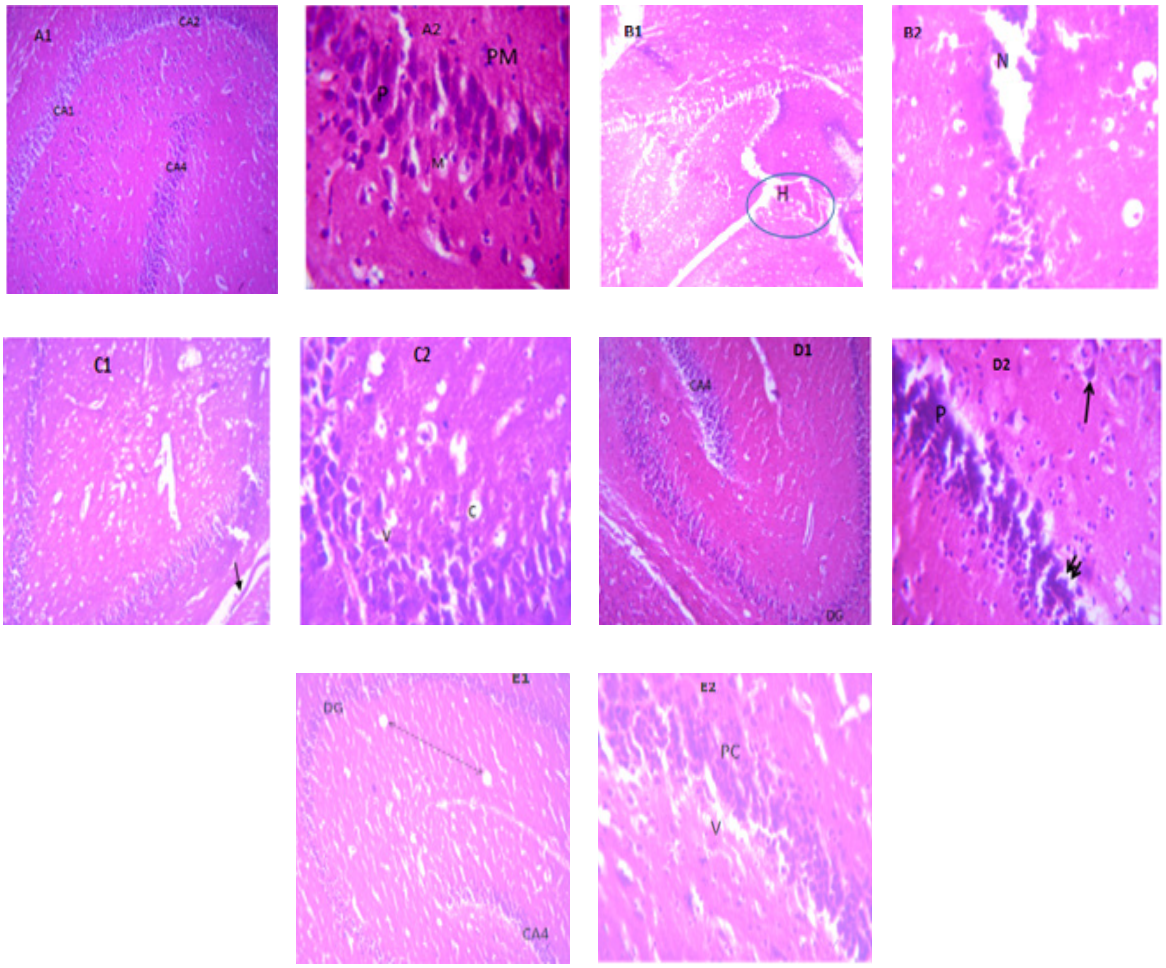


Fig 7: Photomicrograph of the section of the hippocampus as seen under the microscope at a magnification of x400, H & E stain shows; the control group distinct hippocampal layers plexiform layer (PLL), Pyramidal layer (PYL) and multiform layer (MFL) (A1 and 2); ketamine group severe necrosis (N) hemorrhage (H), vacuolations and enlarged nuclei (B1 and 2); low dose group moderate healing, mild vacuolation(V) within the plexiform layer (C1 and 2); medium dose group showed average healing pyknotic cells (P) increased neurons (D1 and 2); high dose group cell regenerations, distinct pyramidal cell (E1 and 2).

A prefrontal cortex section displayed normal histoarchitecture (Figure 8A). At the same time, the ketamine group showed severe degenerations, microcystic space, inflammation of cells within the cystic area, loss of granular cells, and necrosis, as seen in Figure 8B. The extract low-dose group displayed mild regeneration, few microcystic spaces, vacuolated cell layer, pyknotic neurons, and charred neuronal group in Figure 8C.

In contrast, the medium and high-dose groups improved to near-normal cells (Figures 8D and E).

Microscopic Examinations of the Prefrontal Cortex

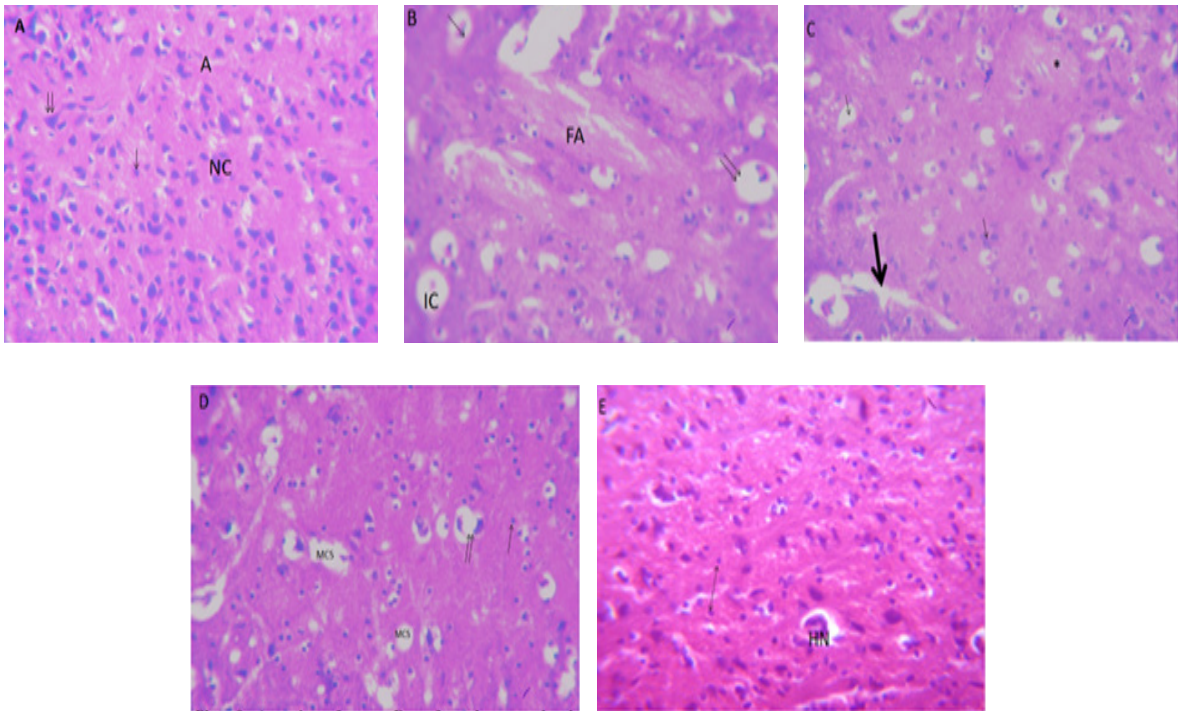
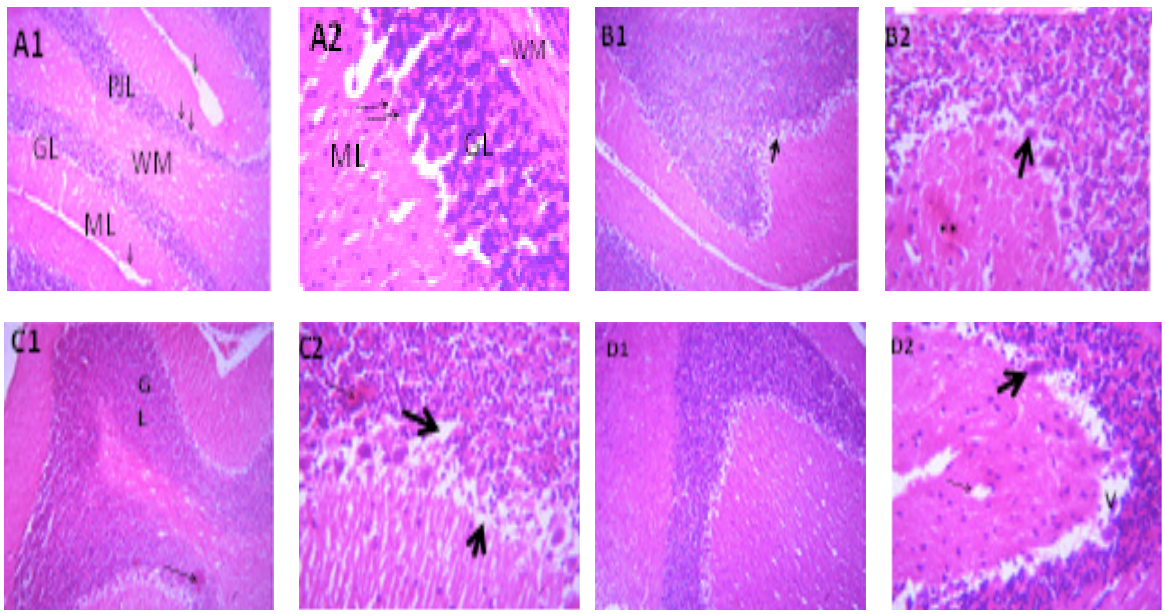


Fig 8: Photomicrograph of section of hippocampus as seen in under the mocroscope at a magnificantion of x400, H & E stain showing; control group with normal neuronal cell (NC) of the prefrontal cortex (A); ketamine group with sever degeneration, sever microcystic spaces (MCS), moderate focal areas of coagulative necrosis (FACN) and inflammatory cell (IC)within the cystic area (B); low dose group with mild regeneration, moderate microcystic spaces (CS) (C); medium dose group with mild regeneration, moderate microcystic spaces (MCS) and hypertrophied neuron (HN) (D); high dose group with healing, cell regeneration, few hypertrophied neurons (HN) and generally normal histoarchitecture (E).

Histologically, the ketamine group showed vacuolation of the Purkinje layer characterized by a decrease in the population of the Purkinje cells, necrotic cells, and hemorrhage scattered around the tissue (Fig. 9B). The low and medium doses of the extract didn't show many effects as mild necrosis, mild vacuolation, and few microcystic spaces are still showing in both granular and Purkinje layers (Figures 9C and D). In contrast, the high dose improved with visible blood vessels and intact cell layers (Figure 9E).

Microscopic Examinations of the Cerebellum



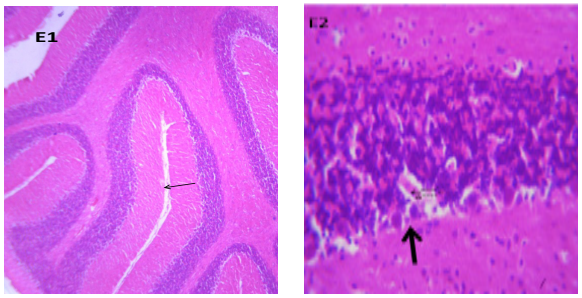


Fig 9: Microscopic sections of H & E cerebellum as seen of various groups showing X40; control with normal granular layer, (GL), molecular layer (ML), Purkinje layer (double arrows), blood vessels (arrow) and white matter (WM) (A1 and 2); Ketamine group with vacuolations (thin arrow), necrotized cell (thick arrow), and mild hemorrhage (double star) (B1 and 2); low dose with mild necrosis (thin arrow), mild vacuolation (thick arrows) within the layers (C1 and 2); medium dose with vacuolations (V), microcystic space (thin arrow) (D1 and 2); and high dose with blood vessel (arrow), intact purkinje cell layer (thick red arrow) and neuronal increase (E1 and 2).

Discussion

During this experiment, physical observations such as staggering, hallucination, deep sleeping, initial staggering upon waking, and vigorous movement after waking up may result from brain alterations (Figures 7-9). Reduction in time spent is a sign of increased anxiety levels in rodents, as reported by Bombi and Hyejung which agrees with the present research with a reduced time spent in the open arms by the untreated rats (Fig. 1b and c) [20]. In the low dose, time spent in open arms increased significantly, which might be a pointer to the extract's anxiolytic property [30, 31]. The more time the animals spend in the open arms, the freer it is and less anxious to explore their environments which the extract can offer. The animals in the ketamine group spent significant time in the closed arms, which implies anxiety-related behaviors, while the reduced time spent in the closed arms by medium and high-dose animal's points to the anxiolytic effect of the extract (Fig. 1d) [20]. Rearing and grooming are both referred to as abnormal verticality behaviors in rodents [32]. Ketamine, from this research, does not increase abnormal verticality behaviors, as seen in figures 2a-d. The extract caused an increase in these abnormal verticality behaviors as the number and time spent in Rearing and grooming increased significantly in figures 2a-d. Fear is one of the characteristics of all animals exhibited, and it makes them hide from open spaces to avoid endangering their lives [31]. According to Rodgers et al., stretching, head dip, and freezing behaviors measure the level of fear contextualization in rodents and their environmental risk assessment [32]. The reduction recorded in the time spent stretching by the animals in the ketamine group might be a pointer that ketamine, used by individuals as one of the social drugs, is capable of intoxication. This intoxication, by implication, reduces the level of fear and the tendency of the individual to assess and carefully monitor the environment before making any move or decision. In other words, it is termed a hype drug as corroborated by the results of the head dip and time spent freezing, and this was restored by the extract (Fig. 3). There was a persistent fear response to the original context, and the extract seemed to have an improved context-dependent freezing behavior [31, 33]. In the OFT, line crossing is one of the best ways to measure and ascertain the level of locomotive and exploratory activities and impairments, which can directly measure the alterations within the cerebellum [34]. In this research, ketamine caused a significant increase in line crossed within the time under review (Fig. 4a). This increase in line crossing is directly proportional to the irrational behavior caused by ketamine. It might lead to erratic reactions to specific issues by their daily users. The line-crossing movement was significantly reduced by the extract of C. longa in a dose-dependent manner, as seen in Figure 4a [35]. The animals spent more time close to the center of the OFT apparatus, implying reduced fear contextualization.

GSH serves as a significant antioxidant militating against numerous reactive oxygen species and hydrogen peroxide with a vital role in maintaining sulfhydryl groups in their reduced form, according to Lund et al., which makes depletion of the reduced form of GSH in the mitochondria cause a severe deficit in their role against oxidative damage leading to an increase in lipid peroxidation, which toxicity is one of the agents of depletion of these all-important substances in the body [36, 37]. Ketamine constitutes one of those agents capable of increasing oxidative stress by depleting GSH, according to Omar et al., which agrees with this current research as the ketamine group shows a significant decrease in GR, GSH, and GPO as seen in figures 5a-c [38]. Depletions of brain biomarkers might be the reason for the alterations seen in the Hippocampus (Fig. 7b). The increase recorded in the extract groups (Fig. 5a-c) might be a very interesting pointer to the antioxidant and anti-inflammatory properties of C. longa in restoring brain functionality by rebuilding the glutathione concentration. This increase in concentration increases the activities of GST and GSH cleansing properties in the body of free radicals [39]. GST is a plasma enzyme primarily implicated in detoxifying chemicals, such as drugs, which use GSH as an agent of conjugation [40]. As seen in figures 6a-c, ketamine administration led to a significant increase in the concentration of GST, GOT, and GPX, which agrees with previous studies like Türkan et al., Maseko et al., Ergün et al. [41-43]. The extract could normalize the enzymatic activities of GST, GOT, and GPX and enhance detoxification in conjunction with GSH, as was evident in brain histoarchitecture in figures 7, 8, and 9 [44].

The microscopical examination of the hippocampus, prefrontal cortex, and cerebellum shows several alterations, which fluctuations in brain markers might have caused due to ketamine toxicity. Constant alteration in biochemical markers causes lipid peroxidation, which increases ROS generation and then damages neuronal cells [45]. Researchers have implicated hippocampal and prefrontal cortex lesions in anxiety, contextual fear, and spatial memory impairment [46]. In contrast, lesions in the cerebel-

lum are implicated in locomotive impairment or loss of movement memory and motor learning, which agrees with the current research (Figures 1, 3, and 4). Substances with antioxidant and anti-inflammatory properties can restore tissue histology and enhance cell or neuronal regeneration at all levels, and Curcuma longa possesses these properties. Treating the rats with C. longa brought about some healing and cell regeneration ranging from mild to near normal neurons, as seen in figures 7c-e, 8c-e, and 9c-e. This healing may have resulted from restoring the enzymatic activities, which reduces depletion, as was also evident in the improvement in fear, anxiety, and locomotive activity (Figures 1, 3, and 4). The extract helped in restoring locomotion and reducing anxiety levels in the animals.

Conclusion

Ketamine as anesthesia causes hallucinations, induces anxiety and vigorous movement on waking from a long sleep. It also causes depletion of brain biomarkers and plasma enzyme activities, ameliorated by Curcuma longa, probably leading to restoring the tissues' integrity. In addition, C. longa may also act as a promising agent for removing ROS.

Competing Interest

The authors of this article declare no conflicting interest in this article now or in the future.

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