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Attenuating Roles of *Curcuma longa* on Ketamine-induced Neurotoxicity on Hypothalamic-Pituitary-Ovarian Axis of Wistar Rats

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ABSTRACT

The hypothalamic-pituitary-ovarian (HPO) system is mostly affected by toxins, which can cause infertility in mammals. This research investigated the role of *Curcuma longa on* Ketamine toxicity in the HPO axis of rats. Twenty-five female Wistar rats were assigned into five groups of five rats after seven days of acclimatization. Group A received water and served as control. Groups B, C, D, and E received 10 mg/kg of Ketamine intraperitoneally at intervals of 2 days for five times each, and Groups C, D, and E received 250 mg/kg, 400 mg/kg, and 600 mg/kg of *Curcuma longa* orally for 14 days. Blood samples were used to study hormonal concentrations and oxidative stress markers. The result showed a significant decrease in weight in the ketamine untreated group. The malondialdehyde (MDA) (5.78±0.04), superoxide dismutase (SOD) (15.60±0.40), and catalase (CAT) (28.82±0.28) enzyme concentrations were also lowered in the ketamine group but showed improvement in the treated groups. The serum concentration of estrogen (E2), follicle-stimulating hormone (FSH), ant-diuretic hormone (ADH), luteinizing hormone (LH), lactate dehydrogenase (LDH), and cortisol were all lowered by ketamine compared to control at $p \leq 0.05$, which was improved by the extract. The alterations caused by ketamine toxicity were ameliorated by *C. longa*. Ketamine is a toxic drug with increased MDA and Lowered antioxidants such as SOD, but *C. longa* may be very productive in reducing these toxic effects.

Keywords: Axis; *Curcuma longa*; disorders; ketamine; hypothalamo-pituitary; ovarian

INTRODUCTION

The hypothalamic-pituitary-ovarian (HPO) axis functions as a unit to enable procreation by leveraging the cyclic synthesis of gonadotropic and steroid hormones[1,2,3]. The ovary is crucial in producing the steroid hormones required for follicular growth and oocyte maturation[4]. This axis controls the hormonal environment necessary for oocyte development and fertilization[5]. Whenever diseases arise at any point along the axis, the intricate regulation may suffer[6]. According to the World Health Organization, ovulation abnormalities are the primary cause of infertility[7]. Previous studies reported that about 10 percent of ovulation abnormalities are due to group I disorders such as hypogonadotropic hypogonadism, panhypopituitarism, autoimmune or viral hypophysitis, pituitary adenomas, and histiocytosis[8]. Group II accounts for 85% of ovulatory abnormalities by abnormal body mass index (BMI), and endocrinopathies entail disruption of the HPO axis[9]. Similarly, oocyte depletion is a severe consequence of ovarian

insufficiency, also known as ovarian failure, which is included in group III[10,11]. Due to ovarian hormones' neuroprotective roles and their facilitation of neurogenesis, neuronal differentiation, survival, and cognitive function, the HPO system also plays a crucial part in maintaining healthy brain function[12]. Kisspeptin is a neuromodulator that modifies the release of GnRH and eventually governs the activities of the HPO axis[13,14]. Several lines of evidence suggest that estradiol, progesterone, and testosterone, all produced in the hippocampus, can affect various neural pathways in addition to the negative feedback loop controlling HPO axis activity [15-17]. Again, recent findings established that, in complement to ovarian hormones, peripherally generated membrane proteins also control the HPO activity[18]. The heterodimers paracrine protein complexes activins and inhibins have opposing biological effects on the release of FSH; whereas activins are produced throughout the body, inhibins are only made in the gonads[19,20]. Furthermore, inhibins are responsible for suppressing FSH secretion and inactivating and regulating the effects of activins.[19,21]. In contrast, activins are essential for increasing FSH secretion, activating the HPG axis, and promoting neural plasticity[21]. In combination with inhibin, a third protein complex called follistatin controls the action of activin. These proteins work with circulating ovarian hormone levels to prevent the beginning and ending of HPO axis activity, according to Master *et al*.[22] and Cui *et al*.[23]

Ketamine is a receptor complex antagonist of N-methyl-D-aspartic acid, which evokes amnesia, loss of consciousness, immobility, and analgesia[24]. Further, ketamine has become one of the controversial drugs for use in children due to its neurotoxicity, as reported by Savic *et al*.[25] and Zhao *et al*.[26]. In addition, it causes hallucinogenic effects in people [26-28]. However, ketamine is widely used as a recreational drug with a severe and potentially long-lasting impact, such as nycturia, dysuria, and severe bladder pain [29]. Again, evidence exists that thirty-three percent (33%) of ketamine users always complain of 'K-cramps in addition to memory impairment caused by its constant use [30,31]. According to Tan *et al*.[32] Chronic intake of ketamine has been implicated in genital dysfunctions, but the mechanism of this action is yet to be elucidated.

Turmeric possesses anti-inflammatory properties beneficial in treating various ailments like cancer, Alzheimer's disease, *Staphylococcus aureus*, anemia, cancer, diabetes, digestion, food poisoning, gallstones, indigestion, parasites, poor circulation, staph infections, and wounds[33]. Additionally, turmeric removes mucus in the throat, watery discharges like leucorrhea, and pus in the eyes, ears, or wounds[34]. A recent report suggested that hot water extracts of the dried rhizome taken orally in Ayurvedic medicine reduced inflammation[35]. Thus, the increase in the use of ketamine among teenagers as a social drug has spurred the study. To the best of our knowledge, the current study is the first to administer turmeric specifically to treat the harmful effects of ketamine on the hypothalamic-pituitary-ovarian axis, thereby exploring the ameliorating potentials of *Curcuma longa* in ketamine neurotoxicity on the Hypothalamo-Pituitary-Ovarian Axis of adult female Wistar rats.

MATERIALS AND METHODS

Ethical clearance

This research considered and conformed to the OECD guidelines for testing chemical usage in experimental animals[36]. The test protocols adopted for the experiment were based on the principles of laboratory animal care required and approved by the Animal and Biological Science Ethical Committee (ABSEC) for research at the Alex Ekwueme Federal University Ndufu Alike, Ikwo, Ebonyi State (Reference Number: AE-FUNAI-2021/0021343). At the end of the experiments, animals were humanely euthanized by cervical dislocation to reduce suffering.

Collection and preparation of extract

The turmeric cloves were collected from the AE-FUNAI farm and identified by Mr Festus Ogiji of the Department of Biology and Biotechnology of

David Umahi Federal University of Health Science, Uburu, Ebonyi State. They were washed, peeled, and dried for two weeks before grinding. The powder was soaked in plastic filled with water for 48 hours to extract the active ingredients and sieved using a cheesecloth and then with number one Whiteman filter paper. A sticky paste was obtained by evaporating the filtrate at 40^0C, which was stored until required.

Induction of Ketamine

30 ml of Ketamine was procured from the pharmacy section of Alex Ekwueme Federal University, Abakiliki (AE-FUTHA), Ebonyi State. The chemical batch number was 5C80115, manufactured on Mar-18-2022, Expires Oct-2024, and NAFDAC Reg.No:A4-8208.

Research design

Healthy female albino Wistar rats, one to two months old, weighing 150 -250g, were maximized in this study. The rats were obtained from Alex Ekwueme Federal University Animal House and housed as 5 rats per cage under the same laboratory conditions (temperature 22 ± 25°C, 12 h light: 12 h dark cycle) for one (1) week of acclimatization. Rats were fed with standard commercially available rat chow (pellets) and water *ad libitum*. The rats were housed at Alex Ekwueme Federal University Ndufu Alike and used for study. Group A received water and served as the Control (A), B received 10 mg/kg of Ketamine only and served as the ketamine group, C was given 10 mg/kg of Ketamine and later treated with 250 mg/kg of *Curcuma longa* (CL low dosage), D received 10 mg/kg of Ketamine and treated with 400 mg/kg of *Curcuma longa* (CL medium dosage). E received 10 mg/kg of Ketamine and was treated with 600 mg/kg of *Curcuma longa* (CL high dosage). Ketamine was administered intra-peritoneal at an interval of 2 days, five times, while *Curcuma longa* was given orally for 14 days in the early hours of the morning daily.

Estimation of antioxidants

The serum oxidative and antioxidants activities measured, such as superoxide dismutase (SOD), Malondialdehyde (MDA), and Catalase CAT using the ELISA method as well as specific commercial kits (Teb Pazhouhan, Razi, Iran), were as described by Yin *et al*[37].

Estimation of sex hormones

The blood sample collected into a sample bottle was kept at room temperature ($20\text{-}27^0$) until it was separated. The sample was centrifuged at 3000 minutes per hour (mph) for 20 minutes, and the serum was decanted and transferred into a new bottle. The serum was kept at -20^0C until it was required for the hormonal assay. The hormones, namely estrogen, follicle-stimulating hormone (FSH), and luteinizing hormone (LH) levels, were measured by homologous specific double antibody radioimmunoassay using material supplied by the National Hormone and Pituitary Program (NHPP, Rockville, MD), respectively as described by Winter and Faiman[38] and Abraham[39].

Estimation of stimulating hormones

Cortisol hormone was measured using techniques from Nieman *et al*.[40], which utilizes mass spectrometry methods as a powerful structural characterization technique that overcomes the antibody-antigen cross-reactivity problem of immunoassays. The antidiuretic hormone was determined using the method described by Lee[41], which is based on the over-hydrated rat under ethanol anaesthesia, while Lactate Dehydrogenase was assayed. At the end of the experiment, the rats were euthanized by cervical decapitation after using chloroform to reduce animal suffering. The brain and ovaries were harvested while the brain was fixed in phosphate buffer solution, the ovaries were fixed in Bouin's fluid for 24 hours and then re-fixed in 10% formol saline and processed for histological examination.

Data analysis

The data were analyzed by the use of GraphPad Prism version 8.0.1, and a Two-way analysis of variance (ANOVA) was used in comparing means, to establish a significance level at $p<0.05$. The standard error of the mean (SEM) was demonstrated in the tables and charts. The means were compared using the Turkey multiple comparison test.

RESULTS

Effect of *C. longa* on the weight of the animals

In the current study, the weight change decreased significantly in the ketamine group compared to the control at p≤0.05, while the weight decreased in *C. longa* treated groups. Furthermore, it is negative in both low dose (Ld) (- 8.60±1.43) and high dose (Hd) (- 4.40±0.19), respectively, while it is positive in medium dose (Md) (10.40±0.83) and the weight decreased significantly at p≤0.05.

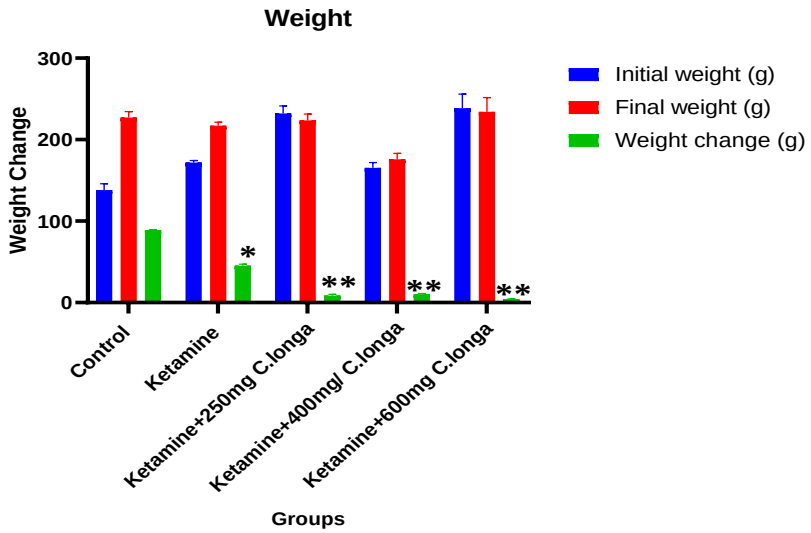
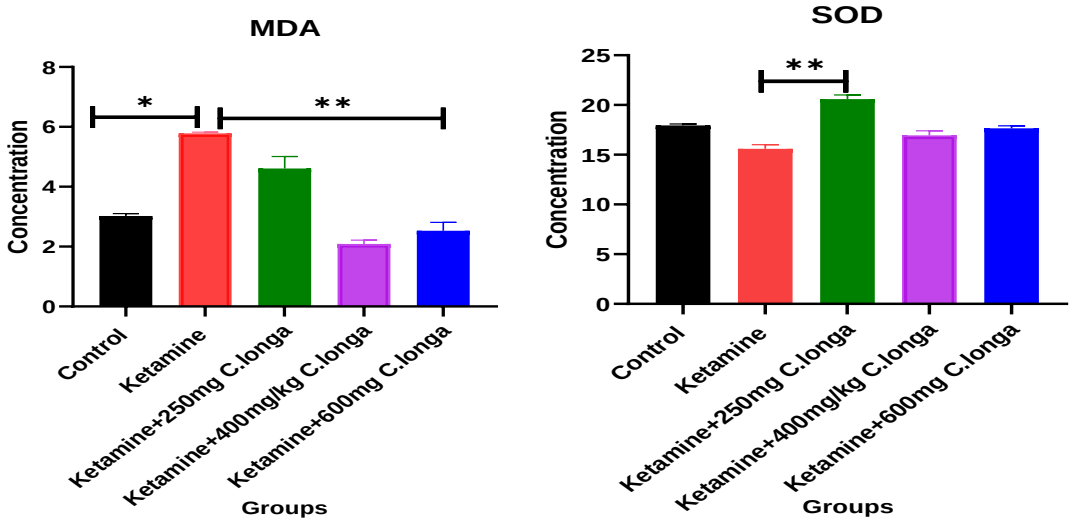


Fig. 1: Effects of C. *longa* on the animal weight. *****Significant decrease in weight compared to A at p≤0.05, ******Significant decrease in weight compared to A at p≤0.05

Effect of *C. longa* on oxidative stress markers

In Table 2 below, induced Ketamine caused a significant increase in Malondialdehyde (MDA) at p≤0.05. There was a dose-dependent decrease in MDA levels in the treated groups. Ketamine untreated group also showed a reduced superoxide dismutase (SOD) and Catalase (CAT) enzyme concentration level, as seen in Figure 2 below, which was also increased on administration of *C. longa*.



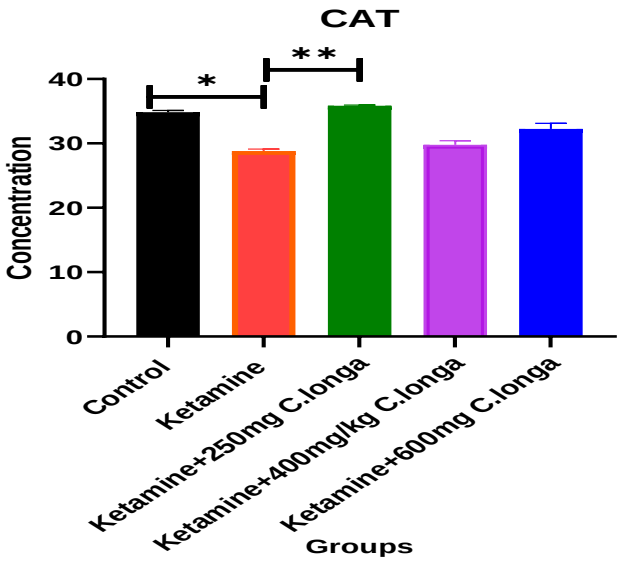
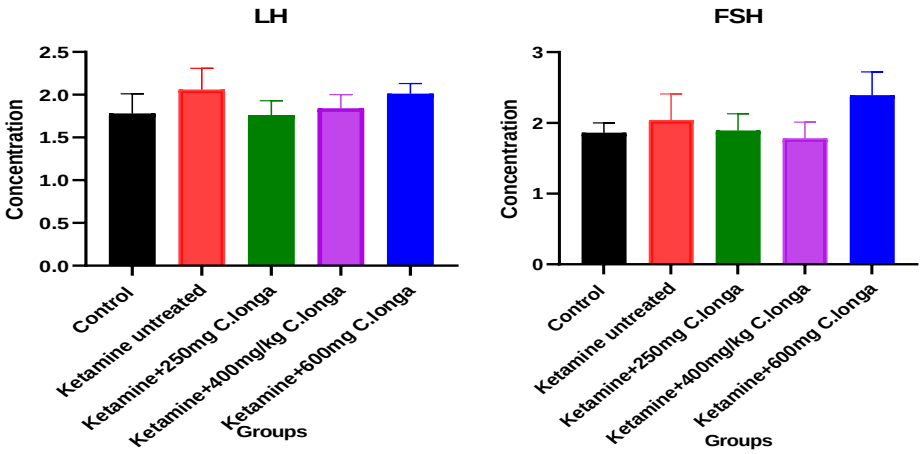


Fig. 2: The effect of *C. longa* on ketamine-induced oxidative stress and antioxidant markers in adult Wistar rats. *Significant decrease compared to control at p≤0.05; **Significant increase compared to ketamine untreated at p≤0.05. SOD-superoxide dismutase, MDA- malondialdehyde, CAT-catalase

Effect of *C. longa* on sex hormones

In this research, Ketamine **(**group**)** caused a rise in Luteinizing hormone (LH), follicle-stimulating hormone (FSH), and a significant increase in estrogen (E2) compared to the control group at p≤0.05. At the same time, *C. longa* lowered the concentration of the sex hormones intermediate level (2.01±0.12) compared to the ketamine untreated group at p≤0.05, as seen in Figure 3 below.



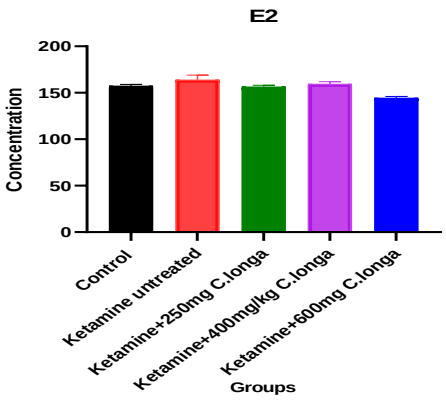


Fig. 3: The effect of *C. longa* on ketamine-induced sex hormones in adult Wistar rats. * Significant increase compared to control group at P≤0.05; **Significant decrease compared to ketamine group at P≤0.05 LH-Luteinizing hormone, FSH-Follicle stimulating hormone, E2-Estrogen

Effect of *C. longa* on stimulating hormones

In the present research, there was a significant increase in the level of Cortisol (17.78±0.03), Lactate Dehydrogenase Hormone (LDH) (453.00±4.04), as well as Antidiuretic Hormone (ADH) (5.16±0.10) in the ketamine group compared to the control group. Furthermore, the efficacy of *C. longa* was shown in Md (19.44±0.18) and Hd (19.44±0.18) groups with a significant increase in their blood concentration. In contrast, LDH increased significantly in treated groups at p≤0.05. The extract (*C. longa*) also lowered the blood concentration of ADH but not at a significant level, as shown in Figure 4 below.

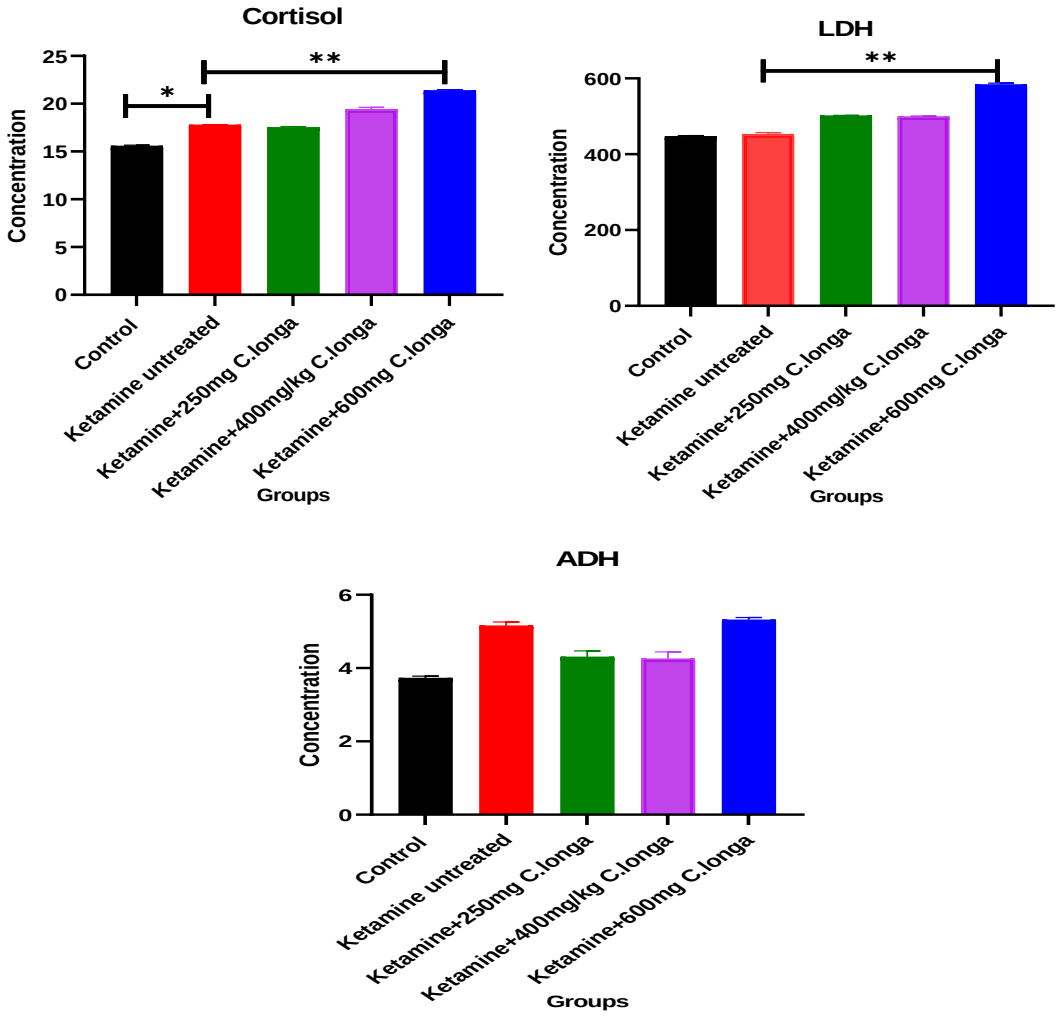


Fig. 4: The effect of *C. longa* on ketamine-induced stimulating hormones in adult Wistar rats. * Significant increase compared to control group at p≤0.05; **Significant increase compared to ketamine group at p≤0.05.

Microscopic observations

Examining the hypothalamus, the control group showed normal neurons, blood vessels, and well-preserved histoarchitecture. In contrast, the ketamine group showed various alterations, such as enlarged vessels, necrotized nuclei, microcystic space, and pyknotic nuclei, as seen in Figures 5A and B. The groups treated with extract starting from Low dose showed regenerating nuclei and numerous neurons as a sign of healing; the medium dose showed normal nuclei, healthy neurons, blood vessels, and multiple neurons, and

the high dose group with nucleated neurons, normal nuclei, and healthy cell nuclei as seen in figure 5C-E respectively. The micrograph of the pituitary gland, as presented in Figures 6A and B below, showed a control group with normal chromophilic cells, basophilic cells, and chromophobes. In contrast, the ketamine group showed several alterations, such as necrotized chromophobes, hemorrhages scattered around the tissue, necrotized basophil, and fatty changes due to toxicity. The low dose group showed restored chromophil and better features; the medium dose group with disappearing hemorrhage, neuron, and chromophils; and the high dose group with healthy acidophilus, healthy basophils, and healthy chromophobes as seen in figures 6C-E below. The ovary from the ketamine group showed several distortions, including severe hemorrhage, necrotized primary follicles, reduced primordial follicles, and atretic follicles (Fig. 7B), while the extract presented near regular ovarian sections.

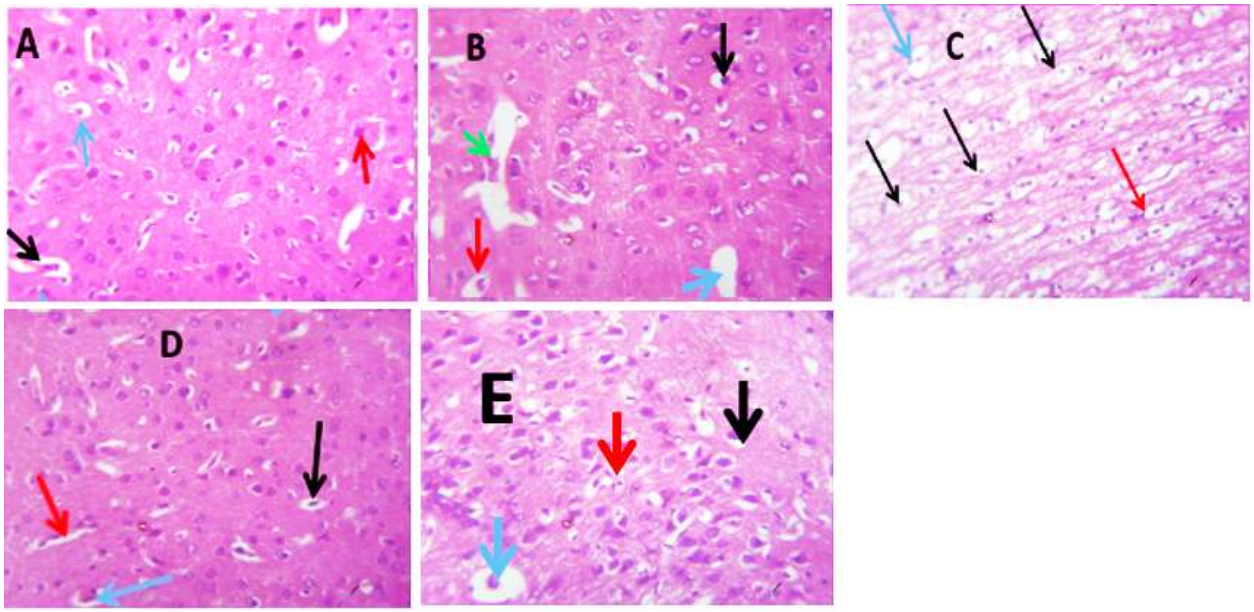


Fig. 5: Micrographs of hypothalamus section showing (A) control group with normal neurons (red arrow), blood vessels (black arrow) and well-preserved histoarchitecture, (B) ketamine group enlarged vessel (green arrows), necrotized nuclei (black arrow), microcystic space (blue arrow) and Pyknotic nuclei (Red arrow); (C) Low dose group with regenerating nuclei (blue arrows), blood vessel (red arrow) and numerous neurons (black arrows); (D) medium dose group with normal nuclei (black arrows), healthy neuron (blue arrow), blood vessel and numerous neurons and (E) high dose group with a nucleated neuron (red arrow), normal nuclei (blue arrow) and cell healthy nuclei (black arrow). Mag X400, H & E.

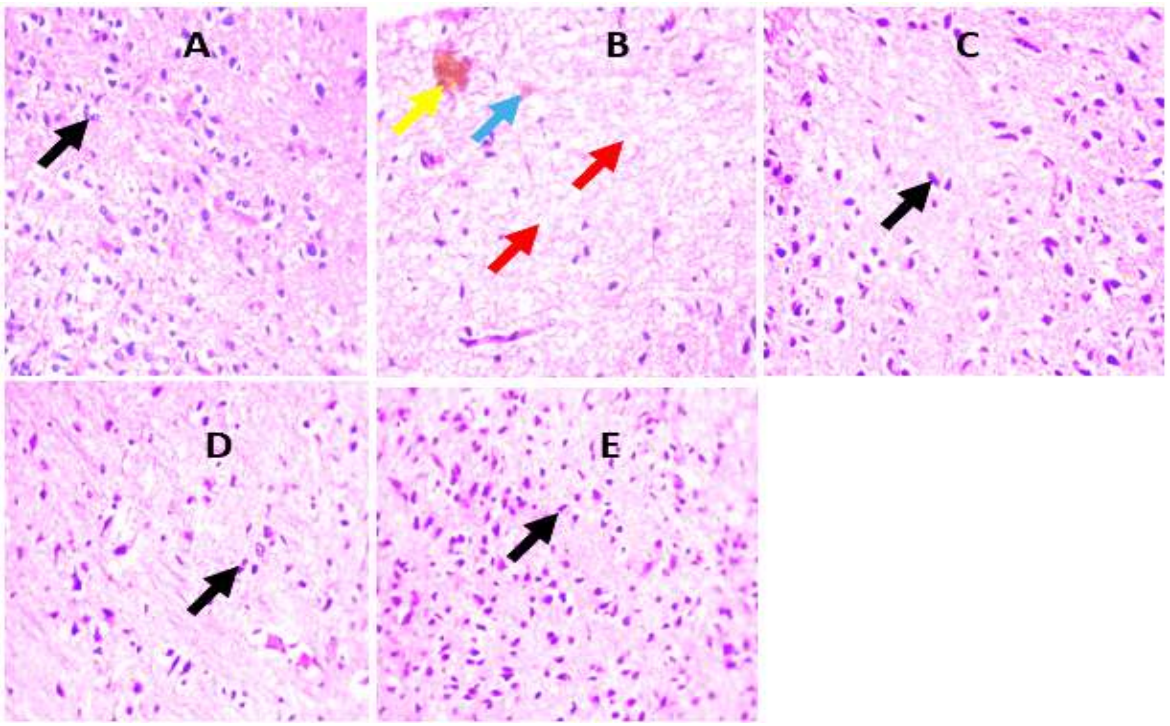


Fig. 6: Micrographs of pituitary gland showing (A) control group with normal chromophilic cells, basophilic cells (black arrows), chromophobes; (B) ketamine group with necrotic area (yellow arrow), hemorrhage (blue arrow), fatty changes affecting the better features; (D) medium dose with disappearing hemorrhage, healthy neuron (black arrow), and (E) high dose group with; healthy acidophils (black arrows), healthy basophils, healthy chromophobes. Mag X400.

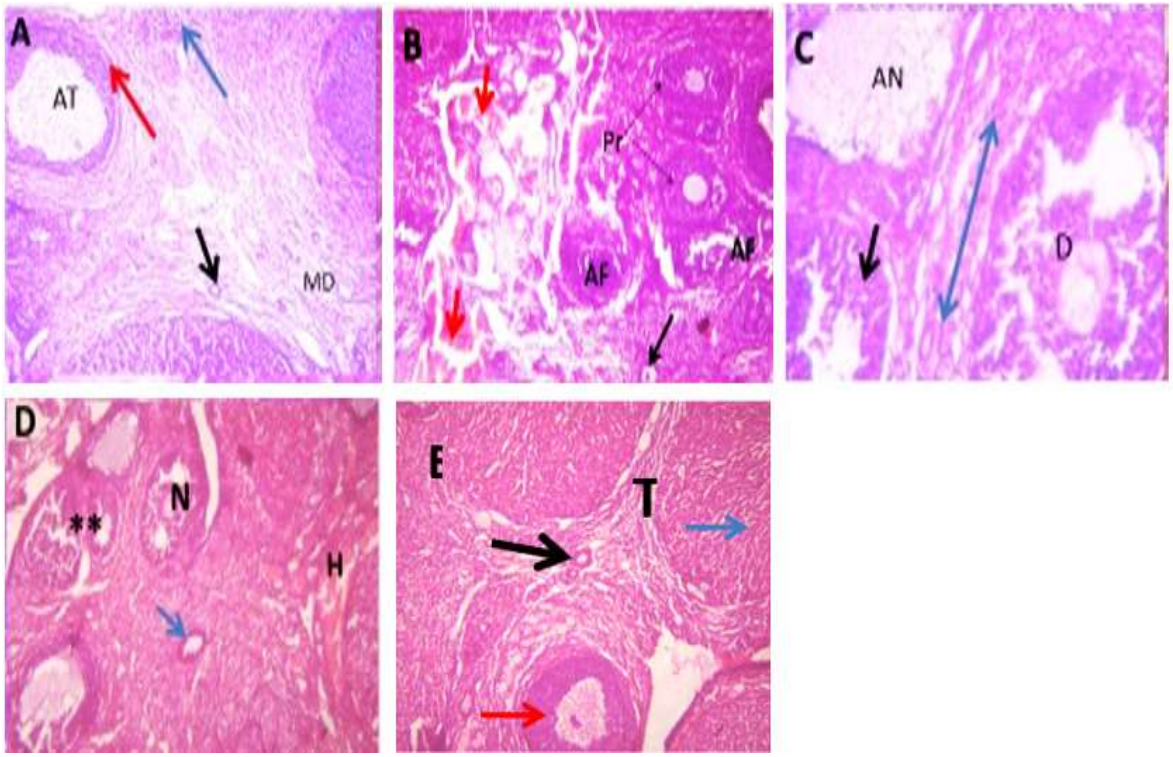


Fig. 7: Micrograph of ovary showing (A) control group with normal ovarian follicles at various stages of development such as primary follicles (red arrow), the primordial follicle (blue arrow), antrum (AT), ovarian medulla (MD), medulla cell (black arrow); (B) ketamine group with severe hemorrhage (thick

red arrow), necrotized primary follicles (Pr), reduced primordial follicle (thin black arrow) atretic follicle (AT); (C) low dose of extract with mild distortion of the primary follicle (D), medulla with regenerating cells (double head arrow), zona granulosa cells (black arrow), pale looking antrum; (D) medium dose with hemorrhagic theca interna and external (H), necrotized primary follicle (N), mild atretic follicle (double star), young primary follicle arrow); (E) high dose of extract with primary oocyte (red arrow), mild hemorrhage (black arrow), theca interna (blue arrow), theca externa (T). Mag X400.

DISCUSSION

Increased reactive oxygen species (ROS) adversely affect the reproductive system, and this detrimental effect can be explained by several mechanisms[42]. In the current study, Ketamine was found to cause lipid peroxidation, translating to an increase in ovarian MDA levels, which might have raised ROS levels, causing weight loss. The *C. longa* significantly decreased MDA level as a sign of its reversal effects. The SOD and CAT levels all decreased in the ketamine untreated due to increased ROS in the blood as an indirect effect of increased MDA, which would have altered the body's antioxidant level. *C. longa* extract was observed to have caused a significant increase in the concentration of SOD and CAT. The effects seen may be attributed to the anti-inflammatory and antioxidant properties of *C. longa*.

Adenohypophysis gonadotropin cells are the primary target site for GnRH, which releases FSH and LH into the circulatory system to regulate gametogenesis. Ketamine has proven to alter this axis by causing significant changes in serum concentrations of LH, FSH, Estrogen (E2), ADH, LDH, and cortisol. On this premise, we put forward that Ketamine is capable of altering the reproductive and genital system by destabilizing the equilibrium of HPO, and this it does by hiking the stimulating hormone and production of steroid hormones. However, the results from this study contradict those of Qi *et al*.[43], who reported a dose-dependent decrease in LH and FSH in response to Ketamine. Similarly, Paulis *et al*.[28] reported just a reduction in FSH and LH both in male rats. The increased serum estrogen (E2) levels might be a partial indicator or response to increased concentration of the gonadotrophic hormones and directly point out the harmful effect of Ketamine on ovarian follicles.

Further, Ketamine has been implicated in inducing lipid peroxidation and cell apoptosis, which may be the mechanisms on which the anesthesia acts on the follicles[44]. The effect of Ketamine on LH, FSH, and E2 may be attributed to the direct depressant/activation of the pituitary gland. Also, the lowering of these serum hormonal levels of *C. longa* dose-dependently suggests its potential to restore the equilibrium of the HPO axis. The body is continually subjected to stress, and the drug can stress every system and keep it active.

Cortisol acts on various organs, such as the reproductive and sympathetic nervous systems[45]. The corticotrophin-releasing hormone (CRH) is released by the paraventricular nucleus (PVN) of the hypothalamus, stimulating the anterior pituitary to release adrenocorticotropic hormone (ACTH) that works on the adrenal cortex to release cortisol[46,47]. Additionally, the hypothalamus continues stimulating the hypothalamic pituitary adrenal axis (HPA) for the adrenal cortex to release cortisol as long as the body interprets the stimuli as dangerous, enabling the body to maintain its heightened alert[48]. The body immediately gets energy from cortisol's catabolic processes[49]. The effects of Ketamine on the hypothalamus would have caused a significant surge in the blood concentration level of cortisol. Thus, the inference can be made that since Ketamine alters the HPO axis, it affects the body’s circadian rhythm[50]. The *C. longa* could not lower the plasma level of cortisol within the period under consideration but up-surged it, especially in the medium and high doses. The hypothalamus produces an antidiuretic hormone (ADH) crucial for maintaining the osmotic equilibrium of the body [50,51] by triggering increased reabsorption of fluids back into the body in the collecting duct and distal tubule during transportation[51,52]. The

histological alteration of the hypothalamus led to the disruption of the HPO axis, which would have caused a surge in the ADH that was decreased by low and medium doses of *C. longa*, but it was not significant enough, and the high dose increased it, an indication that Ketamine can be effectively used in regulating the blood concentration of ADH but at low and medium doses. The enzyme oxidoreductase class, including lactate dehydrogenase (LDH), is a crucial component of the anaerobic metabolic pathway[53]. The enzyme catalyzes the reversible transformation of lactate into pyruvate and vice versa[54]. Typically, it is used to track or identify interior tissue damage or monitor the exact condition responsible for the harm[55]. It has been established that high lactate dehydrogenase levels in the circulatory system frequently suggest tissue injury[56]. The increased plasma level of LDH caused by Ketamine in this study may partly indicate the extent of tissue damage. Unfortunately, the extract couldn't lower the surge under consideration.

Microscopically, sections of the hypothalamus, present normal histoarchitecture with all the cells and blood vessels preserved[57]. In contrast, the ketamine untreated showed necrotized nuclei and vessel enlargement. The effects of the ketamine were seen to be reduced by the administration of the extract with healthy nuclei. The section of the pituitary gland from the control showed normal chromophilic cells, basophilic cells, and chromophobes[57] while the ketamine untreated with necrotized chromophobes and hemorrhage. scattered within the tissue. The treated rats showed healthier histoarchitecture with little or no necrosis in a dose-dependent manner. The micrographs of the ovary from the control showed normal ovarian follicles at various stages of development[15]. At the same time, the severe hemorrhage, necrotized follicles, and atretic follicles seen later would have been due to the effects of the administered ketamine. This finding is consistent with the report of Qi *et al*.[43,58] in which they found out that ketamine is very toxic to the reproductive system and produces fewer antioxidants. The decreased level of antioxidants due to the presence of ketamine invariably increases the level of reactive oxygen species (ROS) and oxidative stress markers leading to the damage seen in the ovaries. The alterations caused by ketamine were seen to be ameliorated on the administration of the extract, which translated to an increased number of follicles at various stages of maturity.

CONCLUSION

Ketamine, as an anesthetic, can hamper the HPO system and impair reproduction. *C. longa* is a spice that might be used to alleviate toxicity induced by Ketamine on the Hypothalamic-Pituitary-Ovarian axis.

Authors contributions

UOO, OAE, OOS, and AOI conceived the topic; UOO, AOI, OC, OOS, OTF, and AEU conducted the practical; UOO, OAE and OOS analyzed the data. All authors conducted the literature search and proofread the final copy of the work.

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