Curcumin improves cognitive function by restoration of neurotransmitter balance, attenuate oxidative stress, inflammation and improves hippocampal integrity in intestinal ischemia/reperfusion injury induced-cognitive deficit in aged female Wistar rats

ABSTRACT

Cognitive deficits, more common with age, affect memory and other functions and can result from various health issues. Intestinal ischemia-reperfusion injury (IIRI) causes systemic inflammation that can damage the brain, particularly the hippocampus, leading to cognitive decline. The hippocampus, critical for memory, is highly susceptible to damage from stress, inflammation, and neurodegeneration. Studies in aged female rats show significant cognitive impairment. Curcumin, an active ingredient found in turmeric has been shown to possess antioxidative, anti-inflammatory, antiapoptotic and well as neuroprotective properties was used in this study. The aim of this study is to investigate the effect of curcumin on intestinal ischemia reperfusion injury induced-cognitive deficit in aged female Wistar rats.

Forty female Wistar rats between 200-250 kg were used in this study, they were weighed and randomly divided into four groups n=10. 150ml/g of D-Galatose was administered for eight weeks to induce aging. The animals pretreated with 100mg/kg for twenty-one days prior to the induction of IIRI. To induce IIRI, the abdominal region of the animals was shaved prior, an abdominal incision was made, then a gentle pressure was exerted to expose the intestine after which the ileocecal junction was located, five centimeter was measured from the ileocecal junction proximally and distally using infusion line, then three centimeter was anchored in between the measured intestine. It was twisted clock wisely at 720^{o}, to induce ischemia for two hours and closed with 2.0 chromic suture, after two hours, the animals were opened and the intestine was untwisted for reperfusion to occur, after seventy-two hours of reperfusion, behavioral assessment (Novel Object Recognition (NOR) test, T-Maze, and Y-Maze) to investigate the behavioral functions. Animas were sacrificed and the brain was collected, the hippocampus was isolated.oxidative stress parameters (SOD, MDA, GSH, catalase), and inflammatory markers (TNF-α, MPO, Nitric oxide) were assessed. Caspase-3 activity was also measured to evaluate apoptosis, and histological examination of the hippocampus was performed. Data were analyzed using one-way ANOVA followed by Tukey's post hoc test with statistical significance set at $p<0.05$.

The result shows that there was an increase in % Alternation in IIRI group when compared to CUR+IIRI ($p<0.001$) but with no significant difference in the number of entries in Y-Maze test, this shows hyperactive exploratory behavior indication inability to remember already visited arm but stable no of entries indicates cognitive deficit which not disrupted locomotive activities. There was a reduction in the neurotransmitter level (AchE, Serotonin and Dopamine) in IIRI groups which was revered in the CUR+IIRI groups ($p<0.001$). curcumin also ameliorated oxidative stress (OS) by increasing antioxidant in the hippocampal tissues, which could be as a result of its antioxidant property. Inflammatory markers (TNF-α, MPO) were also significantly reduced in CUR+IIRI group when compared to IIRI group ($p<0.001$), which suggests curcumin has a modulatory effect on inflammatory cascade. Caspase-3 activity was reduced, indicating reduced apoptosis. Histological assessment shows that curcumin preserved hippocampal architecture, whereby reducing IIRI-induced cognitive deficit in aged female Wistar rats.

This study showed that curcumin demonstrates significant neuroprotective effects against IIRI-induced Cognitive deficit.

Keywords: Intestinal ischemia/reperfusion injury, Aging, Behavioral, Neurotransmitters, Hippocampus, Curcumin.

1.0 Introduction

Cognitive deficit is characterized by deficiencies in executive function, language, memory, attention, and visuospatial abilities. It can greatly affect capacity to execute daily activities and independence. It can range in severity from mild to severe (Kirova*et al*., 2015). As people age, cognitive deficiencies become more common (Murman, 2015). About 15-20% of elderly female individual of 65 years of age and above suffer from mild cognitive impairment (MCI), which is frequently seen as a prelude to dementia (Anderson, 2019). A variety of conditions, including neurodegenerative diseases, vascular disorders, traumatic brain damage, infections, metabolic disorders, and psychiatric disorders, can result in cognitive loss (Brett *et al*., 2022).

A well-known model of systemic inflammatory response syndrome is intestinal ischemia-reperfusion (I/R) injury,which is the temporary occlusion of bloodflow to the intestine followedby re-establishment of the blood flow to the intestine (Arumugam *et al*., 2004; Arumugam *et al*., 2006), this condition could occur as a consequence of various conditions like cardiac insufficiency, necrotizing enterocolitis, trauma, atherosclerotic thrombus, embolism, or surgical complications, ischemia in the intestines is a relatively common occurrence in humans (Edwards *et al*., 2004; Oldenburg *et al*., 2005). Damage to the intestinal mucosal epithelium and villi, significant impairs local microvasculature, increased vascular and mucosal permeability resulting in edema, leukocyte plugging of capillaries, and systemic sequelae such blood acidosis, sepsis, multiple organ damage (MODs), and systemic inflammatory responses (SIRs) are among the consequences of an I/R injury (Hsieh *et al*., 2011).Increased brain permeability brought on by inflammation enables proinflammatory mediators to penetrate brain and interact directly with neural cell extracellular receptors (Huang *et al*., 2021). This may cause cerebral inflammation, which in turn alters the hippocampus, the structure of the brain involved in learning, memory, and cognitive functions, ultimately resulting in cognitive deficit (Skelly *et al*., 2019). Ischemic events, such as stroke, can cause severe harm to the hippocampal region, resulting in the death of neurons and a decline in memory function. These events also disrupt blood flow, which further exacerbates neurodegeneration in the hippocampal region (Farooqui, 2010; Dhungana, 2014). Due to the hippocampus's ongoing battle to play a vital role in cognition, persons who are impacted frequently experience difficulties remembering recent experiences or picking up new knowledge (Lane *et al*., 2015). MODS and localized damage to a number of essential organs, such as the kidney and liver, have been directly linked to systemic inflammatory diseases that can induce encephalopathic symptoms (Zhou, 2023).

Furthermore, ischemia damage from vascular diseases might impair neural transmission and brain integrity (Levit *et al*., 2020). Other causes that lead to cognitive impairment include oxidative stress, neurotransmitter imbalances, genetic factors, and inflammatory responses (Rekatsina*et al*., 2020; Teleanu*et al*., 2022; Leyane*et al*., 2022).

The hippocampuscan be severely damaged by oxidative stress, apoptosis, and inflammation, which can ultimately result in cognitive deficiencies (Gu *et al*., 2021). Reactive oxygen species (ROS) generated due to excessive oxidative stress, which damages neurons, interferes with mitochondrial function, and reduces synaptic plasticity, a crucial component of memory formation (Beckhauser*et al*., 2016). In addition to decreasing cognitive capacities, oxidative

damage can also cause apoptosis, or programmed cell death, which results in the loss of neurons and synaptic connections (Reis *et al*., 2022).

The brain's immune cells, known as microglia, are activated by chronic inflammation and generate inflammatory cytokines that damage neurons and compromise the blood-brain barrier (Gullotta *et al*., 2023). According to Gomez-Arboledas*et al*. (2021) this inflammation may cause excessive synaptic pruning as well as neurodegeneration. According to Escudero-Lourdes (2016), oxidative stress, apoptosis, and inflammation increase the risk of neurodegenerative diseases, which includes cognitive impairment, by causing neuronal death, reduced synaptic function, and gradual cognitive decline.

Reduced physiological and biochemical organ functions are hallmarks of aging which is a biological transformation (Dharmarajan, 2021). Additionally, it has a negative impact on cognitive processes, leading to a decline in coordination and locomotor activity as well as learning and memory impairment (Nakanishi *et al*., 2021). Numerous lines of evidence point to a progressive decline in learning and memory with age, and that this age-related deficit also affects activities involving spatial learning and memory. Numerous research (Belviranl*et al*., 2013; Foster, 2012) have suggested that changes in the morphology and function of the hippocampus formation are the cause of the age-related reduction in spatial learning ability.

Research has indicated that cognitive abilities in adult female rats are significantly reduced when compared to younger rats, as seen by observations made in a variety of maze models, including the Morris water maze, T-Maze, and Y-Maze. Laboratory rats can be used to estimate human life expectancy, with 13.8 rat days equal to one human year (Quinn, 2005; Sengupta, 2012). Rats live an estimated 2-3.5 years (Pass and Freeth, 1993; Sengupta, 2012).

Asian medicine has traditionally employed curcumin, a yellow pigment derived from the rhizome of the plant Curcuma longa, as a traditional therapeutic treatment to treat a variety of illnesses (Jovanovic *et al*., 2001; Aggarwal and Harikumar 2009). Previous research on humans and laboratory animals has shown that curcumin has antioxidant, anti-inflammatory, antiapoptotic, antiproliferative, anticancer, antidepressant, immunomodulatory, and neuroprotective properties (Xu *et al*., 2005; Bala *et al*., 2006; Jagetia and Aggarwal 2007; Zhao *et al*., 2008). According to epidemiologic data, supplementing with curcumin appears to reverse several forms of cognitive impairment in rats and mice models. Regular consumption of curcumin also improves cognitive performance in healthy aged adults (Ng *et al*. 2006; Reeta *et al*. 2009; Tian *et al*. 2012). Studies have investigated the effect of curcumin on neurological disorder such as AD, however, there are limited study addressing cognitive deficit, a major clinical feature of AD in aged individual hence, the need for this study.

Methodology

3.1 Experimental Animals

Forty (40) female Wistar rats (200-250g) were purchased from Animal house of Ladoke Akintola University of Technology (LAUTECH), Ogbomoso, Oyo State, Nigeria.Ogbomosho, Oyo State, Nigeriabefore the onset of the study. They were acclimatized for two weeks and kept throughout the experiment in well aerated plastic cages in the animal house (temperature 28-31^{0}C; photoperiod: 12-h natural light and 12-h dark; humidity:50-55%) of Faculty of Basic Medical Sciences (FBMS), LAUTECH, were fed with pelletized feed obtained from commercial dealer in

Ogbomoso and watered *ad libitum*. The animal handling procedure was carried out in accordance with guidelines for the use and care of laboratory animals approved by LAUTECH's animal care and use research ethical committee.

Ethical Approval

Ethical approval was obtained from Faculty of Basic Medical Sciences, LAUTECH Ogbomoso with the approval number: ERCFBMSLAUTECH:056/08/2024.

3.2 Drugs and Reagents

All drugs and reagents used were of high analytical grade. D-galactose (Pan ReacAppliChem, Castellar del Vallès, Barcelona, Spain. Product code: A11311), curcumin (Cenik Chemicals, Sully Penarth, United Kingdom). Procaine penicillin (Anhui Chengushi Pharmaceutical CO., Ltd. China), Ketamine (Swiss Parenterals Ltd. India), Xylazine (BiovetaKomenskeho, Pharmaceutical, Czech Republic), normal saline, distilled water (Department of Pure and Applied Chemistry, LAUTECH, Oyo, Nigeria), buffered formalin (Department of Anatomy, FBMS, LAUTECH, Oyo, Nigeria), phosphate buffer saline (Department of Science Laboratory Technology, LAUTECH, Oyo, Nigeria).

3.3 Materials

Plastic cages, feeds, wood shavings, drinkers and feeders, weighing scale, surgical gloves, latex gloves, needle holder, universal bottles, plain bottles, EDTA bottles, razor blade, ethanol, sanitizer, methylated spirit, tissue paper, cotton wool, dissecting board, dissecting kits, 2.0 chromic sutures, sterile infusion set (tourniquet), paper tape, centrifuge, sensitive scale, incision set, ruler, normal saline, paper tapes, permanent markers, homogenizer, foil paper, Pasteur pipette, Syringes (insulin, 2ml and 5ml), Eppendorf bottles, methylated spirit, stop watch,

3.4 Experimental Design

Forty (40) female Wistar rats were divided into four groups of ten (10) animals each: **Group 1**; Curcumin (CUR), **Group 2**; Sham operated (SO), **Group 3**; Intestinal ischemia-reperfusion injury (IIRI) and **Group 4**; Curcumin and Intestinal ischemia-reperfusion injury (IIRI) (CUR + IIRI).

The treatments were given before the induction of IIRI. Intestinal ischemia was induced for 90 minutes while intestinal reperfusion was maintained for 72 hours.

The groups and their treatments are described below.

Group 1: Curcumin: Rats were treated with curcumin at the dosage of 100mg/kg

Group 2: Sham Operated (SO); Rats were subjected to mid-line abdominal incision only. involving mobilizations and replacement of intestine into the abdomen.

Group 3: Intestinal Ischemia Reperfusion injury (IIRI)

Group 4: Curcumin + Intestinal ischemia reperfusion injury (IIRI) (CUR + IIRI) group: Rats were treated with curcumin at the dosage of 100mg/kg orally for 21 days surgical induction of IIRI, after 21 days the animals were surgically induced with intestinal ischemia for 120 minutes

and intestinal reperfusion was established after 90 minutes of ischemia, the animals were sacrificed 72 hours after reperfusion.

3.5 Surgical procedure for inducing intestinal ischemia reperfusion injury

i. Animals were shaved at the abdominal region and cleaned with normal saline; then fasted for 12 hours before the surgical induction of Intestinal ischemia.
ii. They were weighed and anaesthetized with Ketamine and Xylazine intraperitoneally (ketamine at 50mg/kg; xylazine at 10mg/kg) (Lorenzini *et al*., 2012).
iii. The animals were placed on a dissecting board and pinned down at the limbs.
iv. A ventral midline laparotomy incision was made to open the abdominal cavity.
v. Operating scissors was used to locate the small intestine and the superior mesenteric artery.
vi. An infusion set of 5 cm was measured with a ruler and then cut to the appropriate size for measurement.
vii. The intestine was lifted exposing the superior mesenteric artery: Some of the small intestine was measured from the proximal end of the ileocecal junction and 5cm to the distal end of the gut.
viii. A small piece (3cm) was cut out from the infusion set (tourniquet), cut open, and wrapped on the measured part of the intestine and was sutured together lightly, taking care not to puncture the organ or any vessels.
ix. Using the region wrapped with the infusion tube as a hand-hold, the gut was twisted clockwise 360 degrees.
x. After twisting, the exposed gut was pushed back gently whilst ensuring that the twisted part is not loosened, and the abdominal region was closed with 2.0 chromic suture.

After 90 minutes ischemia, paleness of the intestine was observed.

The intestine is then untwisted to restore blood flow and animals were sacrificed after 72 hours.

3.6 Mode of Sacrifice, and Tissue Collection

3.6.1 Mode of Sacrifice

Seventy-two (72) hours after reperfusion, the animals were anesthetized using ketamine (50

Blood was collected through cardiac puncture with the use of 2ml syringe and needle and introduced into EDTA bottles.

3.6.2. Tissue Collection

• The hippocampus was harvested *a*nd cleared of adherent tissue.

• The harvested tissue was labelled appropriately and kept in universal bottles.

• The tissue was maintained at cold temperature after which it was homogenized

3.6.3. Preparation of Tissue homogenate

The hippocampalwere harvested and cleared of adherent tissue. The harvested tissue was placed in a foil paper and weighed immediately with sensitive weighing scale (Lisay, China).The hippocampaltissue was washed in cold normal saline to remove blood stain. Some of the tissues were collected into sample bottles containing formaldehyde and labelled appropriately for

histological examination.Other hippocampal and tissue werehomogenized. For preparation of tissue homogenates,a specific weight of hippocampaltissue was homogenized in a universal bottle containing cold phosphate buffer (p^H = 7.2) in the ratio of 1:5. The homogenates were centrifuged at 3000 revolutions per minutes for 15 minutes with cold centrifuge. The supernatants were collected into Eppendorf bottles and refrigerated at -4 ^oC for further assays.

3.7 Determination of hippocampal and weights

The weight of the hippocampus was measured using weighing scale (Lisay, China).

3.8 Behavioral evaluation

3.8.1 Novel objects recognition (NOR) test

The novel object recognition test is a behavioral test for spatialworking memory in rats. It consists of two sessions; firsttrial (T1) and the second trial (T2). The two trials were separatedby an inter-trial interval of 2 h. In T1, each rat was put inthe test box (22.5 × 24.5 × 11.0 cm), in which two identical objects (truncated cone: diameter, 4.5 and 6.0 cm; height,3.5 cm) were placed at two adjacent corners, and the timespent exploring each object was measured for 5 min. Duringtraining, the animals explored between 40 and 60 % of thetime on each object. Immediately after T1. In T2, a novel object replaced theobject explored less by the mouse in T1, and the time spentexploring the familiar object (F) and the novel object (N) wasrecorded for 5 min. Objects were made of odorless plasticand similar in size. Between each trial, the objects and the fieldwere cleaned. The total time spent sniffing or touching eachobject with the nose and/or forepaws were recorded.Recognition memory index was calculated by a discriminationindex (%) using the method of Adeniyi *et al*. (2024).

Memory Index (%)= ([(Tnovel - Tfamilar)) / (Tnovel + Tfamiliar)] x 100).

Biochemical evaluation

Determination of acetylcholinesterase (AChE) activity

The AChE enzymatic assay was determined using a modificationof thespectrophotometricmethod of Ellman *et al*. (1961) aspreviously described by Akinyemi *et al*. (2016). The reactionmedium (2 mL final volume) contained 100 mmol/L of K+-phosphate buffer, pH 7.5, and 1 mmol/L of 5,5′-dithiobisnitrobenzoicacid. The method is based on the formationof the yellow anion, 5,5'dithio-bis-acid-nitrobenzoic,measured by absorbance at 412 nm during 2-min incubationat 25 °C. The enzyme (40–50 µg of protein) was preincubatedfor 2 min. The reaction was initiated by adding0.8 mmol/L of acetylthiocholine iodide. All samples were runin triplicate, and enzyme activity was expressed in micromoleacetylthiocholine (ACh) hydrolysed per hour per milligramof protein. Serotine and dopamine were assayed using a their respective colorimetric assay kit from bio vision and ratELISA kit from Fine test with a catalog no:ERO 77. The manufacturers'procedures for assays were strictly followed.

2.9 Biochemical Analysis

Brain lipidperoxidation (MDA) levelwas estimated, brain superoxide dismutase activity level,brain reduced glutathione (GSH) level and brain catalase activity level.The extent of lipid peroxidation in the brain was determinedquantitatively (Wills, 1966). Briefly, the samples were mixed with 1 mLof 10 % trichloroacetic acid and 1 mL of 0.67 %, thiobarbituric acidthen heated

in boiling water bath for 15 min after which butanol (2:1v/v) was added to the solution. The amount of malondialdehyde (MDA)was measured by reaction with thiobarbituric (TBA) acid at 532 nmusing UV Spectrophotometer. The values were calculated using themolar extinction coefficient of MDA-TBA adduct at 532 nm is 155(mM−1 cm−1).

Superoxide Dismutase Activity (SOD) was assessed by NitroblueTetrazolium (NBT) method based on the principle that NBT undergoes aphoto-reduction (which is blue colored formazan) when exposed tolight by superoxide radicals. It competes with the enzyme SOD for superoxideanions. With the presence of SOD in the reaction mixture, NBTproduces a lesser quantity of coloured complex as compared to control.

Hippocampal tissue homogenate (500 µL) was mixed with chloroform(300 µL) and ethanol (500 µL). The mixture was centrifuged at 18,000× g for 30 min. 50 µL of supernatant was mixed with 900 µL of SODreagent (0.1 mmol/L xanthine, 0.1 mmol/L EDTA, 50 mg bovine serumalbumin, 25 mmol/L NBT and 40 mmol/L Na2CO3) (pH 10.2). Further,twenty-five units of xanthine oxidase was added to the mixture andincubated for 20 min at 25 °C. The reaction was stopped by adding 1 mLof CuCl2 (0.8 mmol/L) and absorbance was recorded at 560 nm(Pal*et al*., 1993).

The reduced glutathione (GSH) content in the mice brain wasinvestigated spectrophotometrically at 412 nm. Briefly, in a test tube,the supernatant of the brain homogenate and trichloroacetic acid (10 %w/v) were mixed in 1:1 ratio and then the test tubes were centrifugedat1000xg for 10 min at 4□. The supernatant (0.5 mL) was mixed with0.3M disodium hydrogen phosphate (2 mL) and 0.25 mL of 0.001Mfreshly prepared DTNB [5,5′-dithiobis (2-nitrobenzoic acid) dissolved in1% w/v sodium citrate] was added and the absorbance was measuredusing a spectrophotometer at 412 nm. A standard curve was plottedusing 10−100 µM of the reduced form of glutathione and the resultswere expressed as micromoles of reduced glutathione per mg of protein(Das *et al*., 2019).

The activity of catalase enzyme was assessed through a spectrophotometerat 240 nm. Briefly, 1 mL of the brain homogenate was takenin a test tube and 1.9 mL of the phosphate buffer (50 mM, pH 7.4) wasadded. The reaction was initiated by the addition of 1 mL of 30mMH_2O_2. The mixture of 2.9 mL of phosphate buffer and 1 mL of H_2O_2without the brain homogenate was taken as blank. The decompositionof H_2O_2resulted in the reduction of absorbance, which was recorded at240 nm against the blank. The unit of catalase activity was expressed asthe amount of enzyme that decomposes 1 µM of H2O2 per min at 25 °Cusing molar coefficient of 43.6 M−1 cm−1 and the activity was expressedin term of unit/mg proteins (Das *et al*., 2019).

Hippocampal homogenates were used to assess superoxide dismutase (SOD) activity spectrophotometrically using the protocol of Obay*et al*, (2008).

Measurement of nitric oxide (NO)

NO content in tissue homogenates was estimated in a medium containing 400 µL of 2 % vanadium chloride (VCl3) in 5 % HCl, 200 µL of 0.1 % N-(l-naphthyl)ethylenediaminedihydrochloride, 200 µL of 2 % sulfanilamide (in 5 % HCl). After incubating at 37 °C for 60 min, nitrite levels, which corresponds to an estimative of levels of NO, were determined spectrophotometrically at 540 nm, based on the reduction of nitrate to nitrite by VCl3. Hippocampal nitrite and nitrate levels were expressed as nanomole of NO/mg of protein.

Myeloperoxidase (MPO) activity was assess as described by Pulli *et al.* (2013). TNF-α andCaspase 3 were also accessed.

Histological Analysis

The brains were stored in 10 % formaldehyde and fixed in methanol/chloroform/acetic acid solution (6:3:1) and dehydrated in ethanol. The dehydrated material was clarified with xylene and embedded in Paraplast. Then paraffin embedded coronal sections were cut (3 µm thickness) using a microtome and stained with hematoxylin (H) and eosin (E), after which it was mounted onto silane-coated slides for microscopic examination at 100x magnification.

2.12 Statistical analysis

Data were expressed as mean ± standard error of mean (Mean ± SEM). Analysis was performed with Graph Pad Prism, Version 7.0 (Graph Pad software, Inc., USA) was used to compare within group and Tukey's Post-test was used for multiple comparison P-Values less than 0.05 were considered statistically significant.

3.0 Result

Results obtained from behavioral study (NOR) shows that there was statistically significant decrease in in both discrimination index and spatial short-term memory in intestinal ischemia reperfusion (IIRI) group when compared with Cur treated groups. In discrimination index, there is a significant decrease in IIRI when compared with CUR+IIRI ($p<0.05$). Spatial short-term memory shows that there was a statistically significant decrease in sham when compared with IIRI group ($p<0.05$), there was a statistically significant decrease in IIRI when compared with CUR+IIRI group ($p<0.05$) (Fig. 1A and B).

AchE, Serotonin and Dopamine were increased in CUR+IIRI groups when compared with IIRI group. There was a statistically significant increase in AchE level in sham group when compared with IIRI ($p<0.05$), Also in CUR+IIRI group when compared with IIRI group ($p<0.001$). There was a statistically significant increase in AchE level in sham group when compared with IIRI ($p<0.05$). There was a statistically significant increase in Serotonin level in Sham when compared with IIRI group ($p<0.001$) and CUR+IIRI when compared with IIRI group ($p<0.001$). there was a statistically significant increase in dopamine level in CUR+IIRI when compared with IIRI ($P<0.05$) (Fig. 2A, B and C).

The biochemical parameters obtained from the hippocampus includes CAT and SOD, the result showsthat they were significantly decreased in IIRI groupwhen compared with sham and CUR+IIRI group ($p<.0.001$)while MDA significantly increased in IIRI group when compared with CUR+IIRI group ($p<.0.001$). GSH shows no significant difference across the groups (Fig. 3A, B, C and D).

TNF-α, MPO and NO were significant increased in IIRI group when compared with CUR+IIRI groups. There was a statistically significant increase in TNF-α in IIRI when compared with CUR+IIRI ($p<0.05$). MPO were decreased in sham and IIRI+CUR groups ($p<0.05$; $p<0.05$) when compared with IIRI ($p<0.05$).NO shows that there was a statistically significant increase in IIRI when compared to sham and CUR+IIRI groups ($p<0.001$;$p<0.001$) (Fig. 4A, B and C).

Caspase 3 were increased sham groups when compared with IIRI group ($p<0.01$). Also, there was a statistically significant increase in IIRI group when compared with CUR+IIRI group ($p<0.05$) (Fig. 5A).

4.0 Discussion

The Novel Object Recognition (NOR) test is commonly used to assess cognitive functions, particularly recognition memory, which falls within the broader domain of cognitive abilities such as spatial and short-term memory. Intestinal ischemia-reperfusion injury (IIRI) is known to induce systemic inflammation and oxidative stress, which can extend to the brain, especially the hippocampus (Yang *et al*., 2020). The hippocampus plays a critical role in memory and cognitive functions. Consequently, the damage caused by IIRI likely resulted in neuronal injury, synaptic dysfunction, and reduced neurogenesis, leading to impaired cognitive performance, as demonstrated by the low or negative discrimination index observed in the NOR test.

The improved discrimination index in the CUR+IIRI group indicates that curcumin treatment alleviated the cognitive deficits caused by IIRI. Curcumin likely exerted neuroprotective effects on hippocampal neurons by counteracting the harmful consequences of inflammation and oxidative stress triggered by IIRI. Additionally, curcumin may have promoted synaptic plasticity and enhanced neurotransmitter systems involved in learning and memory, thus improving the processing and retention of new information.

Acetylcholinesterase (AChE), serotonin, and dopamine are key neurotransmitters involved in cognitive function, mood regulation, and memory. Their balance and function are critical for maintaining normal cognitive processes, and disruptions in their levels can lead to significant impairments, particularly in the context of conditions such as intestinal ischemia-reperfusion injury (IIRI).

AChE is responsible for breaking down acetylcholine, a neurotransmitter that facilitates learning and memory. Elevated AChE activity reduces acetylcholine levels, leading to impaired cholinergic transmission and cognitive dysfunction (Ozdemir *et al*., 2019). In the IIRI group, the decrease in AChE activity suggests that cholinergic transmission was disrupted, likely due to oxidative stress and neuronal damage caused by IIRI. This impairment negatively affects cognitive functions such as learning and memory. However, curcumin, with its potent antioxidative and anti-inflammatory properties, likely helped restore neuronal integrity and normalize AChE activity (Ashafaq*et al*., 2023). By preserving acetylcholine levels and enhancing cholinergic signaling, curcumin supports cognitive function, particularly in memory and learning (Agrawal *et al*., 2017).

Serotonin, another crucial neurotransmitter, plays a significant role in mood regulation, cognition, and memory. A reduction in serotonin levels is often associated with mood disorders and cognitive deficits (Jenkins *et al*., 2016). In the IIRI group, decreased serotonin levels can be attributed to oxidative stress and neuroinflammation, which damage serotonergic neurons and disrupt neurotransmitter synthesis and release (Muraleedharan and Ray, 2024). This disruption contributes to both mood disturbances and cognitive decline. Curcumin's neuroprotective effects help counteract these detrimental processes, preserving serotonergic neurons and maintaining serotonin levels (Winiarska-Mieczan*et al*., 2023). The increase in serotonin levels in the curcumin-treated

IIRI group suggests that curcumin supports serotonergic function, which is essential for maintaining mood stability and cognitive performance.

Dopamine is similarly vital for cognitive processes, including motivation, reward, and attention (Aarts *et al*., 2015). Reduced dopamine levels are often linked to cognitive impairments and neurodegenerative conditions like Alzheimer's disease (AD) (Klein *et al*., 2019). The drop in dopamine levels observed in the IIRI group is likely a result of oxidative stress and inflammation, which disrupt dopaminergic neurons and their signaling pathways (Sivandzade*et al*., 2019). This decrease can negatively impact motivation and other cognitive functions. Curcumin's antioxidative and anti-inflammatory properties help preserve dopaminergic neurons, maintaining dopamine levels and supporting cognitive health (Bhowmick *et al*., 2021). In the curcumin-treated group, the increase in dopamine levels highlights curcumin's role in protecting dopaminergic function, thereby aiding in cognitive processes such as attention and motivation.

Curcumin exerts its neuroprotective effects by targeting multiple neurotransmitter systems, including acetylcholine, serotonin, and dopamine. Through its antioxidative and anti-inflammatory properties, curcumin helps preserve the integrity of cholinergic, serotonergic, and dopaminergic neurons, maintaining neurotransmitter balance and supporting cognitive functions such as learning, memory, and mood regulation. These effects suggest curcumin's potential therapeutic role in conditions like IIRI and neurodegenerative diseases, where cognitive function is compromised (Sharma *et al*., 2017; Khayatan*et al*., 2024).

Oxidative stress, driven by an imbalance between reactive oxygen species (ROS) and antioxidant defenses, is a key factor in the pathogenesis of numerous neurodegenerative diseases, including Alzheimer's Disease (AD), where cognitive decline is a primary symptom (Bai *et al*., 2022; Ekundayo *et al*., 2024). In this study, intestinal ischemia-reperfusion injury (IIRI) was employed as a model to investigate oxidative stress and its effects on cognitive function, alongside the neuroprotective potential of curcumin, a polyphenol known for its strong antioxidant and anti-inflammatory properties (Tsai *et al*., 2021).

The results demonstrated a significant increase in oxidative stress in the IIRI group, as evidenced by decreased levels of catalase (CAT) and superoxide dismutase (SOD), two key enzymatic antioxidants responsible for neutralizing harmful ROS. The decrease in these enzymes indicates that the antioxidant defense mechanisms were overwhelmed, leaving cells vulnerable to oxidative damage. This vulnerability was further supported by the elevated malondialdehyde (MDA) levels in the IIRI group, which reflected increased lipid peroxidation, a hallmark of oxidative damage to cellular membranes.

In contrast, curcumin treatment in the CUR+IIRI group led to a noticeable reversal of these oxidative stress markers. CAT and SOD levels increased, suggesting that curcumin mitigated oxidative damage by reducing ROS levels, thereby allowing these antioxidant enzymes to recover and function effectively. This protective effect of curcumin was further underscored by the significant reduction in MDA levels, indicating a lower rate of lipid peroxidation and, thus, less cellular membrane damage.

The stabilization of oxidative stress markers with curcumin treatment highlights its potential to preserve neuronal health by preventing the cascade of oxidative damage that contributes to neuroinflammation and cognitive decline. Interestingly, while other markers of oxidative stress showed significant changes, reduced glutathione (GSH) levels remained relatively unchanged between the groups, suggesting that curcumin's antioxidant effects may target specific pathways rather than inducing a broad shift in overall antioxidant capacity.

By reducing oxidative stress and lipid peroxidation, curcumin not only protects neuronal integrity but also helps prevent the progression of neuroinflammation, a key process involved in diseases like AD. These findings suggest that curcumin's antioxidant and anti-inflammatory properties make it a promising therapeutic candidate for mitigating cognitive decline associated with oxidative stress-related neurodegenerative diseases.

Tumor Necrosis Factor-alpha (TNF-α), myeloperoxidase (MPO), and nitric oxide (NO) levels highlights the intricate relationship between neuroinflammation, oxidative stress, and cognitive function. These parameters are interconnected, as both TNF-α and MPO are key drivers of inflammatory and oxidative damage, while NO, though essential for normal signaling, exacerbates neurotoxicity when overproduced.

The increased levels of TNF-α, MPO, and NO in the intestinal ischemia-reperfusion injury (IIRI) group reflect a heightened inflammatory and oxidative response in the hippocampus, a brain region essential for learning and memory. TNF-α, a pro-inflammatory cytokine, amplifies neuroinflammation, contributing to neuronal damage and cognitive deficits, as seen in neurodegenerative conditions like Alzheimer's Disease (AD) (Montgomery and Bowers, 2012; Onyango *et al*., 2021). MPO, an enzyme that generates highly reactive oxidative species during inflammation, further compounds this damage by promoting oxidative stress (Hawkins and Davies, 2021). NO, while necessary in small amounts for physiological processes, becomes harmful in excess, contributing to oxidative damage and neurotoxicity (Tewari *et al*., 2021). The combined elevation of these markers in the IIRI group underscores the widespread damage inflicted on the brain by inflammatory and oxidative processes.

Curcumin's effects on these parameters in the CUR+IIRI group demonstrate its ability to disrupt this cascade of inflammation and oxidative stress. By reducing TNF-α levels, curcumin alleviates the inflammatory response, thereby limiting the harmful effects of chronic neuroinflammation on neuronal function and structure. The reduction in MPO levels indicates a decrease in oxidative stress, suggesting that curcumin protects neurons from oxidative damage that can impair cognitive functions. Finally, curcumin's ability to lower NO levels suggests its effectiveness in reducing neurotoxicity, further preserving neuronal health and preventing cognitive decline.

Taken together, the reduction of TNF-α, MPO, and NO in the CUR+IIRI group reflects curcumin's broad protective effects. Its antioxidative and anti-inflammatory properties act synergistically to mitigate the damaging effects of IIRI-induced neuroinflammation and oxidative stress, preserving hippocampal neurons and maintaining cognitive function. This multifaceted action makes curcumin a promising therapeutic candidate for neurodegenerative diseases like AD, where inflammation and oxidative damage play central roles in disease progression and cognitive decline.

The blood-brain barrier (BBB) is a selective barrier that protects the brain from harmful substances. Excessive nitric oxide (NO) can impair the BBB by forming peroxynitrite (ONOO-), a potent oxidant that damages endothelial cells and increases BBB permeability. This damage allows inflammatory cytokines and oxidative agents to enter the brain, worsening neuroinflammation and oxidative stress, which contribute to cognitive deficits seen in conditions like Alzheimer's disease (AD) (Sanchez-Cano *et al*., 2021; Shandilya *et al*., 2022).

Intestinal ischemia-reperfusion injury (IIRI) leads to systemic inflammation and increased oxidative stress, compromising BBB integrity. Elevated NO levels in the IIRI group indicate BBB disruption, which exacerbates neuroinflammation and neuronal damage. This observation is consistent with findings that traumatic injury or ischemia-reperfusion injury (IRI) can compromise the BBB and disrupt normal neuronal function, potentially leading to neurodegenerative diseases (Hausburg *et al*., 2020; Khan *et al*., 2012).

Curcumin, known for its antioxidative and anti-inflammatory properties, helps restore BBB integrity by reducing NO levels and, consequently, the formation of peroxynitrite. This action protects BBB endothelial cells from oxidative damage, preventing the influx of harmful substances into the brain. Research supports that curcumin can prevent BBB disruption through multiple mechanisms, such as inhibiting iNOS expression, reducing water content in the brain, preventing the absorbance of Evans blue dye after ischemia, and blocking NF-κB activation (Pan *et al*., 2000; Ferri and Kroemer, 2001; Jiang *et al*., 2007; Zhu *et al*., 2022).

Curcumin inhibits NF-kB activation, a key regulator of inflammatory responses, reducing the production of pro-inflammatory cytokines and oxidative stress markers. By downregulating NF-kB, curcumin maintains BBB integrity and protects against cognitive deficits.

Caspase-3 is a pivotal enzyme in the execution of apoptosis, responsible for cleaving various cellular components to facilitate cell death (Lossi*et al*., 2018). Increased caspase-3 expression is a hallmark of apoptotic processes and signifies significant neuronal loss (Snigdha *et al*., 2012).

Intestinal ischemia-reperfusion injury (IIRI) induces systemic inflammation and oxidative stress, leading to neuronal apoptosis in the brain (Ren *et al*., 2017). The elevated caspase-3 expression observed in the IIRI group highlights enhanced apoptotic activity in the hippocampus, a region critical for memory and learning. This increased apoptosis in the hippocampus can lead to substantial cognitive deficits, which are characteristic of neurodegenerative diseases such as Alzheimer's Disease (AD) (Wang *et al*., 2021).

Curcumin, known for its strong antioxidative properties, helps neutralize reactive oxygen species (ROS) and mitigate oxidative stress (Singh *et al*., 2020). By reducing oxidative damage, curcumin protects neurons from apoptosis (Tiwari and Chopra, 2012). Additionally, curcumin exhibits anti-inflammatory effects by lowering levels of pro-inflammatory cytokines such as TNF-alpha (Makuch *et al*., 2021). This reduction in inflammation decreases the signaling pathways that activate apoptotic processes, leading to lower caspase-3 expression (Arjumand *et al*., 2011).

Curcumin also modulates cell survival pathways, notably by activating the PI3K/Akt pathway, which enhances cell survival and inhibits apoptosis, thus decreasing caspase-3 activity (Wang *et al*.,

2019). Its ability to inhibit NF-kB activation, a key regulator of inflammatory responses, further reduces the production of pro-inflammatory cytokines and subsequently diminishes the activation of apoptotic signaling cascades, including those involving caspase-3 (Patel *et al*., 2020). Additionally, curcumin's enhancement of survival pathways contributes to reduced caspase-3 expression by promoting cell survival and inhibiting apoptotic mechanisms (Fiorillo *et al*., 2008; Jin *et al*., 2015).

Histological examination of the hippocampus in the IIRI group reveals significant histopathological alterations, including the presence of apoptotic neurons and dystrophic changes. These findings indicate that IIRI induces considerable neuronal damage, likely due to oxidative stress and inflammation, leading to cell death and a loss of functional neurons in the hippocampus, which is crucial for cognitive function. These observations are consistent with the study by Oyeleke *et al*. (2022), which also reported neurodegeneration due to amyloid beta injection.

In disparity, the histological analysis of the CUR+IIRI group shows reduced neuronal apoptosis and a lack of dystrophic changes, suggesting that curcumin's anti-inflammatory and antioxidant properties mitigate the damage caused by oxidative stress and inflammation. This supports the protective role of curcumin in preserving neuronal integrity and cognitive function, consistent with findings by Yang *et al*. (2020), who observed similar neuroprotective effects with melatonin in an IIRI model.

The histological changes observed in the IIRI group, particularly increased apoptotic neurons and vascular congestion, contribute to cognitive deficits. Damage to neurons impairs hippocampal function, leading to cognitive impairment. Curcumin's ability to reduce neuronal apoptosis and maintain normal hippocampal structure indicates its effectiveness in counteracting IIRI-induced damage through its anti-inflammatory and antioxidant mechanisms. By protecting the hippocampus from oxidative stress and inflammation, curcumin helps preserve cognitive function despite the damage caused by IIRI.

5. Conclusion

Intestinal ischemia-reperfusion injury (IIRI) leads to significant cognitive impairment due to oxidative stress and inflammation, causing neuronal damage, disrupted neurotransmitter function, and increased apoptosis in the hippocampus. Curcumin, with its antioxidant and anti-inflammatory properties, effectively mitigated these effects by restoring neurotransmitter balance, reducing oxidative stress markers, and protecting hippocampal neurons from apoptosis. This resulted in improved cognitive performance and hippocampal integrity in the curcumin-treated groups.

6. References

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1. Behavioral Test (Novel Object Recognition (NOR) Test)

Fig. 1A

1B

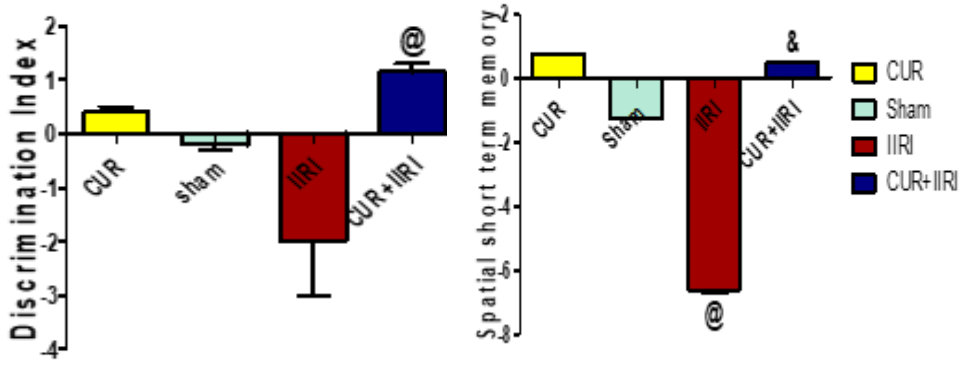


Fig. 1A and B: Effects of curcumin on intestinal ischemia reperfusion injury induced-cognitive deficit on discrimination index and spatial short term memory in aged female Wistar rats.

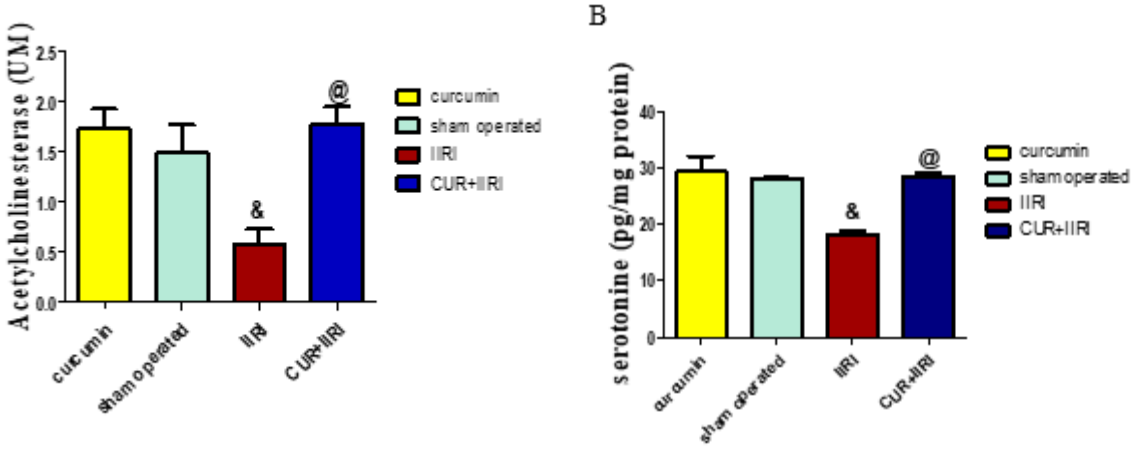
@represents significance at $p<0.05$; $p<0.001$ when compared with IIRI; CUR+IIRI

&represents significance at $p<0.001$ when compared with CUR+IIRI

2. Brain Neurotransmitters

2A

B



C

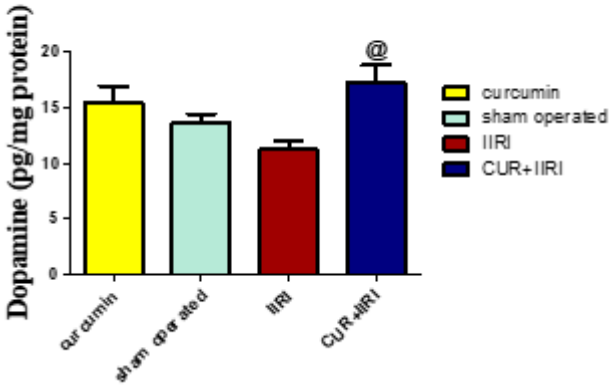


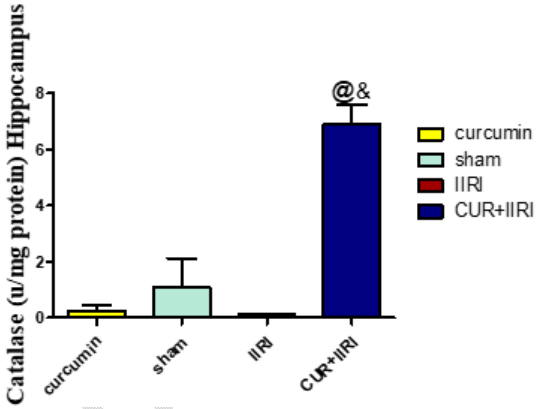
Fig 2. Fig. 2A, B and C: Effects of curcumin on intestinal ischemia reperfusion injury induced-cognitive deficit on acetylcholinesterase, serotonin and dopamine in aged female Wistar rats.

@repersents a significance p<0.01; p<0.001; p<0.05 when compared with IIRI

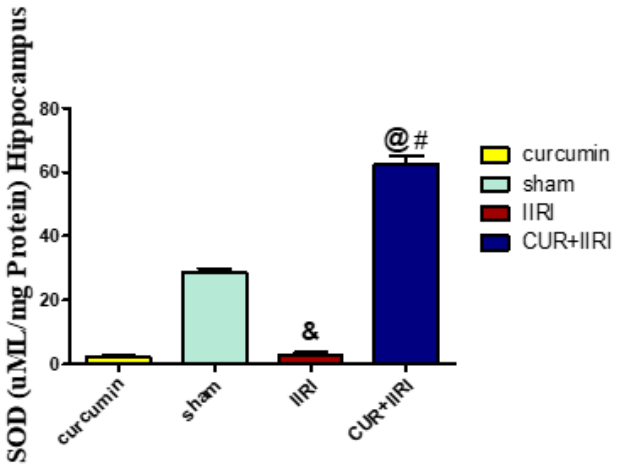
&represent a significance p<0.05; p<0.001 when compared with sham

3. Antioxidants parameters

A



B



C **D**

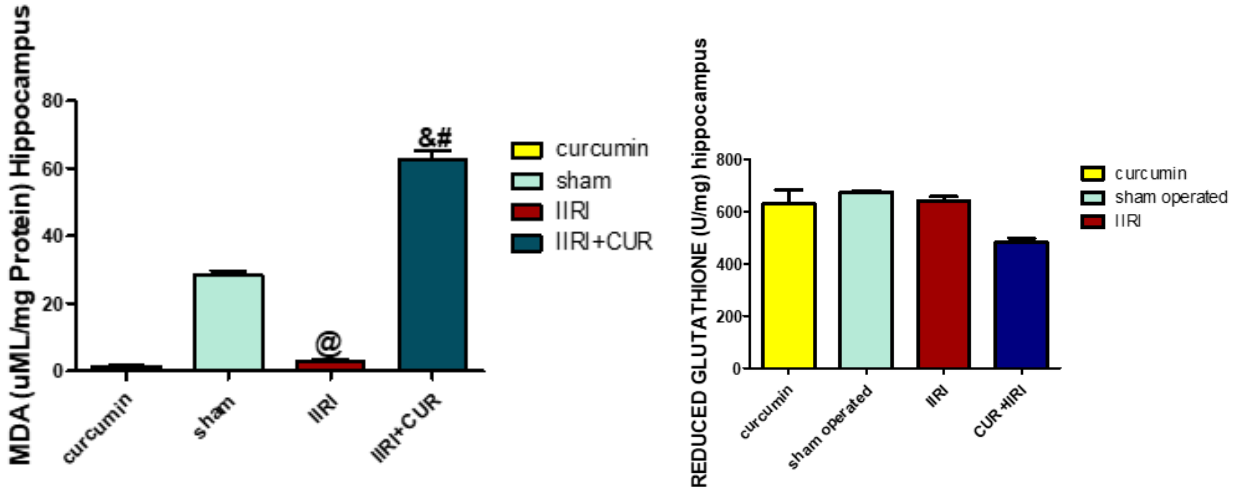


Fig. 3A, B, C and D: Effects of curcumin on intestinal ischemia reperfusion injury induced-cognitive deficit on CAT, SOD, MDA and GSH in aged female Wistar rats.

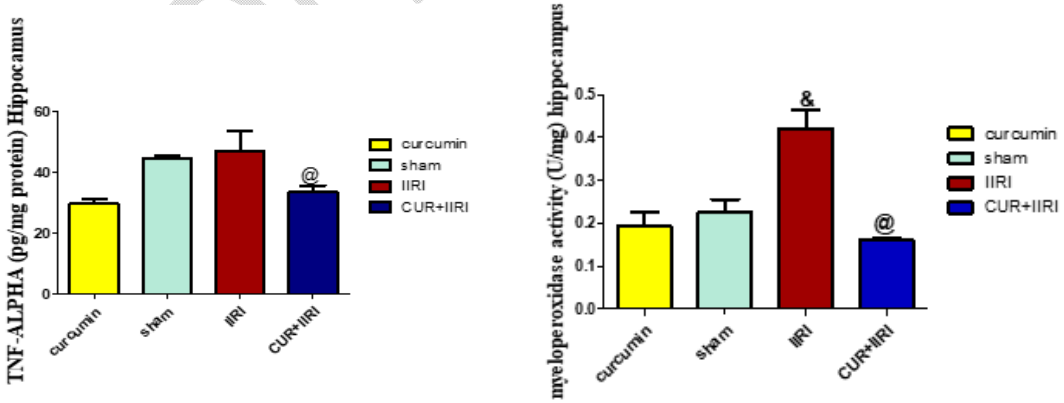
@represents a significance p<0.001; p<0.001; p<0.001 when compared to CUR+IIRI and IIRI.

&represents a significance p<0.001; p<0.001; p<0.001 when compared with CUR+IIRI

#represents a significance p<0.001; p<0.001 when compared with IIRI

4. Inflammatory Markers

A **B**



C

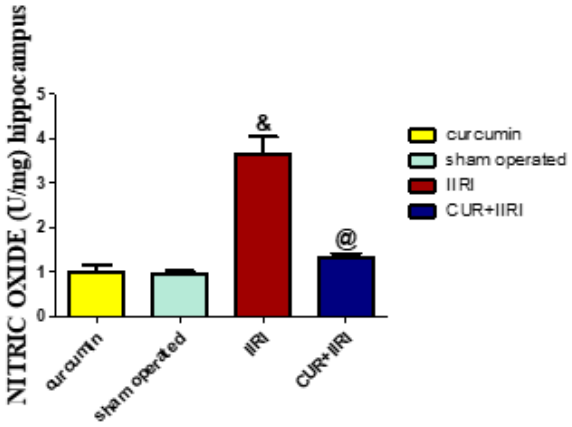


Fig. 4A, B, and C: Effects of curcumin on intestinal ischemia reperfusion injury induced-cognitive deficit on TNF-α, MPO and NO in aged female Wistar rats.

&represents a significance p<0.05 when compared to sham

@represents significance p<0.05; p<0.05; p<0.001 when compared to IIRI

5. Apoptotic Markers

A

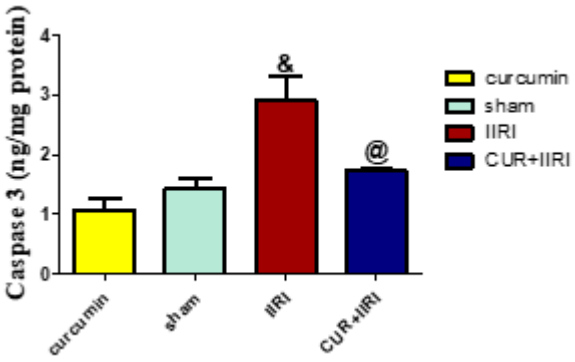


Fig. 5A: Effects of curcumin on intestinal ischemia reperfusion injury induced-cognitive deficit on caspase 3 in aged female Wistar rats.

&represents a significance p<0.01 when compared to sham

@represents a significance p<0.05 when compared to IIRI

6. Histology (H&E)

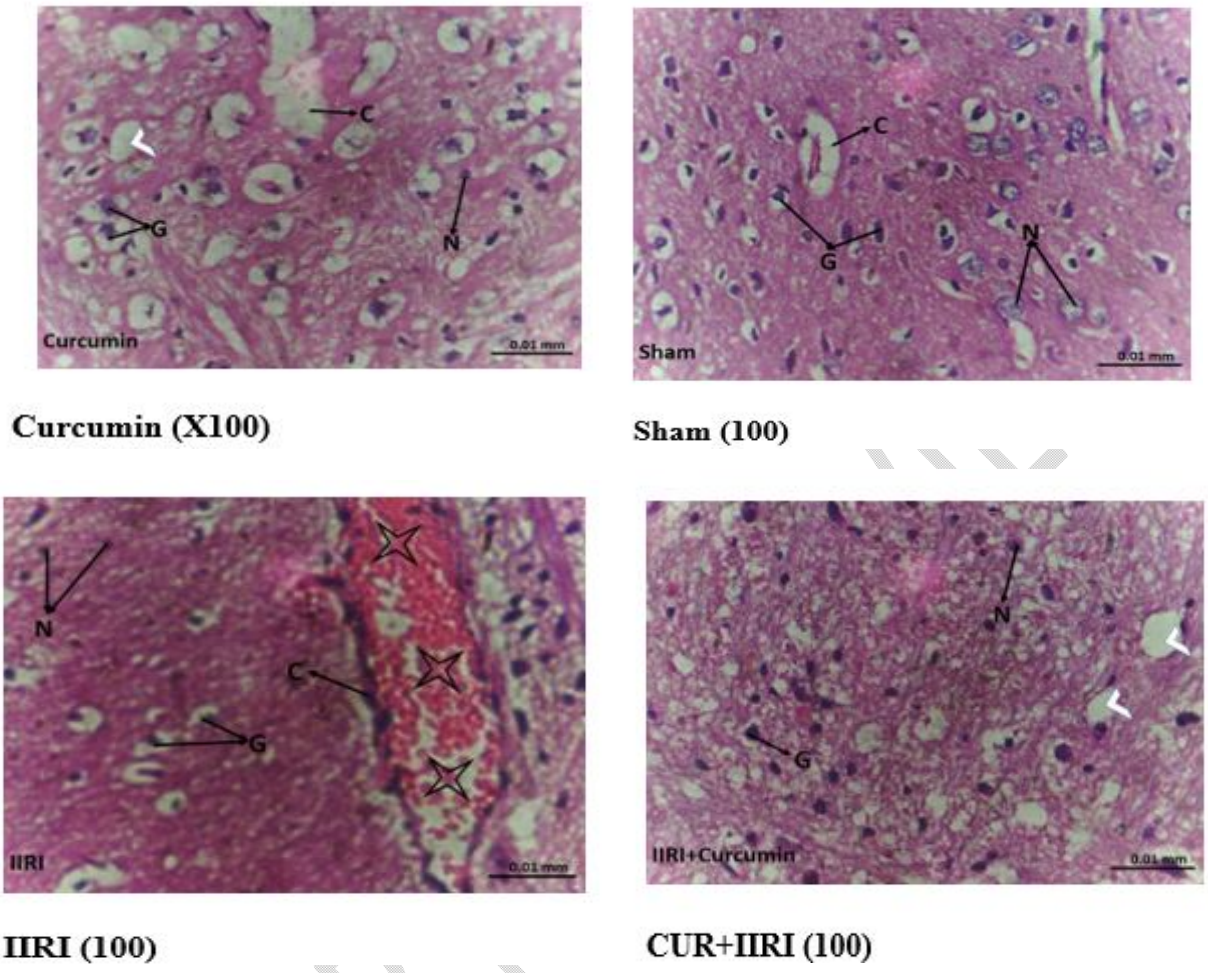


Fig. 6: Effects of curcumin on intestinal ischemia reperfusion injury induced-cognitive deficit on histology of the hippocampus in aged female Wistar rats.

N represents Apoptotic neurons; G represents Glial cells and C represents capillary.