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CURCUMIN MITIGATES STRESS INDUCED DEPRESSION AND HIPPOCAMPAL DAMAGE THROUGH UPREGULATION OF BDNF EXPRESSION AND ADULT NEUROGENESIS.

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ABSTRACT

Chronic stress, recognized as a major precipitant of depression, has been linked to various neural alterations, including cell death, neuronal atrophy, and compromised hippocampal neurogenesis and plasticity. This study aim to scrutinize curcumin's influence on glucocorticoid hormone secretion and its subsequent effects on the structural integrity and neurogenesis of the hippocampal neurons. A total of 30 adult albino Wistar rats, each weighing between 200-250 g, were utilized for the study. The rats, excluding those in the control group, underwent a 42-day regimen of modified Chronic Unpredictable Stress (CUS) to induce depressive-like states. After inducing CUS, these rats were categorized into six groups, each receiving different oral treatments for two weeks. The treatments included 30 mg/kg body weight of curcumin, 20 mg/kg body weight of fluoxetine, or a combination of both, along with a control group that received distilled water and an olive oil treated group. The rats were tested for behavioural despair using the forced swim test and their blood samples were obtained for serum corticosterone test. Afterwards, the rats were anesthetized, transcardially perfused and the hippocampus dissected and prepared for histopathological study. The study's multi-faceted approach encompassed behavioral, biochemical, and histological evaluations. Behavioral despair, gauged through the forced swimming test, displayed a marked reduction in the curcumin-treated rats compared to controls ($p<0.05$). Additionally, curcumin significantly lowered serum corticosterone levels, aligning them closely with the control levels. Histomorphological analysis of the hippocampus showed that the curcumin-treated rats exhibited substantially less neurodegeneration, as evidenced by fewer cytoplasmic vacuolations and more intact neuronal structures. Increased cell proliferation and BDNF level were also observed in curcumin treated rats. This study has illuminated a multifaceted approach through which curcumin mitigates hippocampal neurodegeneration, thus showing possible therapeutic potential of curcumin in ameliorating depressive symptoms,

INTRODUCTION

Depression is a widespread disorder that is affecting over 120 million people worldwide as reported by the World Mental Health (WMH) and the 4th edition of Diagnostic and Statistical Manual of Mental Disorders, (DSM-IV).Major depressive disorder (MDD) has been shown to be a foremost cause of disability worldwide; with lifetime population prevalence as high as 20% (Kessler et al., 2005). MDD is a medical condition that

consist of mood and affect abnormalities, neuro-vegetative functions (like appetite and sleep disturbances), cognition, inappropriate guilt and feelings of worthlessness, and psychomotor action (retardation or agitation) (WHO, 2012).

One of the etiologies of depressive behavior has been shown to be stress related. A major mechanism in which the brain responds to chronic and acute stress is by stimulation of the hypothalamic-pituitary-adrenal (HPA)axis. The activity of the HPA axis is controlled by several brain pathways, including the hippocampus which causes an inhibitory influence on hypothalamic Corticotropin Releasing Factor-containing neurons through a polysynaptic circuit and the amygdala which causes a direct excitatory influence (McEwen, 2000). Severe or chronic exposure to stress, and consequent exposure to high cortisol levels during both early life and adulthood, can cause cell death, neuronal atrophy, and can affect hippocampal neurogenesis and plasticity (McEwen and Gianaros, 2011).

Neurotrophic factors regulate neural growth and differentiation during development as well as the plasticity and survival of adult neurons and glial cells (Nestler et al., 2002). The neurotrophic hypothesis of depression states that a shortage in neurotrophic sustenance may lead to the resolution of depressive symptoms. Work on this hypothesis has been channeled to brain-derived neurotrophic factors (BDNF), one of the most common neurotrophic factors in the adult brain. Brain-derived neurotrophic factor is the most abundant and widely distributed neurotrophin in the central nervous system, involved in neuronal survival, growth and proliferation (Martinowich and Lu, 2008). Neurogenesis and neuronal plasticity are compromised in major depression, with subsequent neurodegeneration. This results in stress-induced alterations to the number and shape of neurons and glia in brain regions of depressed patients (Duman, 2009) and decreased proliferation of neural stem cells (Eyre and Baune, 2012).

BDNF levels are low in people with major depression (Duman, 2009). Currently in clinical practice, many pharmacological interventions are used in treatment of depression, such as tricyclic antidepressants, selective serotonin reuptake inhibitors and monoamine oxidase inhibitors (Duval et al., 2006; Karrouri et al.,2012). Fluoxetine, known by trade names Prozac and Sarafem among others, is a selective serotonin reuptake inhibitor (SSRI) class of antidepressant (Altamura, 1994). However, the existing treatments were not effective to all patients, and also accompanied with unwanted side effects (Mojtabai, 2011). Thus, it is necessary to develop a more effective and safer pharmacological intervention with less or no side effects. There has also been interest in applying various nutraceuticals for the treatment of depression (Sarris et al., 2011). In the domain of mental health, natural and complementary therapies are a popular option sought by the general community.

For centuries, turmeric has been used in Ayurvedic medicine and this integrates the medicinal properties of herbs with food (Porro and Panaro, 2023) Curcumin is a polyphenol, and is one of the curcumoids found in turmeric. Curcumin is endowed with multifaceted medicinal properties. For instance. It has antioxidant, antibacterial, anti-inflammatory, antiviral and antifungal actions and has also shown to be non-toxic to humans even at high doses. (Hewlings and Kalman, 2017)

The present study evaluates the effect curcumin exerts on the glucocorticoid hormone secretion during chronic unpredictable stress and its resultant influence on the histomorphology of the hippocampal neurons and neurogenesis.

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METHODOLOGY

Procurement and Authentication of Test Drug

100% curcumin supplement was purchased from Good Nature Pharmaceutical Company, New York, USA. Fluoxetine tablets (Proza brand) were purchased from E-blend Pharmaceutical outlet, Port Harcourt, Nigeria.

Animal Ethics: All procedures involving the use of animals in this study followed the guiding principles for research involving animals as recommended by the declaration of the research ethics and guiding principles in the Use and Care of Animals, and was in conformity with the international acceptable standards. The ethics approval of this research work was obtained from the University of Port-Harcourt Ethical Committee, with the approval code (UPH/CEREMAD/REC/ MM83/027).

Experimental Animals

A total of 75 adult albinoWistar rats (35 male and 30 female) were used. The rats were purchased from the Animal research house of the Faculty of Basic Medical Sciences, University of Port-Harcourt, Rivers State. The rats were kept in clean cages in the animal research house at the faculty of Basic Medical Science, University of Port Harcourt, Rivers State, Nigeria and allowed to acclimatize for two weeks under standard laboratory conditions of temperature 23-34^{0}C with a photoperiodicity of approximately 12 h light alternating with 12 h of darkness. They were fed with commercially available chow (Livestock feed, Nnewi) and allowed unrestricted access to water.

Determination of LD_{50} of Curcumin

A preliminary LD_{50} test of curcumin stock was done using the Lorke's procedure (Lorke, 1983; Enegide et al., 2013). The animals were grouped into 5 different groups of 3 animals each and administered the curcumin stock at different doses; 20, 50, 100, 300 and 500 mg/kg body weight. The control group received distilled water. After treatment, all animals were observed for behavioral changes and mortality at least once daily for 2 weeks.

Induction of Chronic Unpredictable Stress (CUS) model of Depression in rats (Lopez et al., 1997)

50 albino Wistar rats were depressed using a modified Chronic Unpredictable Stress (CUS) method as described by Lopez et al (1997). This was done by subjecting the rats to chronic unpredictable stress for 42 days. The stressors used were as follows; cold shock, heat shock, tilted cage (45°), wet beddings, cage illumination, predator presence, isolation and restraint. The schedule used for inducing depression in rats in this study is shown in the Table below.

Table 1: Schedule for induction of chronic unpredictable stress model of depression in rats.

DAYS	Stressors	Duration	Days	Stressors	Duration
DAY 1	Heat shock	<60 s	DAY 22	Predator presence	12 h
DAY 2	Tilted cage (45^o)	24 h	DAY 23	Isolation	24 h
DAY 3	Wet bedding	12 h	DAY 24	Restraint	300 s
DAY 4	Cage illumination	24 h	DAY 25	No stress	2 h
DAY 5	Predator presence	12 h	DAY 26	Cold shock	60 s
DAY 6	Isolation	24 h	DAY 27	Heat shock	<60 s
DAY 7	Restraint	300 s	DAY 28	Tilted cage (45^o)	24 h
DAY 8	No stress	24 h	DAY 29	Wet beddings	12 h
DAY 9	Cold shock	60 s	DAY 30	Cage illumination	24 h
DAY10	Heat shock	<60 s	DAY 31	Predator presence	12 h
DAY11	Tilted cage (45^o)	24 h	DAY 32	Isolation	24 h
DAY12	Wet beddings	12 h	DAY 33	Restraint	300 s

DAY13	Cage illumination	24 h	DAY 34	No stress	24 h
DAY14	Predator presence	12 h	DAY 35	Cold shock	60 s
DAY15	Isolation	24 h	DAY 36	Heat shock	<60 s
DAY16	Restraint	300 s	DAY 37	45^{o} Tilted cage	24 h
DAY17	No stress	24 h	DAY 38	Wet beddings	12 h
DAY18	Cold shock	60 s	DAY 39	Cage illumination	24 h
DAY19	Heat shock	<60 s	DAY 40	Predator presence	12 h
DAY 20	Tilted cage (45°)	24 h	DAY 41	Isolation	24 h
DAY 21	Wet bedding	12 h	DAY 42	Restraint	300 s

Procedure for Inducing CUS Model of Depression in Rats

Isolation: Isolation is one of the stressors that could cause anxiety and depression in rats. Here, the animals were removed from their cages and separated from the group of rats that they have been staying with, and kept alone in a cage each for a period of 24 h (Lopez et al., 1997).

Cage illumination: Rats are nocturnal animals and they prefer dark or dimly-lit niche to highly illuminated ones. Cage illumination changes the normal photoperiod of the rats and provides constant and direct cage illumination. The rats were exposed to bright light for 24 h. This aims at depriving the rats from sleep both during the day and at night (Lopez et al., 1997).

Predator Presence: Cats are predators to rats and they were used to instill fear in the rats. The cat was kept in a cage close to the rat's cage where the rats could see and perceive the presence of their predator for a period of 30 min (Lopez et al., 1997).

Cold Shock: The rats were exposed to a cold shock by immersing them in cold water of about 4°C for less than a minute. Afterwards, they were dried with a warm towel and put back inside their cages (Lopez et al., 1997).

Heat Shock: The rats were exposed to heat shock by placing them on a hot plate and hot water for less than a minute. Afterwards, they were dried with a warm towel and put back inside their dry cages (Lopez et al., 1997).

Wet Bedding:10 ml of water was poured on the beddings of the animals in their cages and left for a period of 24 hours (Lopez et al., 1997) to keep them uncomfortable and stressed. Afterwards, the animals were dried and kept in dry cages.

Cage Tilt: The cages of the rats were tilted to 45°, for 24 h(Lopez et al., 1997) to keep the animal stressed and uncomfortable.

Experimental Design

A total number of 36 adult albino Wistar rats weighing about 200-250 g were used for this study. The rats were grouped into six with 6 animals per each group;

Group A (Control group) – received distilled water only through oral gavage.

Group B (Depressed group) – received distilled water only through oral gavage after Chronic Unpredictable Stress of 42 days.

Group C (Olive oil group) – received 0.8ml/kg b.wt of virgin olive oil through oral gavage for 42 days after Chronic unpredictable stress of 42 days

Group D (Curcumin group) – received 30 mg/kg b.wt of curcumin through oral gavage for 42 days after Chronic unpredictable stress of 42 days.

Group E (Fluoxetine group) –received 20 mg/kg b.wt of fluoxetine through oral gavage for 42 days after Chronic unpredictable stress of 42 days.

Group F (Fluoxetine+Curcumin) - received 30 mg/kg b.wt of curcumin and 20 mg/kg b.wt of fluoxetine through oral gavage for 14 days after Chronic unpredictable stress of 42 days.

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Porsolt's Test for Depression in Rodents (Forced Swim Test)

This task is used to assess depression-like behavior (or a form of learned helplessness). Animals that were considered more depressive tend to give up sooner and more completely when faced with a high degree of adversity.

A two Liter beaker was filled with 1500 mL of room temperature water and placed in an isolation chamber. Water was changed after each rat was tested. The isolation chamber was placed in front of a digital camera which was connected to a computer.

The forced swim test was carried out in an isolation chamber to eliminate external cues from influencing a rat's behavior. A single rat was placed in the water filled beaker for a period of 5-minutes and their behaviour is digitally recorded. The rat's behaviour was analyzed offline to measure immobility time (a measure of depression-like behaviour) with the aid of a desktop containing the saved videos. Rats were judged to be immobile when they made no movements or only those movements necessary to keep their head above water.

Relevant Controls: Care was taken to ensure that the rats in each group had no impairment in motor function, motor coordination, motor endurance, and balance so as not to have a biased result.

Serum Corticosterone Assay

Procedure: Corticosterone level served as a marker for endocrine stress response. Blood samples were obtained from the depressed rats through cardiac puncture (Gross et al., 2020) and checked for serum corticosterone level using a commercially prepared Corticosterone Eliza kit (Biovendors, Brno, Czech Republic). All samples were subjected to a single assay run before centrifugation for 20 minutes at the speed of 2000-3000 r.p.m. The serum was immediately removed and assayed for corticosterone level. The assay procedure used was as described by Gross et al., 2020.

Perfusion Fixation and Animal Sacrifice

24 hours after the last exposure to stressors and subsequent treatments, the rats in each group were anesthetized under mild anesthesia (chloroform) and perfused transcardially first with (0.9 %) saline containing heparin and then by 4 % paraformaldehyde in 0.1 M phosphate-buffered saline (PBS), pH 7.4. Brains were removed from the cranium shortly after perfusion (Ziabreva, et al., 2003).

Tissue Preparation

After the dissection of the brain from the cranium, the tissues were processed. This involves fixation, dehydration, clearing, paraffin embedding, sectioning, dewaxing, staining and mounting. The prefrontal cortex and the hippocampus were located on the brain tissue, cut longitudinally into two equal halves and fixed for duration of one hour in 10% formal saline. This was done to maintain the morphological tissue integrity (Williams et al., 2006).

Tissue samples were dehydrated in ascending grades of alcohol ranging from 70%- 80%- 95%-three changes of absolute alcohol (100%). The essence of dehydration is to remove water from the tissue thereby hardening the tissue (Williams et al., 2006). The samples were cleared using three changes of xylene each. Clearing removes alcohol from the tissue thus, increasing the refractive and optical index of tissue (Williams et al., 2006).

The tissue samples were embedded in molten paraffin wax using embedding moulds and then allowed to cool to form solid tissue blocks. Trimming of tissue blocks was done first to expose the tissue surface and with the help of a rotary microtome, sectioning was accomplished at 5 microns per section. Sections were then floated on a water bath and picked with a frosted glass slide. Immediately, sections were placed on a hot plate to dry for about 30 minutes (Williams et al., 2006). Afterwards, clearing of the slides was done using xylene.

Histological Staining (Haematoxylin & Eosin)

Tissue staining was done using hematoxylin and eosin stains (H and E) This stain produces a contrast between the nucleus and the surrounding cytoplasm. The Hematoxylin stains the nucleus dark blue while the eosin stains the cytoplasm pinkish red. This stain shows the cytoarchitectural arrangement of the neurons and their structure in the brain tissue (Feldman and Wolfe, 2014).

Cell Proliferation Assay

Cell proliferation assay was done using Ki-67 immunostaining**.** Following serial sectioning of the tissue blocks, the sections were deparaffinized in xylene twice for 5 min each and rehydrated by running them through graded alcohol. The slides were washed in running water for 5 min and subjected to antigen retrieval in citrate buffer pH 6.0, microwaved at medium high 750W for 10 min and allowed to cool at room temperature for 20 mins. The slides were then washed in TBS for 5 min. Endogenous peroxidase in the brain tissue was blocked with 1% Hydrogen peroxide in methanol for 15 mins and washed in TBS pH 7.4 Thrice for 5 mins each. The tissue sections were then incubated with 5% normal goat serum for 45 mins. The serum was tapped off the sides and the slides were incubated with primary antibody Ki-67 1:2000 overnight at 4^0C. On the 2nd day, the slides were washed in TBS pH 7.4 thrice for 5 min and incubated with biotinylated secondary antibody (goat anti-rabbit 1:1000) for 30 min. The slides were then washed in TBS pH 7.4 thrice for 5 min and incubated with DAB working solution for 5 min. The slides were rinsed in running tap water for 5 min and counter-stained with Hematoxylin. The slides were also hydrated through graded alcohol, cleared in xylene and mounted with Entellen. Positive control used was the brain tissue (subventricular area of lateral ventricle)

Assay for Hippocampal BDNF Concentration

BDNF concentration was measured using the quantitative sandwich enzyme linked immunosorbent assay (ELISA) kit (RayBio, USA) in accordance with manufacturer's protocols. Flat bottom 96-well plates coated with anti-human BDNF were used to analyze BDNF level. All samples were assayed in duplicate. The immobilized antigen and antibody were incubated with 70 µl of antibody cocktail containing biotinylated anti-Human BDNF and concentrated HRP-conjugated streptavidin for 10 min. After incubation, 50 µl of each standard and sample were added into the appropriate wells followed by incubation for 2 hours. The solutions were discarded by adding and washing with 300 µl of wash buffer 4 times. After washing, 100 µl of tetramethylbenzidine (TMB) one-step substrate reagent was added to each well and incubation in the dark room was done for 30 minutes by gentle shaking.

Color development was stopped by the addition of 0.2 M sulfuric acid, and then the absorbance of 450 nm of each well's contents was measured immediately by a Microplate Reader (BMG Labtech; Ortenberg, Germany). All incubations were performed under room temperature and it took 2 hours and 40 minutes for total assay time excluding the washing process. BDNF concentrations were expressed as µg/ml.

Statistical Analysis

Data obtained from this study was analyzed using Statistical Package for the Social Sciences (SPSS IBM version 23.0) and Microsoft Excel 2019 edition. Values were expressed as mean and Standard Deviation in descriptive statistics. One-way analysis of variance (ANOVA) was used to compare the differences between the groups followed by Tukey's*post hoc* test. Confidence interval was set at 95%, therefore $p < 0.05$ was considered significant.

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RESULTS

The Effect of Curcuminon Behavioral Despair in Depressed Rats Using Forced Swimming Test

In this task, more depressive animals give up and stop struggling sooner and more completely, whereas less depressive animals tend to swim vigorously and attempt to climb out of the cylinder for a greater period of time. From this study, it was observed that the duration of struggling was significantly higher in the control group (2.95 s) than the curcumin treated group (0.33 s), depressed group (0.28 s), the curcumin plus fluoxetine treated group (0.23 s) and the olive oil treated group (0.16sec) indicating the absence of behavioral despair and hopelessness in the control group. There was no significant difference between the control group (2.95 s) and the fluoxetine group (0.36 s). The duration of struggling was significantly higher in the curcumin treated group (0.33 s) and the fluoxetine treated group (0.36 s) than the depressed group (0.28 s), the curcumin plus fluoxetine treated group (0.23 s) and the olive oil treated group (0.16 s). The duration of immobility was significantly lower in the control group (0.13 s) than the curcumin treated group (0.23 s), the curcumin plus fluoxetine treated group (0.39 s), olive treated group (0.40 s), fluoxetine treated group (0.48 s) and depressed group (0.77 s) which had the highest duration of immobility. There were no significant differences in the duration of immobility in the fluoxetine treated group (0.48 s), olive treated group (0.40 s) and curcumin plus fluoxetine treated groups (0.39 s) (Figure 1).

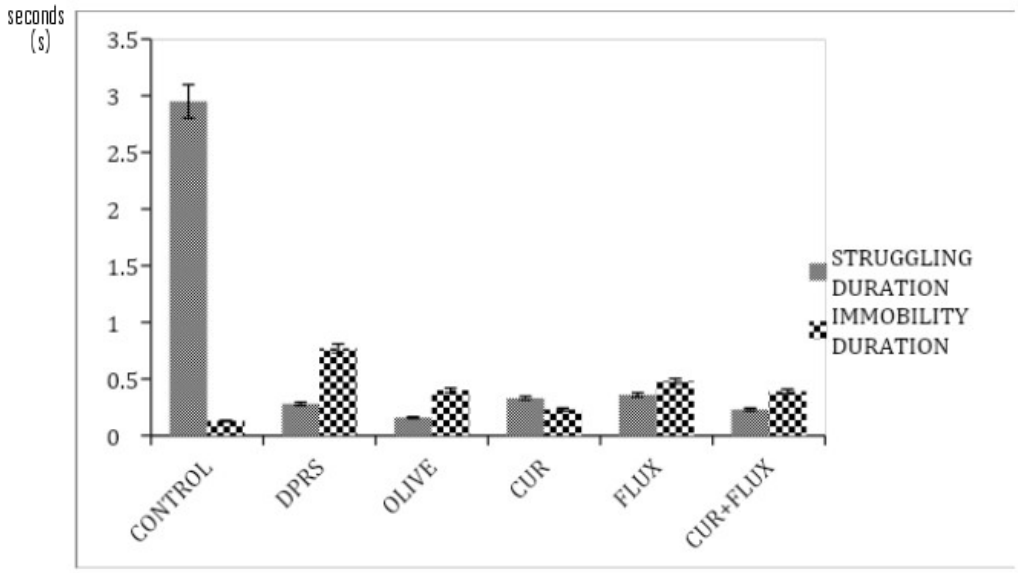

Figure 1: The effect of curcumin treatment on behavioral despair in depressed albino Wistar rats. *Significantly different from control (P<0.05).

The Effect of Curcumin Treatment on Corticosterone (Cort) Level in Depressed Albino Wistar Rat

From this result it shows the effect of curcumin treatment on serum corticosterone (CORT) level in depressed albino Wistar rats. It was observed that CORT level was significantly lower in the control group (77.85 ± 18.7 mg/dl) than in the depressed group (213.26 ± 34.5 mg/dl). It was also observed that there was no significant difference in the CORT level between the control group (77.85 ± 18.7 mg/dl) and the curcumin treated group (62.5 ± 3.41 mg/dl), the curcumin plus

fluoxetine treated group (57.16 ± 4.97 mg/dl), the olive oil treated group (70.7 ± 7.07 mg/dl) and the fluoxetine treated group (66.25 ± 5.85 mg/dl).

Table 1: The effect of curcumin treatment on serum corticosterone level in depressed albino Wistar rats.

Groups	Control	CUS	Olive	Curcumin	Fluoxetine	Cur + Flux
Corticosterone (mg/dl)	77.85± 18.7	213.26 ± 34.5*	70.7 ± 7.07	62.5 ± 3.41	66.25± 5.85	57.16± 4.97

***Significantly different from control = P<0.05

The Histomorphological Assessment of the CA3 Region of the Hippocampus of Curcumin Treated Depressed Rats.

The micrograph of the CONTROL shows that the neurons appear intact with no visible sign of neurodegeneration. The cells appear intact with a centrally located nucleus and no cytoplasmic vacuolations present as seen in a healthy brain tissue. The CUS shows the histomorphology of the CA3 region of the hippocampus of the depressed rats with no treatment. In the micrograph of the CUS group, there were numerous observable neurons that appear enlarged with cytoplasmic vacuolations, eccentrically located nucleus and plasma membranes which appear to have lost integrity. This is indicative of hydropicneurodegeneration and could possibly lead to neuronal apoptosis. In the curcumin treated group (CUR), the neurons appear fairly intact with less cytoplasmic vacuolation.

In the fluoxetine treated group (FLUX), there were less neuronal cytoplasmic vacuolations observed. This was also observed in the curcumin and fluoxetine combined treatment group (CUR +FLUX). In the olive oil treated group (Olive), there were more neuronal cytoplasmic vacuolations observed just as seen in the depressed group this indicating neuronal degeneration.

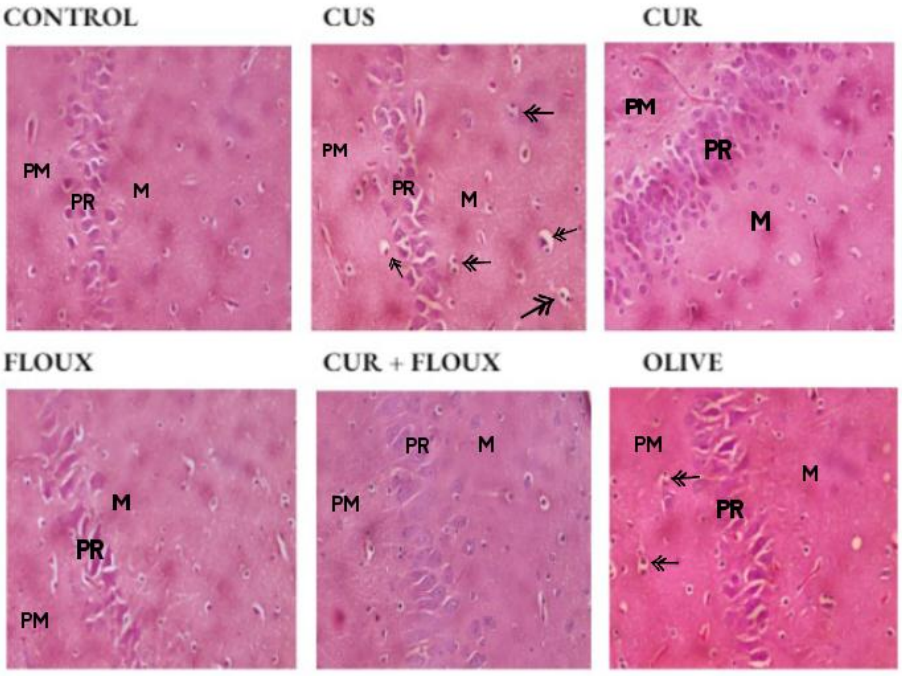

Figure 2: Micrograph plates showing the histomorphology of the CA3 region of the hippocampus of curcumin treated depressed rats (x400).

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The Effect of Curcumin on the Ki67 Expression of Neuronal Proliferation in the Subgranular Region (SGR) of the Dentate Gyrus in the Brain of Depressed Rats.

The control group (CONTROL) showed significantly increased expression of Ki-67 positive cells in the sub-granular (G) layers and the polymorphic layer (PO) of the dentate gyrus indicating presence of neuronal cell proliferation. In the subgranular region, the CUS group showed significantly reduced expression of ki67 positive neurons thus indicating the down regulation of neuronal cell proliferation. The curcumin treated group (CUR) shows high intensity of Ki-67 expression and increased Ki-67 positive cells in the sub-granular (G) layers and the polymorphic layer (PO) of the dentate gyrus indicating presence of adult hippocampal neurogenesis. (See Plate CUR). The fluoxetine treated group (FLUX), the curcumin plus fluoxetine (CUR + FLUX) combined treatment group and the Olive oil treated group (OLIVE) showed moderate intensity of Ki-67 expression and less Ki-67 positive cells in the sub-granular (G) layers and the polymorphic layer (PO) indicating presence of adult hippocampal neurogenesis.

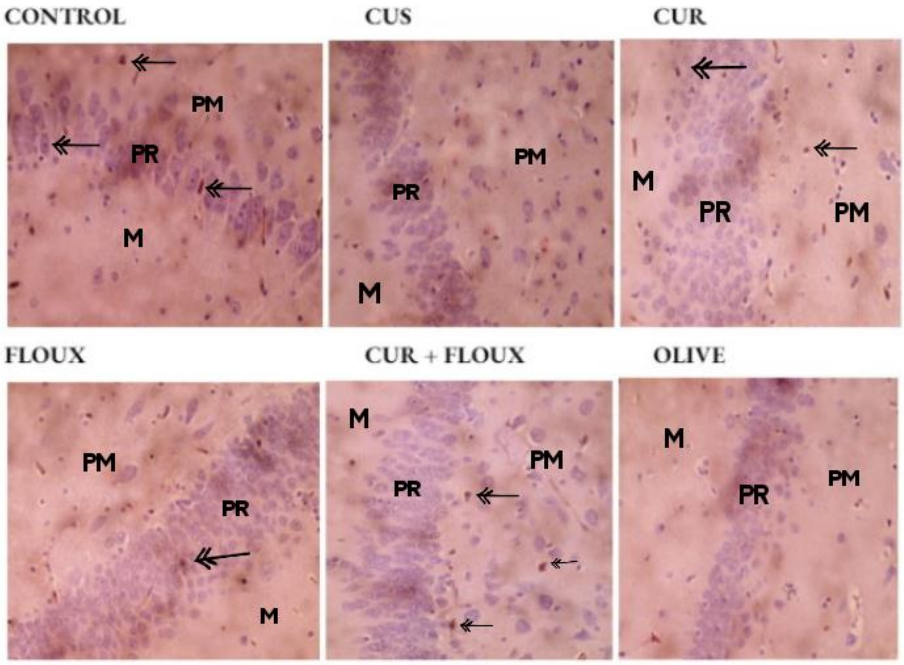

Figure 3: Micrograph plates showing the effect of curcumin on the ki67 expression of neuronal proliferation in the SGR of the dentate gyrus in the control group (CONTROL), curcumin treated group (CUR), the fluoxetine treated group (FLOUX), the curcumin plus fluoxetine treated group (CUR +FLOUX) and the olive oil treated group (OLIVE).

The Effect of Curcumin on the Brain Neurotrophic Factor (BDNF) on the Brain of Depressed Rats.

From this study, it was observed that the level of brain neurotrophic factor (BDNF) was significantly higher in the control group compared to the depressed group (CUS) ($P<0.05$). The BDNF was also significantly higher in the curcumin treatment group (CUR), the fluoxetine treated depressed rats (FLUX) the olive oil treated depressed rats (OLIVE) and curcumin plus fluoxetine treated group (CUR +FLUX) compared to the depressed group (CUS) ($P<0.05$).

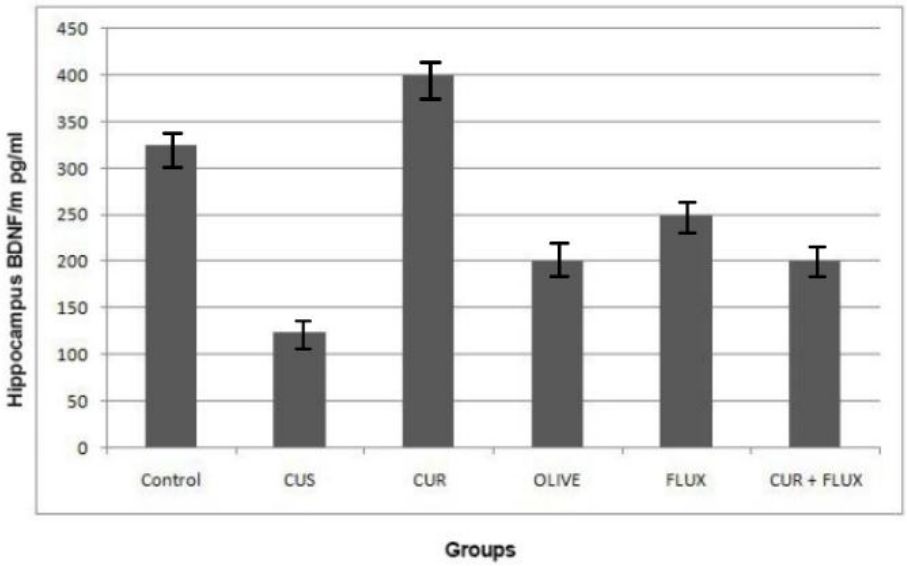

Figure 4: The effect of Curcumin on BDNF level in depressed rats. Each value represents mean±SD, Values marked with asterisk (*) differ significantly from control value (*$p < 0.05$). While those marked with (#) differ significantly from depressed group (CUS) (#$p < 0.05$). CUS: Chronic Unpredictable Stress model of Depression

DISCUSSION

Stressful life events have been shown to reduce the level of adult neurogenesis and have been noted to be an essential element in the development of several neuropsychiatric disorders such as depression. Neurogenesis is influenced by a range of neurotrophic factors such as BDNF in the CNS. BDNF plays a key role in regulating a wide range of neurogenic functions such as neuronal growth, survival and proliferation. This present study shows a significant reduction in the hippocampal BDNF level in the depressed group in comparison with that of the control group indicating that MDD is associated with low BDNF levels in the brain. The present study showed very low expression of Ki-67 positive neurons in the neurogenic areas of the brain (sub granular region of the dentate gyrus and the subventricular region of the lateral ventricles of the brain) of depressed rats in comparison with the rats in the control group. This indicates that depression is associated with reduction in adult neurogenesis. The reduction in the rate of adult neurogenesis could be as a result of decrease in the level of the BDNF which promotes neurogenesis the brain. BDNF has been associated with depression (Lopesti et al, 2015). This agrees with the findings of Duman (2009) and Kim and Lee (2010) which emphasizes that the decreased level of BDNF affects neuronal survival, growth and proliferation, thus impeding the process of adult neurogenesis in the brain. The present study is also in agreement with Veena et al (2009a) and Veena et al (2009b) which reported that chronic restraint stress for 3 weeks suppressed neuronal proliferation as well as decreased the survival of new-born cells in adult rat hippocampus while acute restraint stress for 2 h did not show any major changes an adult neurogenesis in the brain of the rats (Pham et al., 2003). The present study is comparable with the findings of Malberg and Duman (2003), supporting our results. Heine et al. (2004) and Luo et al. (2005) also stated that rats subjected to chronic and intense unpredictable stress in adulthood also exhibited prolonged inhibition of cell proliferation in the dentate gyrus of their brain. Heine et al. (2004) and Luo et al (2005) showed that after chronic stress, there

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was increase in the cell cycle inhibitor p27kip1 which parallels the reduction in proliferation and cell death indicating that more cells had initiated cell cycle arrest and that the granule cell turnover had thus showed down. This is in line with the outcome of the present study which shows that chronic exposure to stress induced depression resulted in degeneration of the hippocampal neurons. This could be due to the consequent exposure to high cortisol levels during chronic stress exposure which has been shown to cause cell death, neuronal atrophy, and can affect hippocampal neurogenesis and plasticity (McEwen and Gianaros, 2011).

Several polyphenols have been reported to possess the ability to stimulate neuronal growth and proliferation (Lopresti, 2015). From the present study, it was observed that the curcumin treated group significantly elevated the tissue BDNF level compared to the depressed group, an evidence for its antidepressant-like effect (Sen et al., 2008). This is in line with work by Radbakhsh*et al*.(2023) which showed that chronic administration of curcumin resulted in a dose-dependent increase in hippocampal BDNF level. From the present study, it was observed that curcumin, fluoxetine and curcumin plus fluoxetine treated groups significantly elevated the tissue BDNF level as well as the expression of the ki67 positive neurons compared to the depressed group.

The present study showed that depressed rats treated with curcuminshowed increased neuronal proliferation at the sub granular region of the dentate gyrus and the sub ventricular region of the lateral ventricles of the brain. This could be as a result of curcumin's ability to increase BDNF level in the CNS, thus upregulating adult neurogenesis. The present study also agrees with Hung *et al*. (2011) which showed that curcumin was able to significantly ameliorate the decreased BDNF level in rats after repeated corticosterone injections. Mu *et al.*, (2007) also reported that curcumin reversed the reduced BDNF and Serotonin receptor A mRNA expression across the hippocampal subfield. Thus, it could be deduced that curcumin ameliorated the impaired adult neurogenesis processes in depressed subjects.

CONCLUSION

The outcome of the present study has shown that chronic and unpredictable exposure to stress could result in depressive-like behaviors. Stressful conditions result in high levels of CORT in rodents which in turn down regulates the BDNF level and consequently impedes neuronal proliferation in the neurogenic regions of the brain (SGR of dentate gyrus). This study also shows that the administration of curcumin mitigated the hippocampal degeneration by upregulating the brain tissue level of BDNF and enhancing neuronal proliferation.

Notably, curcumin administration led to an increase in BDNF levels, a critical element enhancing neuronal proliferation within neurogenic brain regions. These findings illuminate curcumin's potential as an effective antidepressant agent. By improving hippocampal integrity and function in depression-induced rats, curcumin shows promise in modulating neurotrophic factors and stress hormones, crucial elements in the pathophysiology of depression. This study also underscores the therapeutic potential of curcumin in ameliorating depressive symptoms, opening avenues for future research in natural compound-based treatments for depression

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