ORIGINAL ARTICLE

Anti-depressant Activities of *Theobroma cacao* Extract on Reserpine-induced Depression in Female Wistar Rats

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Abstract:

Background: Depression is one of the most common types of neurological disorder, which is a marked pattern of disturbances in emotional behavior, memory and hedonic processing. *Aim and Objectives:* To investigate the role of ethanolic extract of *Theobroma cacao* seed on the prefrontal cortex of female Wistar rats following reserpine-induced depression. *Material and Methods:* Thirty-six (36) female Wistar rats were used for this study. They were divided into six (A - F) groups (n = 6). Group A - control, Group B - 0.2 mg/kg reserpine, Group C - 10 mg/kg fluoxetine, Group D - 500 mg/kg *Theobroma cacao* seed extract, Group E - 0.2 mg/kg reserpine + 500mg/kg *Theobroma cacao* seed extract, Group F - 0.2 mg/kg reserpine + 10mg/kg fluoxetine. Animals were euthanized via cervical dislocation after the last day of administration and the prefrontal cortex and hippocampus were excised and fixed in 10% formalin solution for routine histological processing while the part used for biochemical assay were homogenized in phosphate buffer before centrifugation. *Results:* Morphological alteration and reduced population of prefrontal cortex and hippocampus neurons, reduced protein synthesis, poor behavioral patterns, reduced neurotransmission and induction of oxidative stress in reserpine exposed animal. *Conclusion: Theobroma cacao* seed extract was able to mitigate these aberrations.

Keywords: Depression, Reserpine, Prefrontal Cortex, Hippocampus, *Theobroma cacao*

Introduction:

Depression is a serious medical condition that affects negatively such as thinking, feelings and actions. Depression causes feeling of sadness and general loss of interest in day to day activities which can culminate into emotional and physical problems leading to suffering, functional ability at home, work or school [1]. According to World Health Organization, depression is one of the major factor contributing to Disability Adjusted Life Years (DALY) and recent research have suggested that depression will become the second greatest contributor to the DALY by 2020 [1-2]. DALY referred to as the loss of one year of healthy life i.e. a measurement of gap between current health status and an ideal health situation [1]. Depression is one of the main risk factor for suicide, representing a significant public health concern [3].

Theobroma cacao (cocoa bean) seed contains numerous phytochemical components such as flavonols, procyanidins; Cocoa also contain stimulants such as theobromine and caffeine [4] which are under preliminary studies for potential beneficial properties on a biological system [5].

Hence, the research study aimed to unravel the anti-depressant efficacies of *Theobroma cacao* seed extract on induced depression model in Wistar rats. An anti-hypertensive drug (Reserpine), a Rauwolfia indole alkaloid that acts as a sympatholytic and sedative agent but research and clinical trials have shown that reserpine has depressive properties [6-7]. The mechanism of action of reserpine is depletion of monoamine neurotransmitters from nerve endings which may result in poor neuronal excitation and communication leading to depression [8-9]. Therefore, reserpine was used to induce depression in the experimental female Wistar rats.

A widely known anti-depressant, Fluoxetine (FLU), it's a selective serotonin reuptake inhibitor was administered to animals to compare anti-depressive properties of *Theobroma cacao* seed extract on the depression induced female Wistar rats. FLU prevents the reuptake of serotonin into presynaptic neurons, thereby maintaining increased serotonin levels in the synaptic region and promoting repeated stimulation of post-synaptic serotonin receptors. FLU treatment has been reported to cause increase adult cortical and hippocampal neurogenesis from neural progenitor cells [10-11]. Chronic FLU treatment also induces the reversal of neuronal maturation i.e. dematuration, in the hippocampus, amygdala, and cerebral cortex of rodents such that neuronal cells revert to a state featuring multiple molecular and electrophysiological characteristics of immature neurons [10,12].

Material and Methods:

Ethical Review

This research study was conducted in the Department of Anatomy, Benjamin Carson (Snr) School of Medicine, Babcock University, Ilishian Remo, Ogun State, Nigeria. All rules and regulations in the guide, care and use of animals in research and teaching are abide and approved by Babcock University Health Research Ethics Committee (BUHREC380/19), Ilishian Remo, Ogun State, Nigeria.

Acquisition of Experimental Substances

Reserpine and fluoxetine were acquired from Sigma Chemical Co., USA, while *Theobroma cacao* seeds were acquired from a cocoa plantation in Ilishan-Remo, Ogun State, Nigeria. *Theobroma cacao* seeds were identified and authenticated by taxonomists from the Department of Botany, Babcock University, Ilishian Remo, Ogun State, Nigeria.

Preparation of Experimental Substances

Reserpine was dissolved in glacial acetic acid (1%) and then diluted with appropriate Distilled Water (DW). Vehicle consisted of the same amount of acetic acid and water as in the reserpine solution [13]. Flunisan tablets containing 20 mg of fluoxetine-hydrochloride was purchased from Hemofarm, Serbia and Novartis Pharmaceuticals UK, respectively. Flunisan was crushed, and dissolved in DW and 1N HCl, respectively [14]. Solutions were filtered using Whatman No. 42 filter paper and concentrations of FLU were determined using ultra performance liquid chromatography analysis [15].

Theobroma cacao seed was left to dry in the laboratory at ambient temperature (30 ± 2°C) for 10 days. *Theobroma cacao* seeds were thereafter pulverized with a laboratory mechanical grinder. The obtained fine powders were then stored until needed. A 100 g of the powdered sample was subjected to crude extraction methods.

Study Design:

Thirty-six (36) female Wistar rats were purchased from the animal house Babcock University, for the experiment. They were placed in plastic cages with net covers for ventilation. The rats were bred at the Department of anatomy animal house, Babcock University. After two weeks' acclimatization period, the animals were divided into six (A - F) groups (n = 6).

Group A - control group (CTR),

Group B - 0.2 mg/kg Reserpine (RES) for 14 days,

Group C - 10 mg/kg Fluoxetine (FLU) for 14 days,

Group D - 500 mg/kg *Theobroma cacao* seed ethanolic extract (CSE) for 14 days,

Group E - 0.2 mg/kg RES for 7 days + 500 mg/kg CSE for 7 days,

Group F - 0.2 mg/kg RES for 7 days + 10 mg/kg FLU for 7 days.

RES was administered intraperitoneally (IP) while FLU and CSE were administered via oral gavage using oral cannula.

Excision of target tissue

After the last day of administration, the experimental animals were euthanized using cervical dislocation. Prefrontal cortex and hippocampus were thereafter harvested and fixed in 10% formol-saline for routine histological processing using Haematoxylin & Eosin (H&E) stain to highlight the general microstructure prefrontal cortex and hippocampus, while cresyl fast violet stain to highlight protein synthesis in the brain.The whole brain was placed in phosphate buffer and homogenized after which it was subjected to centrifugation at a resolution of 300 rpm for 10 minutes. Cleared supernatants were aspirated into plain bottles and refrigerated at 4°C before biochemical analysis.

Neurobehavioral Tests

The neurobehavioral tests were carried out in a room with a quiet atmosphere between the hours of 10 am to 2 pm and all events were filmed and observed critically with a canon camera.

Forced Swim Test (FST)

This was used to check depression in rat by forcing them to swim. The rat was placed in a cylinder; 40 cm tall in height and 20 cm wide in diameter. The animals were left for about 5 minutes each and the time taken for each rats to struggle for surviving, by nosing above water. Usually it takes more than 2 minutes for non-depressed rats to take a survival method and less than 2 minutes in depressed rats.

Open Field Maze (OFM)

This test was used for measuring anxiety and exploration as well as locomotion due to it large open area. The open field maze was cleaned with 70% ethanol before test began. The experimental animal was placed at the corner of one of the four corners of the box of the apparatus and allowed to explore the apparatus for 5 minutes. After 5 minutes, the apparatus was cleaned with 70% ethanol before the next test began. The behaviors were scored in accordance to the number of lines crossed and rearing. The numbers of lines crossed and rearing were used to measure locomotion activity and also anxiety and exploration. Therefore, the higher frequency of these behaviors are the high exploratory behavior and low anxiety behavior.

Biochemical assay

The supernatants collected from the brain homogenate were used for Malondialdehyde (MDA) and serotonin assay. The oxidative stress

marker and neurotransmitter were assessed by an enzyme-linked immunosorbent assay kit (Minneapolis, Minnesota, USA).

Data analysis:

Data collected were analyzed using two-way Analysis of Variance (ANOVA) followed by Tukey's (HSD) multiple comparison test with the aid of GraphPad Prism v.6 (GraphPad Software, Inc., La Jolla, CA, USA). Data were presented as means ± SEM (standard error of mean). P value less than 0.05 ($p<0.05$) was considered statistically significant.

Results:

Behavioral test

The immobility period during the FST was significantly higher ($P<0.05$) in RES group compared to control and other experimental groups. During the OFM, number of lines crossed and rearing reduced significantly ($P<0.05$) in RES group compared to control animals (Fig. 1A-C).

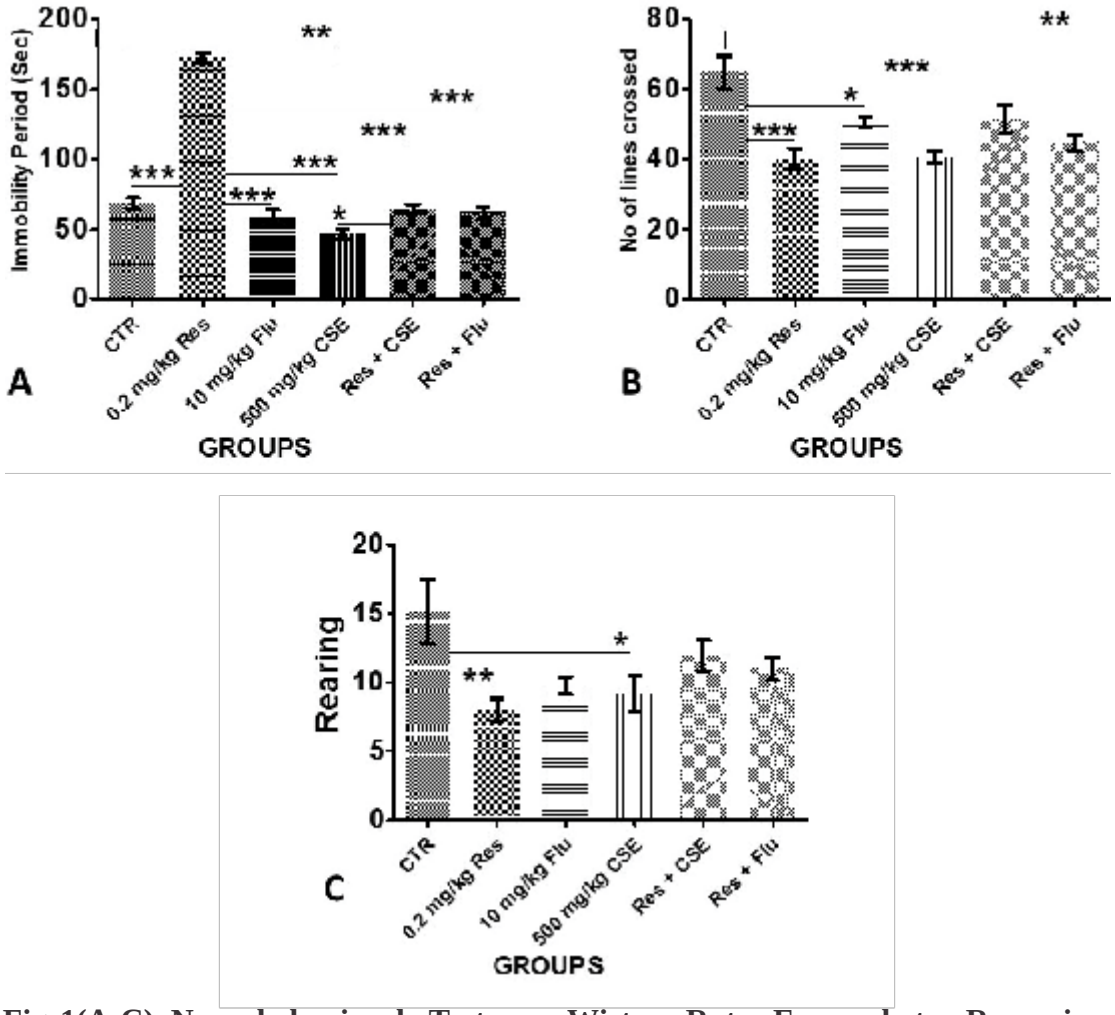

Fig. 1(A-C): Neurobehavioral Test on Wistar Rats Exposed to Reserpine, Theobroma cacao Seed Extract and Fluoxetine
Fig. 1A: Immobility Period (FST),
Fig. 1B and Fig. 1C: Number of Line and Rearing (OFM).

Histological observation

The photomicrographs of the prefrontal cortical and the hippocampal regions of animals exposed to reserpine, *Theobroma cacao* seed extract and fluoxetine revealed normal morphology, orientation and population of prefrontal neuronal cells in CTR, FLU and CSE groups while degeneration and clustering of neuronal cells were noticeable in RES group. Less neuronal degeneration and dispersal was noticed in groups given concurrent RES + CSE RES + FLU (Fig. 2). RES showed reduced population and staining intensity of protein producing Nissl cells in the prefrontal cortex and hippocampus compared to control CTR, FLU and CSE groups. The staining intensity of Nissl cells in RES + CSE and RES + FLU was similar to that of control group but reduced population of protein producing Nissl cells (Figs. 3, 4, 5).

Oxidative stress and Neurotransmission

MDA concentration significantly increased ($P<0.05$) in reserpine (RES) group compared to control and other experimental groups. No significant differences ($P>0.05$) exist in serotonin levels of control animals compared to experimental animals. Though, serotonin levels were highest in CSE and FLU groups but insignificant compared to control animals (Fig. 6).

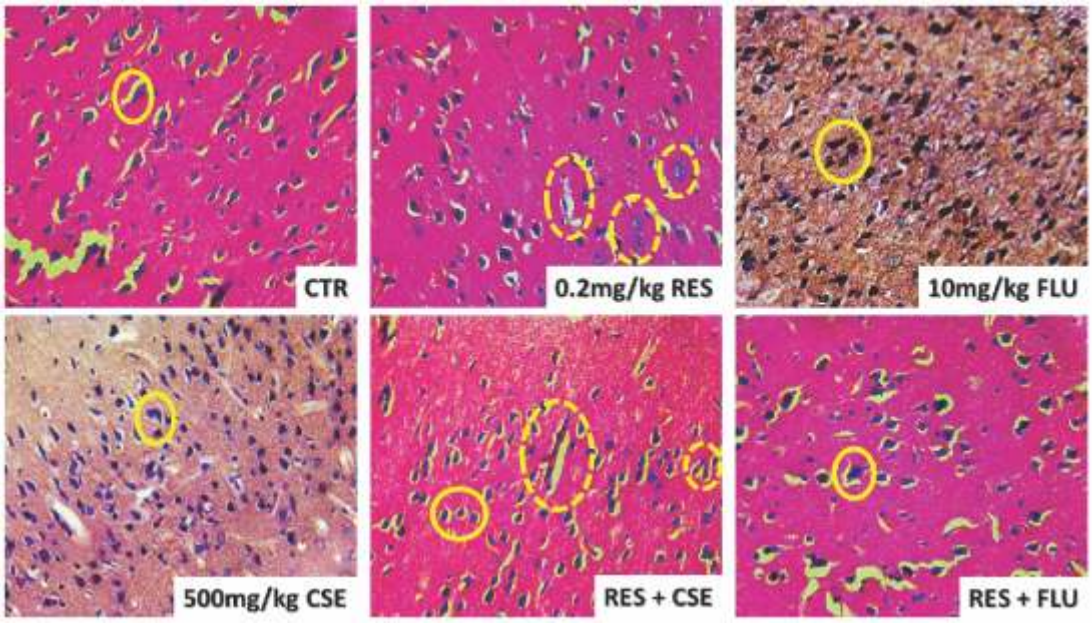

Fig. 2: Prefrontal cortex of Wistar rats exposed to RES, CSE and FLU Mag × 100 H&E

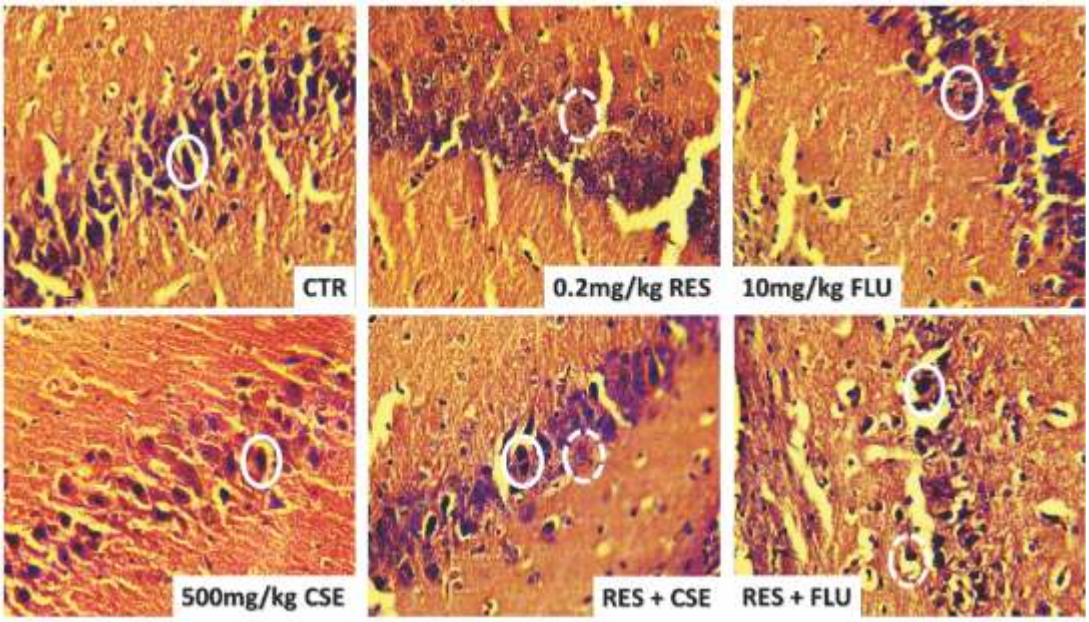

Fig. 3: Hippocampus (Cornu Armonis) of Wistar rats exposed to RES, CSE, FLU Mag × 100 H&E

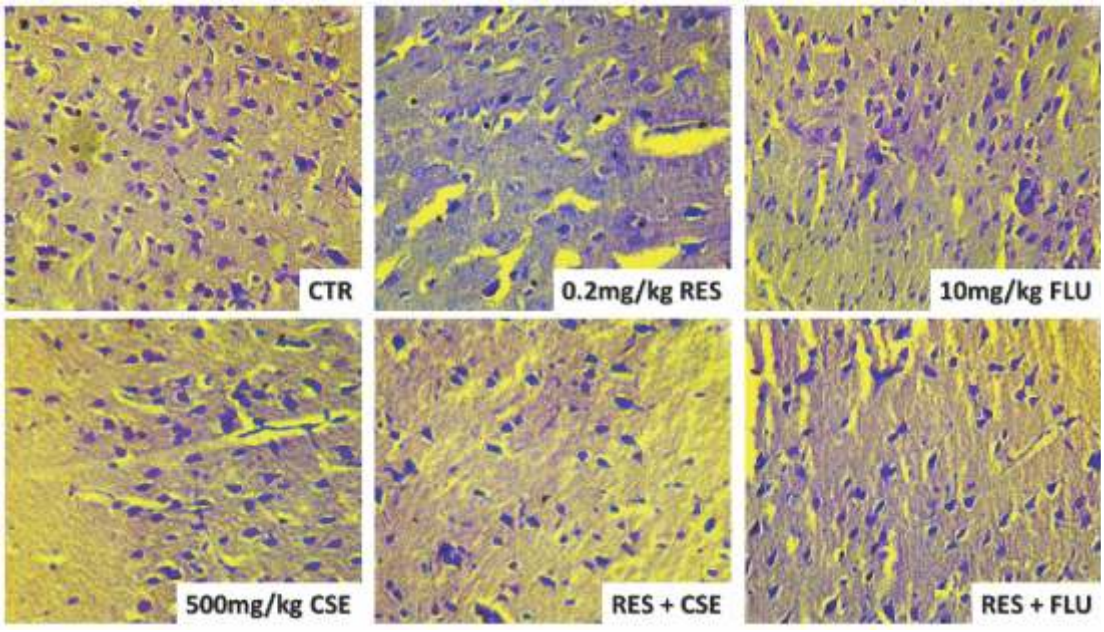

Fig. 4: Prefrontal Cortex of Wistar rats Exposed to RES, CSE and FLU Mag. ×100. Cresyl Fast Violet stain

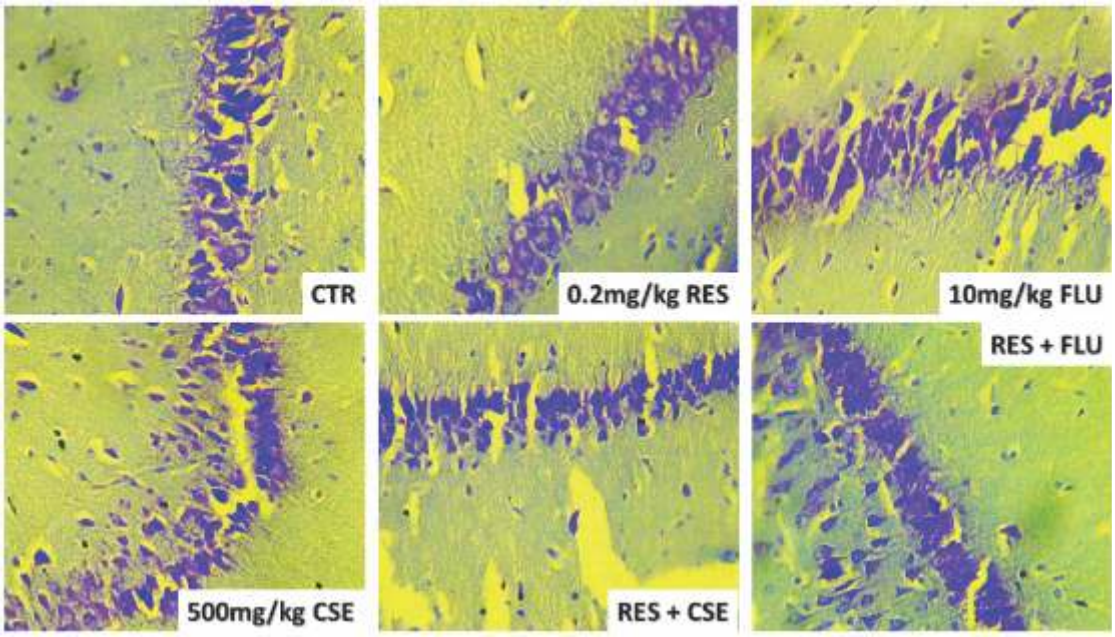

Fig. 5: Hippocampus (Cornu Armonis) of Wistar Rats Exposed to RES, CSE, FLU Mag. × 100 Cresyl Fast Violet stain.

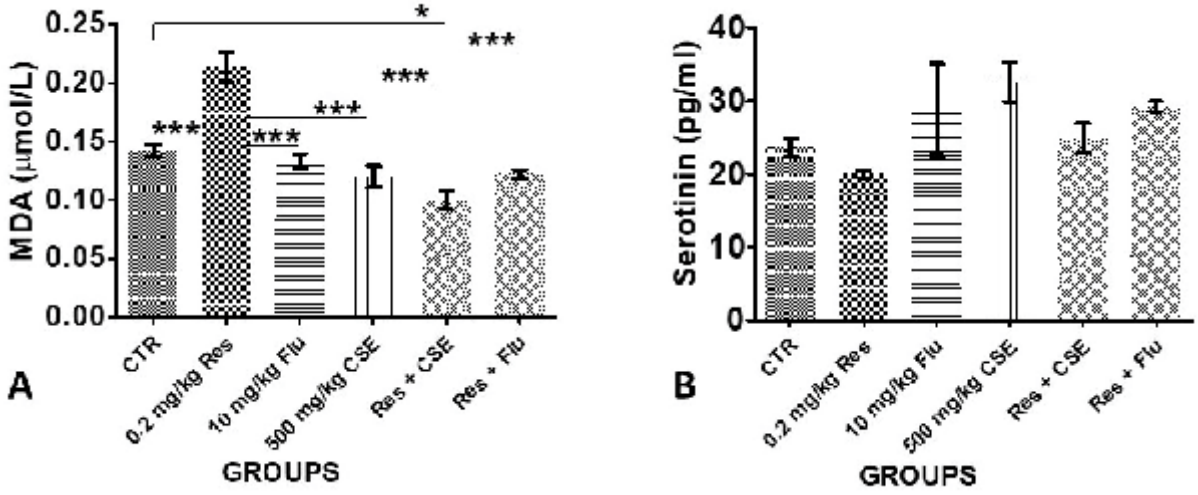

Fig. 6: Biochemical Concentrations in Wistar rats exposed to RES, CSE and FLU Fig. 6A: MDA concentration, Fig. 6B: Serotonin concentration

Discussion:

Epicatechin, the main flavonoid present in *Theobroma cacao* improves various aspects of cognition in animals, likewise induces positive effects on mood and is often consumed under emotional stress in form of chocolates. Flavonoids preserve cognitive abilities during aging in rats and it might likely lower the risk for developing Alzheimer's disease and decrease the risk of stroke in humans. All these properties are of great interest but at present it is not clear when the consumption of *Theobroma cacao* should be initiated to generate beneficial effects on age-dependent cognitive decline and neurodegenerative diseases and many studies have been carried out on *Theobroma cacao* showing the neuroprotective potential [5].

The histological observation from the PFC photomicrographs result shows impaired executive function and inability to form new memory in exposed subjects [6] and [13]. Varying structural distortions such as neurodegeneration and clustering in the PFC and dispersion in the hippocampus of RES treated animals. Also, Nissl stain revealed less staining intensity in PFC neurons of animals treated with RES. At the dose and duration of the CSE tested restorednormal morphology, orientation and population of PFC and hippocampal neurons, and also improved the protein producing Nissls substances due to the deep staining intensity seen in the PFC and hippocampal neurons of CSE groups.

According to the results obtained from the behavioral test our experimental animals were subjected to, CSE repressed the anxiolytic and depressive behaviors of animals exposed to RES to a great extent. Animals treated with CSE were very active, fighting for survival during the FST, immobility periods were at lowest among CSE treated animals likewise with the animals treated with FLU and control animals. During the Y-maze behavioral test, CSE improved explorative instinct and inquisitiveness of experimental animals in the number of lines crossed and rearing behavior of animals.

A possible mechanism of action of RES maybe oxidative stress due to increased concentration of oxidative stress marker, MDA, produced in the brain of animals treated with reserpine only [16]; MDA is usually produced in a biological cell due to gradual degeneration of lipid membrane covering of cells. This support the report of researchers who suggested that RES induces oxidative stress in exposed subjects[16]. CSE appreciably reduced the lipid peroxidation in RES exposed animals, CSE may be said to possess anti-oxidative properties.

The finding from this research also suggested that CSE may possess neuroprotective properties. Though no significant difference was seen in the neurotransmitter levels in all experimental group but CSE had a positive effect on neurotransmission among PFC and hippocampal neurons, as it was able to revert the diminishing effects of RES on serotonin neurotransmitter in the brain of exposed animal. Serotonin; the happy neurotransmitter in the brain, aids communication and impulse transmission among PFC interneurons, reduction in the level of serotonin would hamper excitation and communication of PFC interneurons which may result in depression if unchecked [17].

Conclusion:

Ethanolic extract of *Theobroma cacao* seed revert the debilitating actions of reserpine on the structure and function of the prefrontal cortical and

hippocampal regions of the brainas well, the behavioral pattern of exposed female rats at the dose and duration tested. *Theobroma cacao,* based on the findings in the study, may be said to possess anti-oxidative, anti-depressant and neuroprotective properties.

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