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NEUROPROTECTIVE AND ANTI-INFLAMMATORY EFFECTS OF COMBINE HERBAL THERAPY ON AMITRIPTYLINE-INDUCE STROKE IN RAT

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Abstract

This study investigated the neuroprotective and neurorestorative role of *Khaya senegalensis* (KS) bark and *Tinospora cardiofolia* (TC) leaf extracts on amitriptyline (AMT) induced stroke on the prefrontal cortex in adult Wistar rat. Fifty-fiveadults male Wistar rats weighing 184-254g, were used for this experiment and they were grouped into eleven groups (n = 5), 2 controls (normal and AMT rats) and 9 treatment groups. The experiment was in 2 phases, phase I: rats were divided into 2 main groups, normal control (administered with distilled water) and the other group were to induce stroke, using a single dose of 1.4 mg/kg of AMT for 3 days.Phase II,the rats were subdivided into AMT group, which served as negative control (stroke rats), not treated and 9 treatment groups, treated with KS and TC orally, at doses of 200, 300 and 400mg/kg body weight, respectively, for a period of two weeks. After administration the rats were euthanizedusing cervical dislocation and the brain were collected and fixed in 10% neutral buffered formalin.Serum level of malondialdehyde, a marker of lipid peroxidation, and total antioxidant activities were measured.AMT induced rats showed a significant decrease in oxidative stress marker (SOD, GSH and CAT) while MDA significantly increase, and these deficits were effectively reversed by KSBE and TCLE.Normal histoarchitecture was found in normal control rats while morphological and neuronal degeneration in prefrontal cortex observed in AMT induced rats. On treatment with KS_{LD} (200 mg/kg), KS_{MD} (300 mg/kg), KS_{HD}+ AMT (400 mg/kg); KS_{LD} (200 mg/kg), showed, morphological and neuronal recuperative changes while marked improvement (neurovascular unit and cortical cells) were noted in KS_{MD} (300 mg/kg), whereas KS_{HD} (400 mg/kg) showed vacuolated cortical cells with minimal improvement. In conclusion, combined KS bark and TC leaves at dose 300 mg/kgshown neurorepairs and protective role on neurodegenerative changes in the prefrontal cortex, by regenerating cortical cells.

KEYWORDS: Amitriptyline, Prefrontal Cortex, *Khaya senegalenesis, Tinospora cardiofolia,*Neurovascular unit.

Introduction

Understanding the multiple events (ischemic cascades) surrounding the occurrence of stroke, especially on the neuronalinjury,may assists researchers and clinicianson neurorestorative and neuroprotective strategies to treats and managed stroke.

Stroke is the third most common cause of death and long-term disability. There are over 13.7 million new strokes each year (Stroke Association, 2012;World Stroke Organization, 2019). Globally, studies have shown that one in four people over age twenty - five will have a stroke in their lifetime and over 80 million people currently living who have experienced a stroke. Five and a half-million people die from stroke annually, in 2030. Stroke deaths are expected to increase to 7.8 million globally (Word Health Organization, 2004; Strong *et al*., 2007; America Heart Association, 2015; World Stroke Organization, 2019).

Herbal medicines have been used for the treatment of various disease conditions in the ancient system of medicine. These are nowadays revalued by various researches on different plant species and their effective principles (Legos et al., 2002; Abhishek *et al*., 2013*)*.It has been reported that herbal drugs have fewer side effects and high therapeutic window and moreeconomical, thereforethey are currentlyaccepted widely and they may be use for prophylactic treatment. Conventional treatment for strokehas been reported withadverse reactions after a window period of stroke treatment and alsohavefailed its neuroprotectionclinical trials in stroke**(**Kaste, 2001;Legos et al., 2002; Able *et al*., 2009; Abhishek *et al*., 2013).

Khaya senegalensis A. Juss (Meliaceae) commonly known as African mahogany, is a popular medicinal plant among the Nupes and Yorubas in Nigeria. It belongs to the family Meliaceae (mahogany). The stem bark aqueous extract is traditionally used by Yoruba and Nupe tribes to treat malaria, jaundice, edema and headache (Bickii*et al*; 2000; Ahmed *et al*; 2014; Muhammad and Alhassan, 2016). Studies have demonstrated that *Khaya senegalensis* stem bark extract possesses anti-inflammatory, high antioxidant/ radical scavenge capacity, and boost the immune system by promoting the production of globulins and immunoglobulins thereby chemo preventing diseases like neurodegenerative disorders (Atawodi *et al*., 2009; Kolawole *et al*., 2013;*MacDonald et al.,* 2014; Ahmed *et al*; 2014).

Tinospora cordifolia (Willd.) Miers ex Hook. F. and Thoms belonging to the family Menispermaceae, is a large, deciduous, climbing shrub found throughout India, especially in the tropical parts ascending to an altitude of 300 m, and also found in certain parts of China. It is shrub widely use in folk medicine and in Ayurvedic system of medicine and in botanical formulations in India and China (Shrivastava *et al.,* 2013). Due to free radical scavenging property of *Tinospora cardifolia* against reactive oxygen and nitrogen species, it reduces the expression of iNOS gene (Reddy *et al.,* 2009). It may be an effective therapeutic tool against ischemic brain damage due to attenuation of oxidative stress mediated cell injury during oxygen-glucose deprivation (OGD) and exerts the free radical scavenging effect in both the cytosolic as well as at gene expression levels (Reddy *et al.,* 2009, Sengupta *et al*., 2009; Chaudhari *et al.,* 2013).

Combined approach to treat stroke, is reported to be the most refined method, using neuroprotection agent and thrombolytic agent (Abhishek *et al*., 2013; Legos *et al*., 2002; Green *et al.,* 2008;Fisher *et al.,* 2000;Jonas *et al.,* 1995). And the most

effective method to re-establish cerebral blood flow is thrombolytic therapy; however, this therapy is often at the risk of bleeding (Albers *et al.,* 2008).

Amitriptyline is potentially lethal in overdose, hence its decline in treatment of psychiatric disorders. The main concerns are of cardiac toxicity through cardiac sodium channel blockade and serotonin syndrome (Moore *et al*., 2015).

Amitriptyline possesses a strong action on the serotonin transporters (SERT) and moderate effects on the norepinephrine transporter (NET) causing inhibition in the neuronal uptake of the neurotransmitter serotonin and norepinephrine from the synaptic cleft in the central nervous system. It has negligible influence on the dopamine transporter (DAT) and hence does not affect dopamine reuptake (Tatsumi *et al*., 1997; Gillman, 2007; Kim, 2017).

Several authors had reported that for a given chemical/drug exposure in nervous system, there are multiple potential targets of neurotoxicity working through multiple and unknown mechanisms. And the damage to the CNS results in conversion of microglia and astrocytes into their 'reactive' or 'activated' form. Since Microglia and astrocytes constitute subtypes of glial cells, glia and neurons being the main cellular constituents of the nervous system. Therefore, injury-induced either by chemical/drug exposure results in transformation of microglia and astrocytes into their 'activated' phenotype which is referred to as 'reactive' gliosis or simply, gliosis. Injury may result from trauma, ischemia, infectious and neurological diseases and, more recently, chemical exposures, are all known to have the capacity to induce gliosis. Gliosis has been recognized as a rapid and universal response to all types of brain insults (Callaghan & Sriram, 2005; Callaghan 2005**;** Norenberg, 2005; Fang et al,.2016; Huang *et al.* 2019).

Single therapeutic approach using thrombolytic therapy has been reported as the most effective method to re-establish cerebral blood flow and but often at risk of bleeding after a window period of two weeks (Albers *et al.,* 2008).Therefore, there is a need to attempt the used of combined therapy (thrombolytic and neuroprotection agents) to treat and manage stroke, hence this present study.

Materials and methods

Experimental protocols

All protocols and treatment procedures of the experiment were approved by the Research Ethics Committee of the College of Medicine of the University of Lagos with protocol number CMUL/ACUREC/03/21/362V1. The animals were housed in well-ventilated plastic cages, kept and maintained under standard laboratory conditions in the animal house of the faculty of Basic Medical Sciences of the University of Lagos. They were given feed and water *ad libitum*.

Plants Material

Fresh bark of *Khaya senegalensis* (KS) was gotten from a Forest at Ukelle community of Cross River State while *Tinospora cordifolia* (TC) leaves were gotten from a neatly cultured garden at Okpoma community of Cross River State. The tree bark and leaves were identified and authenticated in the Department of Botany, University of Lagos, Nigeria and stored in theUniversity herbarium with ID No: 8003 and 8004 for TC and KS respectively.

Plant extract preparation

Fresh bark of *Khaya senegalensis* was well cleansed and diced into smaller pieces using a sterile knife to aid the drying process and after which they were air dried at room temperature for a period of four weeks. The

stem bark was then oven dried at 50℃ for 3hrs and thereafter crushed into semi powder using a grinding machine. 180g of coarse powder of bark *Khaya senegalensis*and 160g of *Tinospora cordifolia*was packed into separate thimble and inserted to separateSoxhlet extractor. The Soxhlet was inserted into the quick fit bottom flask containing solvent. The solution was left to concentrate using a rotary evaporator, the dried extract of *Khaya senegalensis* yielded 161g, and that of *Tinospora cordifolia*yielded138g,and were collected and preserved at 4℃ for further use.

Animal grouping, reagent preparation and treatment

Fifty-five adults male Wistar rats weighing 184-254g, were used for this experiment and they were grouped into eleven groups (n = 5), 2 controls (normal and AMT rats) and 9 treatment groups. The experiment was in 2 phases, phase I: rats were divided into 2 main groups, normal control (administered with distilled water) and the other group were to induced stroke, using a single dose of 1.4 mg/kg of AMT for 3 days.

Amitriptyline was purchased from Sigma-Aldrich, USA, and prepared at a dose of 1.4 mg AMT in 20ml of distilled water.

Phase II,the rats were subdivided into AMT group(stroke rats), which served as negative control andwere not treated with any extract and 9 treatment groups, which were treated with KSBEat doses of 200(KS_{LD}), 300 (KS_{MD}) and 400(KS_{HD}) mg/kg bodyweight and TCLEat doses of 200(TC_{LD}), 300 (TC_{MD}), and 400(TC_{HD}), mg/kg body weight orally, as single treatment for a period of two weeks.For combined treatment, KSBE and TCLE were combined at doses of 200(COM_{LD}), 300 (COM_{MD}), and 400(COM_{HD}), mg/kg body weight respectively. And all were administered orally for a period of two weeks.

Animals Sacrifice and Tissues Processing for Basic Histology

At the end of 2 weeks, the rats were sacrifice using cervical dislocation and the brain were collected and fixed in 10% neutral buffered formalin. Fixed tissues were processed for routine paraffin embedding using standard methods, and subjected to routine Hematoxylin and Eosin staining protocols. Stained slides were observed under a Digital Microscope (OMAX 40-2000X 3MP Digital Compound Microscope, USA) and photomicrographs obtained.

Estimation of Oxidative Stress Biomarkers

Superoxide Dismutase activity, reduced Glutathione**,** malondialdehyde, a marker of lipid peroxidation, and Catalaseactivity were measured in the serum. Lipid peroxidation was determined by measuring Malondialdehyde (MDA)formed by thiobarbituric Acid reaction (Chowdhhry and Soulsby, 2013). Catalase activity was estimated by measuring the rate of decomposition of H_2O_2. thesuperoxide Dismutase (SOD) activity was measured by the method of Mistra *et al*. while the method of Eleman *et al*. was adopted to determined Reduced Glutathione (GSH) level.

Determination of Superoxide Dismutase (SOD) activity

Superoxide Dismutase activity was determined by its ability to inhibit the auto-oxidationofepinephrine determined by the increase in absorbance at 480nm as described by Mistra, Fridovich, (1972). The reaction mixture (3 ml) contained 2.95 ml 0.05 M sodium carbonate buffer pH 10.2, 0.02 ml of liver homogenate and 0.03 ml of epinephrine in 0.005 N HCL was used to initiate the reaction. The reference cuvette contained 2.95 ml buffer, 0.03 ml of substrate (epinephrine) and 0.02 ml of water. Enzyme activity was calculated by

measuring the change in absorbance at 480 nm for 5 min. $\sum = 4020M^{-1}\ cm^{-1}$

Catalase activity Determination

Catalase activity was determined according to Sinha*, et al*., (1972). It was assayed colorimetrically at 620nm and expressed as µmoles of H_2O_2 consumed/min/mg protein at 25^0C. The organs of the animal were washed in an ice cold 1.15% KCL solution, blotted and weighed. They were then homogenized with 0.1M phosphate buffer (pH 7.2), putting the organs each into the mortar; laboratory sand was added to it (acid washed sand) and it was blended in the mortar with pestle together. The resulting homogenate was centrifuge at 2500rmp speed for 15mins then it was removed from the centrifuge and the supernatant was decanted and stored -20°C until analysis. The reaction mixture (1.5ml) contained 1.0ml of 0.01M phosphate buffer (pH 7.0), 0.1ml of tissue homogenate and 0.4ml of 2M H_2O_2. The reaction was stopped by the addition of 2.0ml of dichromate-acetic acid reagent (5% potassium dichromate and glacial acetic acid were mixed in 1:3 ratio). $\sum = 40M^{-1}\ cm^{-1}$

Reduced Glutathione Determination

The reduced glutathione (GSH) content of liver tissue as non-protein sulfhydryl was estimated according to the method described by Eliman and Riddles (1959). To the homogenate 10% TCA was added, centrifuged. 1.0ml of supernatant was treated with 0.5ml of Ellmans reagent (19.8mg of 5,5-dithiobisnitro benzoic acid (DTNB) in 100ml of 0.1% sodium nitrate) and 3.0ml of phosphate buffer (0.2M, pH 8.0). The absorbance was read at 412nm.$\sum = 1.34 \times 10^4\ M^{-1}\ cm^{-1}$

Lipid Peroxidation

Malondialdehyde (MDA) an index of lipid peroxidation was determined using the method of Chowdhhry and Soulsby (2013). 1.0 ml of the supernatant was added to 2 ml of (1:1:1 ratio) TCA-TBA-HCl reagent (thiobarbituric acid 0.37%, 0.24N HCl and 15% TCA) tricarboxylic acid- thiobarbituric acid-hydrochloric acid reagent boiled at 100°C for 15 min, and allowed to cool. Flocculent materials were removed by centrifuging at 3000 rpm for 10 min. The supernatant was removed and the absorbance read at 532 nm against a blank. MDA was calculated using the molar extinction coefficient for MDATBA-complex of $1.56 \times 10^5\ M^{-1}CM^{-1}$.

Statistical analysis

Numerical data was expressed as Mean ± standard error of mean; analysis was done using two- way analysis of variance (ANOVA) and graph prism 8 software. Statistical Significance was set at P-value < 0.05

Results

Oxidative stress markers

The effect of KS extract on AMT induced superoxide dismutase (SOD)activity,Glutathione (GSH), Catalase (CAT) level and Malondialdehyde (MDA)level in the prefrontal cortex.

Khaya senegalenesis (KS) group

Oral administration of AMT induced a significant decrease in SOD activity, in stroke-induced animals (AMT group; negative control) as compared to positive control ($p<0.05$).However, AMT - induced SOD deficit was reversed significantly in animals treated with*Khaya senegalenesis* low dose (KS_{LD};200 mg/kg), compared to AMT group (negative control). While treatment KS_{MD}+ AMT and KS_{HD}+ AMT(300 and 400mg/kg) of *Khaya senegalenesis*shown no significantly difference as compared to AMT group (fig. 1a).

AMT administration significantly ($p<0.05$), decreased GSH levelin AMT group; negative control as compared to positive control-group A. when treated with low; 200mg/kg, medium; 300 mg/kg and high; 400mg/kgdose of KS, a significantly reversed of AMT – induced deficit was observed in 200mg/kg+ AMT, while 300mg/kg+ AMT and 400+ AMT shows no significant as compared to positive control (fig. 1b).

CAT level was found to decrease significantly ($p<0.05$) in AMT - induced stroke animals as compared to positive control. KS treated groups (200mg/kg+ AMT, 300mg/kg+ AMT and 400mg/kg+ AMT)shows a significant increase ($p<0.05$) in CAT level as compared to AMT group while CAT level wassignificantly ($p<0.05$) higher in 300mg/kg + AMT and 400mg/kg+ AMT groups, than animals in AMT 200mg/kg+ AMT group as compared to positive control group(fig. 1c).
There was a significant ($p<0.05$) increase in MDAlevel in animals treated with AMT (AMT group) as compared with the positive control.There was a significant decreaseobserved in 200mg/kg + AMT group while a slightly decrease was found in 300mg/kg+ AMTgroup as compared to AMT group. At 400mg/kg+ AMT, MDAshows no significant as compared to AMT group (fig. 1d).

The effect of *Tinospora cardiofolia* (TC)extract on AMT induced superoxide dismutase (SOD)activity,Glutathione (GSH), Catalase (CAT) level and Malondialdehyde (MDA)level in the prefrontal cortex.

Oral administration of AMT induced a significant decrease in SOD activity, in stroke-induced animals (AMT group; negative control) as compared to positive control ($p<0.05$). However, AMT - induced SOD deficit was reversed significantly in *Tinospora cardiofolia* low dose + AMT(TC_{LD} + AMT and *Tinospora cardiofolia* medium dose + AMT(TC_{MD}+ AMT) treated with 200 and 300 mg/kg respectively, compared to AMT group.Dose 200mg/kg (TC_{LD}+ AMT), was significantly high while TC_{HD}+ AMT found to be slightly significant when compared to AMT group (fig. 2a).

On treatment with TC, low; 200mg/kg, medium; 300mg/kg and high, a significant increase was found in 200mg/kg+ AMT,as compared to GSH decrease by AMT in negative control (AMT group). No significant difference found in group, 300mg/kg+ AMT and 400mg/kg+ AMT (fig. 2b).

A significant ($p<0.05$) decreasewas found in CAT level in AMT group as compared to positive control. CAT level increase significantly ($p<0.05$) across the TC treated animals (in 200mg/kg+ AMT, 300mg/kg+ AMT and 400mg/kg+ AMT groups) as compared to AMT group (fig. 2c). CAT level at dose 200mg/kg+ AMT, was significantly ($p<0.05$) highly as compared to AMT group and positive control group (fig. 2c).

TC treated animals in 200mg/kg + AMTand 300mg/kg+ AMT groups shows a significant decrease in MDA level as compared to MDA increase in AMT group. There was no significant ($p<0.05$) difference in MDA level found between 400mg/kg+ AMT group and AMT group (fig. 2d).

The effect of combined*Khaya senegalenesis*and *Tinospora cardiofolia* (COM_{KS} and $_{TC}$)extract on AMT induced superoxide dismutase (SOD)activity,Glutathione (GSH), Catalase (CAT) level and Malondialdehyde (MDA)level in the prefrontal cortex.

On treatment with combined therapy; combined *Khaya senegalenesis*and*Tinospora cardiofolia* ($COM_{KS\ +TC}$)of 200, 300 and 400mg/kg (COM_{LD}+ AMT, COM_{MD} + AMT and COM_{HD}+ AMT) doses respectively.A significant increase, was observed in SOD activity ($p<0.05$), across the groups (200mg/kg + AMT, 300mg/kg + AMT and400mg/kg+ AMT) as compared to AMT group (fig. 3a). And highly significant in 300 and 400mg/kg) as compared to AMT group (fig. 3a)

Treatment with $COM_{KS\ +TC}$, on decrease GSH, showsa significant($p<0.05$)increase inGSHacross the treated groups (200mg/kg+ AMT, 300mg/kg+ AMTand 400mg/kg+ AMT), as compared to AMT group. The increase in GSH was significantly($p<0.05$) higher in 300mg/kg+ AMT and400mg/kg+ AMT group,than 200mg/kg+ AMT group, as compared to GSH decrease by AMT in AMT group (fig. 3b).

$COM_{KS\ +TC}$shows a significant increasein CAT level in 200mg/kg+ AMT as compared to the decrease in GSH induced by AMT while CAT level in300mg/kg+ AMTand400mg/kg+ AMTshows no significant (fig. 3c).

$COM_{KS\ +TC}$on increase in MDA levelin AMT group significantlydecreaseat200mg/kg + AMTanimals.No significant ($p<0.05$) difference observed in MDA level between 300mg/kg+ AMTand400mg/kg+ AMTas compared to AMT groups (fig. 3d).

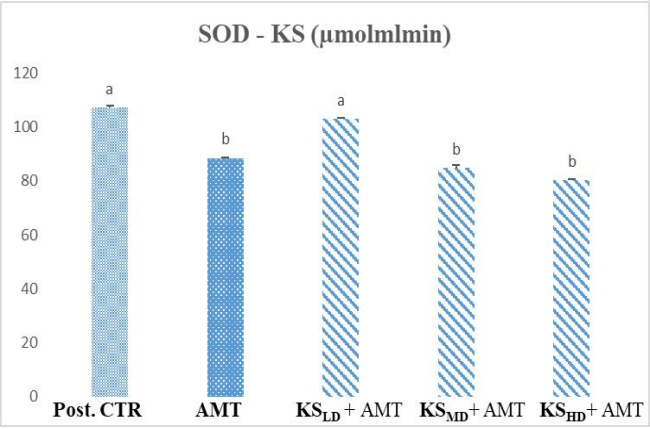

Fig. 1a

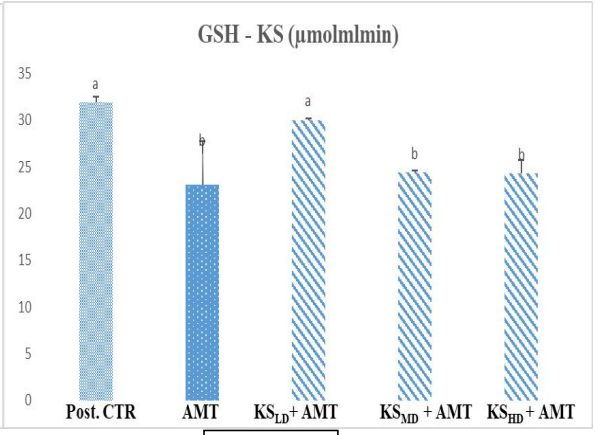

Fig. 1b

Fig. 1a and b:The effect of KS extract on AMT induced SOD and GHS in the prefrontal cortex. Showing, normal control (positive control), AMT group, KS_{LD} + AMT group (KS Low dose; 200mg/kg), KS_{MD} + AMT group (KS Medium dose; 300mg/kg) and KS_{HD} + AMT group (KS High dose; 400mg/kg)P<0.05* = Significantly different from control. Figures are represented as mean ± SD. Level of statistical significance analysis by two- way ANOVA followed by Turkey's post hoc multiple comparison test.

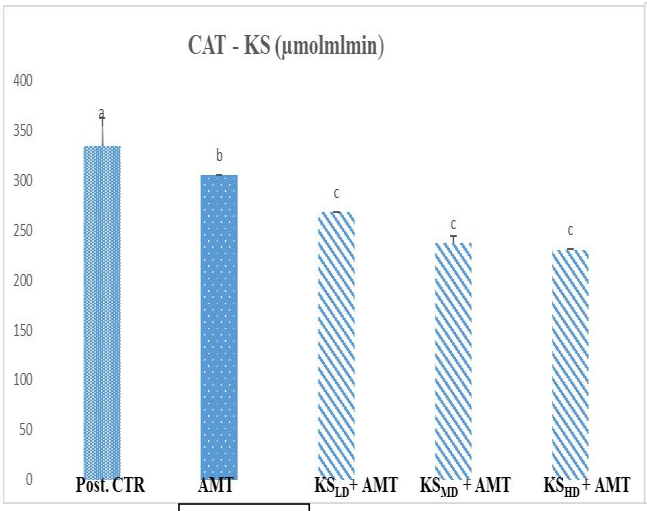

Fig. 1c

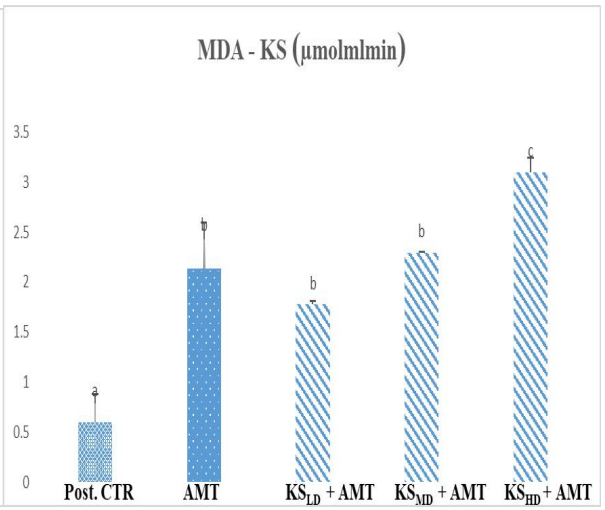

Fig. 1d

Fig. 1c and d:The effect of KS extract on AMT induced CAT and MDA in the prefrontal cortex. Showing, normal control (positive control), AMT group, KS_{LD} + AMT group (KS Low dose; 200mg/kg), KS_{MD} + AMT group (KS Medium dose; 300mg/kg) and KS_{HD} + AMT group (KS High dose; 400mg/kg). P<0.05* = Significantly different from control. Figures are represented as mean ± SD.Level of statistical significance analysis by two- way ANOVA followed by Turkey's post hoc multiple comparison test.

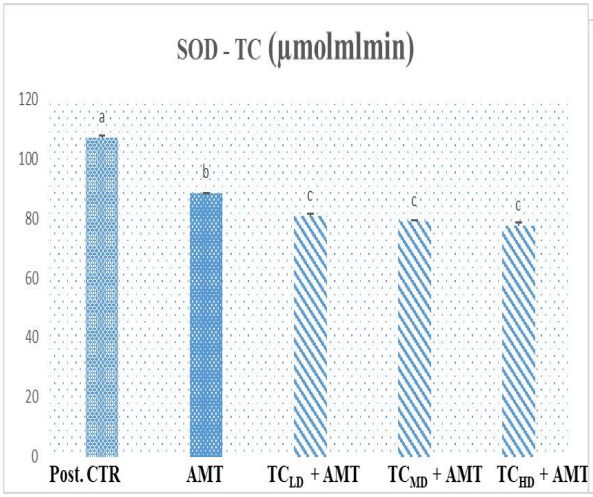

Fig. 2a

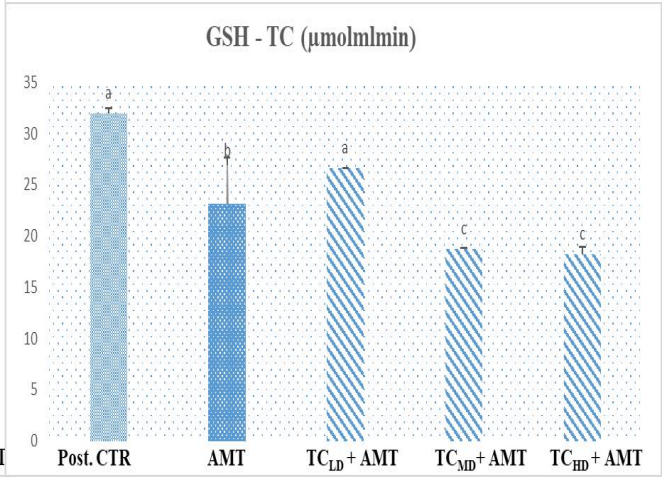

Fig. 2b

Fig. 2a and b:The effect of TCextract on AMT induced SOD and GHS in the prefrontal cortex. Showing, normal control (positive control), AMT group, group TC_{LD} + AMT (TC Low dose; 200mg/kg), TC_{MD} + AMT group (TC Medium dose; 300mg/kg) and TC_{HD} + AMT group (TC High dose; 400mg/kg). P<0.05* = Significantly different from control. Figures are represented as mean ± SD.Level of statistical significance analysis by two- way ANOVA followed by Turkey's post hoc multiple comparison test.

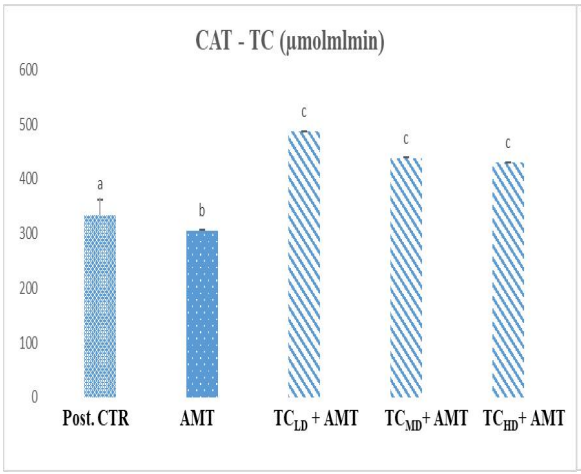

Fig. 2c

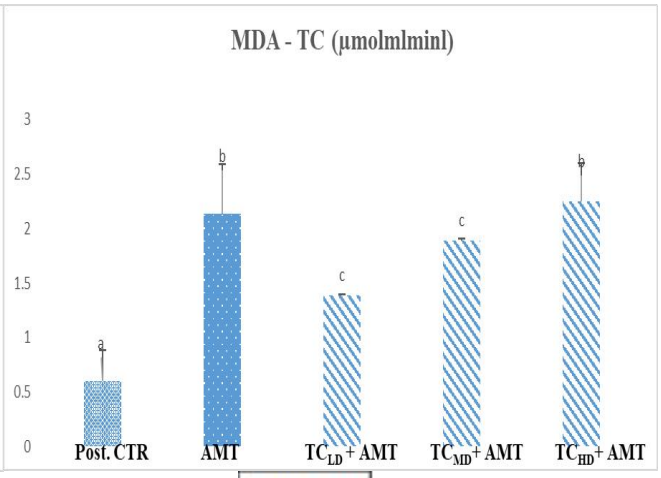

Fig. 2d

Fig. 2c and d:The effect of TCextract on AMT induced CAT and MDA in the prefrontal cortex. Showing, normal control (positive control), AMT group, group TC_{LD} + AMT (TC Low dose; 200mg/kg), TC_{MD} + AMT group (TC Medium dose; 300mg/kg) and TC_{HD} + AMT group (TC High dose; 400mg/kg).P<0.05* = Significantly different from control. Figures are represented as mean ± SD. Level of statistical significance analysis by two- way ANOVA followed by Turkey's post hoc multiple comparison test.

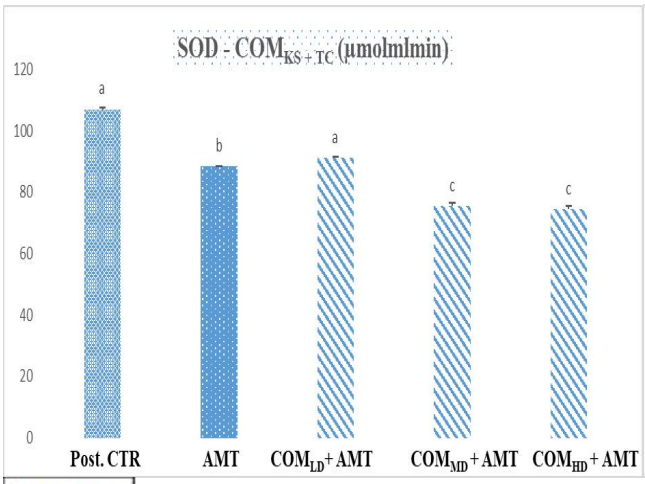

Fig. 3a

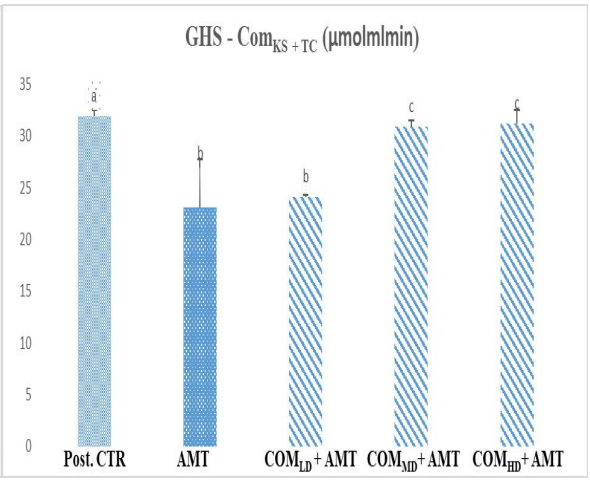

Fig. 3b

Fig. 2a and b:The effect of combined KS and TC extract on AMT induced SOD and GSH in the prefrontal cortex. Showing, normal control (positive control), AMT group, group COM_{LD} + AMT (KS and TC+ AMT - Low dose; 200mg/kg), COM_{MD} + AMT (KS and TC + AMT - Medium dose; 300mg/kg) COM_{HD} + AMT(KS and TC+AMT -High dose; 400mg/kg). P<0.05* = Significantly different from control. Figures are represented as mean ± SD.Level of statistical significance analysis by two- way ANOVA followed by Turkey's post hoc multiple comparison test.

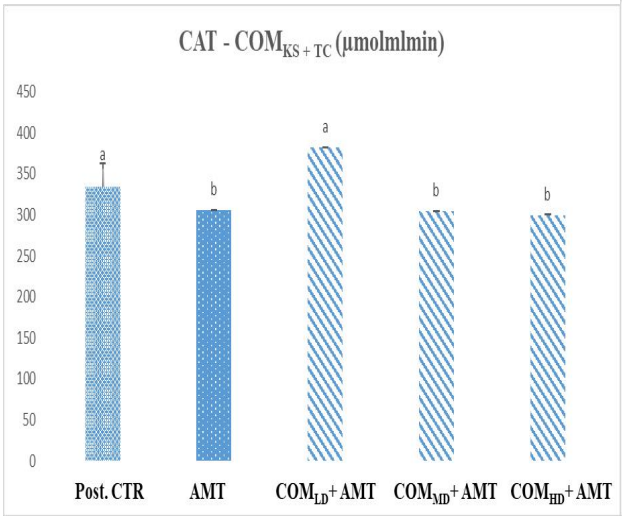

Fig.3c

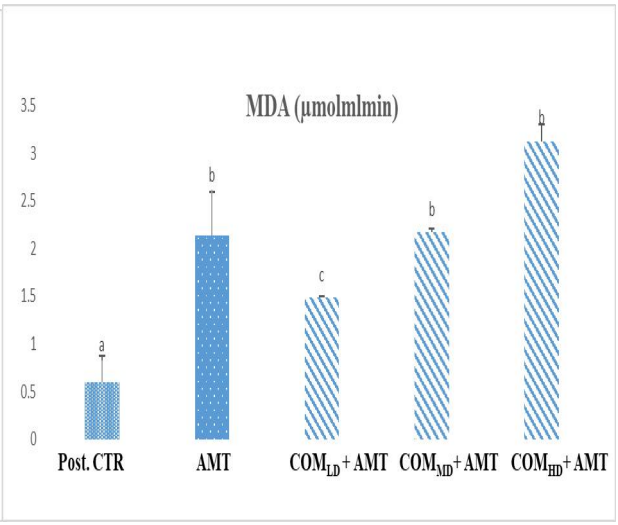

Fig.3d

Fig. 3c and d:The effect of combined KS and TC extract on AMT induced CAT and MDA in the prefrontal cortex. Showing, normal control (positive control), AMT group, group COM_{LD} + AMT (KS and TC+ AMT - Low dose; 200mg/kg), COM_{MD} + AMT (KS and TC + AMT - Medium dose; 300mg/kg) COM_{HD} + AMT(KS and TC+AMT - High dose; 400mg/kg). P<0.05* = Significantly different from control. Figures are represented as mean ± SD.Level of statistical significance analysis by two- way ANOVA followed by Turkey's post hoc multiple comparison test.

Neurohistology of the prefrontal cortex

CONTROLS (Positive & Negative controls

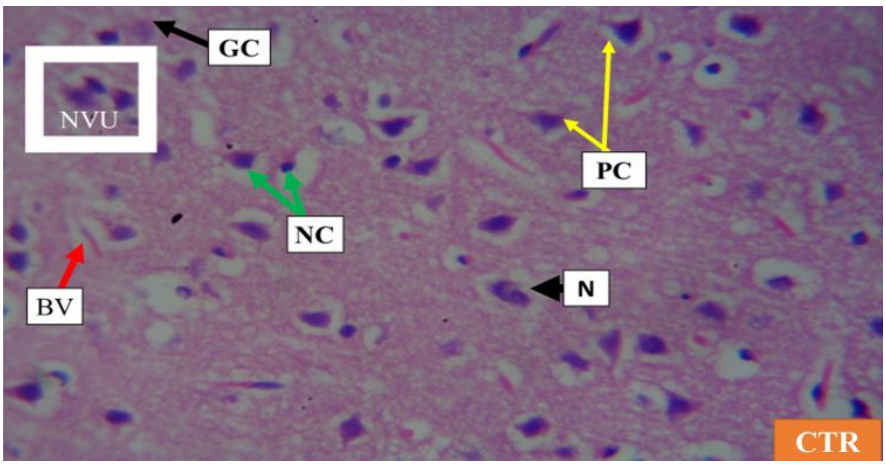

Fig. 4a. A photomicrograph of a section in the prefrontal cortex, group A (positive control). Showing a normal histoarchitecture of cerebral cortex with pyramidal cell (PC), neurovascular unit (NVU), neuron (N), blood vessel (BV), Neuroglial cell (NC), With dense nuclei, granule cell (GC), With pale open face nucleus and blood vessel (BV), (H&E. X400).

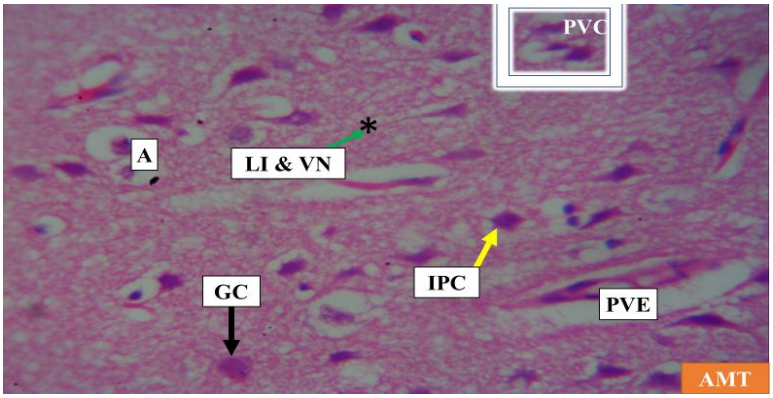

Fig.4b. A photomicrograph of a section in the prefrontal cortex of AMT treated rats, (Negative control) showing degeneration in the prefrontal cortex with evidence of astrocytosis (A), Lymphocytes Infiltrates (LI) and Vacuolated Neutrophils (VN), pyramidal cell with irregular shape (IPC) and surrounded by pericellular halos, Perivascular Edema (PVE), Perivascular Cuffing (PVC) and shrunken Granule cells (GC) deeply stained. (H&E. X400).

KS group (KS+ AMT) results

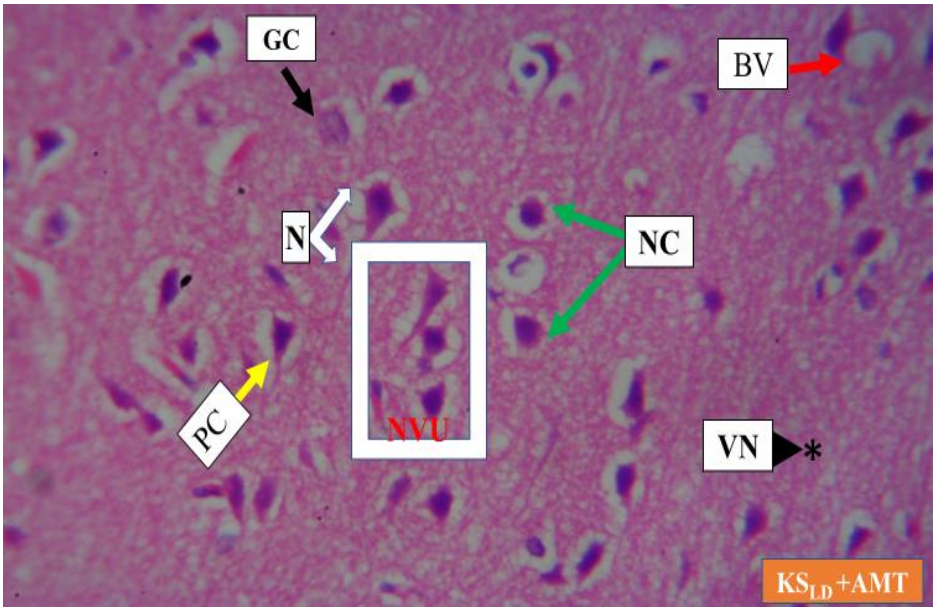

Fig. 4c. A photomicrograph of a section in the prefrontal cortex of KS +AMT treated rats (KS_{LD}; 200mg/kg). Showing less or normal Pyramidal cell (PC), darkly stained nuclei and pericellular halos, neuron (N), Neuroglial cells (NC), deeply stained and vacuolated Neutrophils (*), Granule cells (GC), With pale open face nucleus. The section demonstrates moderate astrocytosis (A), Lymphocytes Infiltrates (LI) and Perivascular Edema (PVE). (H&E. X400).

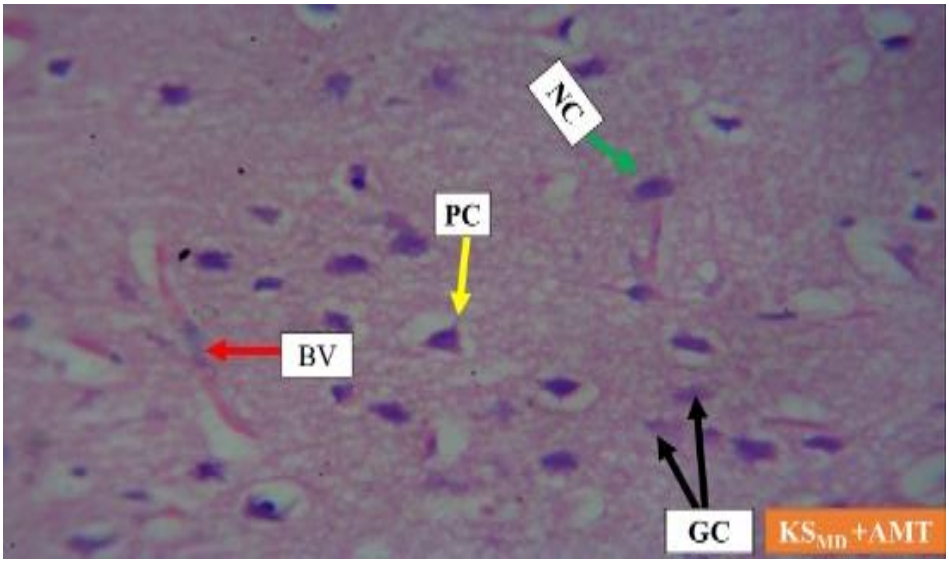

Fig. 4d. A photomicrograph of a section in the prefrontal cortex of AMT and KS treated rats (KS_{MD}; 300mg/kg). Showing Prominence neuron, neurovascular unit (NVN), Pyramidal cell (PC) with pericellular halos, Neuroglial cells (NC) and Neutrophils (*) vacuolated, Granule cells (GC), with open face nuclei and basophil cytoplasm, prominent nucleoli. H&E X400

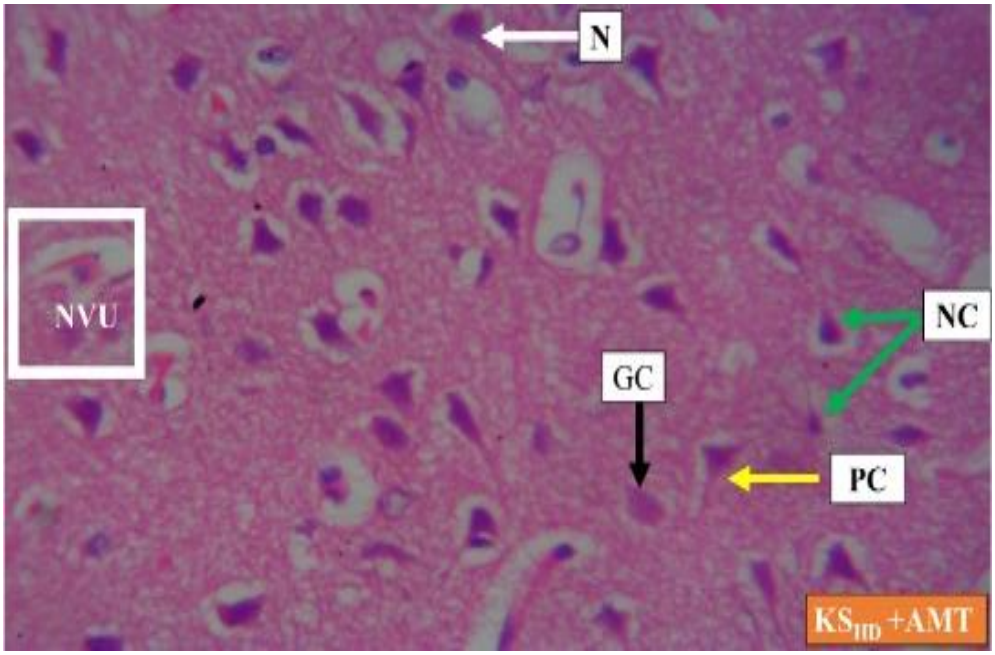

Fig. 4e. A photomicrograph of a sectionin the prefrontal cortex of AMT and KS treated rats (group E; 400mg/kg). Showing normal orientation of neurovascular unit (NVN), blood vessels (BV), Pyramidal cell (PC), Neuroglial cells (NC) and Neutrophils (N) vacuolated, Granule cells (GC). Pyramidal cell (PC), Neuroglial cells (NC) darkly stained and Granule cells (GC) visibly showing open face nuclei and basophil cytoplasm, prominent nucleoli. (H & E X400)

TC group(AMT + TC) results

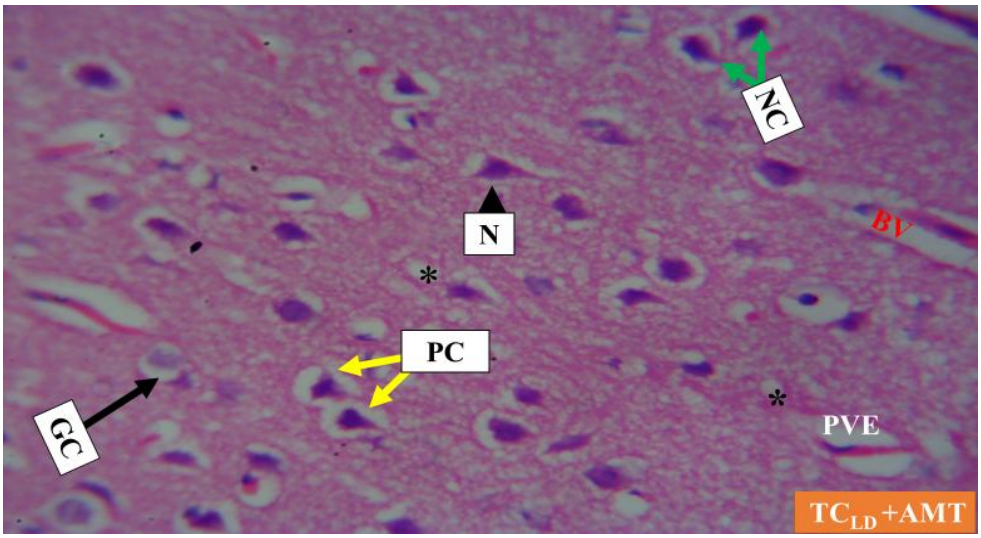

Fig. 4f. A photomicrograph of a section in the prefrontal cortex of AMT and TC treated rats (TC_{LD}; 200mg/kg). Showing intact neurovascular unit (NVU), normal Pyramidal cell (PC), darkly stained nuclei and pericellular halos, neuron (N), Neuroglial cells (NC), deeply stained and vacuolated Neutrophils (arrow head *), Granule cells (GC), With pale open face nucleus. The section demonstrates mild Perivascular Edema (PVE). (H&E. X400).

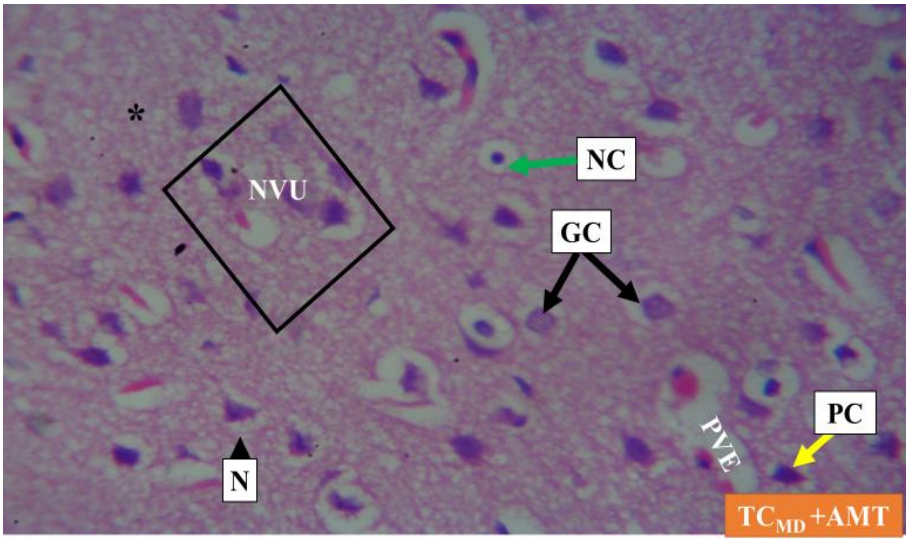

Fig. 4g. A photomicrograph of a section in the prefrontal cortex of AMT and TC treated rats ((TC_{LD}; 300mg/kg). Showing Prominence neurovascular unit (NVN), Pyramidal cell (PC) with pericellular halos, Neuroglial cells (NC) and Neutrophils (N) vacuolated (arrow head), shrunken Granule cells (GC), with open face nuclei and basophil cytoplasm, prominent nucleoli, and moderate Perivascular Edema (PVE). H&EX400

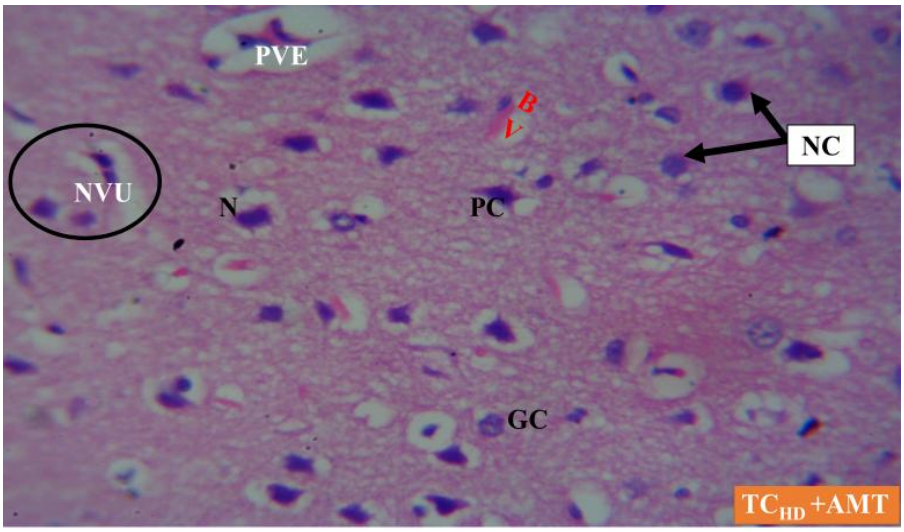

Fig. 4h. A photomicrograph of a section in the prefrontal cortex of AMT and TC treated rats ((TC$_{LD}$; 400mg/kg). Showing normal orientation of neurovascular unit (NVN), blood vessels (BV), Pyramidal cell (PC), Neuroglial cells (NC) and Neutrophils (N) vacuolated, Granule cells (GC). Pyramidal cell (PC), Neuroglial cells (NC) darkly stained and Granule cells (GC) visibly showing open face nuclei and basophil cytoplasm, prominent nucleoli, and moderate Perivascular Edema (PVE).H & E X400

Combine KS and TC + AMT(COM$_{KS+TC}$+ AMT) group

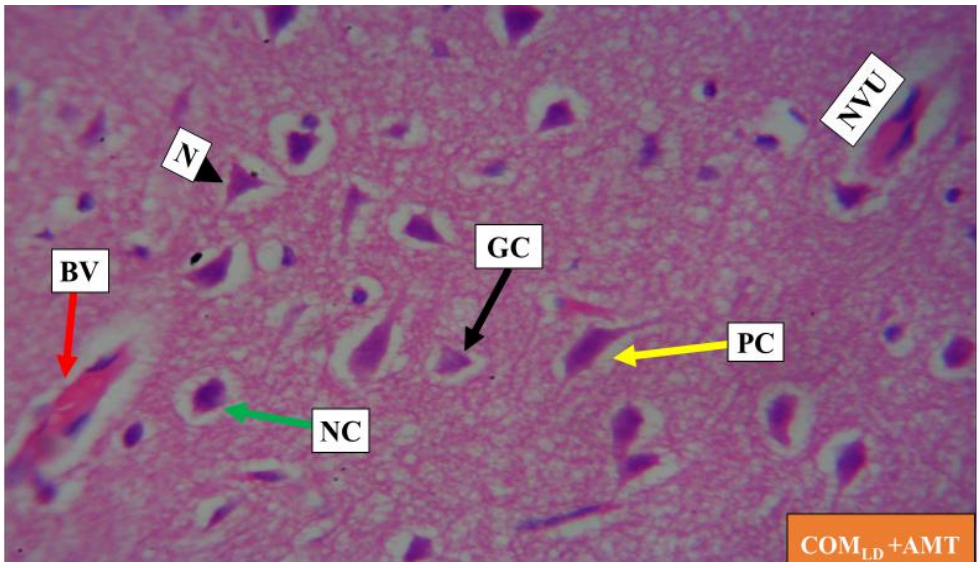

Fig. 4i. A photomicrograph of a section in the prefrontal cortex of AMT and KS & TC treated rats (COM$_{LD}$; 200mg/kg). Showing minimal cellular degeneration in neurovascular unit (NVN), Pyramidal cell (PC), Neuroglial cells (NC) and Neutrophils (N) vacuolated, Granule cells (GC). Pyramidal cell (PC), Neuroglial cells (NC) and, Granule cells (GC) visibly showing open face nuclei and basophil cytoplasm, prominent nucleoli. (H & E X400).

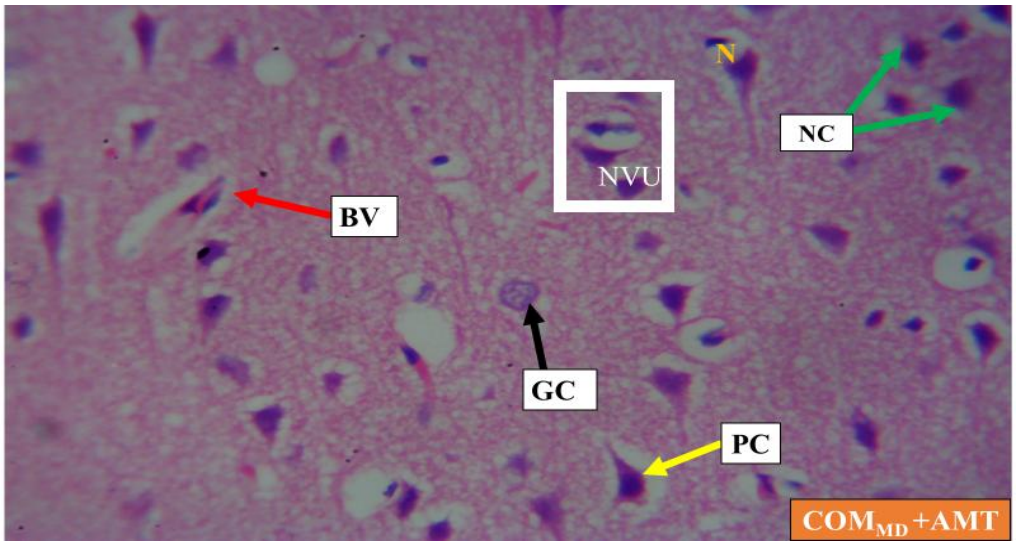

Fig. 4j. A photomicrograph of a section in the prefrontal cortex of AMT and KS & TC treated rats (COM_{MD}; 300mg/kg). Showing normal orientation of neurovascular unit (NVN), Pyramidal cell (PC), Neuroglial cells (NC) and Neutrophils (N) vacuolated, Granule cells (GC). Pyramidal cell (PC), Neuroglial cells (NC) and, Granule cells (GC) visibly showing open face nuclei and basophil cytoplasm, prominent nucleoli and few degenerated cortical cells. (H & E X400).

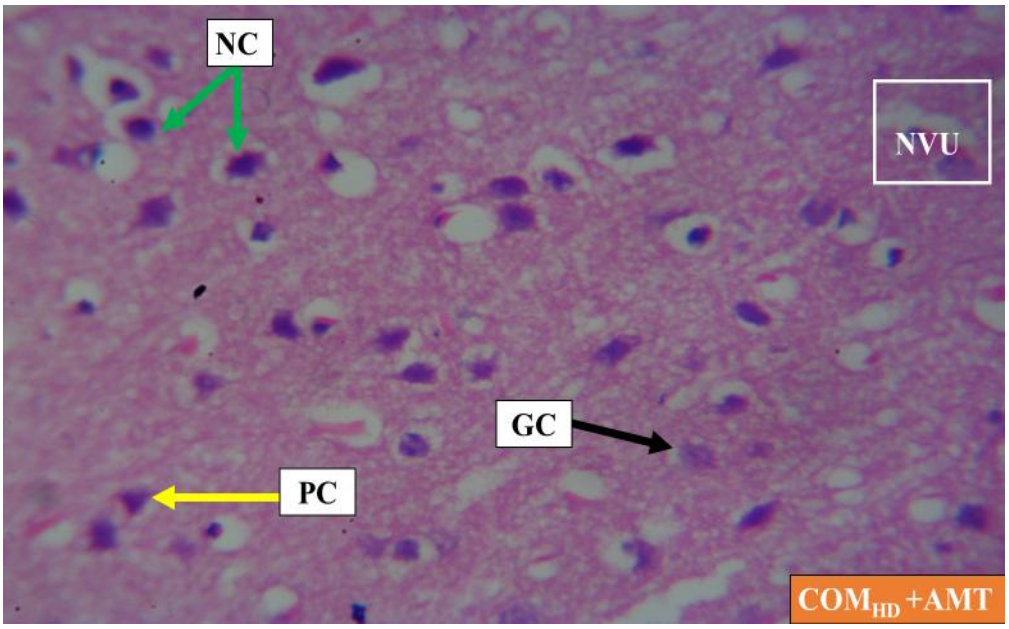

Fig. 4k. A photomicrograph of a section in the prefrontal cortex of AMT and KS & TC treated rats (COM_{HD}; 400mg/kg). Showing normal orientation of neurovascular unit (NVN), Pyramidal cell (PC), Neuroglial cells (NC) and Granule cells (GC) with increase Neutrophils and vacuolated cortical cells. Pyramidal cell (PC), Neuroglial cells (NC) and, Granule cells (GC) visibly showing open face nuclei and basophil cytoplasm, prominent nucleoli. (H & E X400)

Discussion

Single therapeutic approaches used in the treatment of stroke and poor recovery of victims, remained a quest in search of combined approach to manage stroke.

Our present study showed that amitriptyline induced neurodegenerative changes on the prefrontal cortex demonstrates evidence of stroke after three days of induction.On treatment with *Khaya senegalensis* bark and *Tinospora cardiofolia* leave extracts, asignificant improvement in neurohistology of the prefrontal cortex and antioxidant defense system were found. The results of our study further demonstrate the presenceof astrocytosis, lymphocytes Infiltrates and vacuolated neutrophils, pyramidal cell with irregular shape and surrounded by pericellular halos, perivascular edema, perivascular cuffing and shrunken granule cells deeply stained, which were evidence of ischemic insult. This is in line with Vijeyaratnamand Corrin (1974); Archer and Rich,(2000); Liu *et al*; (2010); Lukpata *et al*., (2020), further reported a consistent ischemic insult, in their studies ofadverse pulmonary vascular effects of high dose tricyclic antidepressants: acute and chronic animal studies,that a large acute dose of amitriptyline caused oedema with rupture of capillaries and alveolar epithelium. In our previous study of ethanolic stem bark extract of Khaya Senegalenesis ameliorates cerebral ischemia in Wistar Rats, where occlusion of the middle cerebral artery was carried out to induce-stroke, similar results was found as the present study.

AMT have been reported to induce oxidative stress causing lipid peroxidation. It further shows that AMT poisoning increased level of SOD, MDA and reduced antioxidant capacity of blood (Demling and Lalonde, 1989;Benzie and Strain, 1999; Kadar. and Raja 2019).Vakili *et al*., (2018) in their study also reported that an increased production of reactive oxygen species due to excessive intake of AMT leading to an increase in tissue oxidative stress and tissue structural and functional damage.The results of our present study showed a significant decreasein SOD activity, GSH and CATlevel, in stroke-induced animals (AMT; negative control) as compared to positive control. While there was a significantincrease in MDA in animals treated with AMT (AMT; Neg. CTR) as compared with the positive CTR. Findings from this present study showed that AMT increase lipid peroxidation and reduced GSH level, along with antioxidant enzyme activities (SOD, Catalase).Our results are in agreement with reports of Demling and Lalonde, 1989, Benzie and Strain, 1999; Kadar and Raja, 2019, which works report that acuteAMT increased lipid peroxidation activity, but our result in MDA level disagreed with their MDA result that was found to increase in acute AMT.

Treatment with *Khaya senegalenesis*, on oxidative stress, our present study found that single therapeutic of at dose 200 mg/kg + AMT significantly reversed AMT - induced SOD deficit as compared to AMT treated rat. While treatment 300 mg/kg+ AMT and 400mg/kg + AMT) of *Khaya senegalenesis*shown no significant difference as compared to negative control-AMT group. Single therapeutic of 200 mg/kg+ AMT, 300 mg/kg+ AMTand400 mg/kg+ AMT dose onGHS, significantly reversed of AMT – induceddeficit was observed in 200 mg/kg+ AMT, while 300 mg/kg+ AMT and400mg/kg+ AMT shows no significant as compared to positive control. KS on CAT, 200 mg/kg+ AMT, 300 mg/kg+ AMT and400mg/kg+ AMTgroupsshows a significant increase in CAT level as compared to AMT group. while 300 mg/kg+ AMT and 400mg/kg+ AMT were significantly high as compared to AMT group animals.MDA increase

significantlyin AMT treated animals as compared to the positive control. On treatment with KS, MDA significantly decrease in 200 mg/kg+ AMT while a slight decrease was observed in KS_{LD}+ AMT as compared to AMT group.Our results are consistent with the reports ofGupta and Sharma, 2006;Lompo *et al*., 2007; Kolawole *et al*., 2013; Marius *et al*., 2016, who work reports that KS possesses antioxidant and free radical scavenging activity against oxidative reactions catalyzed by free radicals.

Single therapeutic treatment of TC on oxidative stress in this present study was found significantlyacross all the parameters (SOD, GSH, CAT and MDA). On SOD, a significant($p<0.05$) increased was observed in 200mg/kg + AMT and 300mg/kg + AMT treated animals as compared to animals in AMT group. Dose 200mg/kg + AMT, was significantly high while 300mg/kg+ AMTwas found to be slightly significant when compared to AMT group. GSH significantly increased in200mg/kg + AMT, as compared to GSH decrease in AMT treated animals. TC on CAT level was found to increase significantly in200mg/kg + AMT,300mg/kg + AMTand400mg/kg + AMTas compared to AMT treated animals. TC at dose200mg/kg+ AMT was found significantly highly as compared to AMT treated animals and the positive control. TC on MDA level was significantly high in 200mg/kg + AMT animals as compared to AMT treated animals.This is in with the reports of Stanely and Menon (2001); Rawal *et al*., (2004); Jagetia, and Baliga, (2004);Mukeshwar *et al*., (2012); Rawal *et al*., (2012); Jitendra *et al*., (2014).

Our present study on combined therapy(*Khaya senegalenesis*+ *Tinospora cardiofolia*) on oxidative stress (SOD, GSH, CAT and MDA), showed that athere was a significantincrease, in SOD combined (SOD_{COM}), across the groups (200mg/kg+ AMT,300mg/kg+ AMT and400mg/kg+ AMT) as compared to AMTtreated animals.Dose 300mg/kg+ AMT and 400mg/kg+ AMTof SOD_{COM}, was found significant high as compared to AMT group. In combined GSH (GSH_{COM}), group 300mg/kg+ AMT and 400mg/kg+ AMT was found significant highas compared to GSH decreased in AMT treated animals while combined CAT (CAT_{COM}) and MDA_{COM} treatmen$_t$shows a significant increase in 200mg/kg+ AMT as compared to AMTgroup. This is similar with others studies that have reported that *Khaya senegalensis* bark and *Tinospora cardiofolia* leave haveantioxidant and free radical scavenger properties (Jagetia, and Baliga, 2004; Lompo *et al*., 2007; Atawodi *et al.*, 2009; Mukeshwar *et al*., 2012; Rawal *et al*., 2012; Bhattacharjee and Bhattacharyya, 2013; Jitendra *et al*., 2014).

Several studies have reported that as blood flow within the core area drops below 20% of normal flow rates, metabolic failure leads to anoxic depolarization and activation of the "ischemic cascade" that triggers neuronal death beginning within minutes of ischemic onset. At the ischemic core", brain damage is fast and irreversible as reduced blood flow leads to the activation of proteolytic enzymes, degradation of the cytoskeleton, cytotoxic swelling, and peroxidation of membrane lipids (Dirnagl*et al*., 1999; Hossmann, 1994; Witte *et al*., 2000). It has also been reported that inflammation after brain injury is characterized by the infiltration and proliferation of immune cells in an attempt to eliminate cellular debris and pathogens, and the secretion of chemokines and pro- and/or anti-inflammatory cytokines. The inflammatory response leads to cell death and infarct growth for days after ischemic onset. The key players responding to inflammatory response to strokeare the

leukocytes, monocytes, neurons, and glial cells (Dirnagl*et al*., 1999).

Histomorphological findings of our present study showed cortical degeneration in the prefrontal cortex) which were characterized with evidence of astrocytosis, lymphocytes infiltrates and vacuolated neutrophils, pyramidal cell with irregular shape and surrounded by pericellular halos, perivascular edema, perivascular cuffing, and shrunken granule cells deeply stained, which validated ischemic insultinduced by AMT as compared to positive control, with normal neurohistology of the prefrontal.This is in agreement with otherstudies of Schiling*et al*., 2003; Sicard *et al*., 2006; Elkind, 2007; Kleinig and Vink, 2009; Michael *et al*., 2010.

Khaya senegalensis bark and *Tinospora cardiofolia* leave have shown protective and neurorepairs role on neurodegenerative changes in the prefrontal cortex when administered to AMT stroke – induced rats. The degenerated neurons and cortical cells drastically regenerate. In single therapeutic treatment, in both *Khaya senegalensis* bark and *Tinospora cardiofolia* leave, showed morphological and neuronal recuperative changes in the treatment groups (200 mg/kg+ AMT;300 mg/kg + AMT; and 400 mg/kg+ AMT, as compared to degenerated neurons and cortical cells induced by AMT. In stem bark extract of *Khaya senegalensis* bark, a marked improvement was seen at dose 400mg/kg (KS_{HD} + AMT) than KS_{MD} + AMT; 300 mg/kg. This study suggeststhat *Khaya senegalensis*stem bark extract can effectively protected and repair the degenerative cells and neurons especially in the neurovascular unit, thereby has anti-inflammatory properties. Our findings are in agreement with others studies reported by Lompo *et al*., (1998); Wu *et al*., (2007); Lompo *et al*., (2007);Kolawole*et al*., (2013).

When treated with*Tinospora cardiofolia* leave extract was shown to be effective at dose 200 mg/kg, with evidence of intact neurovascular unit, normal pyramidal cells, neuron, neuroglial cellsdeeply stained and vacuolated neutrophils, granule cells, with pale open face nucleus. The finding demonstrates mild perivascular edema. This is in line with reports of Singh *et al*., (2005);Pingale *et al*., (2010); Aher and Wahi (2010); Onkar *et al*., (2012); Anju *et al*., (2013); Chaudhari *et al*., (2013). These results suggest that, usingsingle therapeutic treatment, with Khaya *senegalensis* stem bark ethanolic extract dose 400mg/kg can repair and restore the degenerated cortical cells and neurons. While single therapeutic treatment, with *Tinospora cardiofolia* leave ethanolic extract at dose 200mg/kg can repair and restore the degenerated cortical cells and neurons.

The findingsin this present study showed that in combined therapeutic (Khaya senegalensis bark and *Tinospora cardiofolia* leave extracts) treatment, have shown morphological and neuronal recuperative changesin200mg/kg+ AMT animals, while marked improvement (neurovascular unit and cortical cells) were noted in 300mg/kg+ AMT). When treated with high dose, 400mg/kg+ AMT, showed vacuolated cortical cells with minimal improvement. The present study suggest that combined therapeutic (Khaya senegalensis bark and *Tinospora cardiofolia* leave extracts) treatment of stroke at dose 300 mg/kg+ AMT;can protective and neurorepairs neurodegenerative changes in the prefrontal cortex, by regenerating pyramidal cell/granule cells and neurovascular unit.

Conclusion

In conclusion, single therapeutic treatment, with *Khaya senegalensis* stem bark ethanolic extract at dose 400mg/kg can repair and restore the degenerated cortical

cells and neurons. While single therapeutic treatment, with *Tinospora cardiofolia* leave ethanolic extract at dose 200mg/kg can repair and restore the degenerated cortical cells and neurons. Therefore, combined therapeutic treatment (*Khaya senegalensis* bark and *Tinospora cardiofolia* leave) at dose 300 mg/kghave shown neuroprotective and neurorepairs role on neurodegenerative changes in the prefrontal cortex, by regenerating pyramidal cell/granule cells and neurovascular unit.

No conflict interest

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