ORIGINAL ARTICLE

ANTI-INFLAMMATORY EFFECTS OF *KHAYA SENEGALENSIS* BARK AND *TINOSPORA CORDIFOLIA* LEAF EXTRACTS ON STRIATAL ISCHEMIC INJURY IN RATS

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ABSTRACT

The striatal synaptic pathways play a crucial role in the functioning of the basal ganglia and are an essential part of the cortical-basal ganglia loops that regulate motor function, emotion, and cognition. The purpose of this study was to examine the potential therapeutic benefits of extracts from the leaves of *Tinospora cordifolia* (TC) and *Khaya senegalensis* (KS) on striatal ischemia injury resulting from amitriptyline (AMT). Eleven groups (n = 5) comprising fifty-five adult male Wistar rats weighing between 184 and 254 g were created: Distilled water (2 milliliters per kilogram) was used as a control. The groups that included AMT (750 mg/kg), KS (200 mg/kg) + AMT, KS (300 mg/kg) + AMT, KS (400 mg/kg) + AMT; TC (200 mg/kg) + AMT; TC (300 mg/kg) + AMT; TC (400 mg/kg) + AMT; COM (200 mg/kg) + AMT; COM (300 mg/kg) + AMT and COM (400 mg/kg) + AMT. Treatment was given orally for a period of 14 days. Rats were neck dislocated to stop the experiment and brain tissues were removed and preserved in 10% buffered formal saline. TNF- α result showed a significant increase ($p<0.05$) by 708.9±28.05δ in AMT-treated rats compared to the control (324.8±7.58). And decreased significantly (328.5±5.24α, 380.4±3.43δ and 375.3±5.50δ) in the treated groups compared to AMT-treated rats. Normal cytoarchitecture of the striatal cells was intact in the control group. AMT rates (Ischemic stroke rats) revealed neurodegenerative changes, characterized by cellular hypertrophy and perivascular edema, and proliferation of reactive astrocytes and microglia. However, the treatment of KS, TC, and COM KS+TC remarkably ameliorates striatal cell degeneration by preserving striatal cell cytoarchitecture, especially with COM KS+TC 400 mg/kg treatment. Findings suggest that COM KS+TC possesses anti-inflammatory properties, which could be of potential benefit in the treatment and management of ischemic stroke.

Keywords: Amitriptyline, Striatal Ischemic, *Khaya senegalenesis*, Tinosporacardiofolia, Neurovascular unit.

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INTRODUCTION

The striatum, the largest and most intricate region of the basal ganglia, combines data from the dopamine system, brain, thalamus, and pedunculopontine nucleus (PPN). The striatal synaptic pathways play a pivotal role in the functions of the basal ganglia and are an essential part of the cortical-basal ganglia loops. They are involved in motor control; learning and memory, action selection, motor skill acquisition, emotion, and the process of determining which actions are worthwhile repeating. Since dopamine influences the synaptic plasticity at striatal dendritic spines, dopamine may be a crucial learning signal for this kind of reinforcement learning. The content of striatum-based learning includes information from the senses, the motor system, the brain, and motivation (1, 2). Due to the intricacy of the cortico-striatal networks, striatal neurons may be uniquely positioned to process convergent inputs from the cortex, control subcortical nuclei through the basal ganglia output nuclei, and/or influence cortical dynamics through the thalamus (1, 3).

Hunnicutt et al (4) reported that the dorsal and ventral striatum are the two areas into which the striatum is commonly split. The tail of the striatum (TS), which is located posterior to the dorsal striatum, has recently been recognized as an additional functionally separate area. According to studies, the dorsal striatum can be divided into sections known as the dorsolateral (DLS) and dorsomedial (DMS). In rats, these regions are equivalent to the putamen and nucleus caudate, respectively, in primates. According to reports, the DLS receives afferents from sensor motor cortical areas, whereas the DMS receives afferents mostly from prefrontal and associative cortices (4, 5). The consensus in studies has been that the dorsal striatum plays a major role in the movement, especially in automatized fine skills and micro-movements that are integrated into action (6, 7). Cortex-basal ganglia loop activities of the striatum have also been demonstrated in other segments of the striatum. The nucleus acumens (the amino acid NAC), located in the ventral region of the striatum (VS), is engaged in goal-related movements, which are movements in which the animal encodes values to the performance of the movement. It receives projections from limbic cortices and the amygdala. (1, 8). Valjent and Gangarossa, (9) reported that the tail of the striatum is mostly supplied with projections from the sensory cortices; it has been demonstrated to be involved in safety learning and avoidance, and it may also serve to sift out irrelevant sensory inputs to prioritize goal-directed activity. Amitriptyline is a tricyclic antidepressant drug with a sedative effect, it's also used in the treatment of depression, posttraumatic stress disorder (PTSD), anxiety disorders, or insomnia. Despite its use in the treatment of depression and other mood disorders, it has been reported that its several mechanisms may cause impaired endothelial function, modifications to fibrinolysis or coagulation, or the encouragement of risk factors for vascular disease (10, 11). Hypothermia, sleepiness, tachycardia, and other arrhythmic abnormalities like bundle branch block, congestive heart failure, and blocking voltage-sensitive sodium channels in the heart convulsions; severe hypotension; stupor, coma polyradiculoneuropathy constipation, and brain have also been linked to overdosage. This is due to its antiadrenergic and antimuscarinic properties (12-14).

The plant known as Khaya senegalensis A. Juss (Meliaceae) is a medicinal herb that has been utilized in traditional medical systems by numerous cultures worldwide, particularly in African, Indian, and Chinese societies. It has been stated that this plant's leaves, stem barks, seeds, and roots can be used to cure a variety of illnesses, including dermatitis, diabetes, arthritis, infections, ulcers, fever, and malaria (15). Extracts from the stem bark of Khaya senegalensis have been reported to exhibit antipyretic, analgesic, and anti-inflammatory effects (16). It is well known for its capacity to scavenge reactive oxygen species and is abundant in polyphenols as secondary metabolites, including flavonoids, lignans, and phenolic acids (17, 18). It has shown a chemo-preventive property against neurogenerative and cardiovascular disorders (19-22). The bitter constituents of Khaya senegalensis, called "calicedrin" in the West African region, are commonly utilized as a bitter medicinal for treating a range of inflammatory diseases (23).

Tinospora cordifolia Miers (Menispermaceae) is widely known for its vast therapeutic value. Its common name in English is Heart leaf moonseed. The Indian medical system employed it to treat a wide range of illnesses and gave it the intriguing Hindi name Giloya, which is a legendary reference to the heavenly elixir that kept celestial creatures youthful forever and prevented them from becoming old. It is also known as Amrit (Sanskrit) and Abb-eHyat (Urdu), which translates to "water of life," in classical Indian literature (24). It has been claimed that heart leaf moonseed *(Tinospora cordifolia*) has anti-inflammatory, antioxidant, anticancer,

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and antidiabetic qualities (25, 26). Its free radicals scavenging properties and desloughing action on macrophages have also been reported (27, 28). The potentiated ability of the Khaya senegalensis and Tinospora cordifolia to attenuate oxidative stress and inflammatory responses may enhance its therapeutic measure in the treatment and management of ischemic stroke.

MATERIALS AND METHODS

Materials from Plants

The freshly harvested Tinospora cordifolia (TC) leaves were gathered from a well-kept garden in Okpoma – Yala, Cross River State, Nigeria, and fresh bark of Khaya senegalensis (KS) was taken from a woodland in Ukelle – Yala. At the University of Lagos, Nigeria's Department of Botany, the tree bark and leaves were recognized and verified. They were then given a voucher in the university herbarium with ID numbers 8003 and 8004, for TC and KS, respectively.

Plant extract preparation

To speed up the drying process, the fresh bark of Khaya senegalensis was cleaned and cut into smaller pieces with a sterile knife. The pieces were then allowed to air dry for two weeks at room temperature. After being oven-dried for 33 hours at 50°C, the stem barks and leaves were crushed with a mortar and pestle to a semi-powder consistency. The coarse powder of Khaya senegalensis bark weighed 120 grams while Tinospora cordifolia leaves weighed 100 g. A rotary evaporator was used to concentrate the extract, which was then stored at 4°C for further use.

Procurement of Drug

The drug (Amitriptyline Hydrochloride) used for the study, was purchased from a reputable Pharmacy in Lagos, Nigeria.

Phytochemical Screening

The stem barks and leaves extracts were subjected to phytochemical Screening based on Sofowora A's, Trease's, and Evans's, respective methodologies.

Animal procurement, Handling, and Experimental Designs

For this study, a total of 55 mature male Wistar rats weighing between 184 and 254 grams were purchased from the Nigeria Institute of Medical Research in Yaba, Lagos, Nigeria. The University of Lagos, Lagos, Nigeria's Department of Anatomy, Faculty of Basic Medical Sciences, College of Medicine, had standard plastic cages housing the animals. Before the study started, the animals were given two weeks for adaptation and were given unlimited access to water and animal feed (growers mash). Animals were weighed and randomly divided into 11 groups with eleven (n = 5) animals each. The experiment was divided into 2 phases. Phase I: induction of experimental stroke, AMT was administered using 1.4 mg/kg of AMT once a day for three days and a control (CTR) group received normal saline (2ml/kg).Phase II Treatment groups: the rats were split into 4 primary groups following the induction of an experimental stroke (AMT group (ischemic stroke rats), which was left untreated, and three treatment groups, KS, TC, and combined KS + TC groups). Each of the treatment groups was further subdivided into 3 groups comprising 200 mg/kg for low 300 mg/kg for medium, and 400 mg/kg for high of the plant extracts.

CMUL/ACUREC/03/21/362V1 was the protocol number of the Research Ethics Committee of the College of Medicine at the University of Lagos, Lagos, Nigeria, which authorized all protocols and treatment methods related to the experiment.

Induction of experimental stroke

Amitriptyline hydrochloride 1.4 mg/100g b.w. as a single dosage was administered daily for 3 days. The drug was dissolved in water, and the dose was calculated by simple proportion based on animal weight, then administered via oral route with the use of a metal oropharyngeal cannula. Close daily food and water monitoring was done after Amitriptyline hydrochloride administration.

Drug Treatment

Drug treatment was carried out 24 hours after the animals were confirmed with stroke. It lasted for 2 weeks as follows:

Group 1: non – stroke + distilled water (normal control)
Group 2: ischemic stroke (AMT) + distilled water
Group 3: ischemic stroke + distilled water + KS KS (200 mg/kg body)
Group 4: ischemic stroke + distilled water + KS KS (300 mg/kg body)
Group 5: ischemic stroke + distilled water + KS KS (400 mg/kg body)
Group 6: ischemic stroke + distilled water + TC TC (200 mg/kg body)
Group 7: ischemic stroke + distilled water + TC TC (300 mg/kg body)
Group 8: ischemic stroke + distilled water + TC TC (400 mg/kg body)
Group 9: ischemic stroke + distilled water + Combined KS +TC (200 mg/kg body)

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Group 10: ischemic stroke + distilled water + Combined KS +TC (300 mg/kg body)
Group 11: ischemic stroke + distilled water + Combined KS +TC (400 mg/kg body)

Force Swimming Test

Force Swimming Test was carried out by the procedure used by Porsolt et al (29-31); Detke et al (32), and Rénéric and Lucki (33). The animals were compelled to swim inside a sealed container that had no escape route.

Animal Sacrifice

Animals were killed by cervical dislocation twenty-four hours following the last injection. The entire brain was removed, preserved in 10% neutral buffered formalin, and prepared for histological examination.

Tissue processing procedure

Standard procedures were followed to prepare fixed tiss ues for routine paraffin embedding and Hematoxylin and Eosin staining.

We examined the stained slides with a digital microscop e Photomicrographs and an OMAX 40-2000X 3MP Digital Compound Microscope (USA) were acquired.

TNF-α ASSAY

The level of TNF-α was measured in rat brain tissue by a manual quantitative ELISA kit for rats (SEA133Ra). TNF alpha was expressed in ng/ml.

Statistical analysis

The findings were analyzed with SPSS and presented as mean ± SEM. ANOVA and Tukey's Post-test were used to assess the statistical significance between the means. P-values less than 0.05 were regarded as statistically significant.

RESULTS

Result of the TNF- α increased significantly ($p<0.05$) in amitriptyline-only treated animals (708.9±28.05δ) compared to normal control (Tab. 2). However, treatment with COMLD + AMT (200 mg/kg KS), COMMD + AMT (300 mg/kg KS), and COMHD + AMT (400 mg/kg KS) significantly decreased TNF- α (328.5±5.24α, 380.4±3.43δ and 375.3±5.50δ) compared to amitriptyline treated animals. The value of COMLD + AMT (200 mg/kg KS) treated group was significantly lower than amitriptyline treated animals.

Histopathological Evaluation results

Figure 1: shows the plates of photomicrographs of the striatum from animals in normal control (CTR), AMT-only treated animals (AMT), and combined KS and TC treated groups (COMLD + AMT (200 mg/kg KS), COMMD + AMT (300 mg/kg KS), and COMHD + AMT (400 mg/kg KS). CTR represents the normal histoarchitecture of the striatum with neurovascular unit (NVU), neuron (N), blood vessel (BV), and Neuroglial cell (NC). Photomicrograph of AMT represents striatum section from AMT-only treated group, showing degeneration in the striatal cells with evidence of mild lymphocytic infiltrates in the neurovascular unit (NVU), moderate degenerated neuron (DN1) to severely degenerated neuron (DN2), capillaries proliferation (CP), cellular hypertrophy (CHT) and perivascular edema and astrocytosis. Photomicrographs COMLD + AMT and COMMD + AMT indicate minimal and moderate degeneration in the striatal cells respectively while normal orientation of the striatal cells was observed in COMHD + AMT group.

DISCUSSION

During ischemic striatal injury, a lot of inflammatory factors come into play. Impairment in the striatum plays a vital cortical function due to its many roles related to the cortical areas, especially in cognition. It receives input not just from motor areas only but also from other areas throughout the cortex.

Amitriptyline (AMT) is an antidepressant tricyclic medication used to treat depression symptoms. Its chronic administration has been reported to be associated with neurologic and heart effects such as arrhythmias, myocardial infarction, and heart block leading to stroke, due to its anti-adrenergic and anti-muscarinic properties (12, 34, 35).

In the current investigation, we evaluated it. therapeutic effects of Khaya senegalensis bark and Tinospora cordifolia leaf extract (combined) on striatal ischemic stroke induced by Amitriptyline. It is reported that Amitriptyline interacts with N-methyl-D-aspartate (NMDA), serotonin, cholinergic, and histaminic receptors. thereby inhibiting norepinephrine and serotonin reuptake, initiating intercellular and vascular adhesion molecules leading to endothelial activation and pro-inflammation. These cause blood components and vessel walls to interact in a pro-thrombotic manner, which encourages thrombogenesis and microvascular clogging (36, 37). According to Liu et al. (38), acute AMT resulted in edema and a dose-dependent, sustained elevation in pulmonary artery pressure. In the present study, degenerated striatal neurons and inflammatory responses were found, which were characterized by mild lymphocytic infiltrations in the neurovascular unit, decrease in striatal neurons, capillaries

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proliferation, cellular hypertrophy, perivascular edema, and gliosis, following administration of AMT. These evidences validate ischemic striatal injury in response to stroke. The results we achieved are consistent with research findings of (39-42) whose studies reported that there is a marked damage to striatal neurons as a result of an ET-1 injection-induced ischemic insult to the striatum. Gliosis in the striatal parenchyma was observed by Ermine et al. (39) Somaa et al. (40); Kamestu et al. (41); and Amat et al. (42).

It was noted in the current investigation that the rats' weight was affected due to the administration of varying doses of *Khaya senegalensis* bark and *Tinospora cordifolia* leaf extract. At normal control, it was seen that the weight was at its highest with a mean and standard deviation of 234.4 ± 23.10. When only AMT was administered, their mean weight dropped significantly to its lowest value at 219.6 ± 19.13 which suggests AMT alone impacts negatively on weight. The administration of the combination of AMT and the extracts at low dosage produced a significant increase in the mean weight to the value of 223.7 ± 18.13, though not up to the value of the normal control, it was evident. A further increase in the administration of the combination to medium dose produced a gradual increase further in weight but not significant at a mean value of 231.5 ± 13.13. Finally, the high dose of the combination resulted in the gradual progression of weight with a mean value of 235.6 ± 19.12. This dose-dependent increase in weight is supported by Bhalerao et al., (43), who reported a similar trend.

It is a well-established fact that the ischemic insult first affects the cerebral blood vessels, whose damage triggers the inflammatory response and early production of many pro-inflammatory genes, including TNF-α and IL-1b, in vascular cells and perivascular microglia-macrophages. These cells also upregulate the expression of adhesion molecules, which, in conjunction with integrins, encourage leukocyte rolling and adhesion to the vessel surfaces (44). In the current investigation, we discovered that following striatal ischemia injury in amitriptyline-treated mice, there was a significant ($p<0.05$) rise in TNF-α in the striatal cells as opposed to normal controls. Glial cell proliferation found in the current investigation indicates that a pro-inflammatory process is expressed as a signal for an elevation in tumor necrosis factor-alpha (TNF-α), which is consistent with the pattern of ischemia damage to the striatum reported by (45–47). The pattern in which striatal ischemic injury where induced differs from each study but similar inflammatory response. Flint et al (47), in their study, characterized it as proliferative alterations in spiny neuron dendrites that resemble those seen in Huntington's disease (HD). Striatal neuronal loss was reported by Vagner et al (45). Intense microglia activation and upregulation of astrocytes were reported by Rafael et al (46). The findings of this present study validated similar results and further presented a significant increase in TNF- α validating the pro-inflammatory process in AMT animals (untreated) compared to the normal control.

However, TNF-α levels in the treated animals were significantly lower ($p<0.05$) in all treatment groups as compared to the untreated AMT rats.

TNF- α decrease found among the treated animals in this study indicate the anti-inflammatory effects of *Khaya senegalensis* bark and *Tinospora cordifolia* leaf extract on inflammatory mediators. *Khaya senegalensis* bark and *Tinospora cordifolia* leaf extract have been reported to possess an inhibitory effect on pro-inflammatory processes such as the macrophage's activation and glial cell proliferation (48, 49). This suggests that decreased TNF- α by *Khaya Senegalensis* bark and *Tinospora cordifolia* leaf extract result in inhibiting the mediating cells of inflammation, thereby reducing further inflammatory cell proliferation. Since TNF- α is a marker that expresses pro-inflammation, the key player in the process is activated macrophages and glial cell proliferation.

Animals treated with *Khaya Senegalensis* bark and *Tinospora cordifolia* leaf extract show a striatal neuronal increase and less glial cell proliferation among the treatment animals (COMLD + AMT, COMMD + AMT, and COMHD + AMT). These anti-inflammatory effects were on dose-dependent mild (low dose), moderately (medium dose), and markedly (high dose). This marked improvement was found among striatal projection neurons in COMHD + AMT animals compared with AMT animals, indicating a high interactive activity between medium spiny neurons (MSN). MSN, is consistent with the work of Gerfen and Wilson, (50), stating that the primary way in which these cells' local axonal collaterals create synapses is with other adjacent medium spiny neurons. The present study's treatment of animals' increased striatal neurons and decreased glial cell proliferation support the anti-inflammatory qualities of Khaya senegalensis bark and Tinospora Cordifolia leaves. This is in agreement with previous studies reporting that Khaya Senegalensis bark and Tinospora Cordifolia leaves suppressed inflammatory mediators (18, 19, 48, 49). Further, the suppressive effects on the inflammatory response

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observed in this present study suggest their antioxidant properties (19, 26), free radical scavenger activities (18, 48), and inhibitory effects on the macrophage's activation and glial cell proliferation (48, 49).

CONCLUSION

Khaya senegalensis bark and *Tinospora cordifolia* leaf extract have been used due to their medicinal purposes in some parts of Africa and Asia for some time now, as it has been found to possess anti-inflammatory properties as well as inhibitory effects on pro-inflammatory processes such as the macrophage's activation and glial cells proliferation, and as such making these plants' extract potent in treating the striatal ischemic injury. Though the combination of these plants for its therapeutic purposes did not produce any noticeable side effects in low, medium, or high doses, hence, more research is advised to determine the highest hazardous, deadly, and acceptable dosages of these plant extracts. Furthermore, treatment of animals with the combination of Khaya senegalensis bark and Tinospora cordifolia leaf extract was observed to improve significant weight gain in the treated animals which suggests that it aids in the accumulation of calories.

REFERENCE

1. Cataldi S, Lacefield C, Shashank N, Kumar G, Boumhaouad S, Sulzer D. Decreased Dorsomedial Striatum Direct Pathway Neuronal Activity Is Required for Learned Motor Coordination. eNeuro. 2022; 11:9 (5): ENEURO.0169-22.2022.
2. Helie, S., Roeder, J. L., and Ashby, F. G. (2010). Evidence for cortical automaticity in rule-based categorization. J. Neurosci. 30, 14225–14234.
3. Tepper J.M. and Plenz D. Microcircuits in the Striatum Striatal Cell Types and Their Interaction, 2004
4. Hunnicutt BJ, Jongbloets BC, Birdsong WT, Gertz KJ, Zhong H, Mao T. A comprehensive excitatory input map of the striatum reveals novel functional organization. Elife. 2016; 28:5: e19103.
5. Graybiel AM. Habits, rituals, and the evaluative brain. Annu Rev Neurosci. 2008; 31:359-87.
6. Thorn CA, Atallah H, Howe M, Graybiel AM. Differential dynamics of activity changes in dorsolateral and dorsomedial striatal loops during learning. Neuron. 2010;10;66(5):781-95
7. Yin H.H., Mulcare S.P., Hilário M.R., Clouse E., Holloway T., Davis M.I., Hansson A.C., Lovinger D.M., Costa R.M. Dynamic reorganization of striatal circuits during the acquisition and consolidation of a skill. Nat Neurosci. 2009;12(3):333-41.
8. Liljeholm, M, O'Doherty, J. P. Contributions of the striatum to learning, motivation, and performance: an associative account. Trends Cogn Sci. 2012;16(9):467-75.
9. Valjent E, Gangarossa G. The Tail of the Striatum: From Anatomy to Connectivity and Function. Trends Neurosci. 2021;44(3):203-214.
10. Girolami A, Cosi E, Tasinato V, Santarossa C, Ferrari S, Girolami B. Drug-induced thrombophilic or prothrombotic states: an underestimated clinical problem that involves both legal and illegal compounds. Clin Appl ThrombHemost. 2017; 23:775–785. doi: 10.1177/1076029616652724
11. Ramot Y, Nyska A, Spectre G. Drug-induced thrombosis: an update. Drug Saf. 2013; 36:585–603. doi: 10.1007/s40264-013-0054-6
12. Umaharan T., Sivayokan S., Sivansuthan S., (2021). Amitriptyline Dependence and Its Associations: A Case Report and Literature Review; Case Reports in Psychiatry Vol. 2021: 1-3 https://doi.org/10.1155/2021/6647952
13. Stahl S.M, Stahl's essential psychopharmacology: Neuroscientific basis and practical applications, Cambridge University Press, 4th edition, 2013.
14. British Medical Association, Pharmaceutical Society of Great Britain, Royal Pharmaceutical Society of Great Britain, Joint Formulary Committee (Great Britain). British National Formulary, British Medical Association, London, 78th edition, 2019.
15. Lalèyè O A F, Ahissou H, Olounladé A P, Azando V B E, Dansou C, Lalèyè, A. (2015) Phytochemical Screening and Evaluation of Antihyperglycemic, Antiradical and Acute Oral Toxicty Activities of Aqueous Extract of Stem Bark of Khaya senegalensis A. Juss (Meliaceae) from Benin. International Journal of Pharmacognosy and Phytochemical Research; 7(3):513-518
16. Lompo M, Nikiema J B, Guissou I P, Moës A J, Fontaine J. The topical anti-inflammatory effect of chloroform extract from Khaya senegalensis stems barks. Phytother Res. 1998; 12:448–450.
17. Bhattacharjee P, Bhattacharyya D. Medicinal plants as snake venom antidotes. J Exp Appl Anim Sci 2013; 1:156-81.

18. Lompo M, Dubois J, Guissou IP. In vitro preliminary study of free radical scavenging activity of extracts from Khaya senegalensis (Meliaceae). J Biol Sci 2007; 7:677-80
19. Atawodi, S.E., Atawodi, J.C., Pala, Y., Idakwo P. Assessment of the Polyphenol Profile and Antioxidant Properties of Leaves, Stem and Root Barks of Khaya senegalensis (Desv.) A.Juss. Elect. J of Bio. 2009;5(4): 80-84.
20. Park, D., Huang, T., Frishman, W. H. (2005) Phytoestrogens as Cardioprotective Agents. Cardiol. Rev, 13 (1): 13-17
21. Mandel, S., Weinreb, O., Amit, T., et al. (2004) Cell signaling pathways in the neuroprotective actions of the green tea polyphenol (-) epigallocatechin-3-gallate: implications for neurodegenerative diseases, J. Neurochem, 88 (6):1555- 1569.
22. Park, E. J., Pezzuto, J. M. (2002) Botanicals in cancer chemoprevention. Cancer Metastasis Rev., 21 (3-4): 231-255.
23. Takin, M.C., Attindehou, S., Sezan, A., Attakpa, S.E. and Baba-Moussa, L. Bioactivity, therapeutic utility and toxicological risks of Khaya Senegalensis. IJPBR, 2013, 1 (4): 122- 129.
24. Singh SS, Pandey SC, Srivastava S, Gupta VS, Patro B, Ghosh AC. (2003). (Chemistry and medicinal properties of Tinospora cordifolia (Guduchi)). Indian Journal of Pharmacology, 35: 83-91.
25. Sankhala L.N., Saini R.K., Saini B.S, (2012). A review of chemical and biological properties of Tinospora cordifolia. International Journal of Medical Aromatic Plants; 2(2): 340-344.
26. Onkar P, Bangar J, Karodi R. Evaluation of the Antioxidant activity of traditional formulation Giloysatva and hydroalcoholic extract of the Curculigoorchioidesgaertn). Journal of Applied Pharmaceutical Science, 2012; 02 (06): 209-13.
27. Srivastava P. (Tinospora cordifolia (Amrita)- A miracle herb & lifeline to many diseases). Int. J. med. Arom. Plants, 2011; 1(2): 57-61.25.
28. Purandare H, Supe A. (Immunomodulatory role of Tinospora cordifolia as an adjuvant in the surgical treatment of diabetic foot ulcers: A prospective randomized controlled study). Indian J Med Sc, 2007; 61: 347-55.
29. Porsolt, R. D., Bertin, A., &Jalfre, M. J. A. I. P. (1977). Behavioral despair in mice: a primary screening test for antidepressants. Archives internationales de pharmacodynamie et de therapie, 229(2), 327-336.
30. Porsolt, R. D., Anton, G., Blavet, N., &Jalfre, M. (1978). Behavioral despair in rats: a new model sensitive to antidepressant treatments. European journal of pharmacology, 47(4), 379-391.
31. Porsolt, R. D., Bertin, A., Blavet, N., Deniel, M., &Jalfre, M. (1979). Immobility induced by forced swimming in rats: effects of agents which modify central catecholamine and serotonin activity. European journal of pharmacology, 57(2-3), 201-210.
32. Detke, M. J., Johnson, J., & Lucki, I. (1997). Acute and chronic antidepressant drug treatment in the rat forced swimming test model of depression. Experimental and clinical psychopharmacology, 5(2), 107.
33. Rénéric, J. P., & Lucki, I. (1998). Antidepressant behavioral effects by dual inhibition of monoamine reuptake in the rat forced swimming test. Psychopharmacology, 136, 190-197.
34. Gupta RK, Gangoliya SS, Singh NK. Reduction of phytic acid and enhancement of bioavailable micronutrients in food grains. J Food Sci Technol. 2015;52(2):676-84.
Haber, S., and Gdowshi, M. (2004). "The Basal Ganglia," in The Human Nervous System, Amsterdam: Academic Press), 675–738.
35. Bansal N, Herzog TJ, Shaw RE, Burke WM, Deutsch I, Wright JD. Primary therapy for early-stage cervical cancer: radical hysterectomy vs radiation. Am J Obstet Gynecol. 2009;201(5): 485:1-9.
36. Connolly, J.B., Roberts, I.J.H., Armstrong J.D, Kaiser K., Forte M., Tully T., O'Kane C.J. Associative learning disrupted by impaired signaling in Drosophila mushroom bodies. Science. 1996; 274:2104–210742.
37. Frijns, C.J.M. and Kappelle, L.J. Inflammatory Cell Adhesion Molecules in Ischemic Cerebrovascular Disease. Stroke; journal of cerebral circulation. 2002; 33:2115-22.
38. Liu, K., Lu, Y., Lee, J. K., Samara, R., Willenberg, R., Sears-Kraxberger, I., Tedeschi, A., et al., PTEN deletion enhances the regenerative ability of adult corticospinal neurons. Nat Neuro, 2010;13(9):1075–1081.
39. Ermine Charlotte M., Somaa Fahad, Wang Ting-Yi, Kagan Brett J., Parish Clare L., Thompson Lachlan H. Long-Term Motor Deficit and Diffuse Cortical Atrophy Following Focal Cortical Ischemia in Athymic Rats. Frontiers in Cellular Neuroscience. 2019;13.
40. Somaa F. A., Wang T. Y., Niclis J. C., Bruggeman K. F., Kauhausen J. A., Guo H., et al. (2017). Peptide-based

scaffolds support human cortical progenitor graft integration to reduce atrophy and promote functional repair in a model of stroke. Cell Rep. 20 1964–1977. 10.1016/j.celrep.2017.07.069

41. Kamestu Y, Osuga S, Hakim AM. Apoptosis occurs in the penumbra zone during short-duration focal ischemia in the rat. J Cereb Blood Flow Metab. 2003; 23:416-22.
42. Amat J. A., Ishiguro H., Nakamura K., Norton W. T. Phenotypic diversity and kinetics of proliferating microglia and astrocytes following cortical stab wounds. Glia 1996;16 368–382.
43. Bhalerao S, Deshpande T, Thatte U. Prakriti (Ayurvedic concept of constitution) and variations in platelet aggregation. BMC Complement Altern Med. 2012;10; 12:248.
44. Michael A. Moskowitz, Eng H. Lo, and Costantino Iadecola (2010). The Science of Stroke: Mechanisms in Search of Treatments; Neuron Review 67: 181-198.
45. Vagner T, Spinelli C, Minciacchi VR, Balaj L, ZandianM, Conley A, Zijlstra A, Freeman MR, Demichelis F, De S, Posadas EM, Tanaka H, Di Vizio D. Large extracellular vesicles carry most of the tumor DNA circulating in prostate cancer patient plasma. J Extracell Vesicles. 2018;7;7(1):1505403.
46. Raphael, I., Gomez-Rivera, F., Raphael, R. A., Robinson, R. R., Nalawade, S., &Forsthuber, T. G. (2019). TNFR2 limits pro-inflammatory astrocyte functions during EAE induced by pathogenic DR2b-restricted T cells. JCI insight, 4(24).
47. Flint, D.H., Tuminello, Joseph and Emptage, M.H. (1993). The inactivation of Fe-S cluster containing hydrolyases by superoxide. Journal of Biological Chemistry. 268. 22369-22376. 10.1016/S0021-9258(18)41538-4.
48. Kolawole O. T., Akiibinu M.O., Ayankunle A. A. and Awe1 E.O. Evaluation of Anti-inflammatory and Antinociceptive Potentials of Khaya senegalensis A. Juss (Meliaceae) Stem Bark Aqueous Extract. British Journal of Medicine and Medical Research 2013;3(2): 216-229.
49. More P, Pai K. Immunomodulatory effects of Tinospora cordifolia (Guduchi) on macrophage activation, Biology and Medicine, 2011;3(2): 134-140.
50. Gerfen, C. R. and C. J. Wilson. The basal ganglia. In Handbook of Chemical Neuroanatomy, 1996;12: Integrated Systems of the CNS, Part III. L. W. Swanson, A. Björklund and T. Hokfelt (eds.). New York: Elsevier Science Publishers, pp. 371–468.

TABLE AND FIGURE

Table 1: The effects of *Khaya senegalensis* bark and *Tinospora cordifolia* leaf extract on weight and Force Swim Test (FST) behavior

Groups	Normal Control	AMT	COM_{LD}+AMT	COM_{MD}+AMT	COM_{HD}+AMT
Weight (g)	243.40±23.10	219.6±19.13$^{\alpha}$	223.7±18.13 $^{\delta}$	231.5 ±13.13 $^{\delta}$	235.6 ±19.12 $^{\delta}$
Number of stops	21.3 ±6.53	9.1±2.49 $^{\alpha}$	9.3±2.45	10.4±2.50	12.3±4.53$^{\delta}$
Immovable time 5 min (seconds)	18.4±1.49	56.3 ±22.57$^{\alpha}$	31.4 ±13.42 $^{\delta}$	27.2 ±11.22 $^{\delta}$	21.3 ±48.09 $^{\delta}$

Values are mean±SD. α significant difference compared to Normal Control and δ significant difference compared to AMT. One ANOVA followed by multiple comparison tests.

Table 2: The Effects of *Khaya senegalensis* Bark and *Tinospora Cordifolia* leaf Extract on Tumor Necrotic Factor Induced by Amitriptyline.

Group	Dosage	TNF-α 2 weeks
Normal Control	Normal saline	324.8±7.58
Amitriptyline (AMT) Hydrochloride	750 mg/kg AMT	708.9±28.05$^{\alpha}$
Ischemic stroke + distilled water + Combined low dose(COM_{LD} + AMT)	200 mg/kg KS	328.5±5.24$^{\delta}$
Ischemic stroke + distilled water + Combined medium dose (COM_{MD} + AMT)	300 mg/kg KS	380.4±3.43 $^{\delta}$
Ischemic stroke + distilled water + Combined high dose (COM_{HD} + AMT)	400 mg/kg KS	375.3±5.50 $^{\delta}$

Values are mean±SD. α significant difference compared to Normal Control and δ significant difference compared to AMT. One-way ANOVA followed by multiple comparison tests was used.

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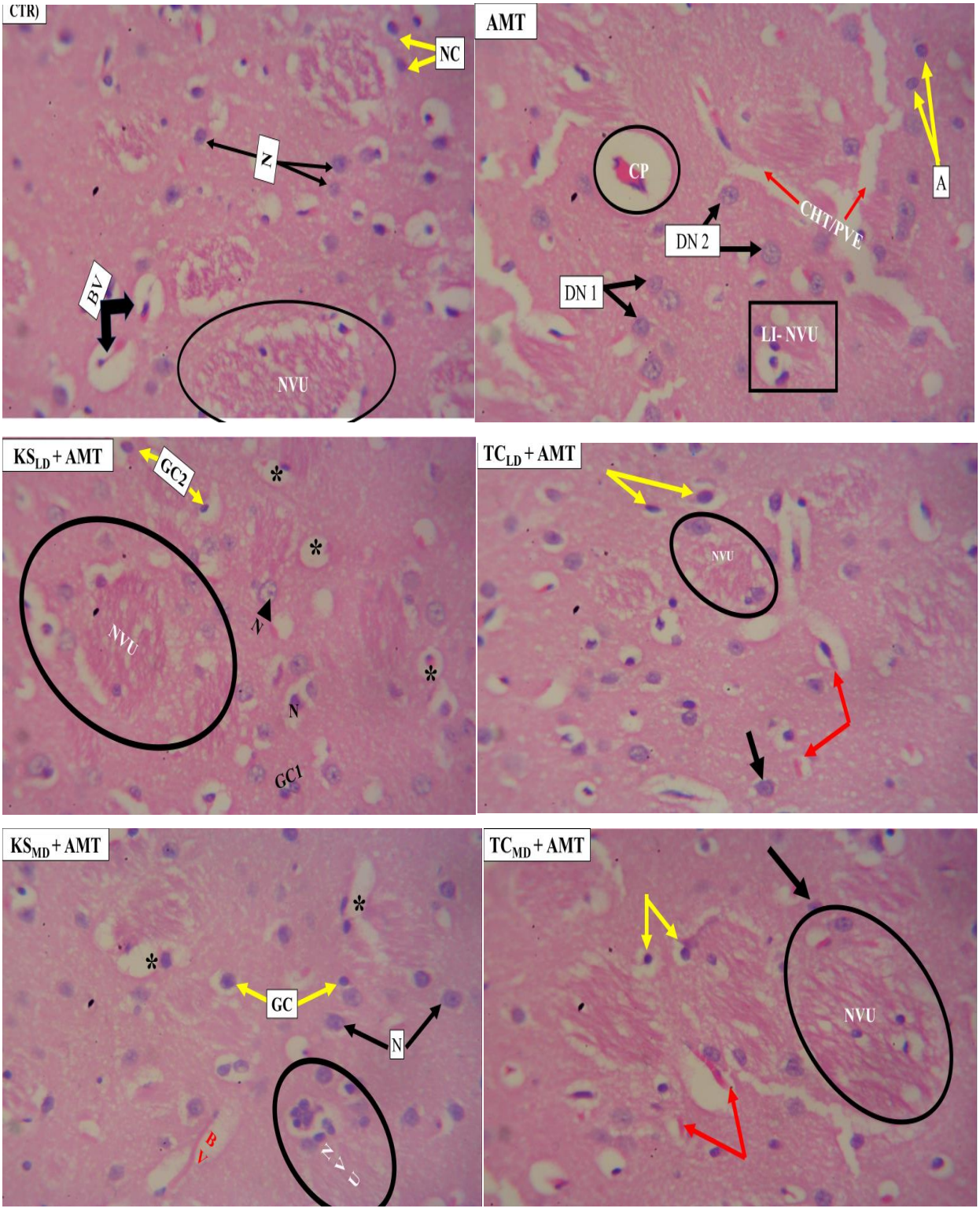
Effects of effects of *Khaya senegalensis* bark and *Tinospora cordifolia* leaf extract on Tumor Necrotic Factor (TNF-α) induced by amitriptyline.



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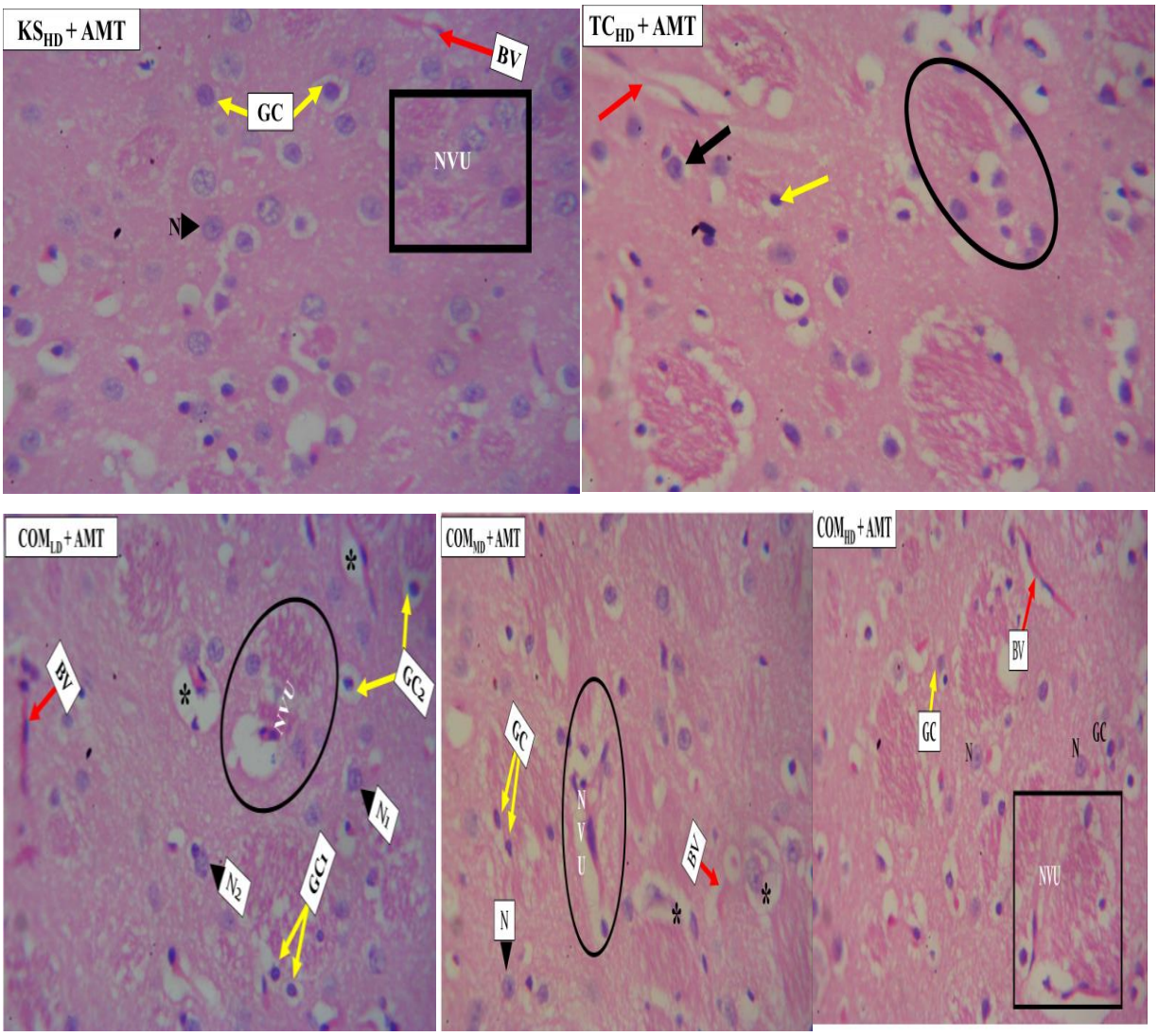

Figure 1: Cross Section of the Striatum: CRT (normal control) animal received normal saline showing intact striatal cells. (AMT) Striatum of animals induced with AMT for 3 days revealed degeneration in the striatal cells (marked neuronal loss and gliosis). (COM_{LD} + AMT) animals treated with 200 mg/kg of combined KS + TC showed a mild increase in striatal neurons and a decrease in glial cells. In COM_{MD} + AMT, animals treated with 300 mg/kg of combined KS + TC show a moderate increase in striatal neurons with a reduction in gliosis. COM_{HD} + AMT, animals treated with 400 mg/kg of combined KS + TC show a marked increase in striatal neurons and intact glial cells. *Legend*: neuron (N), glial cells (GC), blood vessel (BV), Neuroglial cell (NC). neurovascular unit (NVU), degenerated neuron (DN1), and degenerated neuron (DN2).

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