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PRELIMINARY INVESTIGATION OF THE ACTIVITY OF AQUEOUS *CUCUMIS SATIVUS* FRUIT EXTRACT ON THE PREFRONTAL CORTEX IN WISTAR RATS

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ABSTRACT

Cucumis sativus Linn. is reported to have a wide variety of medicinal uses due to its abundance of carbohydrates, proteins, minerals and secondary metabolites. This study investigated the possible alterations in the prefrontal cortex of adult Wistar rats following the administration of aqueous Cucumis sativus fruit extract (ACSFE). Eighteen (18) Wistar rats, randomly divided into three groups (n=6), were used for this study. Group A served as control while Groups B and C received 250 mg/kg and 500 mg/kg of ACSFE respectively. At the end of the administration, the rats were weighed and the Y-maze test was utilized to evaluate spontaneous alternation of the experimental rats. Thereafter, the cerebri were harvested for antioxidant [Superoxide Dismutase (SOD), Catalase (CAT) and Glutathione (GSH)], lipid peroxidation [Malondialdehyde (MDA)] and histological evaluations. Results showed that there were no significant differences in the final body, absolute whole brain weights and spontaneous alternation of the extract-treated Groups when compared to the control. The histological sections of the prefrontal cortex of the rats in control Group were similar to the extract-treated groups showing normal pyramidal cells with open-face nuclei, basophilic cytoplasm and normal neuroglia cells. Taken together, ACSFE, at these doses, is not toxic to the prefrontal cortex of Wistar rats.

Keywords: *Cucumis sativus*, Neurobehavioural, Prefrontal cortex, Wistar rats

INTRODUCTION

The brain is divided into several regions including the prefrontal cortex (PFC), the region of the cerebral cortex covering the anterior part of the frontal lobe and is particularly well-developed in humans (Molnár *et al.*, 2019). The PFC has been implicated in executive functions such as planning, problem deciphering, and social behaviour. It processes information which helps to guide thoughtful and purposeful actions of individuals (Ong *et al*., 2019). In addition, evidence from neuropsychology and neuroimaging indicates that the PFC is a key site for working memory function and participates directly in the storage of information (Elhalal *et al.*, 2014). Some disorders affecting the prefrontal cortex include Alzheimer's and Parkinson's disease, Ischemic stroke, anxiety disorders, Traumatic brain injury, schizophrenia, Dementia, inflammation, and Attention deficit

hyperactivity as well as Autism Spectrum Disorders (Xu *et al.,* 2019).

Traditional medicinal practice has been known for centuries in many parts of the world for the treatment of various human ailments (Yuan *et al*., 2016). Owing to their accessibility and affordability, the use of medicinal herbs has become an important part of daily life despite the progress in modern medical and pharmaceutical research (MazidMohd *et al.,* 2012). *Cucumis sativus* (Cucumber) is a popular creeping vine plant in the Cucurbitaceae family that produces cucumiform fruits eaten as vegetables. It is an annual monoecious plant that has high water content and is low in calories, fat, cholesterol, and sodium (Sood *et al.,* 2012). *Cucumis sativus* fruit promotes healthy hair growth and is useful for skin problems, sunburn and also for curing swelling under the eyes (Akhtar *et al*., 2020). Its juice is efficient in softening the skin texture and its fruit is considered important for maintaining blood pressure and efficient in weight loss (Kashif *et al.,* 2008). Traditionally, the seeds are used to expel intestinal worms and tapeworms (Shrivastava and Roy, 2013). *Cucumis sativus* contains various phytochemical constituents like tannins, cardiac glycosides, terpenoids, carbohydrates, flavonoids, resins, saponins and phytosterols (Sood *et al.,* 2012).

Cucumis sativus is reported to have pharmacological properties such as antioxidant, anticancer, anti-inflammatory, analgesic, antihepatotoxic, antidiabetic, antifungal, antibacterial, antidiarrheal, and thrombolytic effects (Sari *et al.,* 2021). Studies show that flavonoids from medicinal plants possess neuroprotective effects on the brain, such as protection of neurons, suppression of neuroinflammation and promotion of memory, learning and cognitive functions (Vauzour *et al.,* 2008). There is a paucity of information on the activity of *Cucumis sativus* on the histoarchitecture of the PFC and, accordingly, this study was designed to investigate possible alterations in the prefrontal cortex of adult Wistar rats following the administration of aqueous *Cucumis sativus* fruit extract.

MATERIALS AND METHOD

Experimental Animals

Adult Wistar rats for the study were bred at the Animal House, Department of Anatomy, University of Benin, Benin City, Edo State, Nigeria. They were kept in polypropylene cages at room temperature. The rats were fed with standard rat chow (Bendel livestock feed, Edo state, Nigeria) and had free access to water throughout the entire study period of twenty-eight days. They were weighed weekly before commencement and throughout the research using a digital weighing scale calibrated in grams and recorded to the nearest whole number. Protocols for this experiment followed the guide for the care and use of laboratory animals (National Research Council of the National Academics, 2011).

Plant Material

Fruits of *Cucumis sativus* were obtained from a farm at Isiohor and authenticated at the Department of Plant Biology and Biotechnology herbarium unit, University of Benin, with Herbarium number UBH-C567. A known weight **(**2.8 kg) of *Cucumis sativus* were then carefully washed with clean water, chopped into pieces and crushed in an electric blender. The juice from the combined mixture was sieved through a cheesecloth and the residue was discarded while the aqueous filtrate was chilled and then freeze-dried to produce a 45.88g aqueous extract.

Acute Toxicity Study

This study was carried out according to a previously described method of Enogieru and Momodu, 2022 (with little modification). Briefly, three Groups, A1, B1 and C1, containing three rats each were given single doses of 10, 100 and 1000mg/kg bodyweight of aqueous *Cucumis sativus* fruit extract respectively. The rats were then observed for 72 hours to monitor behavioural changes and possible mortality. Following the expiration

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of 72 hours, three new Groups, A2, B2, and C2, containing two rats each were administered with single doses of 1600, 2900, and 5000mg/kg bodyweight of aqueous *Cucumis sativus* fruit extract respectively. The rats were also observed for 72 hours for possible behavioural changes and mortality.

Research Design

A total of Eighteen (18) Wistar rats were used for this study. They were randomly assigned into three Groups (A, B, and C) of six rats each. Rats in Group A served as control and received 1ml of sterile water, while rats in Groups B and C received 250 mg/kg and 500 mg/kg bodyweight of *Cucumis sativus* extract respectively. All administrations were done orally using an orogastric tube. The experimental period lasted for twenty-eight (28) days.

Neurobehavioral Test (Y-Maze)

Animals were gently placed individually in the Y-maze apparatus, which consisted of three identical arms (33×11×12cm each) in which the arms were symmetrically separated at 120° (Dall‟igna *et al.,* 2007). Precisely, each rat was placed at the end of arm A and allowed to explore all three arms (labelled A, B, C) freely for 5 minutes. An alternation was defined as entries in all three arms on consecutive occasions. The percentage of alternation was calculated as the total number of alternations/(total arm entries –2), as previously described (Dall‟igna *et al.,* 2007). After each session, the observation chamber was scrubbed with 10% ethanol to eliminate odour before a new session commences.

Determination of relative brain weight

On the completion of the neurobehavioral tests, rats were sacrificed under low-level ether anesthesia. Thereafter the brains of experimental rats were removed and weighed. To reduce the individual body weight differences, the relative weight of the brain (%) was expressed as a percentage of the final body weight at sacrifice. Thereafter, the cerebrum was dissected out and processed for further biochemical and histological investigation.

Evaluation of Biochemical Parameters

After harvesting the cerebrum, it was blotted free of blood, weighed immediately using an electronic weighing balance calibrated in milligrams and recorded to the nearest two decimal places. The harvested and weighed brains were washed twice in cold phosphate-buffered saline (PBS) and homogenized using acid-washed sand and PBS in porcelain mortar and pestle. The homogenate was centrifuged at 10,000g for 15 minutes at 4°C. The supernatant was then collected for the estimation of Superoxide Dismutase, Catalase, Malondialdehyde and Glutathione as previously described (Enogieru and Inegbedion, 2022).

Histological Analysis

The brains of rats from the control and experimental groups were dissected and fixed in 10% buffered formal saline for 72 h. The tissues were processed and stained using the routine methods for histological examination (Drury and Wallington, 1980). Qualitative microscopic examinations were done using a digital camera (Leica CC50) coupled to a Leica DM750 research microscope. The tissue sections were digitally photographed using a scale bar of 25µm

Statistical Analysis

Data was analyzed using the GraphPad prism statistical package (version 7). Statistical significance ($P<0.05$) was determined using analysis of variance (ANOVA), followed by Turkey's multiple comparison post-hoc test. Results were presented as mean ± standard error of mean (mean ± SEM).

RESULTS

Acute toxicity

No behavioural changes and mortality were observed across experimental groups after administration of aqueous *Cucumis sativus* fruit extract at doses from 10 to 5000 mg/ kg body weight. Therefore, it can be deduced that the extract was safe for the experimental rats.

Effect of *Cucumis sativus* on body and brain weights

Table 1 shows the comparison of the initial and final body weight of rats in the control and *Cucumis sativus*-treated Groups. There was no significant difference (P>0.05) in the final body weight of rats treated with 250mg/kg and 500mg/kg when compared to the control Group. In addition, no significant difference was observed in absolute whole brain weight of rats treated with 250mg/kg and 500mg/kg when compared to control.

Table 1: Body and Brain Weights of Experimental Rats in the Control and *C. sativus*-treated Groups.

Groups	Initial BW (g)	Final BW (g)	Absolute whole brain weight (g)	Relative brain weight (%)
Control	162.5 ± 17.55	192.8 ± 7.341	1.825 ± 0.075	0.947 ± 0.016
250mg/kg *C. sativus* (CC1)	164.3 ± 18.04	193.0 ± 8.784	1.800 ± 0.071	0.934 ± 0.022
500mg/kg *C. sativus* (CC2)	164.8 ± 21.86	193.5 ± 9.904	1.800 ± 0.071	0.933 ± 0.029

Values are given as means ± SEM of each group.

Effect of *Cucumis sativus* on spontaneous alternation

Figure 1 shows the effect of the *Cucumis sativus* on spontaneous alternation in experimental rats. No significant difference (P>0.05) was observed in rats treated with 250mg/kg and 500mg/kg when compared to control.

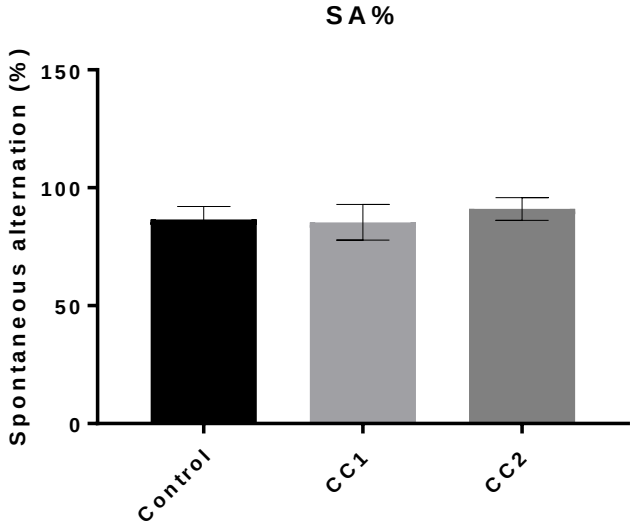

Figure 1: Spontaneous alternation across experimental groups after 28 days. Bars represent the means ± SEM.

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Effect of *Cucumis sativus* on antioxidant enzymes and lipid peroxidation

Figure 2 (A-D) shows the activity of antioxidant enzymes and lipid peroxidation in the prefrontal cortex of rats in the control and *Cucumis sativus*-treated Groups. No significant difference was observed in the activity of SOD, CAT, GSH and MDA of rats treated with 250mg/kg and 500mg/kg when compared to control.

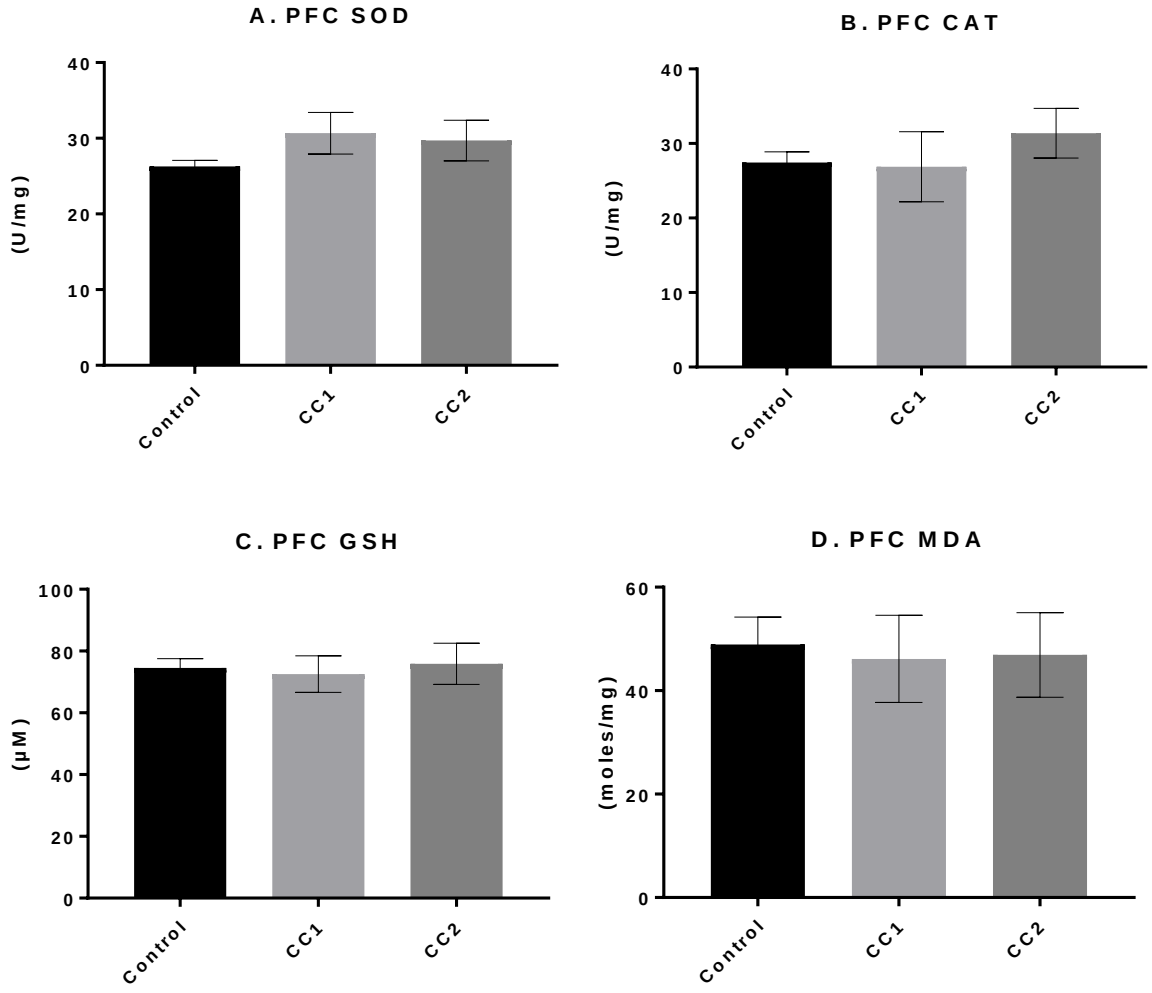

Figure 2: Activity of antioxidant enzymes and lipid peroxidation across experimental groups after 28 days. Bars represent the mean ± SEM.

Effect of *Cucumis sativus* on the histology of the PFC

Photomicrographs of histological sections of the prefrontal cortex of Control Group (Plate A) showed normal Pyramidal cells with open-face nuclei and basophilic cytoplasm (P), vacuolated neuropil (asterisk) and normal neuroglial cells (arrow).These same features were observed in the groups of rats treated with 250mg/kg (Plate B) and 500mg/kg (Plate C).

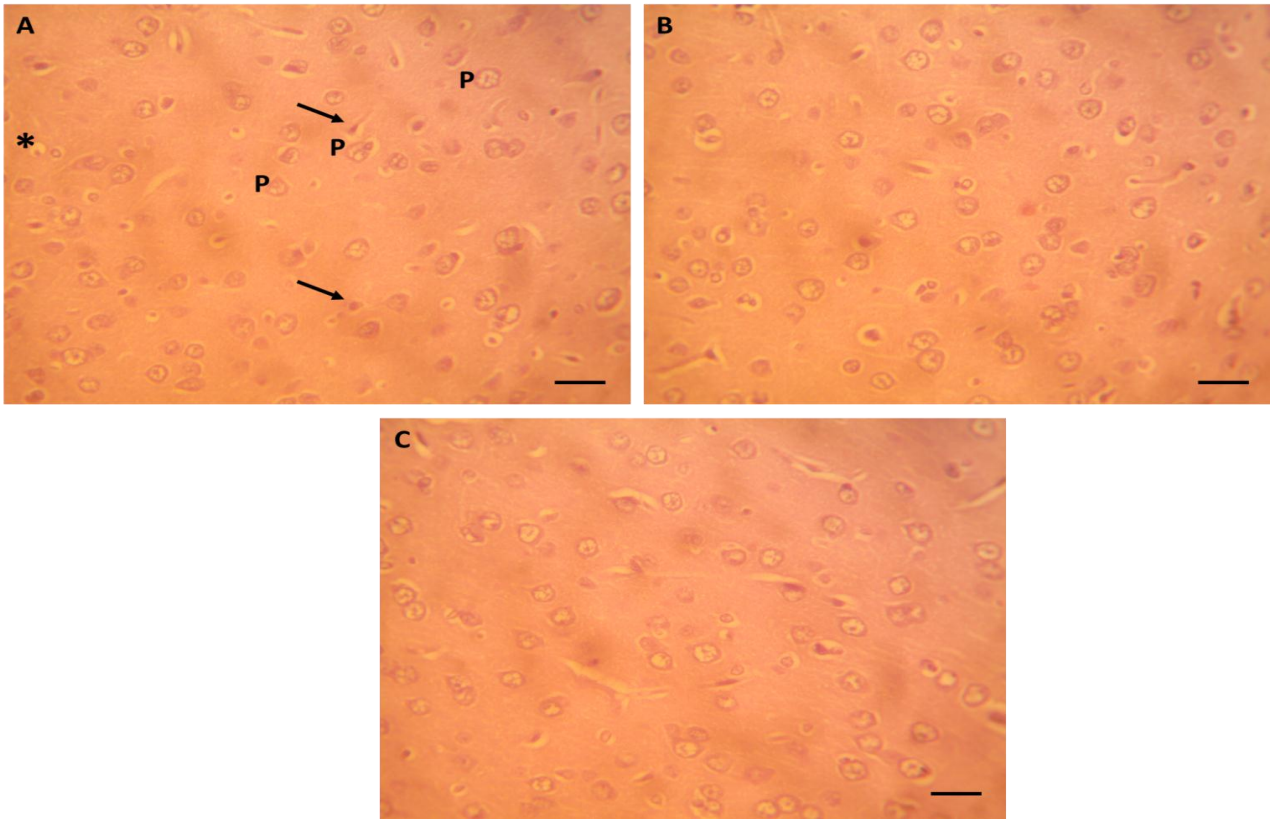

Plate 1: Representative histology of the prefrontal cortex across experimental groups (A) Control (B) 250mg/kg (C) 500mg/kg. Scale bar: 25µm

DISCUSSION

Cucumis sativus is a popular fruit, rich in antioxidants, commonly utilized for many potential health benefits (Abifarin *et al.*, 2019). Over the years, there has been an increased focus on the use of plant-based natural antioxidants due to the possible adverse effects linked to the consumption of synthetic antioxidants. However, there are very few reports demonstrating the effects of these plant-based natural antioxidants on regions of the brain such as the PFC; hence the need for this study.

Findings from this study show that there was no significant difference in the final body weight of rats treated with aqueous *Cucumis sativus* fruit extract, and this is in line with a previous report by Mandey *et al.*, (2020) demonstrating that orally administrated *Cucumis sativus* seed juice did not affect final body weight in broiler chickens. Reports show that organ weight is a key indicator of the effect of drug toxicity, as substantial differences in organ weight between treated and control animals may ensue without any morphological changes (Piao *et al.*, 2013). Consequently, assessment of organ weight changes alongside body weight differences has resulted in the use of organ-to-body weight and organ-to-brain weight to evaluate treatment effects in toxicology studies (Michael *et al.*, 2007). From this study, no significant difference was observed in the relative and absolute brain weights of *Cucumis sativus*-treated rats when compared to control, thus indicating that *Cucumis sativus* does not adversely affect the brain weight of experimental rats.

Spontaneous alternation, a quick and relatively simple test of memory, measures the willingness of rodents to explore new environments. Rodents typically prefer to investigate a new arm of the maze rather than return to one that was previously visited (Enogieru and Omoruyi, 2022). Many parts of the brain including the hippocampus and prefrontal cortex are involved in this task and

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prefrontal cortex lesions are reported to impair spontaneous alternation (Bizon et. al., 2012). Results from this study showed that there was no significant difference in the spontaneous alternation of rats treated with aqueous *Cucumis sativus* fruit extract when compared to the control. This indicates that *Cucumis sativus* did not negatively affect memory in the rats and offers support to the findings from a previous report demonstrating that *Cucumis sativus* maintains and improves learning and memory in experimental animals (Manish *et al.,* 2015).

During routine metabolic functions, free radicals are produced in the body and become highly reactive due to the lone pair of electrons they possess (Krishnamurthy and Wadhwani, 2012). They react with and denature lipids, carbohydrates and proteins. Consequently, important cellular structures and functions are lost eventually leading to various pathological conditions. However, antioxidant enzymes, such as Superoxide dismutase (SOD), Catalase (CAT) and Glutathione (GSH), can stabilize or deactivate free radicals before they attack cellular components, therefore representing an essential defence mechanism against oxidative stress-induced cell damage. In this study, there was no significant difference in the antioxidant enzymes activity in the PFC of *Cucumis sativus*-treated rats when compared to control, thus indicating that *Cucumis sativus* can maintain the integrity of the antioxidant enzymes activity. This finding corroborates with previous reports demonstrating that *Cucumis sativus* regulates the activity of antioxidant enzymes in experimental animals (Olaniyan and Afolabi, 2018; Imosemi *et al.,* 2020). Lipid peroxidation is a process under which free radicals attack lipids containing carbon-carbon double bond(s), especially polyunsaturated fatty acids (Ayala *et al.*, 2014). It is one of the adverse effects of uninhibited oxidative stress in cells, tissues, and organs ultimately leading to oxidative damage. Several reports indicate that high levels of free radicals or reactive oxygen species can inflict direct damage to lipids in a lipid peroxidation process (Ayala *et al.*, 2014). Malondialdehyde (MDA) has been extensively used as a biomarker for lipid peroxidation due to its ability to react with thiobarbituric acid. From this study, no significant difference was observed in the PFC MDA levels of *Cucumis sativus*-treated rats when compared to control, thus indicating that *Cucumis sativus* is safe and does not induce lipid peroxidation in the rats. This agrees with the previous studies demonstrating the anti-lipid peroxidation properties of *Cucumis sativus* (Olaniyan and Afolabi, 2018; Imosemi *et al.,* 2020).

The histology of the prefrontal cortex of *Cucumis sativus*-treated rats and control showed normal pyramidal cells with open-face nuclei and basophilic cytoplasm, vacuolated neuropil and normal neuroglia cells. There were no differences observed in the architecture of the PFC across experimental groups, indicating that *Cucumis sativus* administration did not induce adverse structural alterations to the PFC and maintained its histoarchitecture after its administration. This indicates that *Cucumis sativus* does not alter the histoarchitecture of the PFC and may helpful in protecting regions of the brain against cerebral ischemia, lead acetate toxicity and its associated histopathological lesions as previously reported (Gottemukkala *et al.,* 2018; Imosemi *et al.,* 2020).

Conclusively, findings from this study indicate that aqueous *Cucumis sativus* extract has no toxic effect on the histoarchitecture of the PFC in Wistar rats owing to no significant differences observed in the parameters investigated between *Cucumis sativus*-treated groups and control.

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REFERENCES

Abifarin, T. O., Afolayan, A. J., and Otunola, G. A. (2019). Phytochemical and Antioxidant Activities of Cucumis africanus Lf: A Wild Vegetable of South Africa. *Journal of Evidence-based Integrative Medicine*, 24:1-8

Akhtar, P., Ahmad, I., Jameela, A., Ashfaque, M., & Begum, Z. (2020). Energizing Effectiveness of Cucumber (Khayarain) For Health. A Review Article. *Journal of Emerging Technologies and Innovative Research*, 7(11):906-917

Ayala, A., Muñoz, M. F., and Argüelles, S. (2014). Lipid peroxidation: production, metabolism, and signaling mechanisms of malondialdehyde and 4-hydroxy-2-nonenal. *Oxidative medicine and cellular longevity*, *2014*.

Bizon, J. L., Foster, T. C., Alexander, G. E., & Glisky, E. L. (2012). Characterizing cognitive aging of working memory and executive function in animal models. *Frontiers in aging neuroscience*, 4(19):1-14

Dall‟igna, O.P., Fett, P., and Gomes, M.W. (2007). Caffeine and adenosine A (2a) receptor antagonists prevent beta-amyloid (25-35)-induced cognitive deficits in mice. *Experimental Neurology*, 203:241–245

Drury, R. A. B., and Wallington, E. A. (1980). Carleton's histological technique 5th ed. *New York: Churchill Livingstone*.

Elhalal, A., Davelaar, E. J., & Usher, M. (2014). The role of the frontal cortex in memory: an investigation of the Von Restorff effect. *Frontiers in human neuroscience*, *8*:410.

Enogieru A. B., and Inegbedion G. O. (2022). Attenuation of Oxidative Stress and Cognitive Impairment in Cadmium Chloride-Exposed Wistar Rats Pre-treated with Ethanolic Turmeric Root Extract. *The Journal of Phytopharmacology*, 11(2):118-124.

Enogieru, A. B., and Omoruyi, S. I. (2022). Exploration of Aqueous Phyllanthus amarus Leaf Extract as a Protective Agent in Mercury Chloride-Exposed Wistar Rats: A Neurobehavioural Study. *Journal of Applied Sciences and Environmental Management*, *26*(4):629-637.

Enogieru, A. B., and Momodu, O. (2022). Neuroprotective effects of Zingiber officinale against lead-induced toxicity in Wistar rats. *Nutrire*, *48*(1): 2.

Gottemukkala, M. T., Begum, F., Vangala, S. and Chander, R. T. (2018). Neuroprotective Effect of Cucumis Sativus on Cerebral Ischemia Induced Cognitive Impairment. *Journal of Pharmacology*. 3(3): 32-38

Imosemi, I. O., Owoeye, O., and Ao, M. (2020). Lead-induced oxidative stress in postnatal developing cerebellum of Wistar rats: Role of aqueous extract of Cucumis sativus Linn and vitamin. *MOJ Anat. Physiol*, *7*:104-113.

Kashif, W., Kamran, Q. M., Jilani, M. S. (2008). Effect of Different Nitrogen Levels on Growth and Yield of Cucumber (Cucumis sativus L.). *Journal of Agricultural Resources*, 46 (3):259-266

Krishnamurthy, P., and Wadhwani, A. (2012). Antioxidant enzymes and human health. *Antioxidant enzyme*, *1*:3-18.

Mandey, J. S., Wolayan, F. P., and Cherly K. Y. (2020). Effect of orally administrated of cucumber (Cucumis sativus L.) seed juice on the performance and carcass parameters of broiler chickens. *IOP Conference Series: Earth and Environmental Science*, 492:012025.

Manish, K., Milind, P and Arkash, G. (2015). Amelioration of Diazepam Induced Memory Impairment by Fruit of Cucumis sativus L. in Aged Mice by Using Animal Models of Alzheimer's Disease. *International Journal of Pharmacognosy and Phytochemical Research*. 6(4):1015-1023

Scientia Africana, Vol. 22 (No. 3), December, 2023. Pp 127-136
© Faculty of Science, University of Port Harcourt, Printed in Nigeria *ISSN 1118 – 1931*

MazidMohd., T., Khan, A., Mohammad, F. (2012). Medicinal Plants of Rural India: A review of use of medicinal plant by Indian Folks. *Indo Global Journal of Pharmacological Science,* 2(3):286-304

Michael, B., Yano, B., Sellers, R. S., Perry, R., Morton, D., Roome, N., and Schafer, K. (2007). Evaluation of organ weights for rodent and non-rodent toxicity studies: a review of regulatory guidelines and a survey of current practices. *Toxicologic pathology*, *35*(5):742-750.

Molnár, Z., Clowry, G. J., Šestan, N., Alzu'bi, A., Bakken, T., Hevner, R. F., ... & Kriegstein, A. (2019). New insights into the development of the human cerebral cortex. *Journal of Anatomy*, *235*(3):432-451.

Olaniyan, M. F., and Afolabi, T. (2018). Scavenging antioxidative bioactivities of cucumber (Cucumis sativus) fruit juice in rabbits overdosed with amoxicillin. *Biomedical and Biotechnology Research Journal (BBRJ)*, *2*(4):276-280.

Ong, W. Y., Stohler, C. S., and Herr, D. R. (2019). Role of the prefrontal cortex in pain processing. *Molecular neurobiology*, 56:1137-1166.she

Piao, Y., Liu, Y., and Xie, X. (2013). Change trends of organ weight background data in sprague dawley rats at different ages. *Journal of toxicologic pathology*, *26*(1):29-34.

Sari, T. A., Chandra, B., and Rivai, H. (2021). Overview of Traditional Use, Phytochemical and Pharmacological Activities of Cucumber (Cucumis sativus L.). *Int. Journal of Pharmaceutical Sciences and Medicine*, 6(3):39-49

Shrivastava, A. and Roy, S. (2013). Cucurbitaceae: A Ethnomedicinally Important Vegetable Family. *Journal of Medicinal Plants Studies*, 1(4**)**:16-20.

Sood, A., Kaur, P., and Gupta, R. (2012). Phytochemical Screening and Antimicrobial Assay of Various Seeds Extract of Cucurbitaceae Family. *International Journal of Applied and Pharmaceutical Technology*, 3(3):401-409

Vauzour, D., Vafeiadou, K., Rodriguez-Mateos, A., Rendeiro, C., and Spencer, J. P. (2008). The neuroprotective potential of flavonoids: a multiplicity of effects. *Genes & nutrition*, *3*(3):115-126.

Xu, P., Chen, A., Li, Y., Xing, X., and Lu, H. (2019). Medial prefrontal cortex in neurological diseases. *Physiological genomics*, *51*(9):432-442.

Yuan, H., Ma, Q., Ye, L., & Piao, G. (2016). The traditional medicine and modern medicine from natural products. *Molecules*, 21(5):559.