Original Research Article

Ameliorating Potentials of Lemon Zest on Pons Neurons in Canabis Sativa-Induced Wistar Rat

ABSTRACT

Cannabis sativa, commonly known as marijuana has been indicated for many organs malfunction. It is a common plant ingested or taken by people or individuals for body enhancement. The purpose of this study is to determine the effects of lemon zest on the pons of cannabis sativa induced Wistar rats. The plant was collected from National Drug Law Enforcement Agency (NDLEA), Rivers State, Nigeria and 40 healthy Wistar rats were used for the experiment. The rats were divided into five (5) groups. Group 1 served as control, groups 2,3,4 and 5 served as 250mg/kg lemon zest only, 750mg/kg *cannabis sativa* only, 500mg/kg *cannabis sativa* + 250mg/kg lemon zest and 900mg/kg cannabis sativa +250mg/kg lemon zest respectively. The plant extract was administered for 21 days, and thereafter, the organs were harvested for routine histological tissue processing. Findings from the study revealed that when 500mgkg^{-1} of *cannabis sativa* was used to induce the pons, observation showed neuronal hypertrophy and degeneration with mobilization of several supporting cells. The connective tissue indicated an edematous appearance. It was also observed that when a low dosage (500mgkg-1) of the cannabis sativa was administered alongside 250mgkg-1 extract of lemon zest, well delineated neuronal cells with well-defined nuclei and nucleolus were observed in the tissue. When the dosage of the *cannabis sativa* was increased to 900mgkg^{-1} alongside 250mgkg-1 extract of lemon zest, pockets of edematous connective tissues and blood vessel congestion were still noticeable in the tissue. There was a general recovery (proliferation) of lost cells in groups 4 and 5 respectively, as a consequence of the administration of lemon zest with extract (*cannabis sativa*) on wistar rat. The improved tissue and cellular appearance are indications of the potency of lemon zest in the amelioration of adverse tissue effects introduced by the extract of *cannabis sativa*.

Keywords: Cannabis sativa, Pons, Lemon Zest, Wistar rats

INTRODUCTION

In the health of individuals and communities, medicinal plants and fruits have several vital roles. The medicinal values of these plants and fruits are possible due to the numerous chemical substances contained in them which include phenolic compounds, oleoresins, resins, sesquiterpene, flavonoids, saponins glycosides, alkaloids, fats, and oils, which on the body produce physiological action [1]. Over a long period, naturally occurring constituents used which has the abundant potential for yielding new drugs, and the constituents contain a biologically active substance that demonstrates the biological activity for human health. The naturally occurring antimicrobial substance has greater significant values and has more importance in plant chemotherapy and these substances are found in plants and fruits such as lemon [2].

Cannabis sativa, commonly termed marijuana, weed, pot, and ganja, is one of the earliest known cultivated plants. Its cultivation started with the birth of agricultural farming around 10,000 years ago [3]. It is a multi-purpose crop plant with diverse agricultural and industrial applications which range from the production of paper, wood, and fibre to pharmaceutical uses in industries. Tajammul, *et al* [3] claimed that the first prospect of cannabis sativa as a medical plant was published in 1843 where its use was described for treating patients suffering from tetanus, hydrophobia, and cholera. Consequently, the plant was removed from the medicinal category and recategorized exclusively to the category of drug-type plants.

Niloy, et al. [4] focus on the effects of cannabis on the different cognitive domains, including learning, consolidation, and retrieval. Their study is the first attempt in which significant focus has been imparted on all three aspects of cognition, thus linking to its usage.

Lemon zest contains high amounts of nutrients and provides a wider range of health benefits when consumed as juice [5]. Mainly, the cultivation of lemon is done for the alkaloids which

possess anticancer activities and the antibacterial activities in crude lemon-parts (such as flowers, roots, stems, and leaves) extract and alkaloids also exhibit clinically noteworthy in bacterial-strains. The notable part of lemon peel is the lemon zest. Lemon zest is the outermost layer of the lemon peel known as the flavedo and contains loads of natural oils that are used for various purposes including an influence on the nervous system as well as influence on Cannabis Sativa [6,7,8,9].

The study is aimed to determine the effects of lemon zest on the pons of cannabis sativa induced Wistar rats.

MATERIALS AND METHODS

Experimental Design: This study will adopt the Completely Randomized Design (CRD). This involves two principles which are the principles of replication and the principle of randomization of experimental design.

Collection and Accumulation of Cannabis Sativa: Dried leaves, twigs and seeds of cannabis sativa plant were obtained from the National Drug Law Enforcement Agency (NDLEA), Rivers State command, Nigeria. The characterization and validation of the plant will be done by evidence specialists at NDLEA, Rivers State Command in Port Harcourt.

Animal Collection and Acclimatization: Forty (40) Wistar rats of both sexes were used in this study. The rats were obtained from the animal house in the Faculty of Basic Medical Sciences, University of Port Harcourt, and kept in clean cages under standard laboratory conditions of 12 hours light/dark cycle. They had access to vital pellet feed and distilled water as they were allowed to acclimatize for one week. The rats were allowed to roam in an enclosed space having proper ventilation and temperature throughout the experiment. All the

rats were allowed to feed *ad libitium* for 14 days after which different behavioural tests will be conducted.

Experimental Procedure: The 40 Wistar rats with an average weight of 110g were randomly divided into 5 groups (8 rats each). A low dose and high dose of 250mg/kg and 900mg/kg respectively and a normal dose of 750mg/kg of aqueous marijuana were used according to research conducted by Danladi, *et al.* [10] to induce the rat. The dosage of lemon zest used includes 250mgkg^{-1} following the findings of Juan, *et al.* [11].

Group 1: Control group- This group was given animal feed and distilled water only with no dose of lemon zest or marijuana.

Group 2: Low dose group- This group was served with a low dose (250g) of lemon zest only and no dose of marijuana.

Group 3: Marijuana Induced group: This group was administered a 500mg/kg dose of Marijuana and no dose of lemon zest.

Group 4: This group was induced with 500mgkg^{-1} of marijuana and treated with low dose (250mgkg^{-1}) of lemon zest.

Group 5: This group was served with a high dose (900g) of marijuana but with low dose (250mgkg^{-1}) of lemon zest Lemon.

Tissue Harvesting: The harvesting of tissues was done to extract the organ of choice from the rat which is the pons. The rats were first made unconscious by being exposed to chloroform. While unconscious, the rats were then pinned to a wooden board to keep them

still during dissection. The brains were harvested from their skull and the harvested parts were fixed in Bonin solution

RESULTS

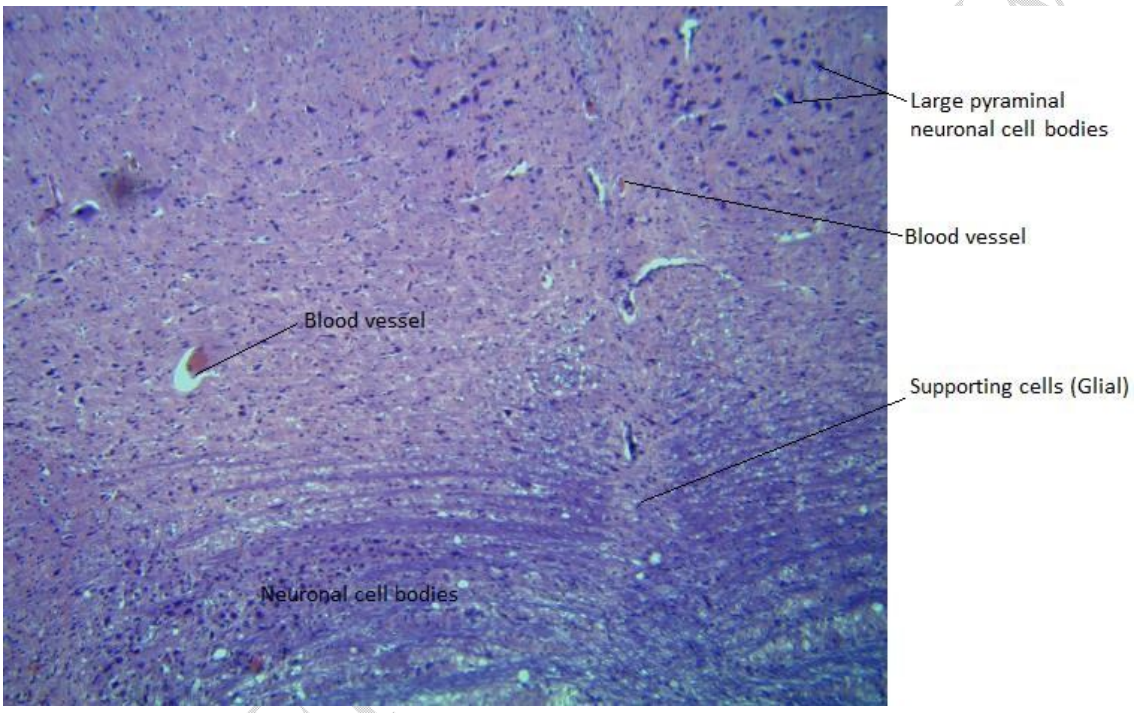

Plate 1: Normal Control showing large pyramidal neuronal cell bodies

The plate 1 above shows the photomicrograph of the pons used for the control experiment at lower magnification showing large pyramidal neuronal cell bodies at the anteromedial area of the pons. Medium size neuronal cell bodies are arranged at the posterior medial area of the pons. Tissue show normal cytoarchitecture. H & E, X100.

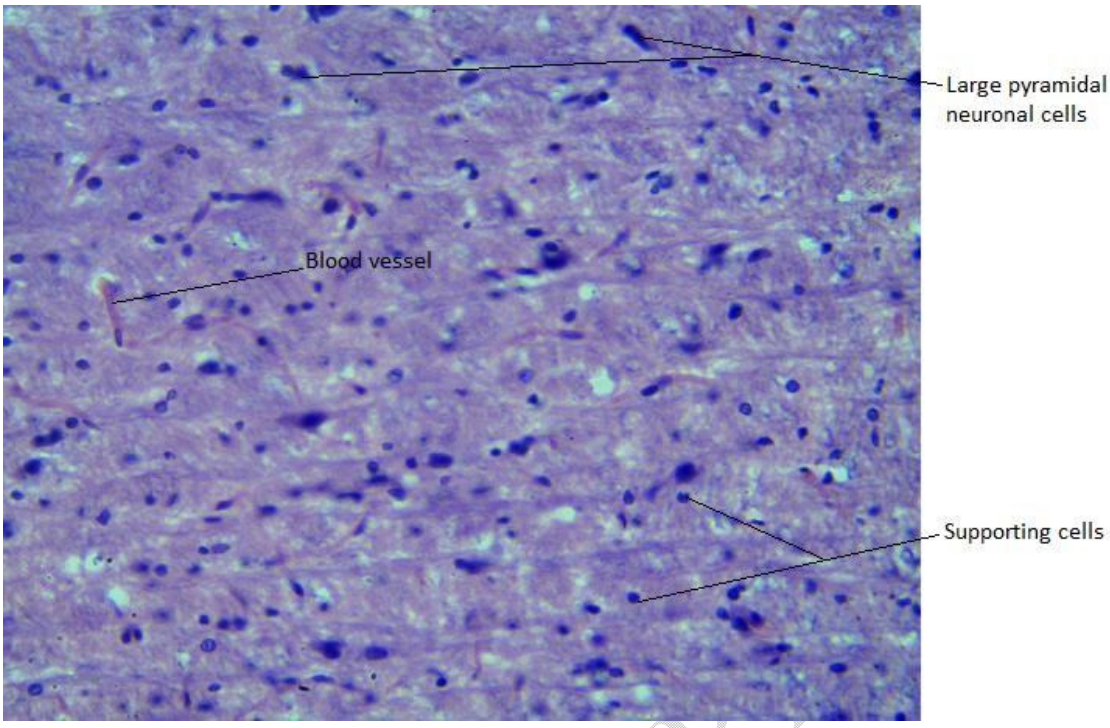

Plate 2: Normal Control showing delineated neuronal and supporting cells

Plate 2 above displays the photomicrograph of the pons showing well delineated neuronal cell bodies and few supporting cells (glial). The connective tissue fibres are well distributed. H & E, X400

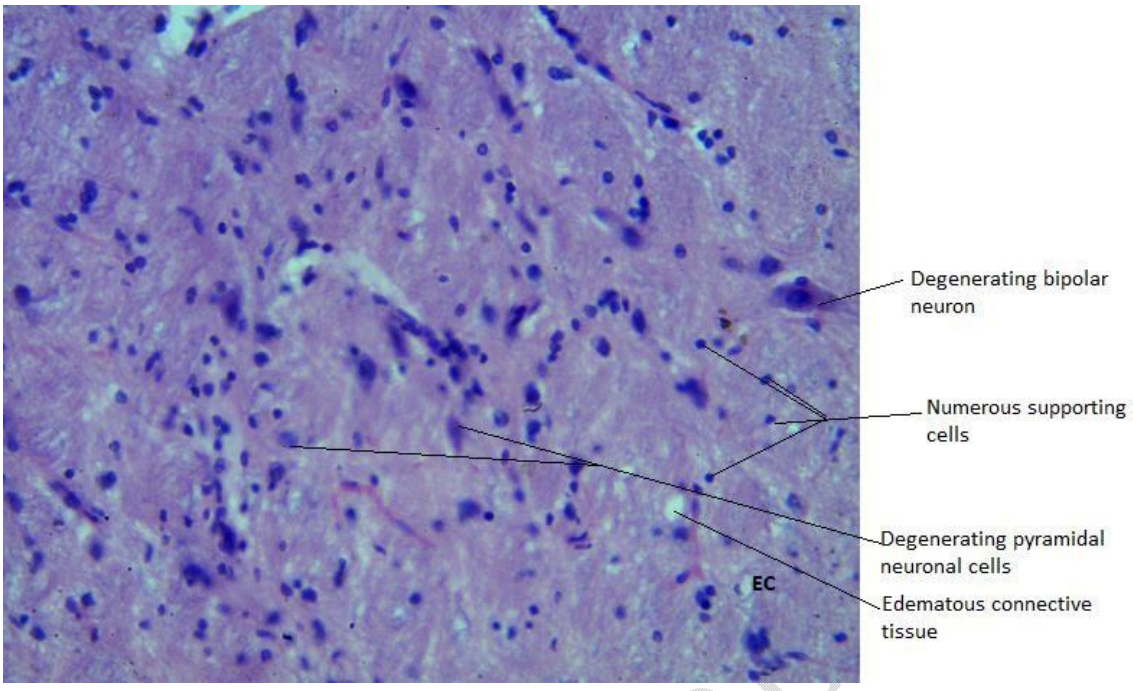

Plate 3: Pons of the Wistar rats after 250mgkg^{-1} of lemon zest induction

The plate 3 above shows the photomicrograph of the pons on few degenerating neuronal cell bodies and numerous supporting cells (glial) after 250mgkg^{-1} of lemon zest was administered. Pockets of edematous connective tissue cells are observed. Pons tissue show mild distortion. H & E, X400.

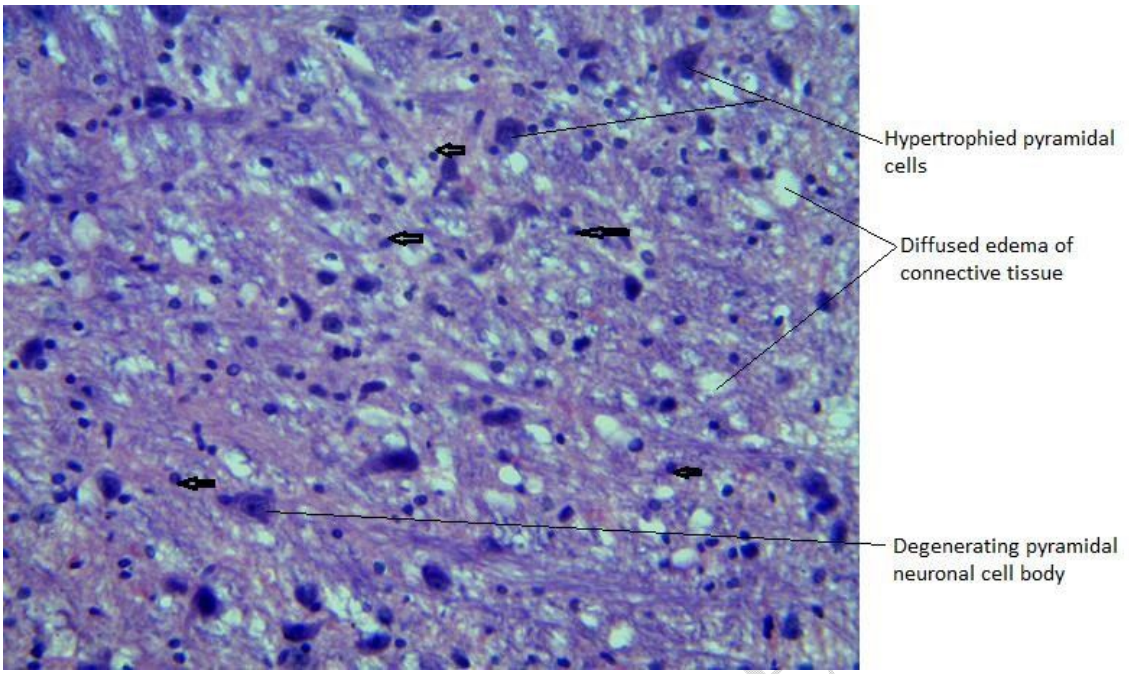

Plate 4: Pon State after the medium dose of *Cannabis Sativa* (500mgkg^{-1}) induction only

Plate 4 above is the photomicrograph of the pons showing several hypertrophied and degenerating neuronal cell bodies alongside numerous supporting cells (glial-arrowed) when a low dose of 250mgkg^{-1} of cannabis sativa was administered to the rat. Diffused edematous connective tissue cells are observed. Pons shows severe distortion of tissue cytoarchitecture. H & E, X400.

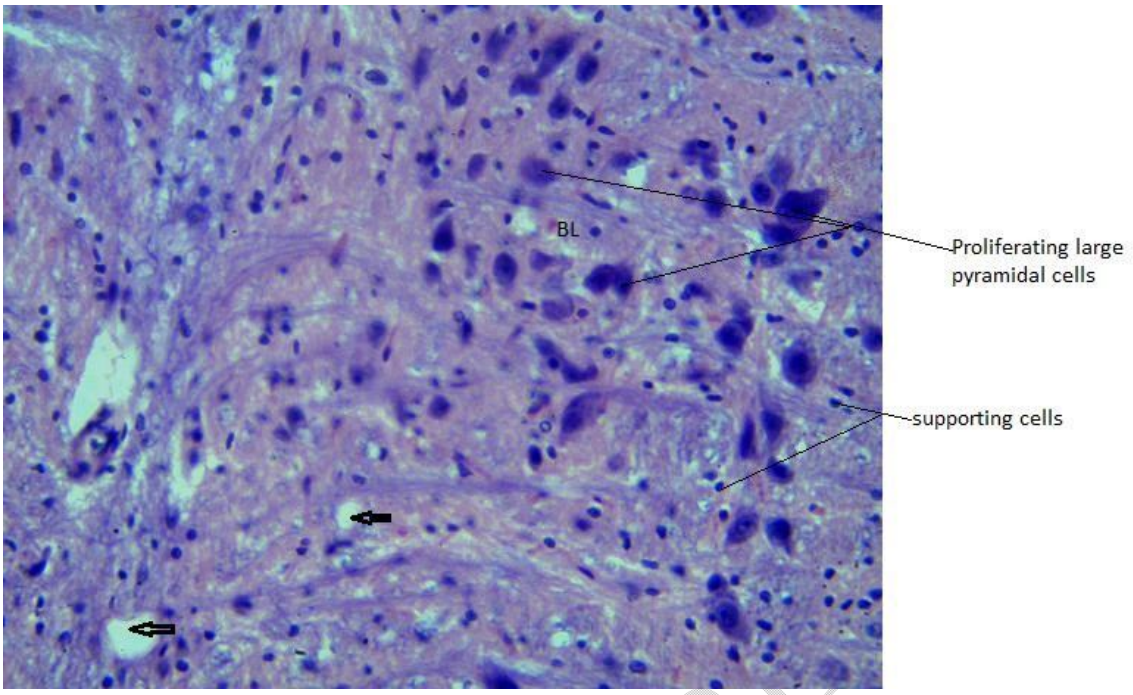

Plate 5: Low dose of *Cannabis Sativa* (250mgkg^{-1}) and a low dose of lemon zest (250mgkg^{1}) application

The plate 5 above displays the photomicrograph of the pons showing proliferation of pyramidal neuronal cell bodies with pockets of supporting cells (glial). Pockets of edematous (vacuolation) connective tissue cells (arrowed) and the presence of congested blood vessel are also observed. Pons shows a recovery state of tissue cytoarchitecture. H & E, X400.

Effects of Lemon Zest on the Histology of Pons of High Dose Cannabis Sativa Induced Wistar Rat

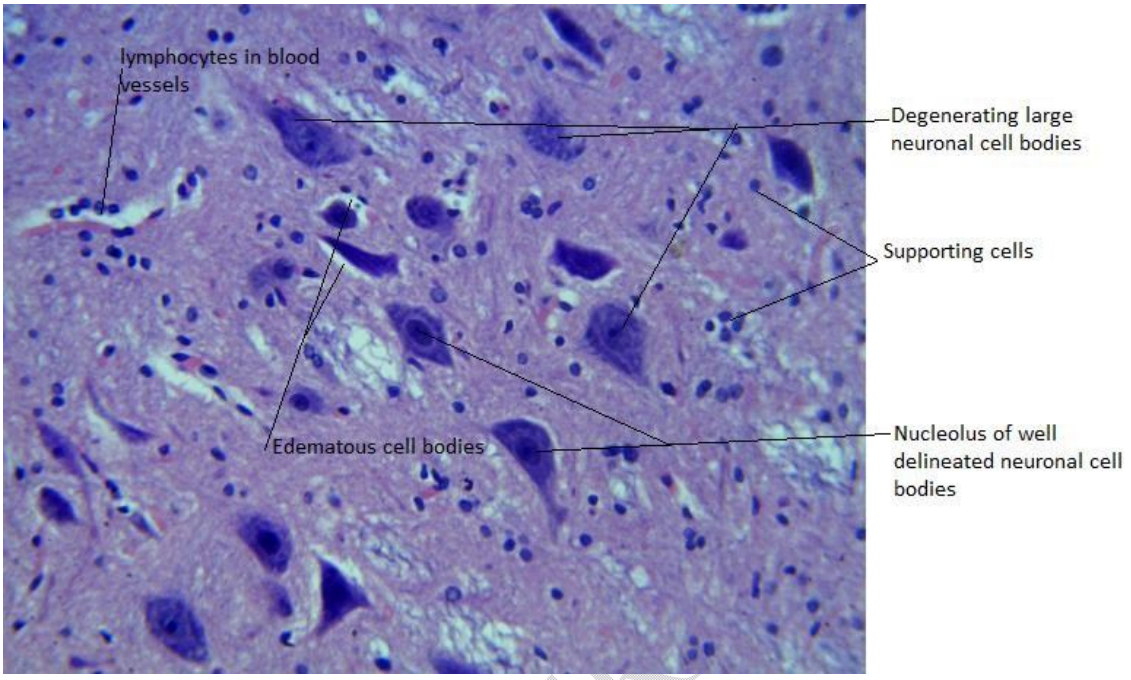

Plate 6: High dose of *Cannabis Sativa* (900mgkg^{-1}) and a low dose of lemon zest (250mgkg^{1}) application

Plate 6 above displays the photomicrograph of the pons showing proliferation and degenerating pyramidal neuronal cell bodies with pockets of supporting cells (glial). Some well delineated neuronal cell bodies with clear nucleolus are outlined in the tissue. The presence of congested blood vessels containing lymphocytic cells is also observed. Pons shows a recovery state of tissue cytoarchitecture. H & E, X400.

Discussion

This study aims at determining the effects of lemon zest on the pons of cannabis sativa induced Wistar rats. The study thus determined the effects of lemon zest on the cytoarchitecture of pons of cannabis sativa non-induced Wistar rats, determined the effects of a low dose of lemon zest on the histology of pons of cannabis sativa induced Wistar rats and examined the impacts of high dosage of lemon zest on histology pons of cannabis sativa induced Wistar rats.

Results in this study displayed the photomicrograph of the pons before marijuana and/or lemon zest were administered. Findings thus revealed that when 250mgkg^{-1} of lemon zest was administered, pons showed few degenerating neuronal cell bodies as well as numerous supporting celles (glaila) as against the normal control which displayed well delineated neuronal cell bodies and few supporting cells since the connecting tissues were properly distributed. When 500mgkg^{-1} of *cannabis sativa* was used to induce the pons, it was observed that the neutrons of the pons were hypertrophied and degenerated and with several supporting cells with edematous collective tissue cells being observed, in tandem with the findings of Akinola et al. [12], Policy, et al. [13], Imam, et al. [14] and Tomas-Roig, et al. [15], their work showed that histological differences occur during the administration of *cannabis sativa* on neuronal cells, leading to perinuclear spaces and vacuolations. It was also observed that when a low dosage (250mgkg^{-1}) of the *cannabis sativa* was administered alongside a low dose of lemon zest, congested blood are observed as well as pockets of edematous connective tissues. When the dosage of the *cannabis sativa* was increased to 900mgkg^{-1}, some well delineated neuronal cells with well-defined nucleolus were outlined in the tissue as once observed in the control experiment. There is also the presence of congested blood vessels carrying lymphatic cells and thus recoveries of tissue cytoarchitecture were observed.

Hence this means that the histological areas of the Photomicrograph of pons treated with lemon zest showed improvement in the degenerating neuronal cell bodies previously noted in the study. This also correlated with the findings of Raimondo et al. [16], who opined that citrus zest results in an increase in Brain Derived Neurotropic Factor (BDNF). This means that lemon zest has neuroprotective effects in individuals since the Wistar rats' brain is similar to that of the humans. The neuroprotective effects observed in this present study agrees with the findings of Shafeeqa et al. [17].

Conclusion

Conclusively, this study has demonstrated the negative effect of cannabis sativa on the neuronal cells of the pons upon administration of different doses of the extract, as demonstrated in group 3. However, this effect was reversed upon administration of lemon zest as established in groups 4 and 5. It is therefore important to note that lemons zest has neuroprotective qualities or the potential to ameliorate adverse effects caused by the extract of cannabis sativa.

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