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HISTOLOGICAL CHANGES IN THE CEREBELLUM OF ADULT WISTAR RATS FOLLOWING ADMINISTRATION OF ETHANOLIC EXTRACTS OF LEMONGRASS AND MANGO BARK

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ABSTRACT

This research work focused primarily on the histological features of Wistar rats cerebellum following the administration of the ethanolic extracts of lemongrass, mango bark and its combined extracts. Twenty adult Wistar rats weighing 180-220g were divided into four groups labeled A, B, C and D. Group A rats served as the control and were fed with rat chow throughout the duration of the experiment. Group B animals received 2000mg/kg of the ethanolic extract of lemongrass, group C animals received 2000mg/kg of ethanolic extract of mango bark while group D animals received combined ethanolic extracts of 1000mg/kg of lemongrass and 1000mg/kg of mango bark orally for two weeks. The animals were sacrificed 24 hours after the last administration using chloroform inhalation method. The cerebellum were excised and preserved using 10% formal saline. Routine paraffin wax tissue processing was carried out and sections stained using Hematoxylin and Eosin. Histological observations revealed in the control rats normal cerebellar cytoachitecture. Cerebellum of the treated groups showed slight degeneration and loss of purkinje cells, which was more in group D when compare with groups B, C and the control group. Result showed that the extract may individually and in combined form cause alterations in the cerebellum cytoarchitecture.

KEYWORDS: Cerebellum, Histology, Ethanolic extract, Lemongrass, Mangobark, Wistar rats.

INTRODUCTION

Traditional medicines have been used to treat malaria for thousands of years and are the source of the two main groups (arteminsinin and quinine derivatives) of modern antimalarial drugs. With the problems of increasing levels of drug resistance and difficulties of being able to afford and access effective antimalaria drugs, traditional medicines have become an important and sustainable source of treatment (Bodeker, and Willcox, 2000).

Mango bark and lemongrass have been used by Nigerians for treatment of malaria (Ishola et al., 2014). Mangifera indica is used medicinally to treat ailments such as asthma, cough, diarrhea, dysentery, leucorrhoea, jaundice, pains and malaria (Agoha, 1981; Madunagu et al., 1990). Every part of mango tree: root, stem, bark, the blossom, raw and ripe mango fruits, and seeds all have curative and medicinal properties. Dried mango flowers, containing 15% tannin, serve as astringents in cases of diarrhea, chronic dysentery, cancer of the bladder and chronic urethritis resulting from gonorrhea. The bark contains mangiferine an astringent employed against rheumatism and diphtheria. It is believed helpful in cases of (Shah et al, 2010).

Lemongrass has been used for several purposes: against coughs, colds, cuts, asthma, bladder disorders, and gastrointestinal problems (Alves et al., 1960). In Nigeria, it is used as antipyretic, and for its stimulating and antispasmodic effects (Olaniyi et al., 1975). Traditionally, lemongrass is used in the treatment of malaria and typhoid fever and recommended for stomach aches (Dean, 2007; Udeh et al., 2001).

Although traditional medicine is widely used to treat malaria and it often more available and affordable than western medicine, it is not without limitations. There are few clinical data on safety and efficacy, there is no consensus, even among traditional healers on which plants preparations, and doses are the most effective. Thee concentration of active ingredients in plant species varies considerably, depending on several factors (Bodeker and Willcox, 2000). Thus the aim of this research is to find out the effect of ethanolic extract of lemongrass, mango bark and their combinations on the cerebellum of adult Wistar rats.

MATERIALS AND METHODS

Breeding of Animals

Twenty (20) adult Wistar rats weighing between 180 - 220g of the same strain were obtained from the animal house of the Department of Animal Science, University of Calabar. They were kept in the animal house of the Department of Human Anatomy for a period of two weeks under standard conditions of temperature 27°C - 30°C, photoperiod of 12 hours natural light cycle and 12 hour dark to acclimatize. The animals were fed with grower mash obtained from Vital Food Company at No. 2 Mount Zion Street Calabar, and water was provided *ad libitum*. Their beddings were changed daily to keep the rat's immediate environment clean and uncontaminated. After the acclimation period, they were randomly divided into four groups labeled A, B, C and D, each group consisting of five rats.

Herbal Preparation

The lemongrass and mango bark were collected from Botanical Garden in Akpabuyo Local Government Area of Calabar, Cross River State. The herbs were authenticated and confirmed by the botanist in the botanical garden of the University of Calabar. The lemongrass leaves were washed in water and dried under a shed. The mango bark was chopped into small pieces and dried. The dried lemongrass and the mango bark were ground to powder separately using electric bender in new Chemistry laboratory, Department of Chemistry, University of Calabar. The Lemon grass powder and the mango bark powder were soaked in 2 liters of ethanol each for 72 hour. The suspension was filtered using 150mm size Whatmann filter paper. The precipitate was allowed to evaporate to dryness at room temperature leaving a crude extract.

Experimental Protocol

Twenty (20) adult wistar rats used for this experiment were randomly divided into four groups labeled A, B, C and D, with group A as the control and group B, C and D as the experimental groups, each consisting of five rats. The ethanolic extract of lemon grass and mango bark were administered orally with the aid of orogastric tube to the animals in the experimental groups respectively for a period of two week.

Extract Administration

Group A: Animals were feed with rat chow
Group B: Animals received 2000mg/kg of ethanolic extract of lemon grass.
Groups C: Animals received 2000mg/kg of ethanolic extract of mango bark.
Group D: Animals received combined 1000mg/kg of the ethanolic extract of lemongrass and 1000mg/kg of the ethanolic extract of mango bark.

Termination of Experiment

The animals were sacrificed 24 hours after the last administration using chloroform inhalation method. The brains were extracted; the cerebellum exercised and preserved using 10% formal saline. Routine paraffin wax histological tissue processing was carried out. The cerebellar sections were stained using Hematoxylin and Eosin staining method.

RESULTS

In the control group (group A), three cell layers were observed from the outside to the inside following Haematoxylin and Eosin staining. These are the molecular layer (ML), purkinje cell layer (PCL), granular layer (GL). The white matter was also seen (WM). The purkinje cells are prominent. The granular layer showed distinct granular cells. The molecular layer consisted of the basket and stellate cells (Plate 1).

The cerebellum of wistar rats that received 2000mg/kg of ethanolic extract of lemongrass (Group B) showed slight degeneration and loss of purkinje cells when compared to the control (Plate 2).

The histological appearance of the cerebellum, of rats adminstered with 2000mg/kg of ethanolic extract of mango bark (Group C) showing decrease cellular population in the molecular layer, loss and increase in the size of purkinje cells (Plate 3).

The cerebellum of rats adminstered with combined 1000mg/kg of the ethanolic extracts of lemongrass and mangobark (group D), showing degeneration and loss of purkinje cells (Plate 4).

Photomicrographs

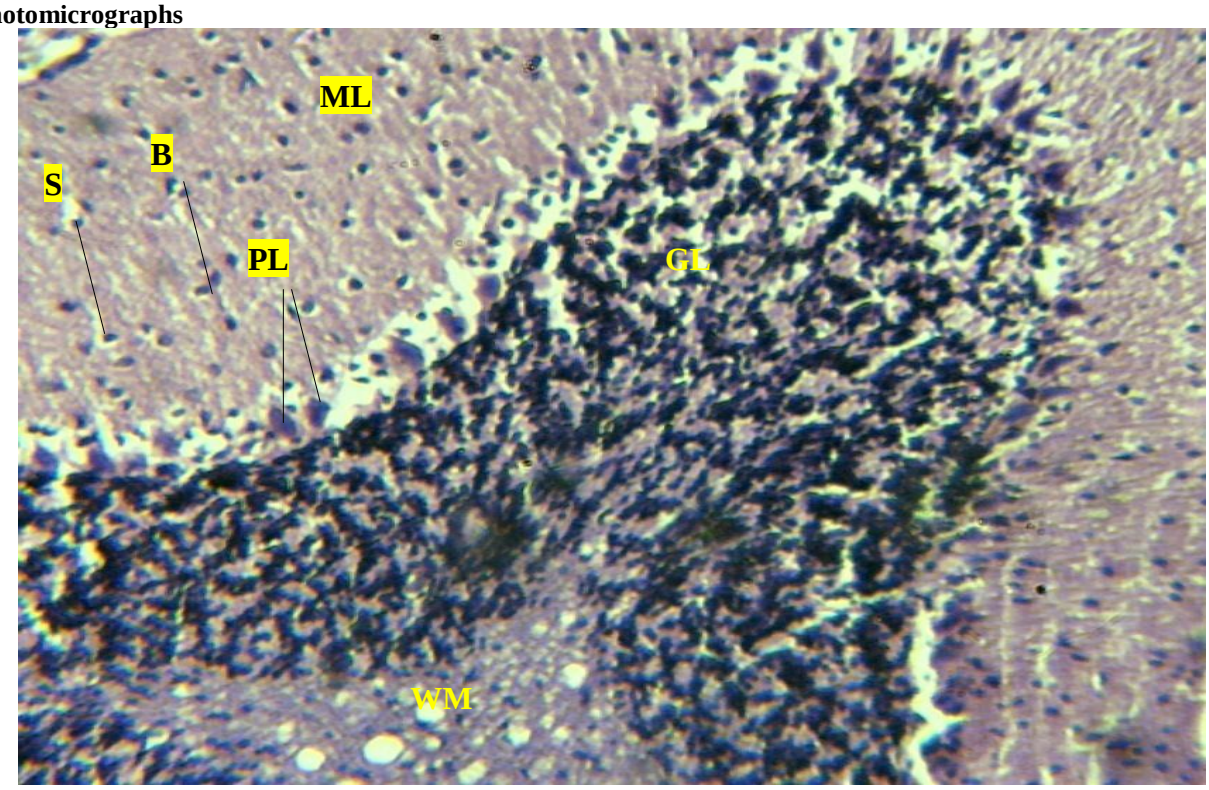

PLATE 1: Photomicrograph of normal histological architecture of the cerebellum of the control showing normal Molecular layer (ML), Granular layer (GL) and the Purkinje layer (PL) with the White matter (WM). Basket (B) and Stellate (S) cells. H & E; Mag. x 100.

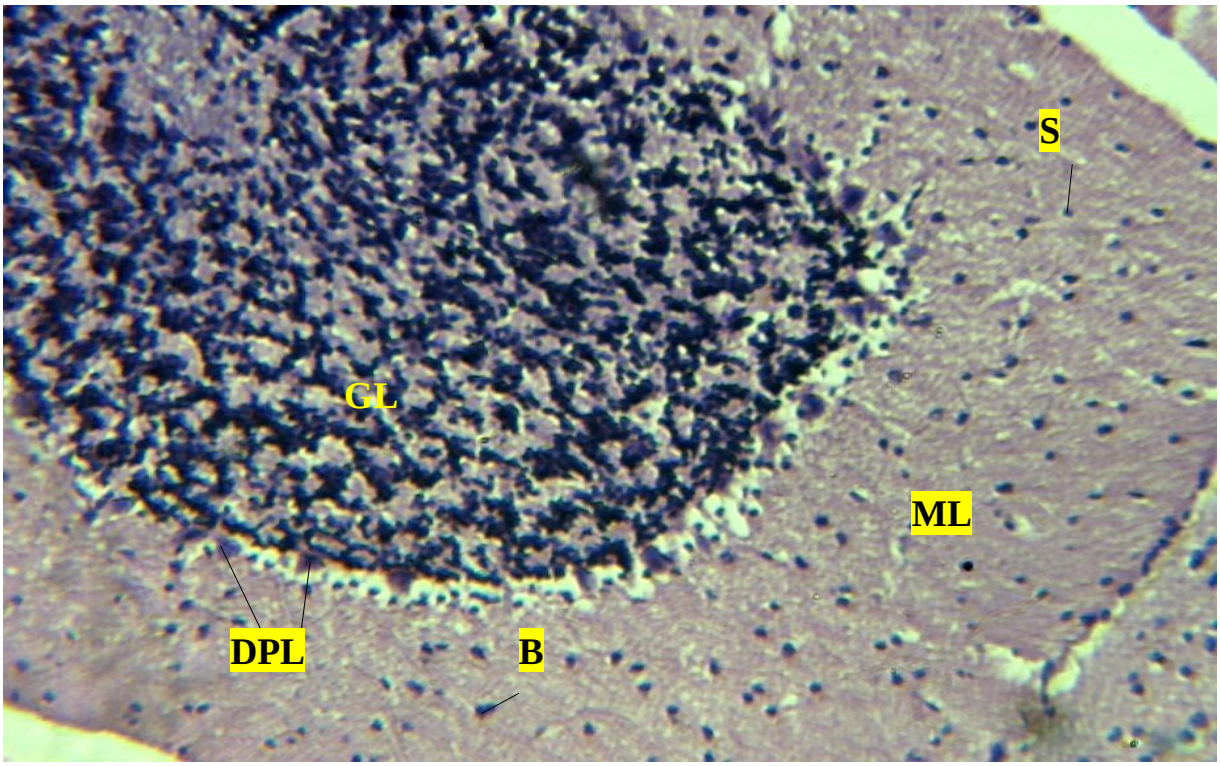

PLATE 2: Photomicrograph of the cerebellum in group B treated with 2000mg/kg of ethanolic extract of lemon grass showing slight degeneration and loss of purkinje cells (DPL). H & E; Mag. x100.

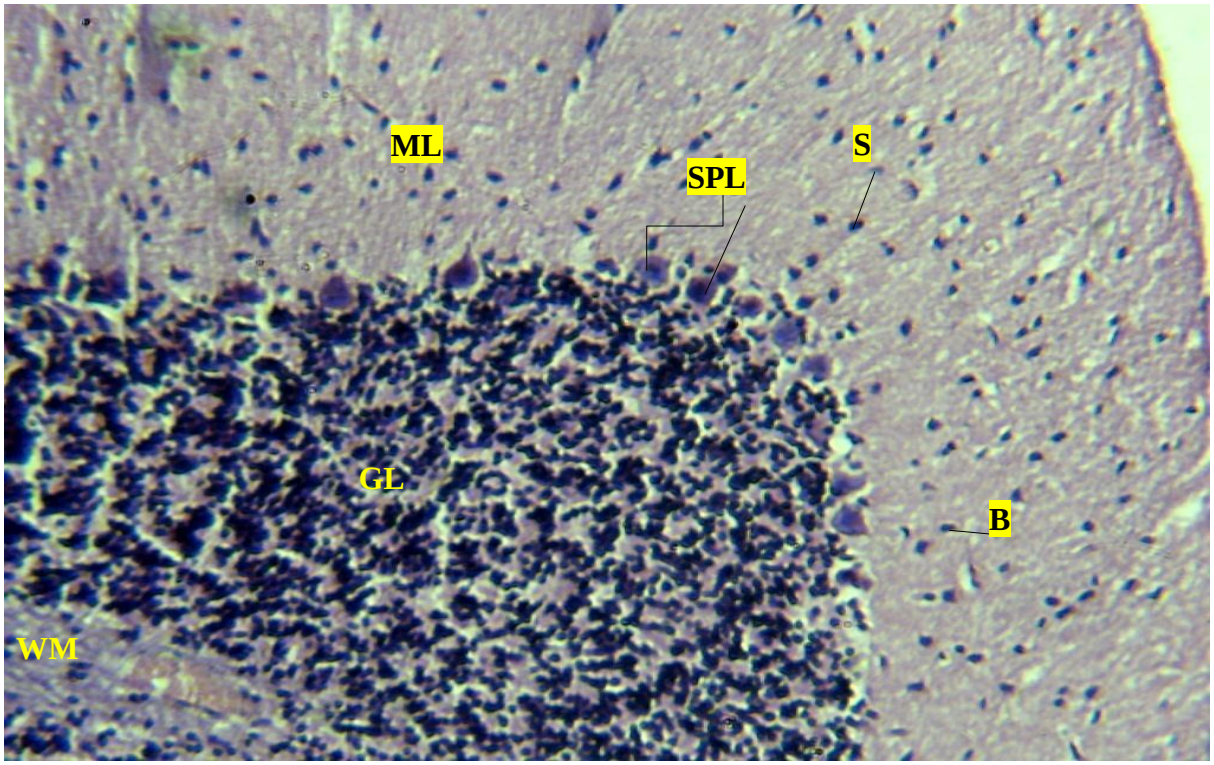

PLATE 3: Photomicrograph of the cerebellum in group C treated with 2000mg/kg of ethanolic extract of mango bark showing decrease cellular population in the molecular layer, loss and increase in the size of purkinje cells (SPL). Hyperplasia of cells in the granular layer. H & E. Mag. x100.

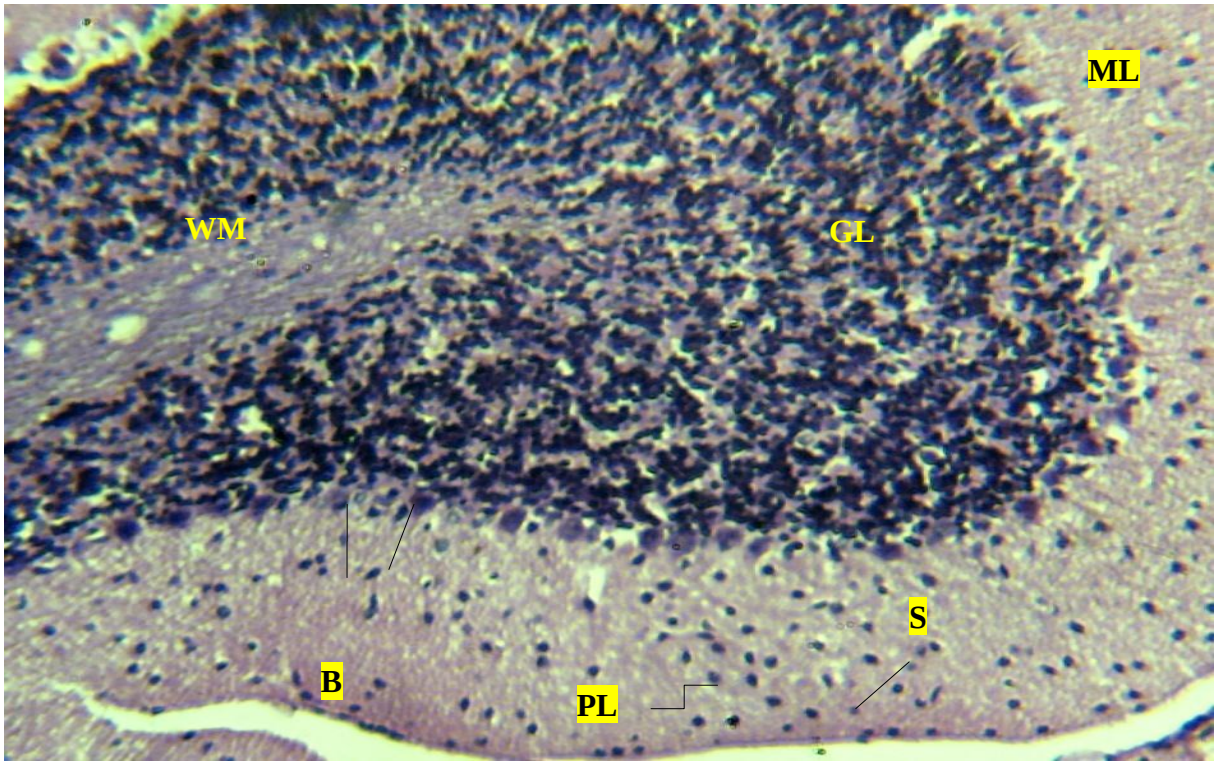

PLATE 4: Photomicrograph of the cerebellum in group D treated with combined 1000mg/kg of ethanolic extracts of lemon grass and 1000mg/kg of mango bark showing degeneration and loss of purkinje cells (DPL). H & E; Mag. x100.

DISCUSSION

The cerebellum is divided into three layers namely, the molecular layer, the purkinje cell layer and the granular layer. The molecular layer of the cerebellum contains two types of inhibitory interneuron: stellate cells and basket cells. Both stellate and basket cells form

GABAergic synapses onto Purkinje cell dendrites. The large, spherical cell bodies of Purkinje cells are packed into a narrow layer (one cell thick) of the cerebellar cortex, called the *Purkinje layer*. Purkinje cells use GABA as their neurotransmitter, and therefore exert inhibitory effects on their targets (Llinas et al., 2004).

In this study, ethanolic extracts of lemongrass and mango bark administered to adult wistar rats orally was found to have slight adverse effects respectively on the histological features of the cerebellum. The sections from the cerebellum of treatment groups showed increase cellular population (hyperplasia) in the granular layer which was more prominent in group C animals that received 2000mg/kg of the mango bark extract. There was also, increase in size shrunken, degeneration and loss of purkinje cells in the purkinje layer as well as decrease molecular layer. The result of this work is in line with the work of Eluwa et al (2014) who reported that ethanolic extracts of lemongrass and mango bark cause cellular hyperplasia of cerebral astrocytes. The hyperplasia may be due to the ability of the lemon grass to cause increase mitotic division resulting to production of new cells. While the degenerated and loss of purkinje cells may result from the effect of ethanolic extracts of lemongrass and mango bark on the cellular synthesis.

Other studies carried out with other medicinal plants were reported to have similar findings. Moses et al. (2013) reported on the cerebellar neurohistology and behavioural effects of Gongronema latifolium and Rauwolfia vomitoria in mice. The cerebellum showed slight hypertrophy of Purkinje cells, with brain matrix loss, increase cellular population, and decrease cellular sizes in the treatment groups. In another study involving the administration of artesunate; histological findings showed degenerated and loss of purkinje cells, cellular hypertrophy with intercellular vacuolation in the cerebellar cortex of treated rats (Ajibade et al., 2012). Administration of ethanolic extract of Cola nitida also showed partial loss of molecular and purkinje cells and neurodegeneration of the cerebellar cell layers (Buraimoh, 2014).

CONCLUSION

This study shows that the ethanolic extracts of lemon grass and mango bark may individually and in combined form may have adverse effect on the histology of the cerebellum and the effect was extract dependent. Hence it consumption should be reduced.

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