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BIOCHEMICAL AND HISTOLOGICAL ASSESSMENT OF THE CEREBELLUM IN ADULT WISTAR RATS FOLLOWING THE ADMINISTRATION OF AQUEOUS EXTRACT OF NICOTIANA TABACUM

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Abstract

Aim: Using an animal model the direct effect of aqueous extract of *Nicotiana tabacum* (ANT) administration on the biochemical parameters and cerebellum in Wistar rats was investigated.

Methods: Twenty four (24) rats were divided into four groups with six rats per group with an average weight of 150 g. Group 1 served as the Control and was administered distilled water (1 ml/kg bw) and Groups 2, 3 & 4 received 1000 mg/kg bwt, 500 mg/kg bwt and 250 mg/kg bwt of (ANT) respectively through oral gavages. The rats were humanely sacrificed after 21 days of treatment. Biochemical and histological studies were done.

Results: Beam Walking Test show increased latencies in treated groupswhen compared to the Control Group. The result showed a significant decrease in CAT level in Groups 2 (43.50±2.12), and 4 (41.50±0.70) when compared with the Control (48.00±1.41) ($p<0.05$). The result of the histological section of the cerebellum of treated groups revealed histoarchitectural distortion; neurodegenerative changes, such as cytoplasmic shrinkage of Purkinje cells and vacuolations in internal granule cell layer.

Conclusion: The result showed that the consumption of tobacco may be harmful to the brain and should be taken with caution.

Key words: *Nicotiana tabacum*, cerebellum, Histological

INTRODUCTION

Smokeless tobacco contain nicotine which is the principal alkaloid contained in tobacco and it is believed to be the primary reason for cigarette smoking in many people particularly as they derive satisfaction and pleasant sensation from inhaling nicotine (Benowitzet al,1983). It is widely consumed through cigarette smoking and tobacco chewing in 30-40% of the world's population. Nicotine, a pyschoactive substsance which is responsible for the development of nicotine dependence among smokers (Perrira et al., 1996). Available evidence showed that nicotine affects many biological activities. For instance, long-term tobacco use can cause prematurely wrinkled skin, gum and tooth loss, loss of taste and smell, weakened immunesystem, stomach ulcers, bad breath, cancer, unwanted weight fluctuation and coronary thrombosis (Sivanandam, 2010). These effects of tobacco are associated with the nicotine content, which is a major constituent of tobacco. Much attention of nicotine researh is centered on its addiction issue and less focus is placed on its potential to cause neurotoxicity. Nicotine is reported to cause postural imbalance in smokers. It may have a great physiological and pharmacological effects (Perrira et al., 1996) and plays crucial role in establishing and maintaining tobacco dependence (Costa et al., 2001). The cerebellum is the central part of the major circuitry that links sensory areas to the motor areas of the brain and is required for coordinationof fine movements. The cerebellum provides connections during movement and is initially involved in motor learning and reflex modification. Cerebellar output is mainly to

those parts of the brain that control movement (Kandel et al., 2000). The rationale for selecting aqueous extract of *Nicotiana tabacum* was based on the fact most people in parts of Nigeria are exposed to this form of smokless tobacco for a long period and very little basic research is focused on its effect on the cerebellum. The aim of the present study was to investigate the effects of aqueous extract of *Nicotiana tabacum* on the biochemical parameters and the histology of the Cerebellum of Wistar rats.

MATERIALS AND METHODS

Animals

Twenty-four apparently healthy adult albino rats of the Wistar strain were used in the study. The animals were housed under conditions of controlled lightening at room temperature in the animal House of the Department of Human Anatomy, Faculty of Medicine, Ahmadu Bello University Zaria. Animal feed and water were provided ad libitum. The animals were randomly divided into four groups of six rats per group with an average weight of 150 g.

Extract preparation

The leaves of *Nicotianatabacum* were collected from Zaria town, Zaria, Kaduna State, Nigeria. The leaves were taken to the Herbarium Unit of the Department of Biological Science, Ahmadu Bello University, Zaria for identification and authentication, and a voucher specimen number was provided as 540**.**Preparation of *Nicotianatabacum* leaves extract was done in the Department of Pharmacognosy, Faculty of Pharmaceutical Sciences, Ahmadu Bello University, Zaria, Nigeria. The method of maceration as reported by Akinpelu and Kolawol (2004), for the preparation of aqueous extract was employed.

Experimental design

Group 1 served as the Control and was administered distilled water (1 ml/kg bw) and Groups 2-4 were the treatment Groups. Groups 2, 3 and 4 received 1000 mg/kg bwt, 500 mg/kg bwt and 250 mg/kg bwt of aqueous extract of *Nicotianatabacum* respectively. All administration were given orally and lasted for a period of 21 days. After the administration, the rats were humanly sacrificed on day 22 and their brains were excised.

Neurobehavioural observations

The motor coordination was studied using the beam walking test. Motor coordination and balance was assessed by measuring the ability of the animals to traverse a narrow beam to reach an enclosed safety platform (Carter et al., 1999; Fan et al., 2008). In this test three parameters were recorded which include; (A) Time spent on platform TP in seconds, (B) Latency: which is the time (in seconds) taken by the animals to traverse the beam TW from point A (starting point) to B (safety Box), it was used to assess learning and memory (C) Number of Foot slips (NFS) which was used to assess the level of balance and coordination.In addition, as described by Abou-Donia et al. (2008), the time taken (latency) until the animal's nose enters the goal box (up to 60 s) was recorded. Rats that fell off the beam or did not entered the goal box were assigned latencies of 60 s. Beam walk scores were based on an average of 3 trials, each separated by 30 minutes per rats

Animal sacrifice

After administration, the rats were humanely sacrificed on day 22 and their brains were excised. Some brains were quickly homogenized and processed for biochemical while some were fixed in Bouin's fluid processed for histological studies.

Biochemical Analysis

Sample collection and preparation of tissue homogenates

The cerebella were collected, and then 1 g of the tissues was immediately homogenized. The tissue homogenates were centrifuged at 3000 rpm for 10 min and the supernatant were utilized for different estimations according to the method of Bradford (1976). MDA level, activity of SOD, CAT and GPx concentration were evaluated.

Histology

The cerebellum was excised and processed for Haematoxylin and Eosin (H & E) and Cresyl Fast Violet (CFV) staining techniques. The tissues were processed and embedded in paraffin wax for routine histologic studies. The brain tissues of 5 µ were sectioned with the Letiz rotary microtome. The sections were mounted, stained with H & E and Cresyl fast Violet methods and examined with the light microscope and the photomicrographs were taken.

Statistical analysis

Results obtained were analysed using Statistical Package for Social Scientist (SPSS version 20.0) and results were expressed as mean ± SEM and

the presence of significant difference between means of the Groups were determined using one way analysis of variance (ANOVA) with Tukey's post hoc test for significance. Values were considered significant when $p<0.05$.

RESULTS

Beam walking Test

The result of the effects of *Nicotianatabacum* administration on motor coordination and balance following 60 seconds of Beam walking in treated Groups of adult Wistar rats show that all the groups had increased time spent on the platform (TP) after administration with Groups 2, 3 and 4 having higher time when compared to the mean training time (Tt) and the Control Group though the increase was not significant as shown in Figure 1.The result shows that all the groups had increased latency time (LT) following the administration of the extracts though the increase was not significance when compared to Tt and Control Group as shown in Figure 2. The results showed that the Number of Foot Slip (NFS) was decreased in Groups 2 and 3 following administration of the extracts when compared to the Control Group though the decrease was not significant as shown in Figure 3.

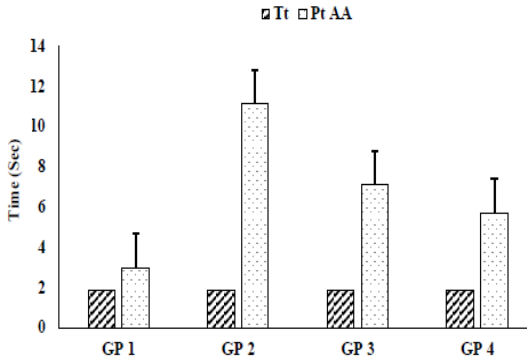

Figure 1: Effect of aqueous extract of Nicotiana tabacum on beam walking test (Time on platform). N=6: Tt= Mean time on platform before administration: Pt AA= Time on platform after administration

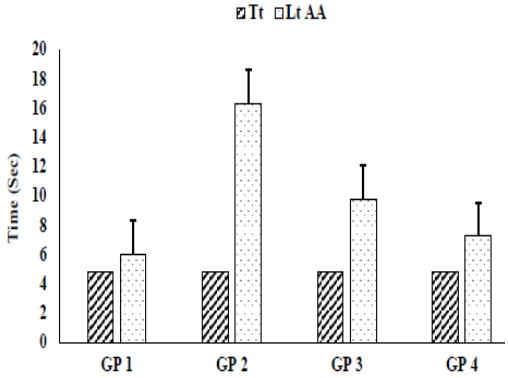

Figure 2: Effect of aqueous extract of Nic otiana tabacum on beam walking test (Latency time). N=6: Tt= Mean training time before administration: Lt AA= Latency time after administration

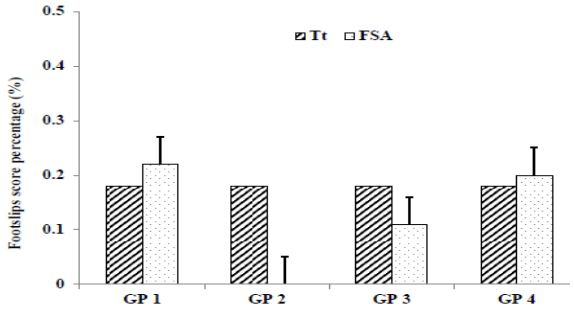

Figure 3: Effect of aqueous extract of Nicotiana tabacum on beam walking test (Number of footslip). N=6: Tt= Mean training time before administration, FSA= Footslip after administration

Biochemical Studies

Biochemical analyses for lipid peroxide levels and antioxidant enzyme activity namely Malondialdehyde, (MDA); superoxide dismutase, (SOD); catalase, (CAT) and glutathione peroxidase, (GPx) were assessed in brain tissue homogenate of Wistar rats. The result showed that there was increased MDA activity in all the treated Groups, though the increase was not significant when compared to the Control Group. The result also showed that the level of CAT in all the treated groups was significantly decreased in Groups 2 and 4 when compared to the Control Group ($P<0.05$). The result of SOD level was significantly decreased in Group 3 when compared to Control Group ($P<0.05$) as shown in Table 1. There was a non-significant increase in GPx level in Groups 2 and 3 and a decrease in group 4 when compared to the Control Group as shown in Table 1.

Table 1: Effect of aqueous extracts of *Nicotianatabacum* on brain tissue oxidative stress markers in adult Wistar rats

Groups	CAT(µg/ml) Mean ± SEM	MDA(µg/ml) Mean ± SEM	SOD(nmol/ml) Mean ± SEM	GPx (µg/ml) Mean ± SEM
1	48.00±1.41	1.00±0.14	2.45±0.21	47.50±2.12
2	43.50±2.12*	1.20±0.14	2.25±0.21	49.00±2.82
3	45.50±2.12	1.15±0.21	2.10±0.14*	49.00±2.82
4	41.50±0.70*	1.25±0.21	2.15±0.07	46.50±2.12

CAT = Catalase, MDA = Malondaldehyde, GPx =Glutathione Peroxidase, SOD = Superoxide Dismutase, *P<0.05

Histological and Histochemical studies of the Cerebellar Cortex of Wistar rats

Histological studies of the cerebellum of Wistar rats in the Control Group showed normal histoarchitecture of the cerebellar cortex. The characteristic appearance of the three cortical layers: an outter molecular layer with distinct neurons and inner granular layer. Between these layers is a layer of flasked shaped Purkinje cells as shown in Figure 4.The cerebellar sections of Groups 2, 3, and 4 treated Wistar rats revealed histoarchitectural distortion of the cerebellar cortex; neurodegenerative changes, such as cytoplasmic shrinkage of Purkinje cells and vacuolations in internal granule cell layer as shown in Figures 4 and 5.

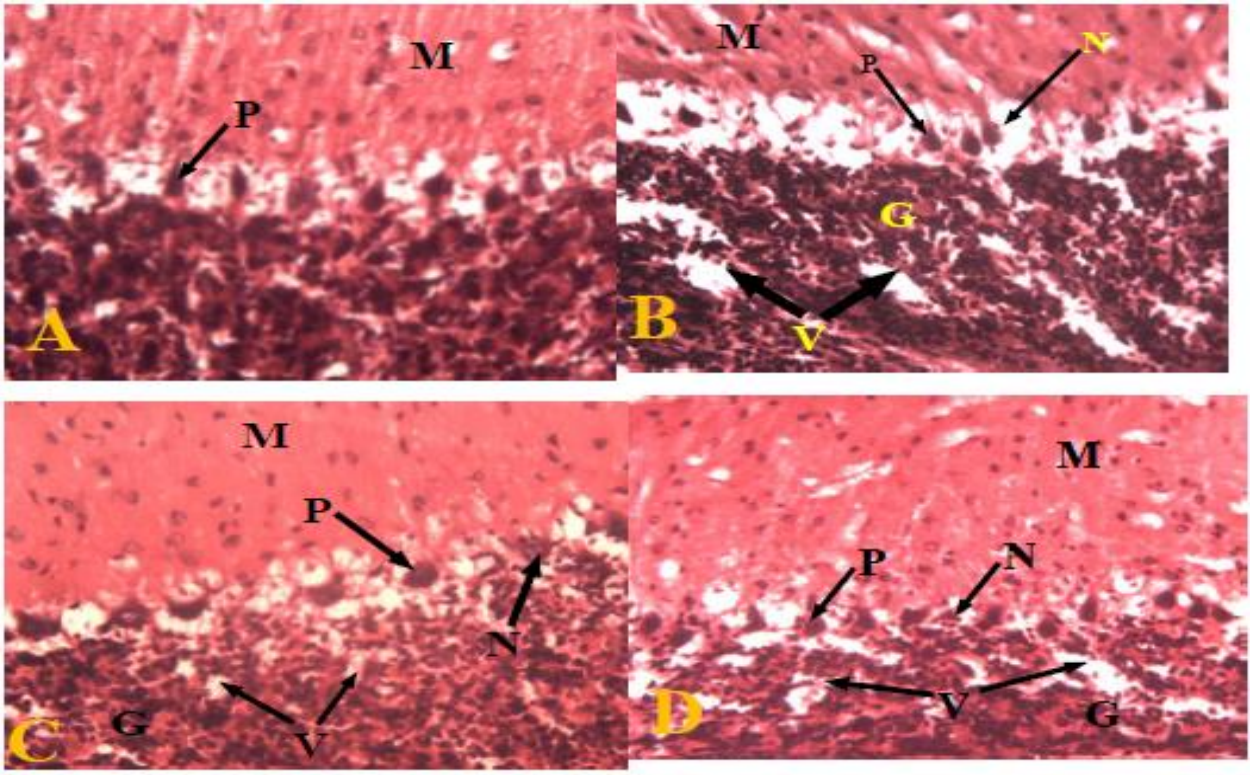

Figure 4: Section of cerebellar cortex of Wistar rat. H&E stain. X250.
A= Second control group showing normal tissue of the cerebellar cortex. Sections B, C and D show mild loss of Purkinje cells, granular layer with vacuolations. The molecular layer shows neuronal degenerations with necrosis.

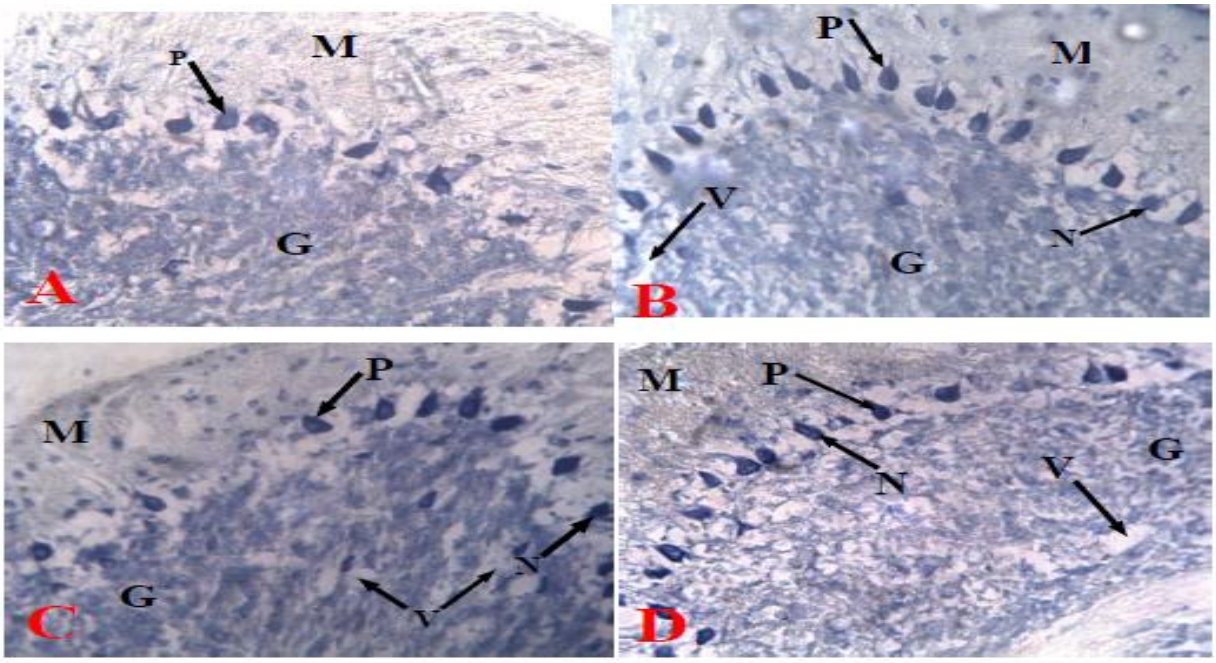

Figure 5: Section of cerebellar cortex of Wistar rat. CFV stain. X250.
A= Second control group showing normal tissue of the cerebellar cortex.Sections B, C and D show mild loss of Purkinje cells, granular layer with vacuolations. The molecular layer shows neuronal degenerations with necrosis.

DISCUSSION

The present study on the effects of aqueous leaves extract of *Nicotiana tabacum* on the cerebellar cortex in Wistar rats was assessed using neurobehavioral, biochemical and histochemical methods. The beam walking test was used to assess motor coordination and balance in the Wistar rats, the control of movement, motor coordination and balance is linked to the cerebellum. Atrophy to the cerebellum is usually accompanied by different ataxia and an unstable gait (Fonnum and Lock, 2000). Across the period of the study, the administration of extracts had no effect on foot-slip score and beam walking ability when compared with the Control. Oxidative stress which is a common pathology which occurs due to imbalance between production and detoxification of reactive oxygen species (ROS) has been implicated in many neurodegenerative diseases (Gilgun-Sherkiet al., 2001; Trushina and Memurray, 2007). Oxidative stress has been implicated in mechanisms leading to neuronal cell injury in various state of the brain. Because of its high oxygen demand, the brain is the most susceptible organ to oxidative damage (Weiss and Fintelmann, 2000). Halliwell et al., (1999) had reported that increased oxidative stress leads to Lipid peroxidation, protein damage and induction of apoptosis.In the present study tissue MDA levels were increased slightly in all treated groups, which suggests enhanced lipid peroxidation, leading to tissue damage and failure of antioxidant defence mechanism to prevent formation of excessive radicals (Himakar et al., 2010). Lipid peroxidation (LPO) is potentially harmful, if uncontrolled it can cause disruption of membrane, lipids and other cell components (Mahboob et al., 2005; Rao and Purohit, 2011). Treatment of rats with *Nicotiana tabacum* in the present study revealed a slight increase in MDA levels, indicative of generation of LPO. SOD is a sensitive index in oxidative damage as it scavenges the superoxide anion from hydrogen peroxide leading to the reduction in the toxic effects (Khan et al., 2012). Our observations indicating decreased SOD levels indicate that aqueous extract of *Nicotiana tabacum*usage may result in oxidative stress and several studies have confirmed that tobacco leaves contain high arsenic and cadmium concentrations which are known to be potential candidates for oxidative stress (Zakiullah et al., 2011; Borgerding et al., 2012; Khilifi et al., 2014). CAT is a key component of the antioxidant defence system and the inhibition of these

protective mechanism enhanced sensitivity to free radical induced cellular damage (Fang et al., 2000; Young and Woodside, 2001). GPx is an important antioxidant enzyme which reacts with hydrogen peroxide thus preventing intracellular damage caused by the free radicals (Lee et al., 2007).In the present study, CAT and GPx activity level was decreased in treated groups. Decreased CAT and GPx levels indicate that aqueous extract of *Nicotiana tabacum*usage may increase the level of reactive oxygen species (ROS), which provoke lipid, protein oxidation (Clarkson, 1997; Li et al., 2006) hence resulting in oxidative stress, studies done by Borgerding et al. (2012) have confirmed that tobacco leaves contain high arsenic and cadmium concentrations which are known to be potential candidates for oxidative stress. In the present study, light microscopic examination of routineH&E stain and Cresly Fast Violet stained sections of Wistar rats cerebellar cortex were studied.The result from the present study showed mild histoarchitectural distortions such as degenerated pyramidal cells, shrinkage of cell body, cytoplasmic and neuropil vacuolations and distorted Purkinje cells. Purkinje and granule cells were the most important targets in the cerebellum for toxic substances (Fonnum and Lock, 2000). Neurodegenerative changes observed in tobacco treated Wistar rats implied treatment related toxicity. These findings were in consonance with the studies related to *Nicotiana tabacum* induced cell injury on cerebellar structures by Omotoso, (2014). Adeniyi (2010) reported that, neuronal damage in the form of shrinkage, atrophy and neuronal necrosis was greatly increased in the brain of rats treated with *Nicotianatabacum*. The results thus support an emerging pattern wherein smokeless tobacco exposure elicits cerebellar damage leading to abnormality of cellular pattern.

Conclusion

The administration of aqueous extracts tobacco leaves mayresult to remarkable alteration or imbalance in the concentration of oxidative stress markers i.e.CAT, SOD, GPx and also alteration in the histologic and histochemical features in cerebellum (Purkinje cells), it shows that tobacco can do more harm than good.

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