The role of Garlic and Honey on Nicotine-Induced Toxicity on the Cerebellum of adult Wistar Rats

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Abstract

Background

Nicotine form the major constituent of consumed tobacco and cigarettes throughout the World resulting in oxidative stress while garlic and honey have been shown to contain numerous natural antioxidants. We investigated the role of combined effects of garlic and honey on nicotine induced Cerebellar and behavioral toxicity in Wistar rats.

Method

: Forty rats were divided into eight groups of five rats each. Group 1 received water only, Group 2 and 3 received 50 mg/kg of nicotine with garlic and honey at (300 mg/kg and 1000 mg/kg) and (600 mg/kg and 2000 mg/kg) respectively. Group 4 received 50 mg/kg of nicotine only, Group 5 received garlic 300 mg/kg and honey 1000 mg/kg before 50 mg/kg nicotine, Group 6 received garlic 600 mg/kg and honey 2000 mg/kg before 50 mg/kg nicotine. Group 7 and 8 received 50 mg/kg nicotine + 450 mg/kg garlic only and 1500 mg/kg honey only respectively. The administration was through oral route and lasted for 21 days respectively. Limb impairment and grip were conducted. After the administration, blood and cerebellum were harvested for lactate dehydrogenase assay, oxidative stress and histopathological analyses.

Result

There was significant decrease in body weight, grip strength and limb impairment in Group 4 with increase in Group 2 compared to the Control ($p < 0.001$). There was significant increase in LDH in Groups 2, 4, 7 and 8 with significant decrease in Group 3 compared to Group 1. The result showed significant decrease in SOD and CAT activities in all the groups compared to Group 1 ($p < 0.001$). Groups 1, 7 and 8 showed normal histology of the cerebellum while Group 4 showed areas of neuronal degeneration with distortions in histology and Groups 3, 5 and 6 showed few neural changes.

Conclusion

The study showed that garlic and honey may be useful in ameliorating nicotine toxicity dose-dependently which may be due to their antioxidant activity.

1.0 Background

The cerebellum is the central part of the major circuitry that links sensory areas to the motor areas of brain and functions in coordination of fine movements (1). In health, it provides connections during movement which is the basis for precision and accuracy and it is initially involved in motor learning and

reflex modification according to Fine *et al.* (3). It is particularly one of the most vulnerable parts of the brain to neurotoxins and poisoning (1) and it forms the main target of drug exposure, drug abuse and environmental toxins (2). Researchers have shown that exposure of organs to tobacco and cigarettes are a major risk factor to various clinical conditions determining their functionality (4, 5) and one of the single greatest preventable cause of death in the world (6). Nicotine is one of the major constituents of tobacco which has been implicated to cause postural imbalance in smokers (7). Studies have shown that tobacco addiction and pattern of its usage is influenced by its nicotine content which is also used to aid smoking cessation pharmacologically (8). When the health impact is made known, most users desire to quit but it becomes difficult due to the addictiveness of nicotine (9). Nicotine replacement therapy is to temporarily replace it in tobacco consumption or to reduce tobacco intake, and thus ease transition from smoking cigarette to complete abstinence (10). Various alternative nicotine sources such as gum, transdermal patch, nasal spray, inhaler and sublingual tablets or lozenges incorporated into tobacco cessation programs and some of the side effects caused by nicotine are common to these products. Serious risks include continued addiction and nicotine poisoning (11). Adulthood exposure has been implicated in neurotoxicity and this is due to the ability of nicotine to generation of free radical in the cell (12).

A good diet is one of the best ways of promoting good health while a poor diet could cause various ailments (13). Despite cultural differences, there are some shared characteristics of healthy dietary patterns and natural medicinal products have been used for millennia in the treatment of numerous diseases (14) and of them, garlic and honey is one of the widely used natural products. A recent study has shown that combined effect of garlic and honey helps in the treatment of respiratory infections (13). Garlic (*Allium sativum*) as a phytomedicine is a specie in the onion genus and allium is one of the most researched plants, with a long history of medicinal uses (15). According to El Demerdash *et al.* (16), garlic contains sulphur, phosphorus, potassium, zinc ions, moderate amounts of selenium, vitamin A, vitamin C and small amounts of calcium, magnesium, sodium, iron, vitamin B complex and allicin. Recent studies have shown that allicin contain anti-mutagenic, anti-oxidative, anti-inflammatory and anti-carcinogenic effects (17). Aqueous garlic extracts exerts its antioxidant action by enzymes, superoxidase dismutase, catalase and glutathione peroxidase and inhibits lipid peroxidation, which causes oxidative stress (18). However, honey has been considered to share some similar properties with garlic (13). Honey is the only insect-derived natural product, and it has nutritional, cosmetic, therapeutic and industrial values (19). Honey consists mainly of sugars and water with fructose as the most abundant sugar as well as vitamins – B complex, vitamin C, ascorbic acid, pantothenic acid, niacin and riboflavin; minerals include calcium, copper, manganese, iron, potassium, phosphorus and zinc (20, 21), amino acids, antibiotic-rich inhibine, proteins and phenol antioxidants (22, 23). It also contains other bioactive substances such as phenolic constituents, flavonoids, organic acids, carotenoid-derived compounds and nitric oxide metabolites (24). Studies have shown that the presence of many flavonoids, phenolic acids, ascorbic acid, tocophrenols, catalase, superoxidase dismutase, reduced glutathione, and peptides, work together to provide a synergist antioxidant effect (25, 26, 27). Research suggests that the polyphenol constituents of honey can quench biological reactive oxygen species and counter oxidative stress while restoring the

cellular antioxidant defense system (28). The present study was designed to examine the effects of garlic and honey on nicotine induced toxicity on behavior and cerebellum of adult Wistar rats.

2.0 Methods

2.1 Collection and Extraction of garlic

The garlic cloves used were procured, peeled, washed and dried under room temperature. The garlic cloves were grinded into fine powder and the powder was soaked in water for 72hours in the ratio of 100 g to 50 ml of water and was stirred every 12 hours. The mixture was filtered to separate the residue from the filtrate first with a sieve and further using No 1 Whitman filter paper. The extract was dried using water bath at a temperature of 40^{o}C till it was concentrated and was preserved in a refrigerator at 4^{o}C until required. The aqueous garlic extract was obtained following the method described by Senapati *et al.* (29)

2.2 Honey and Nicotine

Honey was purchased from Shine foods Inc. and was stored in a cool dry place. Nicotine tablets of 50 mg each was bought from Royal Pharmacy, with brand name Nicotinic Acid with code number PB/DRUGS/1608-B.

2.3 Experimental Animal Care and Management

Ethical clearance was sort and obtained from the Faculty of Basic Medical Sciences Ethics Committee. Forty (40) male Wistar rats of average weight of 170 g were purchased and housed in cages under standardized conditions and allowed free access to food and water *ad-libitum.* The procedures for taking care of research animals were according to the University guidelines. The animals were randomly assigned into eight (8) groups of five (5) rats each after a period of two (2) weeks of acclimatization.

2.4 Experimental Procedure

Group 1 (Control) received water only, Group 2 received nicotine at 50 mg/kg body weight for 2 weeks, garlic at 300 mg/kg body weight and honey at 1000 mg/kg body weight for 1 week. Group 3 received nicotine at 50 mg/kg body weight for 2 weeks, then garlic at 600 mg/kg body weight and honey at 2000 mg/kg body weight for 1 week. Group 4 received nicotine at 50 mg/kg body weight for 2 weeks. Group 5 received garlic 300 mg/kg body weight and honey 1000 mg/kg body weight for 1 week and nicotine at 50 mg/kg body weight for 2 weeks. Group 6 received garlic at 600 mg/kg body weight and honey at 2000 mg/kg body weight for 1 week and nicotine at 50 mg/kg body weight for 2 weeks. Group 7 received nicotine at 50 mg/kg body weight for 2 weeks and garlic at 450 mg/kg body weight for 1 week. Group 8 received nicotine at 50 mg/kg body weight for 2 weeks and honey at 1500 mg/kg body weight for 1 week. The administrations were carried out using oral gavage, once daily for 21 days. The animals were observed daily and humanely sacrificed after the last administration.

2.5 Neurobehavioral Study

The behavior of the experimental animals relative to the cerebellum was studied using the string test method. The method was used to measure grip strength and limb impairment of the animals. The animals were trained before the administration called pretest and the post test was done after the administration before the sacrifice.

2.6 Behavioral studies

2.6.1 Assessment of Grip Strength

Grip strength was assessed using a steel wire, 2 mm diameter and 60 cm length and the rats were allowed to hold-on the wire with their fore paws and were placed at a height of 50 cm over a cushion support. The time taken (latency) by each rat to release the paws and fall was recorded with a cut-off time of 180 s. The latency for the loss of grip was considered as a measure of grip strength according to the method of Tariq *et al.* (30).

2.6.2 Assessment of Limb Impairment

Limb impairment was assessed according to the Method of Yi *et al*. (31), where rats were scored 3 for gripping the wire with both hind paws, 2 for gripping the wire with one hind paw, and 0 for not gripping the wire with either of the hind paws. The results were expressed as the mean of the total scores for animals per group.

2.7 Animal Sacrifice

The animals were sacrificed by cervical dislocation after 24 hours of fasting and blood samples were collected from the apex of the heart for biochemical analysis. The animals were decapitated, skinned and the skull fixed in Bouin's fluid. After 48 hours of fixation, the skull was opened and the cerebellum harvested and further re-fixed in 10% formal saline for histological studies. The tissues were processed, sectioned and stained using H&E and Cresyl fast violet methods.

2.8 Biochemical Analysis

Blood samples collected were centrifuged at 1000rmp for 10 min and the sera were collected and used for the assessment of the following biochemical parameters;

2.8.1 Assessment of Catalase (CAT) activity

The activity of catalase was assayed by the method of Sinha (32). Serum was pipetted into a tube to 0.9 ml of phosphate, 0.1 ml of plasma and 0.4 ml of H_2O_2 added. The reaction was stopped after 15, 30, 45 and 60 seconds by adding 2 ml of dichromate acetic acid mixture. The tubes were kept in a boiling water bath for 10 minutes, cooled and the colour developed was read at 530 nm. The activity of Catalase was expressed as U/ml of plasma.

2.8.2 Assessment of Superoxide Dismutase (SOD) Activity

Superoxide dismutase (SOD) activity was measured using the method of Fredovich (33). Adrenalin (0.01 g) was dissolved in 17 ml of distilled water and 0.1 ml of serum and 0.9 ml of phosphate buffer (pH 7.8) were taken in triplicates in 2.5 ml buffer. A volume, (0.3 ml) adrenaline solution was added and mixed inside the cuvette. The absorbance was taken at 480 nm at 30 seconds interval for five times. The changing rate of absorbance was used to determine superoxide dismutase activity and was expressed as units/mg protein.

2.8.3 Assessment of Lactate Dehydrogenase Activity (LDH)

Lactate dehydrogenase is an oxido-reductase which catalyzes the interconversion of lactate and pyruvate. The protocol measures the reduction of a yellow tetrazolium salt by NADH into a red, water soluble formazan-class dye by absorbance at 492 nm. The amount of formazan is directly proportional to the amount of LDH in the serum. LDH activity was expressed as units/liter (U/l) (34).

2.9 Statistical Analysis

Data obtained were analyzed using statistical Package for Social Sciences (SPSS) version 20 and results were expressed as mean ± SEM and the presence of significant difference between the means were determined using one way analysis of variance (ANOVA). The level of significance was established at $p \leq 0.05$.

3.0 Results

3.1 body weight change

The results of the effect of garlic and honey on nicotine induced toxicity on the body weight of the rats are shown in Table 1. The Control group had 8.30% body weight change at the end of the experiment with significant decrease in percentage weight change in Groups 2, 4 and 5 compared to the Control ($p < 0.05$). Animals in Groups 3, 6, 7 and 8 showed significant increase in percentage weight change when compared to Group 1 ($p < 0.05$). However, there was a significant decrease in the percentage weight gain in Groups 2 and 5 compared to Groups 1 and 4 as shown in Table 1.

Table 1
The effect of nicotine induced toxicity on body weight change of adult Wistar rats

Groups	Final Weight	Initial Weight	Weight Change	% Weight gain
1	224.40 ± 4.80	207.20 ± 2.20	17.20 ± 2.60	8.30*
2	196.00 ± 6.82	191.20 ± 3.31	4.80 ± 3.51	2.51
3	267.60 ± 5.95	234.80 ± 4.16	32.80 ± 1.79	13.97**
4	234.60 ± 6.49	220.60 ± 2.29	14.00 ± 4.20	6.35*
5	248.00 ± 12.56	238.60 ± 2.98	9.40 ± 9.58	3.94
6	250.00 ± 7.45	215.20 ± 1.53	34.80 ± 5.92	16.17**
7	252.40 ± 7.82	227.40 ± 2.62	25.00 ± 5.20	11.00**
8	266.40 ± 6.56	227.40 ± 5.10	39.00 ± 1.46	17.15**
* = $p < 0.05$, ** = $p < 0.01$, when compared to Group 1 and Group 2				

3.2 Neurobehavioural Study

The behavior of the animals was assessed using the latency of the grip strength and limb impairment was assessed by the string test method. The results of grip strength and limb impairment are shown in Tables 2 and 3. The result showed a significant decrease in grip strength in the test compared to the pre-test in Groups 4 and 8 ($p \leq 0.05$). The result of grip strength showed significant decrease in all test Groups compared to animals in Group 2 ($P \leq 0.05$) as in Table 2.

Table 2
The grip strength of the animals during the pre-test and test period

Groups	Pre-test (s)	Test (s)
1	34.07 ± 7.80	17.60 ± 7.80*
2	44.40 ± 12.85	61.00 ± 40.49**
3	39.73 ± 8.19	11.00 ± 1.58*
4	68.93 ± 16.19	53.60 ± 32.34*
5	96.92 ± 21.17	63.50 ± 39.05*
6	51.33 ± 17.72	11.25 ± 4.42**
7	34.53 ± 4.16	17.20 ± 5.15*
8	49.93 ± 9.37	23.40 ± 17.19*
* = $p < 0.05$, ** = $p < 0.01$, when compared to Group 1 and Group 2		

Table 3
Measure of limb impairment of the animals during the pre-test and test period

Groups	Pre-test (s)	Test (s)
1	1.67 ± 0.19	1.00 ± 0.00*
2	2.07 ± 0.22	2.00 ± 0.57**
3	1.92 ± 0.21	1.40 ± 0.24*
4	2.27 ± 0.18	1.60 ± 0.40*
5	2.17 ± 0.17	1.50 ± 0.50**
6	1.58 ± 0.26	1.00 ± 0.00*
7	2.00 ± 0.17	1.80 ± 0.37*
8	1.93 ± 0.21	1.40 ± 0.40*
* = $p < 0.05$, ** = $p < 0.01$, when compared to Group 1 and Group 2		

The result of assessment of limb impairment showed significant decrease in the test compared to the pre-test in Groups 4 and 8 ($p \leq 0.05$). The result of limb impairment test showed a significant decrease in all the Groups in the test compared to Group 2 ($p \leq 0.05$) as shown in Table 3.

Biochemical studies

The result of biochemical analyses of lactate dehydrogenase (LDH) activity, Superoxide dismutase activity (SOD) and catalase (CAT) activity are shown in Table 4. The result showed that LDH concentration was increased in all the Groups compared to Group 1 but the LDH concentration in Groups 6 and 8 was significantly increased compared to Group 2 ($p \leq 0.005$). The result of SOD analyses showed a significant decrease in SOD concentration in Group 2 compared to all the Groups ($p < 0.05$). The result of CAT analyses showed a significant decrease in CAT concentration in Group 2 compared with all the Groups ($p < 0.05$) while CAT concentration was significantly increased in all the groups compared to Group 2 animals ($p < 0.05$) as shown in Table 4.

Table 4
Garlic and honey on nicotine induced changes in biochemical parameters

Groups	LDH(U/l)	SOD(U/mg)	CAT(U/mg)
1	177.98 ± 6.59	11.49 ± 0.01	18.25 ± 0.32aaa
2	198.14 ± 20.16	11.21 ± 0.05*	8.09 ± 0.03***aa
3	141.17 ± 8.21a	11.11 ± 0.03**	11.87 ± 0.18***
4	203.01 ± 5.25*	11.09 ± 0.05**	12.07 ± 0.45***
5	178.55 ± 12.63	11.48 ± 0.04	8.63 ± 0.29***aa
6	237.31 ± 12.63**	11.13 ± 0.07**	13.98 ± 0.78**
7	193.10 ± 10.14	11.46 ± 0.04	12.58 ± 0.53***
8	208.44 ± 9.11**	11.44 ± 0.06	10.96 ± 0.03***
* = p < 0.05, ** = p < 0.01, *** = p < 0.001 when compared to Group 1			
a = p < 0.05, aa = p < 0.01, aaa = p < 0.001 when compared to Group 4			

3.5 Histological studies

The result of histological analyses showed normal architecture of the cerebellum in Group 1 while Group 2 showed degeneration of Purkinje cells resulting in the reduction of the Purkinje cells in the layer as shown in Figs. 1&2 (A&B) when compared to the histology of the cerebellum in the other treated groups which showed reduced degeneration of the purkinje cells due to the administration of garlic and honey resulting in slight distortion of the histology of the cerebellum in the rats as shown in Figs. 3, 4, 5 ,6,7 &8 respectively.

4.0 Discussion

Nicotine has been reported in previous studies to cause a reduction in body weight (35) and the result from the present study showed decrease in body weight in the treated groups compared to the Control which may be due to the reduction in food intake. This agrees with the previous studies of Ijomone *et al.* (35); Omotoso and Babalola, (36). Our results also showed that there was increase in the body weight in Groups 7 and 8 compared to Group 2. This agrees with the works of Farnaz *et al.* (37) and Mohammed *et al.* (38) which showed that garlic and honey increases body weight in experimental animals while animals in Group 5 showed decrease in body weight which suggests that low doses of garlic and honey were not sufficient enough to increase the body weight before the decrease associated with nicotine. The result also showed increase in the body weight of Group 6 which suggests that the high doses of garlic and honey were sufficient enough to increase body weight in the animals.

The string test method can be used to measure grip strength and as a traction apparatus for assessing limb impairment (35). Reduced latency in grip loss was indicative of compromised muscle strength and ability to grasp and hold onto objects (39), while the reduced scores on limb impairment indicate compromised limb function and strength of the animals. The present study showed that there was reduced latency to grip loss in Group 1 and reduced scores on limb impairment. These values suggest that some certain factors could cause the loss of grip and limb impairment in Group 1 and animals in Group 4 showed significant decrease in grip strength and reduced scores on limb impairment. This suggests that there was compromised muscle strength and ability to grasp and hold things in the animals. The loss in grip was also confirmed by the increased concentration of LDH and the neuronal degeneration which showed neurodegeneration in the experimental groups which was characterized by decreased population of Purkinje cells with Purkinje cells undergoing pyknosis and necrosis and loss of Cerebellar white mater. This disagrees with the previous study by Ijonome *et al.* (35) which showed that nicotine produced no major deficit in the motor function and coordination as in animals of Group 2 with increased latency to grip and a slight reduction in the score on limb impairment. These suggest that the low doses of garlic and honey administered after nicotine administration had increased the latency to grip loss. This restoration was confirmed by the increased population of the Purkinje cell layer. The results showed increase in grip loss and a reduced score in limb impairment which suggests that there was a greater compromised muscle strength and ability to grasp and hold. This is confirmed in a section of hippocampus of animals in Group 3 which showed decreased population of the Purkinje cell layer, decreased thickness of the granular layer and necrosis of Purkinje cells while animals in Groups 5 and 6 showed a greater increase in grip loss and reduced score of limb impairment compared to Group 4 which suggests that the administration of low and high doses of garlic and honey before nicotine administration was not protective to counter the toxicity associated with nicotine. The animals in Groups 7 and 8 showed significant increase in grip loss while Group 7 showed slight reduction in limb impairment and Group 8 showed decreased limb impairment. This suggests that the administration of medium dose of garlic had a possible effect of improving limb functionality which can be aligned to the proliferation of Purkinje cells in Group 7 animals. Previous study by Thompson *et al.*, (40) revealed that garlic administration had a reducing effect on the levels of LDH. The animals in Groups 5 and 6 treated with low and high doses of garlic and honey respectively showed a decrease and increase in LDH concentration which suggests that high doses of garlic and honey caused a further increase in cellular damage, probably because the increased concentration of honey could have triggered a reaction which caused further cellular damage as seen in the group treated with honey alone after nicotine intoxication which showed increase in the level of LDH concentration.

SOD concentration were increased in Groups 5 and 6 which suggests that low doses of garlic and honey was more effective in increasing SOD concentration while the activity of CAT was not effective by low doses of garlic and honey as there was significant decrease as the administration of high doses of garlic and honey was more effective. According to previous study by Auza *et al.* (41) which reported that nicotine caused oxidative stress by decreasing the concentration of SOD and CAT in animals. Oxidative stress is a common pathology caused due to the imbalance between production and detoxification of

reactive oxygen species (ROS) and has been implicated in many neurodegenerative diseases (42, 43). Oxidative stress has been implicated in mechanisms leading to neuronal cell injury in various states of the brain. Because of its high oxygen demand as the brain is the most susceptible organ to oxidative damage (44). Cellular damage increases the level of lactate dehydrogenase (LDH) in the blood and the present study showed increase in the concentration of LDH, SOD and CAT activities were shown to be decreased with consequent Cerebellar toxicity in Group 4 compared to Group 1. This toxicity was confirmed in the histological sections which showed necrotic Purkinje cells, nuclear pyknosis of Purkinje cells and a decreased in the thickness of granular layer. The present study thus agreed with the previous study by Auza *et al.*, (41) on the biochemical alterations in the cerebellum brought about by nicotine administration. The harmful effect of nicotine administration has been established to cause a distortion in the histoarchitecture of the cerebellum (41, 45).

5.0 Conclusion

The present study confirmed that nicotine causes neurotoxicity in the cerebellum and the toxicities are seen in reduction in the body weight, reduced latency of grip loss and reduced scores in limb impairment indicating compromised muscle strength and ability to grasp, increased concentration in LDH and reduction in antioxidant activities of SOD and CAT and the distortion of Cerebellar histoarchitecture with decreased Purkinje cell population, granular layer and loss of white mater. While the administration of garlic and honey in combined and single doses had effect in ameliorating the toxicity associated with nicotine by reducing the oxidative stress and causing proliferation of Purkinje cells. These effects are shown to be dose-dependent, as the low doses of garlic and honey administered before nicotine offered a better curative effect to nicotine toxicity.

Abbreviations

ROS
Reactive oxygen species
CAT
Catalase
SOD
Superoxide dismutase
LDH
Lactate dehydrogenase

Declarations

Ethics approval and consent to participate

Ethical clearance was sort and obtained from the Faculty of Basic Medical Sciences Ethics Committee. The procedures for taking care of research animals were according to the University guidelines

Consent for publication

Not required

Availability of data and materials

Data set are available at https://figshare.com/s/e6d145ceacabe737b23d

Competing Interests

The authors declare no conflicts of interest.

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Author contributions

IOA, and ONG conceptualized the study; IOA, ONG, ANL designed the study; IOA, ONG, and ANL collected the data; SA, UOO, EKU, IMU, EAO and GEB conducted statistical analysis, SA, UOO, EKU, IMU, EAO and GEB conducted data interpretation. IOA, ONG and ANL drafted the initial manuscript while IOA, ONG, ANL, SA, UOO, EKU, IMU, EAO and GEB reviewed it for intellectual content. All authors approved the final version for publication and remain in agreement to ensure that questions related to the integrity of any part of the work are resolved.

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Figures

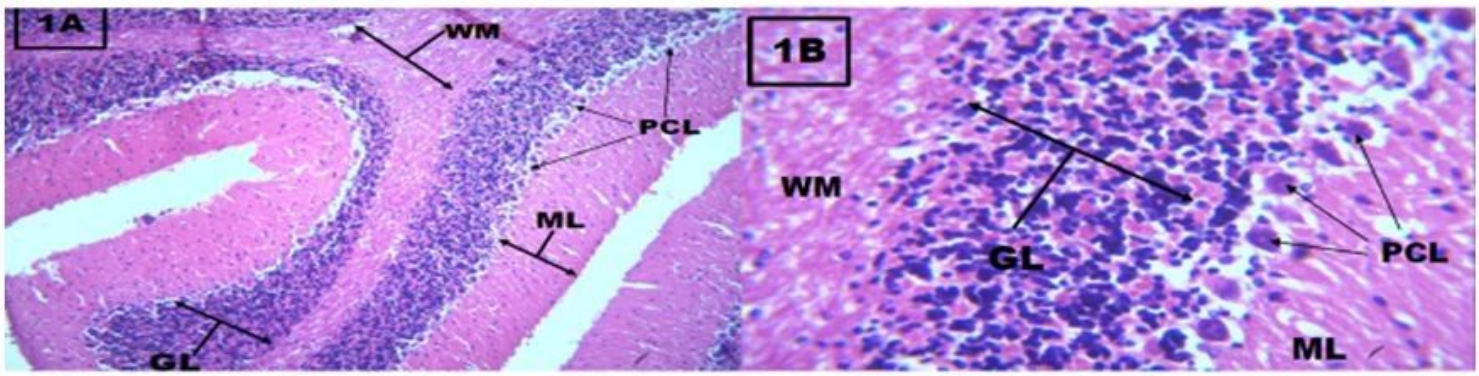

Figure 1

A section of the Cerebellar cortex of Rats in Group 1, showing normal Cerebellum with molecular layer (ML), the Purkinje cell layer (PCL) made up of a single layer of Purkinje cells, the granular layer (GL) and the underlying Cerebellar white matter (WM). (H&E. Mag. X100 and X400; Scale Bar: 5µm =1mm).

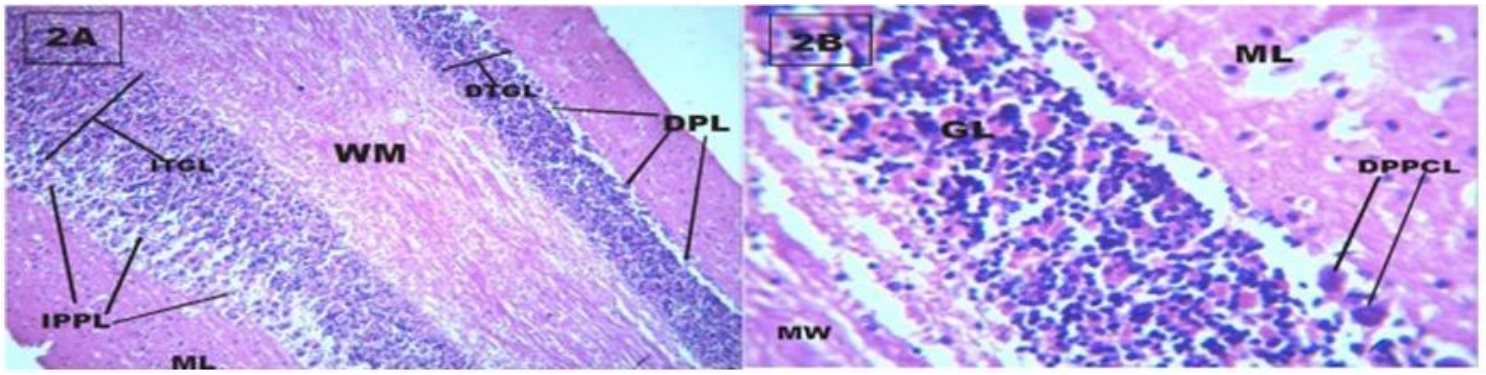

Figure 2

A section of the Cerebellar cortex of Rats in Group 2, showing Cerebellum with decreased Purkinje cell layer (DPPCL) and decreased thickness of the granular layer (DTGL), with increased in the Purkinje cell layer (IPPCL) and increased thickness of the granular layer (ITGL) with increased degeneration of the cerebellar neurons, Molecular layer (ML), and cerebellar white matter (WM). (H&E. Mag. 2A X100; 2B X400; Scale Bar: 5µm =1mm).

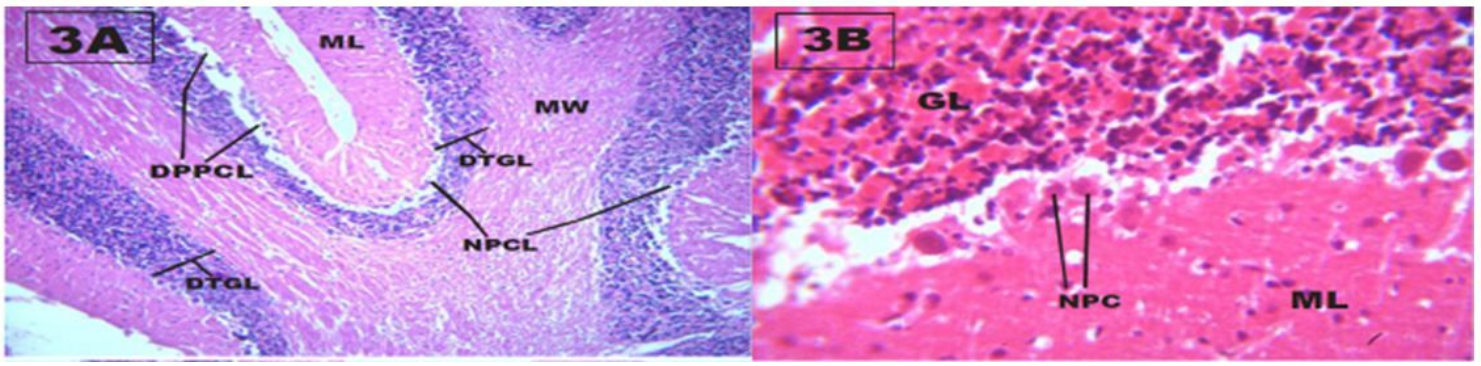

Figure 3

A section of the cerebellar cortex of animals in Group 3, showing altered cerebellar histoarchitecture with degeneration of Purkinje cell layer (DPPCL), with decreased granular layer (DTGL) and necrosis of the Purkinje cell layer (NPCL). Molecular layer (ML), Cerebellar white matter (WM). (H&E. Mag. 3A X100 & 3BX400; Scale Bar: 5µm =1mm).

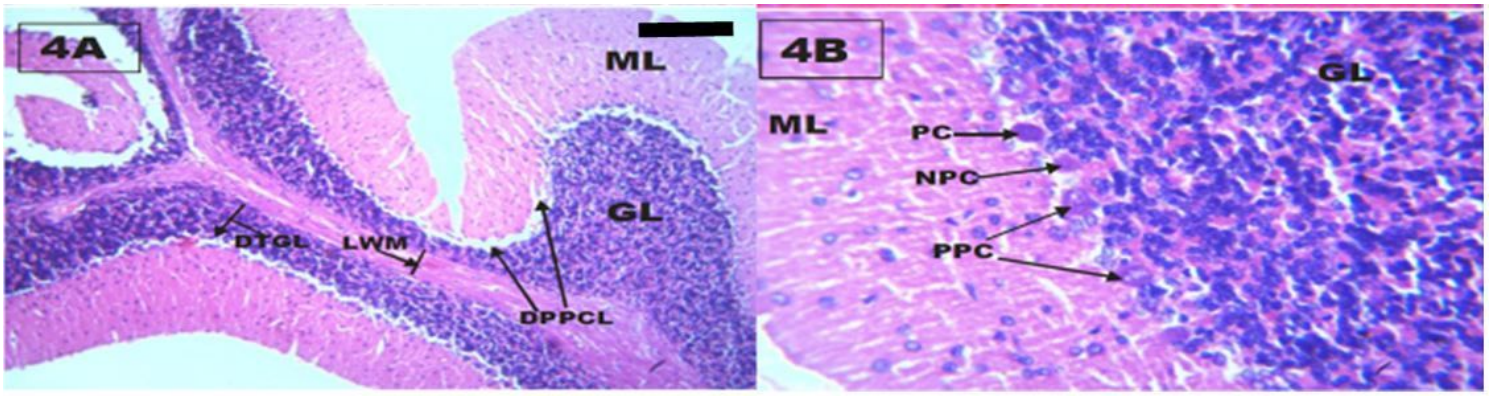

Figure 4

A section of cerebellar cortex of animals in Group 4, showing cerebellar histoarchitecture with decreased population of the Purkinje cell layer (DPPCL), decreased thickness of the granular layer (DTGL) and loss of cerebellar white matter (LWM). Molecular layer (ML) and granular layer (GL). (H&E. Mag.4A X100; 4B X400; Scale Bar: 5µm =1mm).

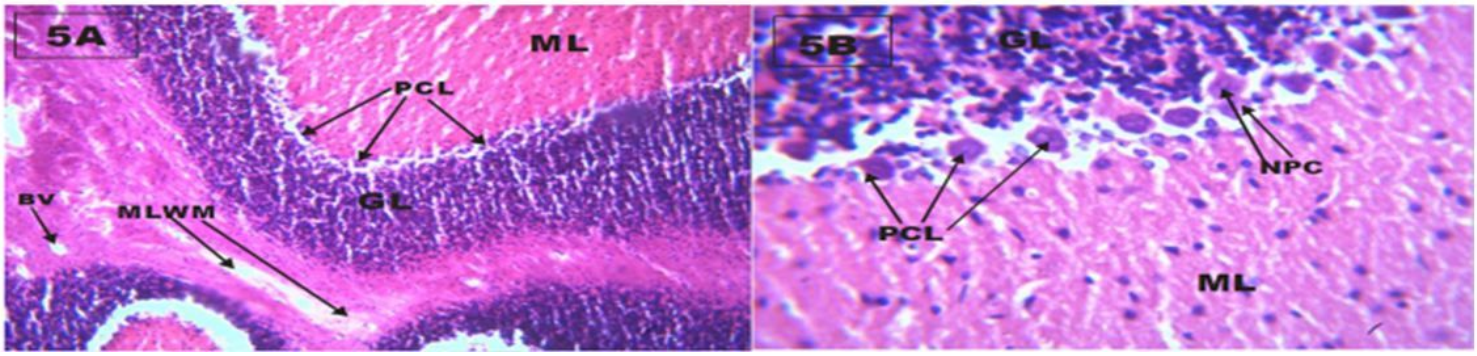

Figure 5

A Section of cerebellar cortex of animals in Group 5, showing cerebellar histoarchitecture with no major alteration in the cerebellum. However, there was presence of few necrotic Purkinje cells (NPC), mild loss of cerebellar white matter (MLWM). Molecular layer (ML), granular layer (GL), Purkinje cell layer (PCL), blood vessel (BV). (H&E. Mag. 5A X100 5B X400; Scale Bar: 5µm =1mm).

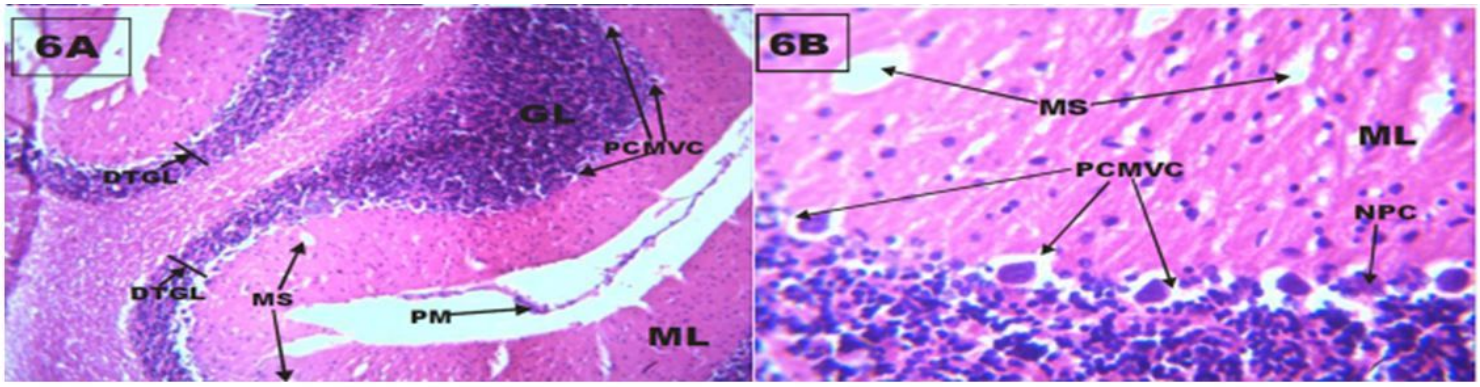

Figure 6

A Section of the cerebellar cortex of animals in Group 6, showing cerebellar histoarchitecture with Purkinje cells undergoing mild vacuolated cytoplasm (PCMVC), decreased thickness of the granular layer (DTGL) and presence of microcytic spaces (MS). Also present are the Molecular layer (ML), granular layer (GL) and Pia matter (PM). (H&E. Mag. 6A X100; 6B X400; Scale Bar: 5µm =1mm).

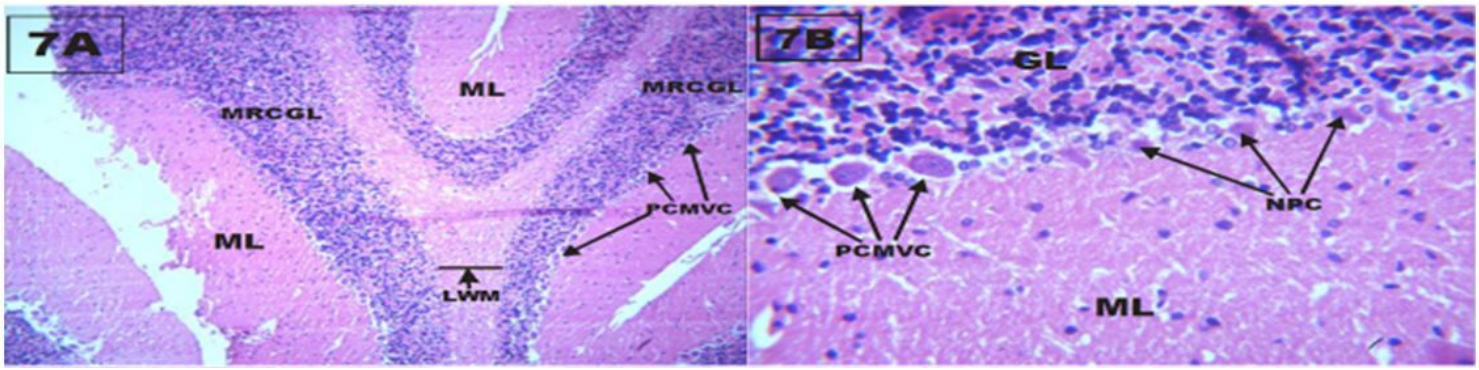

Figure 7

A Section of cerebellar cortex of animals in Group 7, showing cerebellar histoarchitecture with mild reduction in cellularity of the granular layer (MRCGL), and necrotic Purkinje cells (NPC), Purkinje cells with mild vacuolated cytoplasm (PCMVC), loss of cerebellar white matter (LWM) and Molecular layer (ML). (H&E. Mag. 7A X100; 7B X400; Scale Bar: 5µm =1mm).

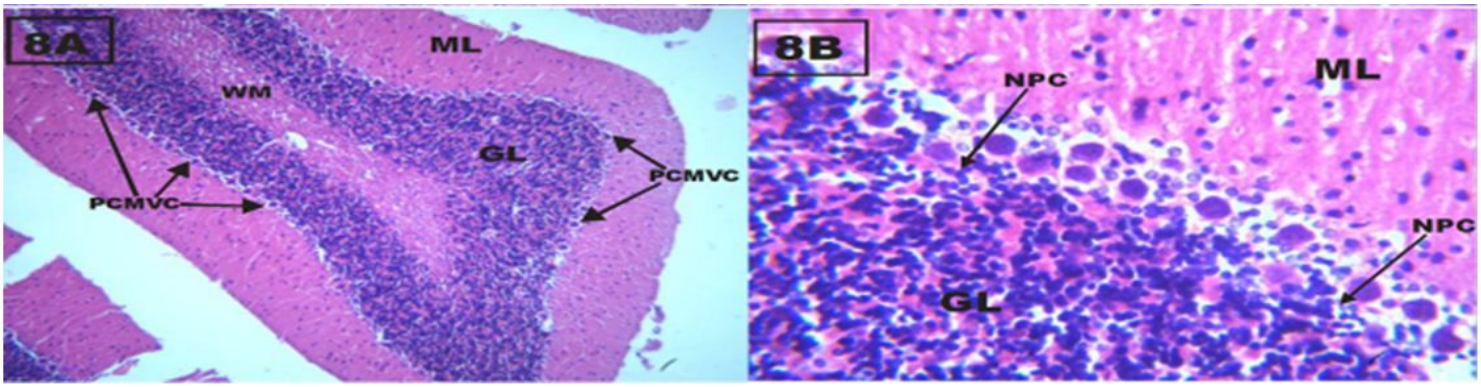

Figure 8

A Section of cerebellar cortex of animals in Group 8, showing no major alteration in the cerebellar histoarchitecture. However, there was presence of few necrotic Purkinje cells (NPC) and few Purkinje cells with mild vacuolated cytoplasm (PCMVC). Also present are the molecular layer (ML), granular layer (GL) and cerebellar white matter (WM). (H&E. Mag. 8A X100; 8B X400; Scale Bar: 5µm =1mm).

Supplementary Files

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