RESEARCHS / INVESTIGACIÓN

Trimethyltin-induced cerebellar damage on adult male Wistar rats.

Trimetil estaño induce daño cerebral en ratas machos adultas Wistar.

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Abstract: This research work was done to investigate the acute toxicological effect of trimethyltin chloride on the cerebellum of Wistar rat. Ten adult male Wistar rats were used for the study. The animals were grouped into two: Group A and B, with five adult male Wistar rats in each group. Group A serves as the trimethyltin (TMT) group, while group B serves as the normal saline (NS) group. 3mg/kg of trimethyltin chloride was administered to animals in the TMT group, while 1.0mls of normal saline was administered to the animals in the NS group via intraperitoneal route for 3 hours respectively. The animals were sacrificed at the Histology Laboratory, University of Ilorin, using 25mg/kg of ketamine administered intramuscularly to anesthetize the animals; followed by perfusion fixation through the heart. The brains were harvested, and the tissues were processed and stained using H & E, and crystal violet stains. The cerebellar cortex and nissl substances of the cerebellum were analyzed and showed a mild distortion in the layers of the cerebellar cortex. Biochemical analysis was undertaken to investigate the disruption of the oxidative status in the animal tissue, using Super Oxide Dismutase (SOD). Oxidative stress was found to increase significantly ($p < 0.05$) in the TMT groups compared with the NS group, because the SOD activity decreased more in the brain homogenates of the TMT group. The result demonstrated that trimethyltin exerts its toxic effect by promoting oxidative stress in the brain and this may affect normal brain functioning and growth.

KeyWords: trimethyltin , Wistar rat, cerebellar cortex, nissl substances.

Resumen: Este trabajo de investigación se realizó para investigar el efecto toxicológico agudo del cloruro de trimetil estaño en el cerebelo de la rata Wistar. Se utilizaron diez ratas Wistar macho adultas para el estudio. Los animales se agruparon en dos: Grupo A y B, con cinco ratas Wistar macho adultas en cada grupo. El grupo A sirve como grupo trimetil estaño (TMT), mientras que el grupo B sirve como grupo salino normal (NS). Se administraron 3 mg / kg de cloruro de trimetil estaño a animales en el grupo TMT, mientras que se administraron 1,0 ml de solución salina normal a los animales en el grupo NS por vía intraperitoneal durante 3 horas respectivamente. Los animales se sacrificaron en el Laboratorio de Histología, Universidad de Ilorin, usando 25 mg / kg de ketamina administrada por vía intramuscular para anestesiar a los animales; seguido por la fijación de la perfusión a través del corazón. Los cerebros se recolectaron y los tejidos se procesaron y se tiñeron con H&E y tintes de violeta cristal. Se analizaron la corteza cerebelosa y las sustancias nissl del cerebelo y mostraron una leve distorsión en las capas de la corteza cerebelosa. Se llevó a cabo un análisis bioquímico para investigar la alteración del estado oxidativo en el tejido animal, utilizando la superóxido dismutasa (SOD). Se encontró que el estrés oxidativo aumentaba significativamente ($p < 0,05$) en los grupos de TMT en comparación con el grupo de NS, porque la actividad de SOD disminuyó más en los homogeneizados de cerebro del grupo de TMT. El resultado demostró que la trimetilestina ejerce su efecto tóxico al promover el estrés oxidativo en el cerebro y esto puede afectar el funcionamiento y el crecimiento normal del cerebro.

Palabras Claves: trimetilestaño, rata Wistar, corteza del cerebelo, nissl sustancias.

Introduction

Trimethyltin (TMT) is a neurotoxin that affects the functions of the Central Nervous System (CNS) causing signs of intoxication such as tremors and hyper excitability in animals[1]. A neurotoxin is a substance that inhibits the function of neurons throughout the brain and the nervous system[1]. Exposure to neurotoxins can cause dizziness, nausea, loss of motor control, paralysis, difficulty with vision, seizures, and even coma or death.

Trimethyltin (TMT) belongs to a member of the organotin class of compound widely used in industry as plasticizers of polyvinyl chloride products[2] and as biocides incorporated in molluscicides, insecticides, fungicides, and bactericides[3]. Individuals working in industries where hazardous chemicals are being produced are at a higher risk of exposure.

The brain is an integral part of the body whose function helps to regulate other parts of the biological system. Any damage or form of stress experienced in the brain may have a serious impact on the entire organism[4]. Exposure of the cerebellum to hazardous chemicals could lead to neurodegenerative diseases and hypoxic damage which can result in damage to Purkinje cells in the cerebellar cortex; leading to the development of cerebellar ataxia in which there is poor coordination of voluntary movement[5].

TMT has been shown to induce neurotoxicity and oxidative stress. Oxidative stress is a disturbance in the balance between the production of reactive oxygen species (free radicals) and antioxidant defenses[6]. It is necessarily a "state in which oxidation exceeds the antioxidant systems in the body secondary to a loss of the balance between them"[7]. TMT can increase the production of reactive oxygen species (ROS), and nitric oxide (NO) which are often associated with the processes of cell apoptosis. The disruption of the oxidative status in the living organism can be prevented by cellular antioxidants[8].

Toxic doses of TMT chloride are capable of disturbing the natural oxidation/reduction balance in cells through mechanisms originating from their complex oxidative/radical

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reactions with endogenous oxidants. These reactions produce effects on cellular antioxidant systems, cellular membranes, and membrane-dependent redox-sensitive enzymatic systems. This may produce a variety of toxic effects, which lead to the cells death[8]. The predicted lethal dose of TMT for humans is probably 3 mg kg^{-1} (15.1 mumol kg^{-1}); however, a lesser dose of this amount may be required to produce neuronal damage[9].

The results of a study carried out by Wang[1] suggested that acute exposure to TMT, induced brain cell apoptosis in the telencephalon, optic tectum and cerebellum, suggesting that TMT exposure in the environment may affect behaviors, sensory and motorial learning based on the observation of cell apoptosis in the cerebral regions of S. marmoratus.

Bioaccumulation of TMT in the biological system may pose a serious challenge to public health. The highly reactive oxidant, superoxide anion (O_2) was investigated in this study, using Superoxide dismutase (SOD). SOD is an antioxidant that is intimately involved in the prevention of cellular damage - a common pathway for cancer, aging and a variety of diseases[10]. Antioxidants interact with free radicals and safely terminate the chain reactions before vital molecules are damaged. The study aims to identify the possible toxicological effect of trimethyltin (TMT) on the histo-architecture and biochemical composition of the cerebellum which may pose a serious challenge to public health.

Materials and methods

Ten adult male Wistar rats were used in the study. Their weights ranged from 150 - 200g. The animals were grouped into two: Group A and B, with five adult male Wistar Rats in each group. Group A serves as the trimethyltin(TMT) group, while group B serve as the normal saline (NS) group. The animals were caged to acclimatize for one day. After that, 3mg/kg of trimethyltin chloride was administered to animals in the TMT group, while 1.0mls of normal saline was administered to the animals in the NS group via intra-peritoneal route for 3 hours respectively. The animals were sacrificed using 25mg/kg of ketamine administered intramuscularly to anesthetize the animals; followed by perfusion fixation through the heart to prevent postmortem effect during the cause of harvesting the brain.

The tissue of the cerebellum for biochemical analysis was homogenized in 5% sucrose, and stored in antifreeze. The tissues for histological analysis were excised after sacrifice and stored in paraformaldehyde. Tissue processing was done on paraffin wax embedded tissue blocks and mounted on a glass slide.

Groupings and Administration of Drugs

A single dose of 3mg/kg of Trimethyltin was administered to the animals in the TMT group, while the animals in the NS group received a single dose of 1.0ml of normal saline via intra-peritoneal route using a needle and syringe.

Table 1. Grouping and Treatments.

Groups (N=10)	Administration of drugs	Route
A (N = 5)	Trimethyltin 3mg/kg (single dose)	Intra-peritoneal
B (N = 5)	Normal saline 1.0ml (NS Group)	Intra-peritoneal

Morphometry and histological techniques

The rats were sacrifice using 25mg/kg of ketamine and all the Interdisciplinary Principles and Guidelines for the Use of Animals in Research, Testing, and Education were observed. The brains were dissected out. The length and width of the brain were measured using a ruler whereas the weight was measured using weighing balance. The tissues were fixed in paraformaldehyde and processed using routine H and E histological techniques, and crystal violet stains.

Superoxide Dismutase Assay

Enzyme activity of superoxide dismutase was assayed according to the method of Mistra and Fridovich[11], using reagent kit produced by Randox Laboratories Ltd.

Statistical Analysis

The statistical analysis was done using Microsoft Office Excel 2007. The student t-test (Paired Two Sample) was used to analyze the differences in the body weight before and after the experiment; while the t-Test for unpaired two samples was used to determine the morphometric differences across the groups and $P < 0.05$ was considered as the level of significance.

Results

Physical Observation and Body Weight Analysis

Physical examination of the animals shows normal activities in both the TMT group (Group A) and in the NS group (Group B). Table 4.0 shows the mean body weights in both groups before and after the experiment. The statistical analyses of the animals' body weight (Table 4.0) show no statistically significant differences ($p > 0.05$) before and after the experiment in group B (NS group), but a statistically significant difference ($p < 0.05$) was observed in the group A (TMT group).

Morphometric Analysis

The gross morphological examination of the brain shows no clear differences in the weight and width of the brain in both groups. Table 4.1 shows statistical analysis of the variables (weight, length, width, and brain/body ratio). The result however, shows no clear difference in the brain/body ratio, brain weight and width in both groups ($p > 0.05$), but a statistically significant difference ($P < 0.05$) in the brain length within the groups.

Histological Examination

Hematoxylin and Eosin staining technique were used to demonstrate the general histo-architecture of the cells, while the crystal violet staining technique was used to demonstrate granulations (endoplasmic reticulum and ribosomes) in the cells. The following results were observed in Fig. 1A and Fig. 1B:

Crystal violet staining is shown in Fig. 2, 3 A and B. The

Groups	Before Experiment Mean ±SEM (body weight)	After Experiment Mean ±SEM (body weight)	P value
A (n = 5)	185.6 ± 18.389	181.96 ± 18.230	0.0311
B (n = 5)	148.4 ± 12.089	135.08 ± 11.977	0.2411

Table 2. Statistical analysis (t-Test: Paired Two Sample) for body weight (g) of the animal.

Variables (Brain)	Group A (n = 5) Mean ±SEM	Group B (n = 5) Mean ±SEM	P value
Weight (g)	0.96 ± 0.196	1.08 ± 0.215	0.6915
Length (cm)	2.42 ± 0.086	2.16 ± 0.068	0.0467
Width (cm)	1.42 ± 0.073	1.22 ± 0.124	0.2112
Brain/Body Ratio (g)	0.005178 ± 0.001	0.005949 ± 0.001	0.5749

Table 3. Statistical analysis of the brain weight, length, width and brain/body ratio.

S/N	Group A Mean ±SEM SOD Activity (Unit/ml)	Group B Mean ±SEM SOD Activity (Unit/ml)	P value
1	705 ± 3.302	778.8 ± 7.067	0.0001

Table 4. Statistical analysis of SOD activity in brain homogenates of the two groups.

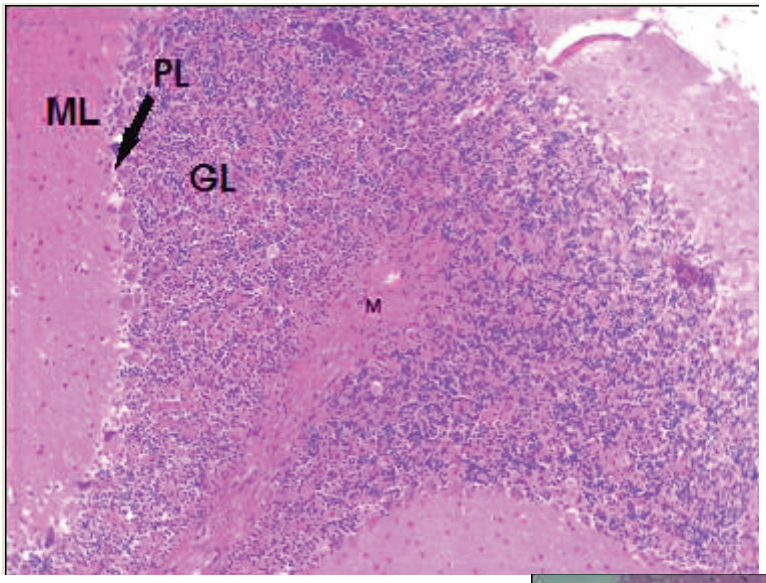

Figure 1A. ANS group (1ml of normal saline), H & E x 100, ML- Molecular layer, PL – Purkinje Layer, GL – Granular Layer, M – Central medulla of white matter.

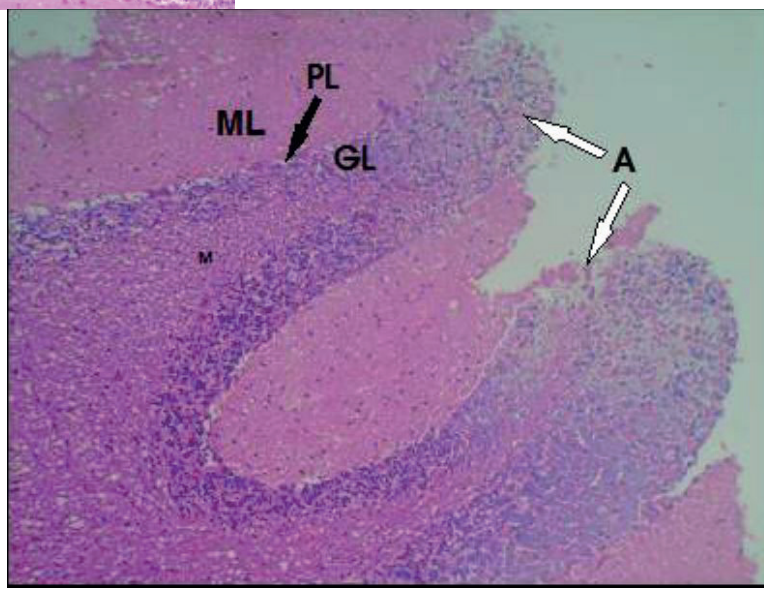

Figure 1B. TMT group (3mg/kg of TMT), H & E x 100, A – A partially stained area of the the cerebellar cortex.

The photomicrograph (Fig. 1A) of the cerebellar cells in group B (1 ml of normal saline) shows the section of the cerebellar cortex consisting of the molecular (ML) and granular layers (GL). In between, them is the Purkinje layer (PL). The central white matter (M) is also visible. The layers of the cerebellar cortex appeared clear as seen in the normal cerebellum. The photomicrograph (Fig. 1B) of group A (3mg/kg ml of TMT) shows an abnormal cellular tissue morphology of the three layers of the cerebellum. A partially stained area in the cerebellar cortex can be seen.

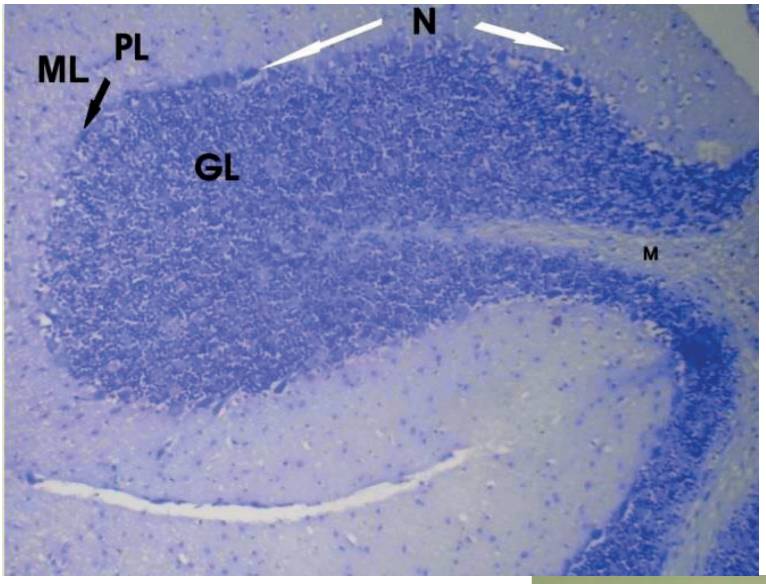

Figure 2A. NS group (1ml of normal saline), CFV x 100, ML- Molecular layer, PL – Purkinje Layer, GL – Granular Layer, M - Central medulla of white matter, N – Nissl substances.

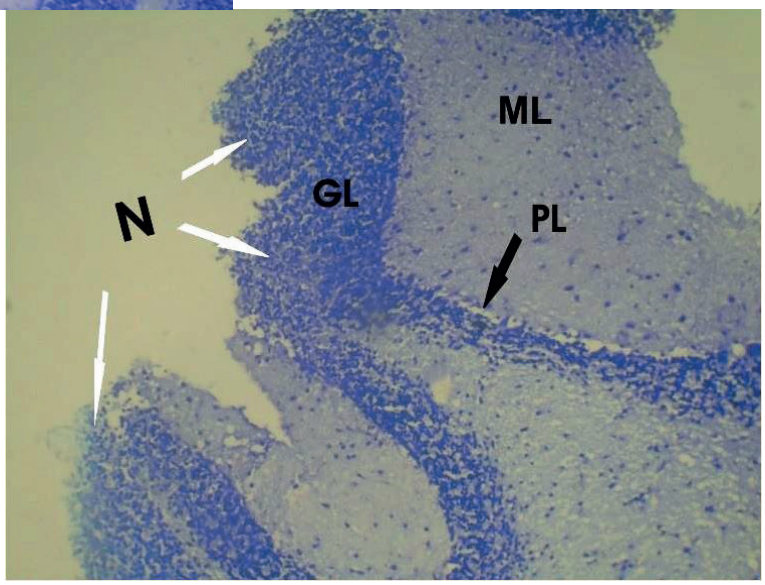

Figure 2B. TMT group (3mg/kg of TMT), CFV x 100, N – Nissl substances in abundance and clustered in areas of distortion.

photomicrograph (Fig. 2 A & 3 B) of group B (1ml of Normal saline) shows nissl substance present as usual in the cerebellar cortex (N). The photomicrograph (Fig. 2 B & 3 A) of group A (3mg/kg of TMT) also shows the presence of nissl substances strongly stained, and clustered mainly in the granular layer of the cerebellar cortex.

Biochemical Examination

Superoxide dismutase (SOD) was employed to assess oxidative stress following exposure to trimethyltin. The biochemical examination has shown that (SOD) activity in brain homogenate of the TMT group decreased significantly compared with the NS group. Oxidative stress was found to increase ($p < 0.05$) significantly. The following results were observed:

The statistical analysis of the biochemical examination shows a statistically significant difference ($P < 0.05$) in SOD activity within the groups.

Discussion

The mechanism of action of trimethyltin chloride has shown to be toxic at 3mg/kg dose following short term administration. A significant decrease in the body weight ($p < 0.05$) is observed in the TMT group.

The histo-architecture of the cerebellar tissues in Group A (TMT group) shows a partially stained area of the cerebellar cortex in the H & E stain and clumping of the nissl substances in the Crystal violet stain, indicating abnormal layers of the cerebellar cortex. It had been reported that TMT administration induces significant behavioral alterations[12] and brain apoptosis in the cerebellum[1]. According to Wang *et al.*[1] TMT exposure in the environment may affect behaviors, sensory and motorial learning.

Cerebellar apoptosis results from exposure of the cerebellum to hazardous chemicals which could lead to hypoxic damage, and may result in damage to Purkinje cells in the cerebellar cortex. This can lead to the development of cerebellar ataxia depicted by poor coordination of voluntary movement[5]. Also, depression, deficits in the ability to experience emotions, and behavioral difficulties are commonly seen in patients with cerebellar lesions[13]. In the present study, the effect produced by TMT chloride on the histology of the cerebellum occurred mildly at the three layers of the cerebellar cortex.

Increased production of reactive oxygen species (ROS) has been linked with increased oxidative stress levels, which is also associated with the process of cell apoptosis[1]. Organisms

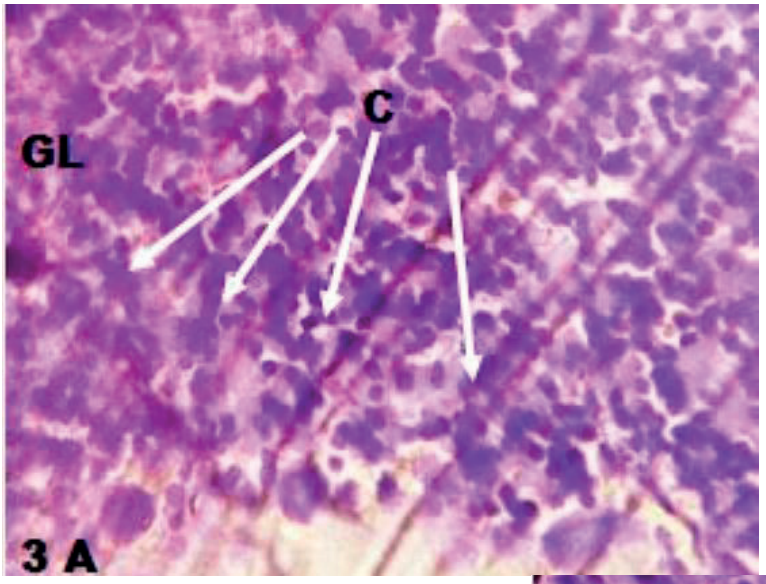

Figure 3A. TMT group (3mg/kg of TMT), CFV x 400, CA - Nissl substances in intensely stained and clustered, GL - Granular layer.

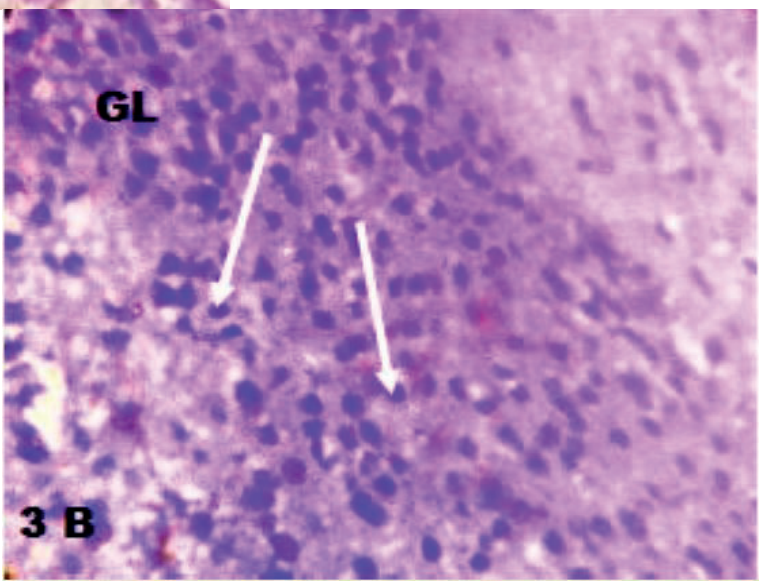

Figure 3B. NS group (1ml of normal saline), CFV x 400, Arrow – Presence of Nissl substances.

have evolved the mechanism to counteract the effect of radicals generated in the biological membrane. This mechanism involves the antioxidant system such as glutathione reductase, glutathione peroxidase, superoxide dismutase (SOD) amongst others. The antioxidant system's functions to modify the highly reactive free radicals to form the less reactive intermediate which no longer pose a threat to the cell[4].

For good biological integrity to be maintained there must be a balance between oxidation and antioxidant's level in the system. Oxidants such as superoxide anions ($O_{2.}$) may attack the membranes of the brain cells thereby causing oxidative stress. The biochemical examination has shown that (SOD) activity in brain homogenate of the TMT group decreased significantly compared with NS group. This decrease may be as a result of the imbalance between oxidants and antioxidants level in favor of the oxidants[4]. Similarly, in a study to investigate the role of oxidative stress in Purkinje cell neurotoxicity of ethanol-treated rat, results showed a decrease in the activities of SOD[14]. SOD is an active enzyme that can cause dismutation of superoxide anions produced during oxidative stress in cells[4].

Metabolism in the brain has been associated with the production of Reactive Oxygen Species (ROS)[4]. Thesignificant increase in ROS in the TMT group compared with the NS group may be associated with trimethyltin poisoning. An important source of ROS is oxidative metabolism of xenobiotics[4]. ROS is controlled by the antioxidant defense systems, which appears to maintain low concentrations rather than complete elimination. Oxidative stress occurs in a cell or tissue when the concentration of ROS generated exceeds the antioxidants capability of that cell[15]. Hence it is suggested that animals in the TMT group experienced oxidative stress.

Conclusions

Short term single dose administration of Trimethyltin (TMT) has no adverse effect on the brain weight of the cerebellum of adult male Wistar rat. However, some changes were observed in the body weight and brain length of the animal, as well as mild cellular distortion in the layers of the cerebellar cortex.

The biochemical analysis suggested an increase in oxidative stress as a result of the production of more reactive oxygen species (ROS) in the TMT group, given that SOD activity in brain homogenate of the TMT group decreased significantly compared with NS group, proving that trimethyltin increases oxidative stress.

Recommendations

It is recommended that individuals in both develop and developing nations who work in industries where TMT is used as plasticizers of polyvinyl chloride products and biocides incorporated in insecticides; should be more cautious with trimethyltin use in the environment, as well as its use with regards to health safety standards especially in humans. Indiscriminate short term exposure to TMT may not have immediate obvious health effect; however, bioaccumulation of TMT in the long-term may be very damaging.

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