

## SOME OF THE EFFECTS OF AQUEOUS EXTRACT OF MISTLETOE ON CADMIUM-INDUCED PREFRONTAL CORTEX DAMAGE IN WISTAR RATS

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### ABSTRACT

Cadmium is heavy metal that enters man's system through various ways. It causes adverse effects on various body tissues by inducing oxidative stress. Mistletoe leaf extract is been used to treat various ailment in folk medicine till present day. This leaf extract contains antioxidants like flavonoids, vitamin C and E. This study was carried out to investigate effects of mistletoe leaf extract on the microanatomy of prefrontal cortex of Wistar rats exposed to cadmium. Twenty-four adult Wistar rats weighing 150-200g were randomly grouped into four, Groups A, B, C and D each containing six animals. Group A animals were not given any injection. Groups B and C animals were injected intraperitoneally with 14mg of cadmium per kg body weight of animals, while group D were not injected also. Groups C and D were administered orally with 40mg of mistletoe aqueous extract per kg body weight of the animals. Whereas Groups A and B were administered orally equivalent volume of distilled water. After four weeks, the prefrontal cortices were excised for H&E and cresyl fast violet staining. Histological section of Group B animals shows the population of both granular and pyramidal cells appeared reduced with dead cell and sparsely stained Nissl substance. Groups A and D histological section shows almost similar cytoarchitecture, the granular and pyramidal cells appeared normal with round and triangular shaped structured respectively whereas Group C shows little densely stained cells with reduced dead cell and the Nissl substance almost similar to Group A. The above findings showed that aqueous extract of mistletoe reduced the adverse effects caused by cadmium on the rat prefrontal cortex.

**Key words:** Cadmium, Mistletoe, Prefrontal cortex, H&E and CFV.

### INTRODUCTION

Cadmium is a chemical element with the symbol Cd and atomic number 48 (Lide *et al.*, 2000). This soft, bluish-white metal is chemically similar to the stable metals (zinc and mercury). Cadmium

occurs naturally in zinc and lead ores and in some rock phosphate fertilizers (McLaughlin & Singh, 1999). It is not essential to human life (Yiin *et al.*, 1999). Nevertheless, man in one way or the other gets exposed to cadmium through his environment and diet. Excess cadmium exposure produces adverse health effects on human beings (Jarup *et al.*, 1998). Since 1989, cadmium has been regarded as a poisonous element (WHO, 1989).

Industrial and agricultural uses of cadmium have led to its widespread dispersion at trace levels into the environment and human food stuffs (Galal-Garchev, 1993). The overall adverse effects of cadmium are dependent on the total levels of exposure (Yiin *et al.*, 1999). Principal factors that determine the level of exposure are: non-occupational exposure, dermal exposure, ingestion and inhalation. While dermal exposure is generally not regarded as being significant, man usually absorbs cadmium into the body either by ingestion or inhalation (Lauwerys, 1986). Even though, inhalation exposure to cadmium does not usually contribute significantly to overall body burden for the non-occupational exposed individual, this is not true of cigarette smokers. In fact, for a smoker, it is estimated that roughly 50% of their cadmium intake arises from cigarettes (Reeves *et al.*, 1997). Occupational exposure to cadmium is mainly by inhalation, which commonly occurs among employees of the construction industry.

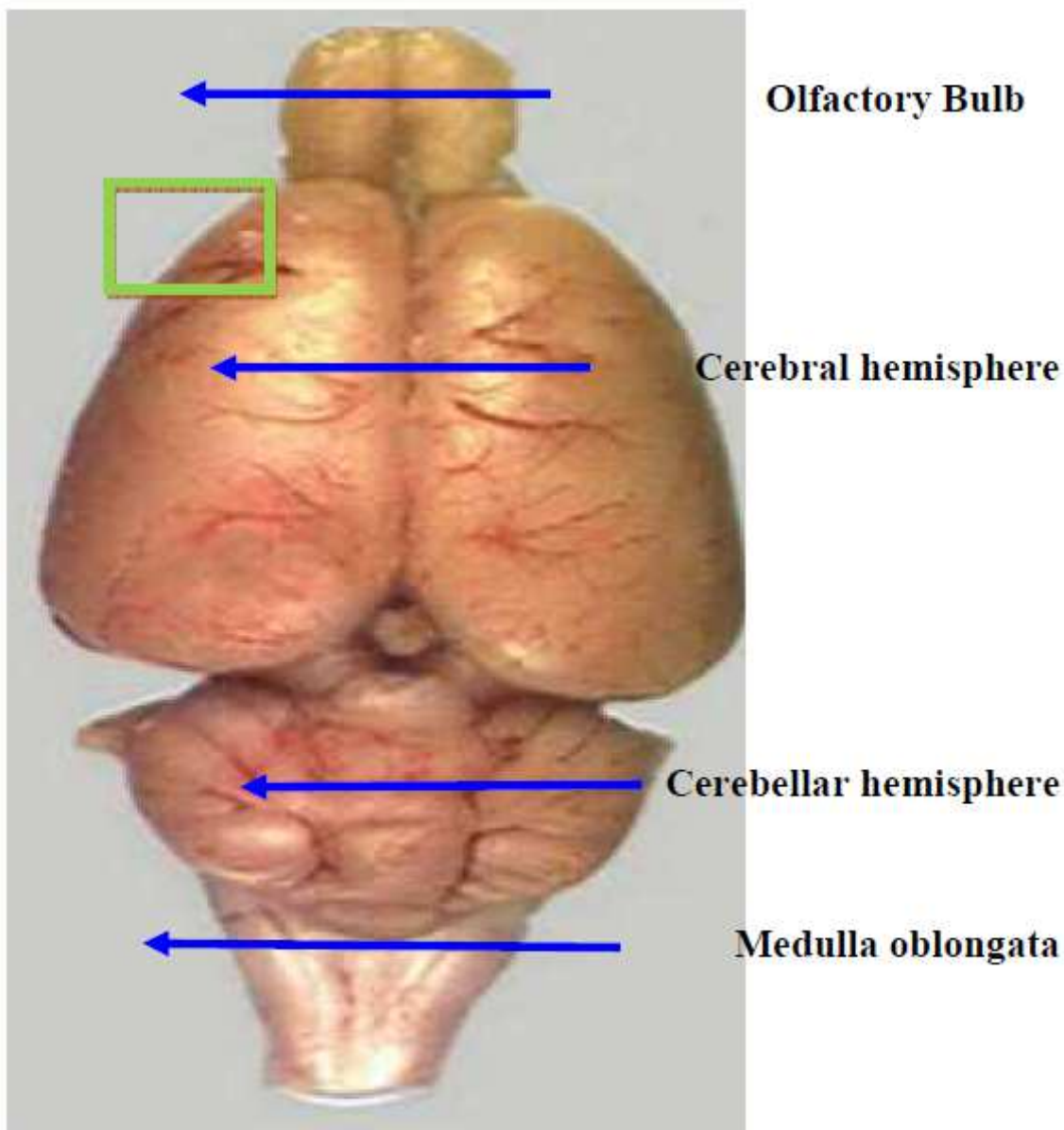
### **Some adverse effects of cadmium on body organs**

Cadmium has been reported causing some adverse effect in some body organs such as kidney, brain, lungs and liver. Cadmium is first transported to the liver through the blood. There, it is bound to proteins to form complexes that are transported to the kidneys. Cadmium accumulates in the kidneys, where it damages the filtering mechanisms. This causes the excretion of essential proteins and sugars from the body and further kidney damage. It takes a very long time before cadmium that has accumulated in kidneys is excreted from a human body. It also causes brain ischaemia as a result of insufficient blood flow to the cerebrum (cerebral oligoemia), heat stress and alters glucose metabolism (Salagapylak, 2010).

### **Organ of study**

#### **Prefrontal cortex**

It is the anterior part of the frontal lobes of the brain, lying in front of the motor and premotor areas (De Young *et al.*, 2010). It is composed of the dorsolateral and ventrolateral areas that receive their major afferents from the mediodorsal nucleus and there are additional contributions from the medial pulvinar, the ventral anterior nucleus and the paracentral nucleus of the anterior intra laminar group of the thalamus (Standring *et al.*, 2008).



**Figure 1:** Illustration of some structures in the brain of rat (Fiala and Spacek, 2007). The green box indicates the area of the prefrontal cortex.

Mistletoe (*Viscum album*) is highly specialized angiosperms of the family Loranthaceae, which is well known as broad host range hemi-parasites of a variety of different gymnosperm and angiosperm (Deeni and Sadiq 2002). It is an evergreen semi-parasitic plant that grows primarily on deciduous trees (Hoffman, 1989). It is widely distributed throughout Europe, North Africa, Australia, Asia and also in Nigeria (Frohne and Pfander, 1984). Mistletoe has been used in the treatment and management of many diseases for many years, both in traditional and complementary medicine in some parts of Africa. It has also been reported to be effective in the management of chronic metabolic disorders such as diabetes (Obatomi *et al.*, 1994). A number of biological effects, such as anticancer, antimycobacterial and antiviral properties, as well as apoptosis-inducing and immunomodulatory activities have been reported for mistletoe (Onay-Ucar *et al.*, 2006). The European mistletoe strengthens the capillary endothelium and reduces blood pressure as well as the heart rate (Obatomi *et al.*, 1994). Cardiotoxic action is thought to be due to its ligands while the

hypotensive action is believed to be due to the presence of choline groups (Lyu *et al.*, 1998). The constituents of mistletoe are: flavonoids, lectins polypeptides, polysaccharides, saponins tannis, triterpines and viscotixins. The major constituents of mistletoe are the flavonoids, lectins (carbohydrate binding proteins), which include viscumin, polypeptides known as viscotoxin (with a basic chemical structure of thionins) and a number of phenolic compounds (e.g. digiallic acid, o-coumaric acid) found in their free states or as glycosides (Duong *et al.*, 2003). Generally, the constituents of mistletoe depend on the type of host plant.

### JUSTIFICATION FOR THE STUDY

It has been shown that cadmium causes brain damage through oxidation that treatment with antioxidant containing substances reduces the destructive effect of cadmium on the tissue (Ige *et al.*, 2009). In view of this fact that mistletoe contains some antioxidants (Duong *et al.*, 2003), this study was conducted to find out if the plant could ameliorate any destructive effects that cadmium may have on prefrontal cortex in rats.

### AIMS AND OBJECTIVES

This study is aimed at investigating some of the effects of oral administration of *Viscum album* extract on the effects of cadmium on the prefrontal cortex. Some of which are:

- To investigate the cytoarchitecture of the prefrontal cortex.
- To demonstrate presence of Nissl substance.

### MATERIALS AND METHOD

The following materials were used for this research:

Rats (24 male Wistar rats), Mistletoe, Cadmium, Normal saline, Cages, Feed/Chow

Formol calcium, Slides, H & E stain, Special stain, Hand gloves, Insulin needles & syringes, Weighing balance, Measuring cylinder, Conical flask, Electric blender, Distilled water.

### PLANT MATERIALS AND PREPARATION OF EXTRACT

The mistletoe was procured from Ladoko Akintola University of Technology Farm in Ogbomoso, Oyo state, Nigeria. It was taken to a botanist in the Department of Plant Biology, University of Ilorin, for identification. Mistletoe aqueous extract was prepared as done by Adeeyo *et al.* (2011). The mistletoe was air dried and grinded into coarse powder using an electric blender. 200g of the powder was soaked in 2000ml of distilled water and was slowly evaporated to dryness in a vacuum using a rotator evaporator. A total yield of 18.2% was obtained. 40g was weighed from the extract were then used to prepared the stock solution after the method of Adeeyo *et al.* 2011 by adding 2000ml of distilled water.

### ANIMAL GROUPING AND TREATMENT

Twenty four Wistar rats weighing between 150-200g were used for the study. They were maintained under standard laboratory condition in the Animal House of the Faculty of Basic Medical Sciences, Ladoko Akintola University of Technology, Ogbomoso. The rats were randomly grouped into Group A, B, C and D each containing six animals. Animals in Groups B and C were each injected intraperitoneally with 14mg of cadmium per kg body weight (Salawu *et al.*, 2009), while animals in Groups A and D were not injected. 72 hours after administering cadmium to rats in Groups B and C, those in group C together with the group D rats were given 40mg per kg body weight of mistletoe aqueous extract orally while an equivalent volume of distilled water were administered orally to

animals in Groups A and B. All the treatments were given daily for four weeks.

### Sacrifice of Animals and Excision of Tissue

Animals were sacrificed by cervical dislocation after administration of the last doses. They were laid supine on the dissecting board and pinned through the fore and hind paw. The skulls of the animals were fractured open with brain forceps and each brain was carefully removed and weighed. Thereafter, the prefrontal cortices were quickly excised. The tissues for histological studies were fixed in freshly prepared formol calcium for 72 hours.

### Processing of Tissues for Histology

#### Tissue Processing

Tissues were processed for Hematoxylin and Eosin staining using Pearse (1980) method.

**Table 1: Composition of formol calcium fixative.**

Reagent	Component	Volume/Mass
Formol calcium	40% formaldehyde	100ml
	Calcium chloride	9g
	Distilled water	900ml

**Table 2: Composition of graded alcohol.**

Graded alcohol (ethanol)	Component	Volume
50% alcohol	Absolute alcohol	50ml
	Distilled water	50ml
70% alcohol	Absolute alcohol	70ml
	Distilled water	30ml
90% alcohol	Absolute alcohol	90ml
	Distilled water	30ml
100% alcohol	Absolute alcohol	

#### Fixation

Tissues were fixed in formol calcium for hematoxylin and eosin and cresyl fast violet methods. This helped preventing autolysis of brain tissue and putrefaction by bacteria.

#### Dehydration

Ethanol was used and the tissues were passed through ascending grades of alcohol, it was firstly dehydrated in 50% alcohol, followed sequentially by 70%, 90 % and absolute alcohol I and II each stage for one hour. This was carried out because the impregnating medium (paraffin wax) is not miscible with water.

#### Clearing

Tissues were passed through two changes of xylene for a period of one hour each to remove the dehydrating fluid to ensure proper impregnation.

#### Infiltration

Molten paraffin wax of melting point 56<sup>0</sup>C was used for infiltration twice in oven at 60<sup>0</sup>C, for one

hour each to remove the clearing agent prior to embedding.

### **Embedding**

Tissues were then placed, after infiltration, in embedding cassettes in an orientated position with the use of forceps. The cassettes were subsequently filled with molten wax at 60<sup>0</sup>C and then cooled to solidification in tap water at room temperature. This solidification provides enough support for the tissues to allow subsequent sectioning.

### **Trimming**

The blocks of tissues were trimmed to suitable size and shape with a knife, leaving about 1-2mm of embedding medium on either side and were mounted on wooden blocks that can be held by the rotary microtome clamps during sectioning.

### **Sectioning**

Ribbons of tissue sections were obtained using a rotary microtome (CE Bright Company Limited, Huntingon England). Microtome section was then set at 5 $\mu$ , while the knife was placed at 45<sup>0</sup> to tissue blocks. The ribbons produced were carefully picked and transferred on 20% alcohol to a water bath set as 51<sup>0</sup>C. The current generated by the reaction of the alcohol with the water ensures the section spreads and flattens thus preventing folding of the section.

### **Picking/lifting of section**

Clean slides with egg albumin were dipped vertically into the warm water bath to lift the sections out at an angle to the plane of the water so that the thin sections could spread out flat on the slides and the egg albumin enhance the sticking of sections to the slides.

### **Dewaxing and drying**

Tissue slides were allowed to dry at room temperature. However, excess was later carefully removed by placing the slides on a hot plate for a short period of time at 58<sup>0</sup>C. The slides were stored in racks prior to staining.

### **Staining Technique**

Two staining methods were adopted in this present study, these are as follows.

- Haematoxylin and Eosin (Pearse, 1980).
- Cresyl Fast Violet Method (Junquiera and Carneiro, 2006) to demonstrate the Nissl substance.

### **Haematoxylin and Eosin (Pearse at al., 1980)**

#### *Procedures*

Sections were:

- (a) Dewaxed in two changes of xylene for 3 minutes each;
- (b) Tissues were Rehydrated by passing them through descending grades of alcohol 100%, 90%, 70%, 50% for two minutes each;
- (c) Rinsed in tap water;

- (d) Stained in haematoxylin for 13 minutes;
- (e) Washed well in running tap water for 5 minute and examined microscopically to confirm sufficient degree of staining;
- (f) Excess stain was removed in acid alcohol (1% concentration of HCl in 70% alcohol) for a 3 seconds as the acid breaks the mordant-dye linkages;
- (g) Washed in running tap water for 8 minutes to regain blue colour (bluing) as observed with naked eye;
- (h) Stained in 1% aqueous eosin for about 3 minutes;
- (i) Excess stain was removed in running tap water and examined with microscope;
- (j) Dehydration was done rapidly in ascending grades of alcohol for 10 seconds each.

The excess eosin stain is removed in low grades of ethanol;

- (k) Mounted in Distrene Plasticizer Xylene (DPX). Clean glass cover slips were used.

The stained tissue slides were stored in racks to dry for histological examination with the aid of an optical microscope.

#### **Cresyl Fast Violet (Junquiera and Carneiro, 2006)**

**Table 3: Staining solution for Cresyl Fast Violet:**

Component S/N	Mass/Volume
Cresyl Fast Violet	Lg
Distilled water	100ml
Acetic acid	0.25 ml

#### *Procedures*

Sections were:

- a) Taken to water;
- b) Stained in Cresyl Fast Violet for 27 minutes;
- c) Rinsed in distilled water;
- d) Placed in 90% alcohol until most of the stains have been removed;
- e) Cleared in xylene;
- f) Mounted in DPX

## Statistical Analysis

Data were analyzed using SPSS (SPSS Inc, Chicago, USA) and Excel 2007 (Microsoft Corporation USA). Data were expressed as mean  $\pm$  standard error of mean (mean  $\pm$ SEM). Mean values were compared using one way analysis of variance (ANOVA), P values less than 0.05 were taken to be statistically significant. All graphs were drawn with excel 2007 (Microsoft corporation, USA).

## RESULT AND DISCUSSION

### Morphological Observation

#### Average Weight/Week (in gram)

The table 4 reveals body weight variations that occurred during the four weeks of daily treatment. At the second week there was a decrease of 6.41% in the body weight of the animals in cadmium only Group, also after the second week their body weight is still declining and by the fourth week there was a decrease of 8.73% compared to initial weight. In Group C of cadmium+mistletoe, there was a decrease of 4.66% at second week, but at fourth week the decrease was 2.21% when compared with their initial body weight. In mistletoe only Group, there was increment in their body weight till week four which is similar to control Group.

Table 5 reveals average brain weight variation that occurred after four weeks of daily treatment. Animals in Groups A and D have the highest average brain weight where Group C were also high but not as seen in Group A and D. Group B animals have the lowest average brain weight.

**Table 4: Average body weight of rats in grams.**

GROUP	A (CONTROL)	B (CADMIUM ONLY)	C (CADMIUM + MISTLETOE)	D (MISTLETOE ONLY)
INITIAL WEIGHT	171.06	172.33	175.24	173.14
WEEK 1	171.56	163.15	166.15	172.87
WEEK 2	172.12	161.28	167.07	173.25
WEEK 3	172.87	158.46	169.15	173.46
WEEK 4	173.34	157.28	171.36	173.97



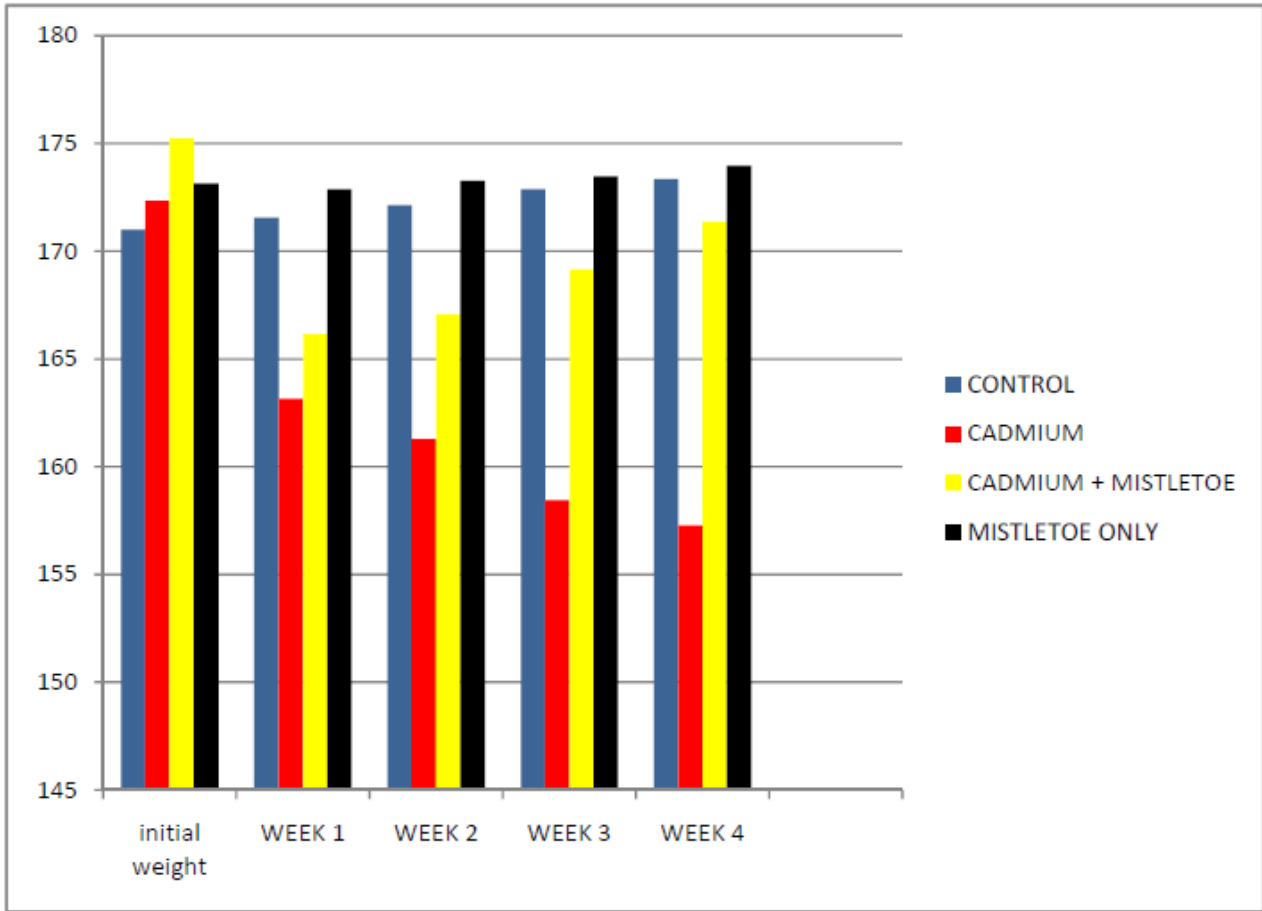
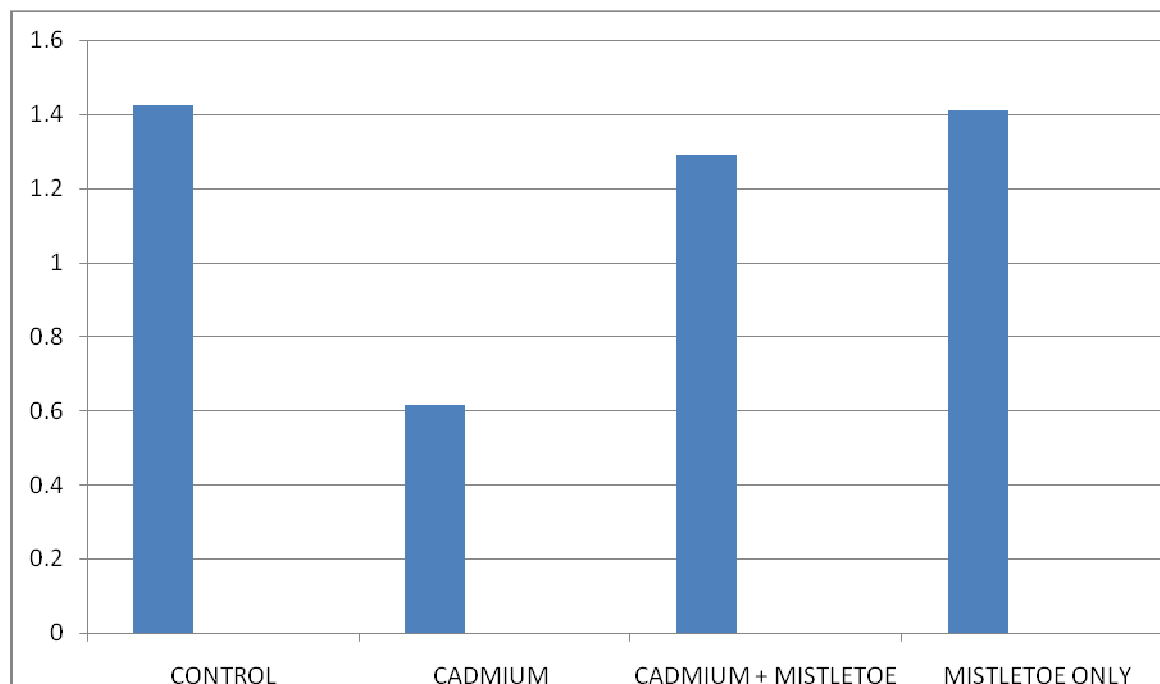


Figure 2: Average body weight of rats in grams.

Table 5: Average brain weight (in gram) after four weeks.

GROUP	A (CONTROL)	B (CADMIUM ONLY)	C (CADMIUM + MISTLETOE)	D (MISTLETOE ONLY)
	1.42	0.62	1.29	1.41



**Figure3: Average brain weight (in gram) after four weeks**

## **Histological findings**

### **Prefrontal cortex**

#### ***Haematoxylin and Eosin staining method of Pearse (1980)***

##### **Histology of the prefrontal cortex of Group A rats (Control) Stain: H&E**

The granular and pyramidal cells appear normal round and triangular shaped body respectively. The cells are densely populated and the cytoarchitecture of the prefrontal cortex was preserved (Figure 4A&B).

##### **Histology of the prefrontal cortex of Group B rats (Cadmium only) Stain: H&E**

Most of the cells present were dead. The cells are scanty and the cytoarchitecture of the prefrontal cortex distorted (Figure 4C&D).

##### **Histology of the prefrontal cortex of Group C rats (Cadmium induced treated with 40mg/kg of mistletoe) Stain: H&E**

Some of the cells were dead while some cells appear normal. Across the prefrontal cortex some areas have distorted cytoarchitecture while some areas have normal cytoarchitecture (Figure 5E&F).

##### **Histology of the prefrontal cortex of Group D rats given only 40mg/kg of mistletoe Stain: H&E**

The granular and pyramidal cells appear normal round and triangular shaped body respectively. The cells are densely populated and the cytoarchitecture of the prefrontal cortex was preserved (Figure 5G&H).

#### ***Cresyl Fast Violet (Junquiera and Carneiro, 2006)***

**Histology of the prefrontal cortex of Group A rats (Control) Stain: CFV**

The cells appeared normal. Granular are seen with their round shaped structure and pyramidal cells with their triangular shape (Figure 6A). No distorted was seen in these cells. The Nissl substance was well demonstrated.

**Histology of the prefrontal cortex of Group B rats (Cadmium only) Stain: CFV**

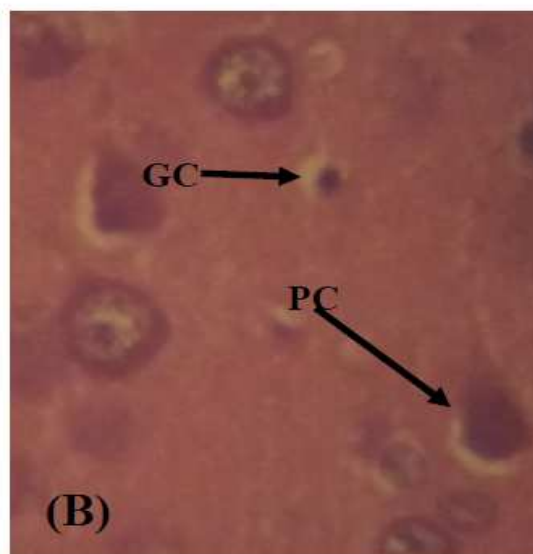
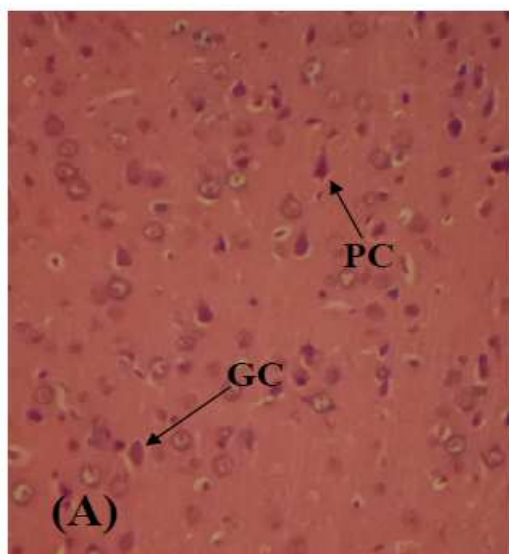
The population of both granular and pyramidal cells appeared reduced. The cytoarchitecture was distorted. Most of the cells were dead. The Nissl substance was sparsely stained (Figure 6B).

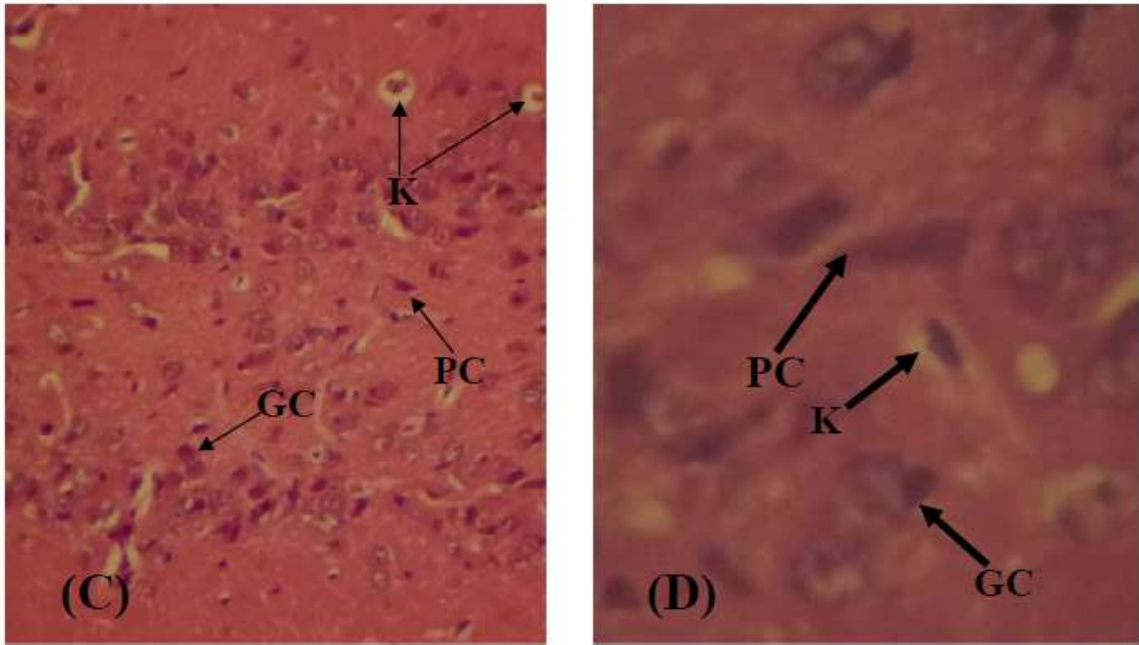
**Histology of the prefrontal cortex of Group C rats (Cadmium induced treated with 40mg/kg of mistletoe) Stain: CFV**

Both granular and pyramidal cells appeared normal with some distorted cytoarchitecture. Some of the cells were dead. Nissl substance was preserved to some extent. (Figure 6C)

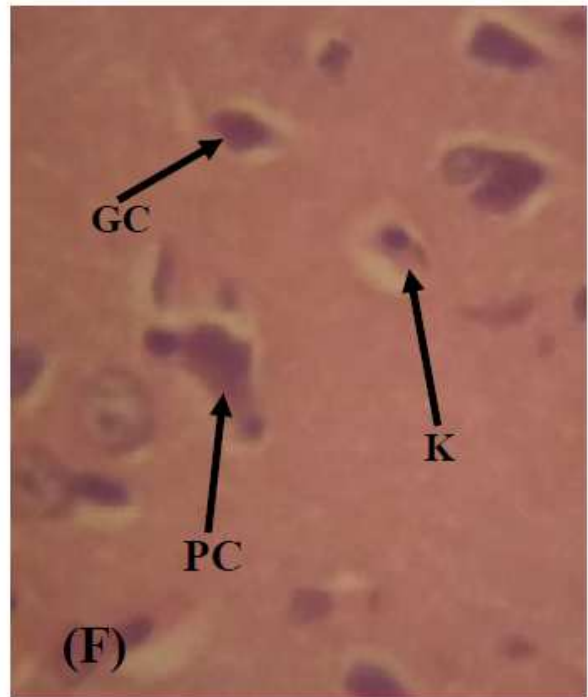
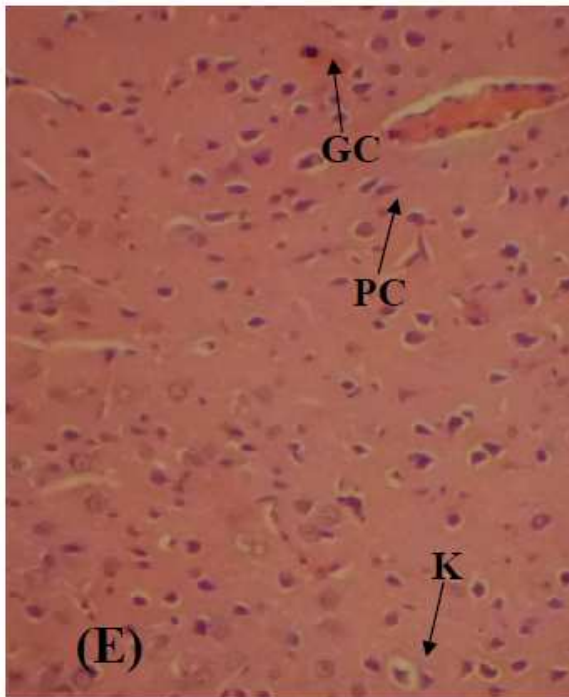
**Histology of the prefrontal cortex of Group D rats given only 40mg/kg of mistletoe Stain: CFV**

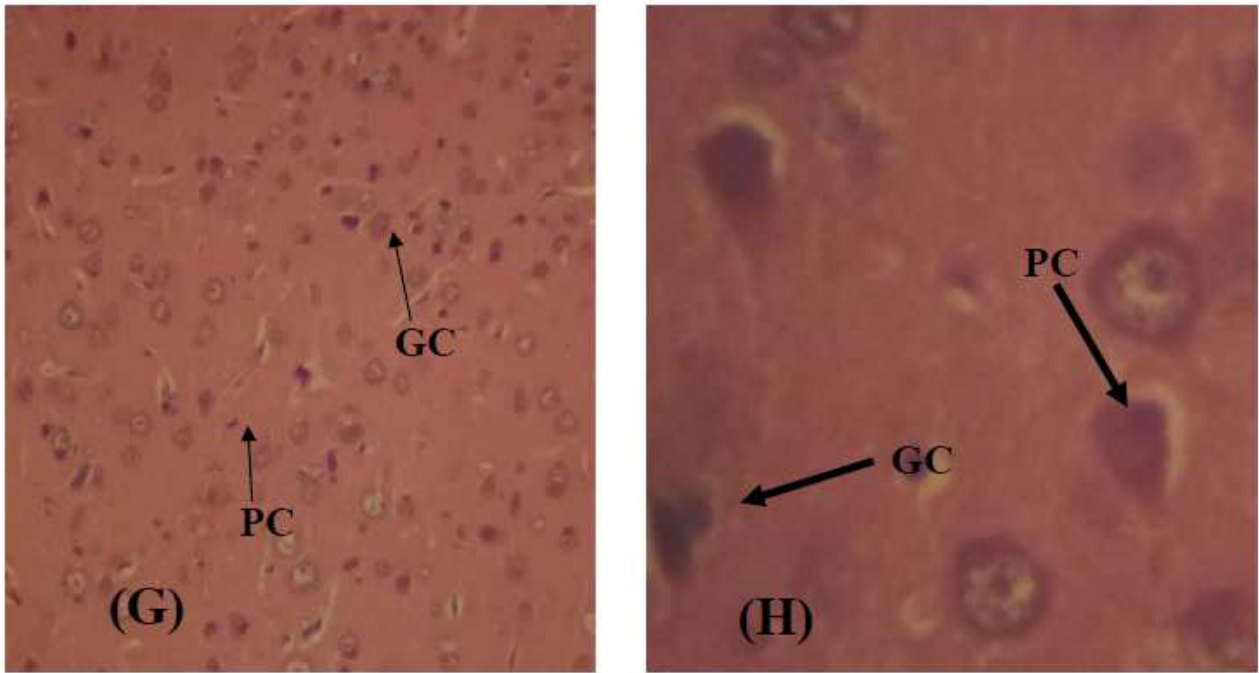
The cells appeared normal. Granular are seen with their round shaped structure and pyramidal cells with their triangular shape (Figure 6D). No distorted was seen in these cells. The Nissl substance was well demonstrated



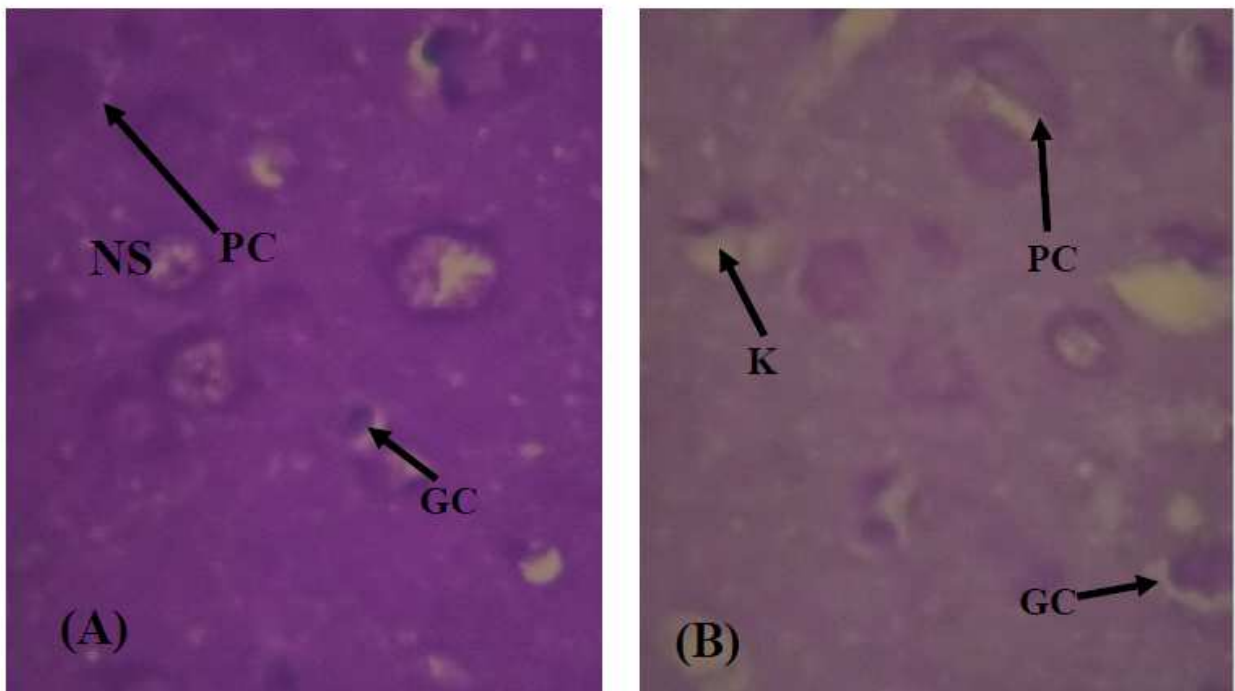


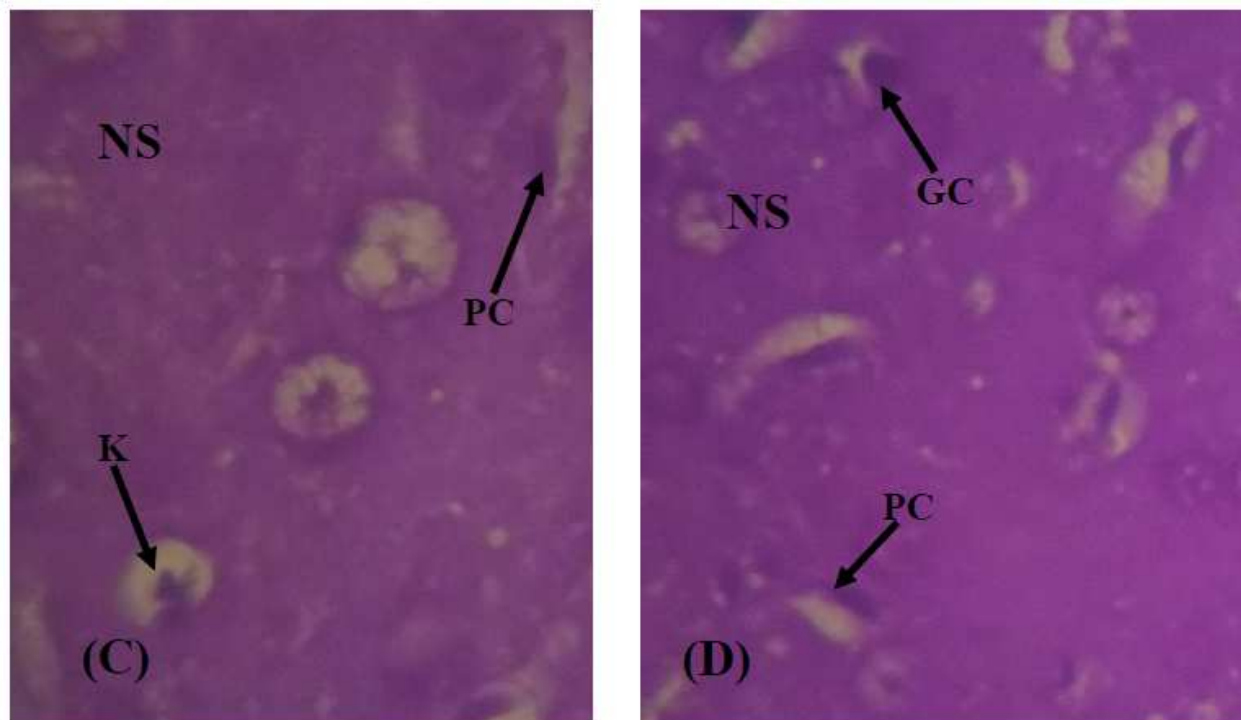
**Figure 4:** Representative Photomicrograph of layer III of the prefrontal cortex showing the distribution of neurons. (GC: Granular cells; PC: Pyramidal cells; K: Dead cells ). (A&B) Control and (C&D) Cadmium induced. H&E stain. Mag.x100 (left) and x 400 (right).





**Figure 5:** Representative Photomicrograph of layer III of the prefrontal cortex showing the distribution of neurons. ( GC: Granular cells; PC: Pyramidal cells; K: Dead cells ). (E&F) Cadmium+mistletoe and (G&H) Mistletoe only. H&E stain. Mag. x100 (left) and x 400 (right).





**Figure 6:** Representative Photomicrograph of layer III the prefrontal cortex showing the distribution of neurons. ( GC: Granular cells; PC: Pyramidal cells; K: Dead cells; NS: Nissl substance). (A) Control ; (B) Cadmium induced; (C) Cadmium+mistletoe and (D) Mistletoe only. CFV stain. Mag. x 400.

## DISCUSSION

The use of plants with medicinal properties for the treatment, cure and prevention of diseases is one of the oldest medicinal methods known in history. At the beginning of the 1990s, the World Health Organization stated that 65-80% of the population of developing countries depended on medicinal plants as their only form of basic health care (Akerle, 1993).

The present study evaluated the possible ameliorative effects of mistletoe on the destructive effects of cadmium on the prefrontal cortex. The histological and histochemical results showed that histological section of Group B animals shows the population of both granular and pyramidal cells appeared reduced with dead cell and sparsely stained Nissl substance. Groups A and D histological section shows almost similar cytoarchitecture, the granular and pyramidal cells appeared normal with round and triangular shaped structured respectively whereas Group C shows little densely stained cells with reduced dead cell and the Nissl substance almost similar to Group A. The control Group A animals shows the granular and pyramidal cells appeared normal with round and triangular shaped structured respectively, the cytoplasm are also intact and well stained with undistorted architecture. In Group B which consists of animals induced cadmium only showed features of dead cells. This might be due to cellular destructive effects of cadmium (Yiin *et al.*, 1999). It has already been documented that cadmium causes cellular destruction even at times cellular death at high concentration (Yiin *et al.*,1999) and cadmium causes cellular destruction through oxidation by releasing of oxygen radicals (Ige *et al.*,2009). Animal in Group C which were induced with cadmium but treated with 40mg/kg of *Viscum album* showed some improvement in cytoarchitecture of the prefrontal cortex as indicated by some reduced dead cells and healthy looking pyramidal cells. These features might be due to presence of flavonoids, lectins, saponins-tannis, tri-pterpine

and viscotoxins in mistletoe (Duong *et al.*, 2003). These constituents have been said to have antioxidant effects, thus possessed ability to remove superoxides and oxygen radicals that are usually implicated in tissue destruction (Gustavo *et al.*, 2007). Flavonoids act as a powerful hydrosoluble antioxidant in biological fluids (Duong *et al.*, 2003). In Group D animals which were given 40mg/kg of *Viscum album* only showed cytoarchitecture of prefrontal cortex almost similar to those seen in Group A animals which were control. This study revealed that mistletoe have a preventive measured on cellular damage as compared with an highly cellular destruction seen in Group B animals, this preventive effect of mistletoe might be due to the to the presence of bioactive substances which might be antioxidant constituent present in the mistletoe (Duong *et al.*, 2003).

Nissl bodies in cells contain ribosomal RNA (rRNA) which is important in protein synthesis for cellular survival. It has been noted that the brain zinc, DNA content, DNA synthetase and thymidine kinase activities and brain weight were reduced by cadmium ingestion (Gupta *et al.*, 1993). Nissl bodies group B animals was significantly reduced as shown in Figure 6B, whereas the Nissl bodies in Group C animals were comparable to those of Group A. This showed that mistletoe given to Group C animals was able to prevent some tissue damage by cadmium. Nissl body in Group D was similar to what was found in Group A.

### CONCLUSION

The results showed a variation in histoarchitecture of the prefrontal cortex of the four different groups of adult Wistar rats even though, there is a basic reversal of the damage induced in the treated group in line with clinical applications of the active constituent found in mistletoe used for treatment.

It is therefore reported that there is a micro anatomical repair of the prefrontal cortex following treatment with *Viscum album* against cadmium induced prefrontal cortex damaged.

The above further added to the fact that some active constituents in mistletoe like antioxidants (flavonoids, lectins, vitamin C and E) could prevent or ameliorate the damage effects that could be posed by oxidative stress.

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