Available online at www.scientiaresearchlibrary.com

Scientia Research Library

ISSN 2348-0416
USA CODEN: JASRHB

Journal of Applied Science And Research, 2014, 2 (3):5-18

(*http://www.scientiaresearchlibrary.com/arhcive.php*)

Some Quantitative Histochemical Findings in the Mistletoe leaf Extract Treated Prefrontal cortex of Wistar rats Expose to Cadmium

Adeeyo O.A[1], Yusuf U.A[1a], AdenowoT.k[2], Badmus O.O[3], Ogunola T.A[4] , Caxton-Martins EA[2a]

[1]Department of Anatomy, Ladoke Akintola University of Technology,Ogbomoso, Oyo – state, Nigeria.
[2]Department of Anatomy, Olabisi Onabanjo University, Ikenne-Remo,Ogun-State, Nigeria.
[3]Department of Physiology, University of Ilorin,Ilorin,Nigeria
[4] Department of Chemical pathology, Ladoke Akintola University ofTechnology Teaching Hospital, Osogbo, Osun-State, Nigeria.

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 aliment 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 injection 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 quantitative enzymes histochemistry for alkaline phosphatase (ALP), acid phosphatase (ACP), lactate dehydrogenase (LDH) and glucose-6-phosphate dehydrogenase (G-6-PDH). The activities of alkaline phosphatase (ALP) was significantly high in Group B $p<0.05$. Groups A (30±1.08µ/L) and D (32±1.06µ/L) were similar and group C (41±0.8 2µ/L) were high but not as seen in Group B (46±0.41µ/L). The acid phosphatase (ACP) was significantly high in Group B $p<0.05$. Groups A (753±1.06µ/L) and D (756±1.08µ/L) were similar and group C (795±1.09µ/L) were high but not as seen in Group B (820±2.35µ/L).The activities of lactate dehydrogenase (LDH) were significantly high in Group B $p<0.05$. Groups A (2868±2.08µ/L) and D (3026±0.95µ/L) were almost similar and Group C (5373±1.08µ/L) were also high but not as seen in Group B (6116±0.73µ/L). The activities of glucose-6-phosphate dehydrogenase (G-6-PDH) were high in Groups A (8428±0.81µ/L) and D (7648±0.01µ/L). The activities were low in Groups B (6116±0.73µ/L) and C (6350±0.82µ/L) when compared to Group (8428±0.81µ/L), but the difference in Group C (6350±0.82µ/L) was not as low as in Group B (6116±0.73µ/L).

The above findings showed that aqueous extract of mistletoe reduced the adverse effects caused by cadmium.

Keywords : Mistletoe, cadmium, prefrontal cortex, ALP, ACP, LDH and G6PDH.

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 oligaemia), 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).

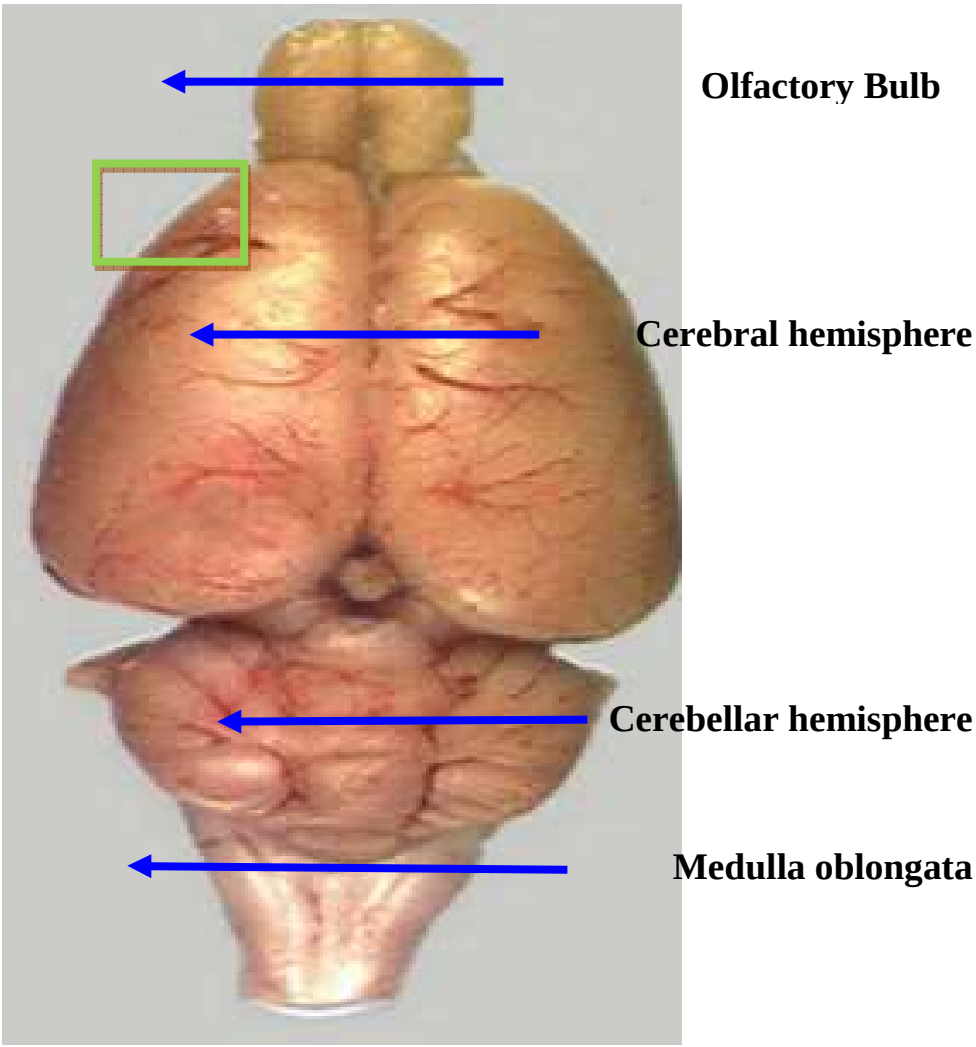

Figure 1: Illustration of some structures in the brain of rat (Fiala and Spacek, 2007). The green box indicates the area of the prefrontal cortex.

MISTLETOE

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). Cardiotonic 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, tri-terpines 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 enzyme quantitative histochemistry of alkaline phosphatase, acid phosphatase and enzymes of carbohydrate metabolism (G6PDH and LDH).

MATERIALS AND METHOD

MATERIALS

The following materials were used for this research:

Rats (24 male Wistar rats), Mistletoe,Cadmium,Normalsaline,Cages ,Feed/Chow,Hand gloves, Insulin needles & syringes, Weighing balance, Measuring cylinder,Conicalflask,Electric blender,Distilled water.

PLANT MATERIALS AND PREPARATION OF EXTRACT

The mistletoe was procured from Ladoke 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, Ladoke 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 and the tissues for enzymes studies were immediately placed in cold 0.25M sucrose solution at the ratio of 1:5 tissues to solution for homogenization.

Enzyme activities techniques

Prefrontal cortex tissues for enzyme studies preserved in cold 0.25M sucrose solution were homogenized using a porcelain mortar and pestle. The homogenates were poured into cold test-tubes and centrifuged at 5000 rpm for 5 minutes using a centrifuge (Model 90-1). The supernatants were collected using Pasteur pipettes and immediately stored in a deep freezer (GC-B207WVQ) at -20^0C, and thereafter assayed using appropriate enzyme activities kits. Activities of alkaline phosphatase (ALP), acid phosphatase (ACP), lactate dehydrogenase (LDH) and glucose-6-phosphate dehydrogenase (G6PDH) were determined through colorimetric method.

Alkaline Phosphatase

The activity of alkaline phosphatase in tissue homogenate was assayed using RANDOX Kit (Lohr and Waller, 1974).

Procedure:

1. 1.0 µl of sample was pipette into test tube labelled 'test'
2. 1.0 µl of distilled water was pipette into a test tubes labelled 'blank'
3. 0.5 µl of alkaline phosphatase buffer substrate was added to each test tube
4. The mixture were incubated at 37°C for 30 minutes
5. 5ml of 0.02M HCL were added to each mixture
6. The absorbance was read at 405nm

Acid Phosphatase

The activity of acid phosphatase in tissue homogenate was assay using RANDOX Kit (Kornberg, 1955, Makarem, 1974, Lohr and Waller, 1974).

Procedure:

1. 1.0 µl of sample was pipette into test tube labelled 'test'.
2. 1.0 µl of distilled water was pipette into a test tubes labelled 'blank'
3. 0.5 µl of acid phosphatase buffer substrate was added to each test tube
4. The mixture were incubated at 37°C for 30 minutes
5. 5ml of 0.02M NaOH were added to each mixture
6. The absorbance was read at 405nm

Lactate Dehydrogenase

The assay for this enzyme was done using an ultraviolet method RANDOX kit (Makarem, 1974).

Principle:

L-Lactate + NAD ⟶ Pyruvate + NADH

Reagents composition:

Buffer/substrate

Phosphate Buffer.....50 mmol/L, pH 7.5

Pyruvate.....0.6 mmol/L

VADH.....0.18 mmol/L

Pocedure:

This was done at a wavelength of 340 nm and temperature of 37 C. 0.05 ml of sample and 3.0 ml of reagent were pipetted into a cuvatte. This was thoroughly nixed and the initial absorbance read at 1minute and then 2 and 3 minutes. This process was used for prefrontal cortex.

Calculation.

U/L (37^{U}C) = 9683 x - A 340 nm/min.

Glucose-6-Phosphate Dehydrogenase

The activity of the enzyme in the prefrontal cortex was assayed using an ultraviolet method RANDOX kit (Lohr and Waller, 1974).*Principle:*Theenzyme activity was determined by measurement of the rate of absorbance change at 340 nm due to the reduction of NADP

G-6P + NADP ⟶ Gluconate – 6 – P + NADPH + H

Reagent 1(R1)	Component	Concentration (mmol/L)
	Triethanolamine	31.7
	EDTA	3.2
Reagent 2 (R2)	NADP	0.34
Reagent 3 (R3)	Glucose-6-Phosphate (G-6-P)	0.68

Table: 1 Reagents used for G-6-PDH study

Preparation:

The physiological reagents used are presented in the Table 4 below:

The assay was performed with NADP and glucoce-6-phosphate (G-6-P) under physiological conditions at 37^{0}C against air at wavelength of 340 nm and at light part of 1cm. 500uL of buffer R_1 was pipetted into a test-tube, 25uL of solution R_2 was added and 125uL of homogenate was added to the solution. The contents were shaken and incubated for 10minutes at 37^{0}C. Thereafter, 13uL of solution R_3 was added and readings were taken after 5 minutes.

Calculation:

Mu/m1 = 841 x ΔA340/min.

Where ΔA = change in absorbance/5mins.

Statistical Analysis

Data were analyzed using SPSS (SPSS Inc, Chicago, USA) and Excel 2007 (Microsoft Corporation USA). Data were expressed as mean ± standard error of mean (mean ±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).

RESULTS AND DISCUSSION

Morphological Observation

Average Weight/Week (in gram)

The table 5 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 6 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 2: 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 3: 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

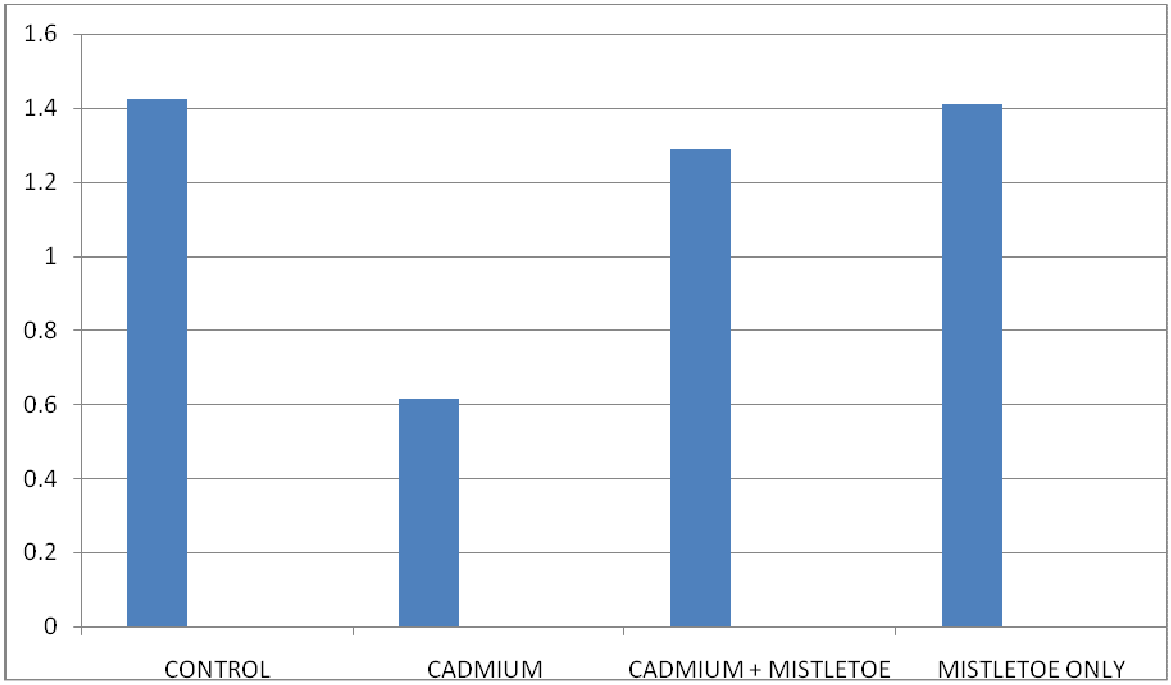

Figure3: Average brain weight (in gram) after four weeks

Quantitative Histochemical Observation (µ/L)

Prefrontal cortex

Table 4: Level of activity of alkaline phosphatase (ALP), acid phosphatase (ACP), lactate dehydrogenase (LDH) and glucose-6-phosphate dehydrogenase (G6PDH).

GROUP	ALP	ACP	LDH	G6PDH
A (CONTROL)	30±1.08	753±1.06	2868±2.08	8428±0.81
B (CADMIUM	46±0.41*	820±2.35*	7441±1.22*	6116±0.73*

ONLY)				
C (CADMIUM + MISTLETOE)	41±0.82*	795±1.09*	5373±1.08*	6350±0.82*
D (MISTLETOE ONLY)	32±1.06	756±1.08	3026±0.95	7648±0.01

Data are means±SEM; *significantly different from control, P<0.05.

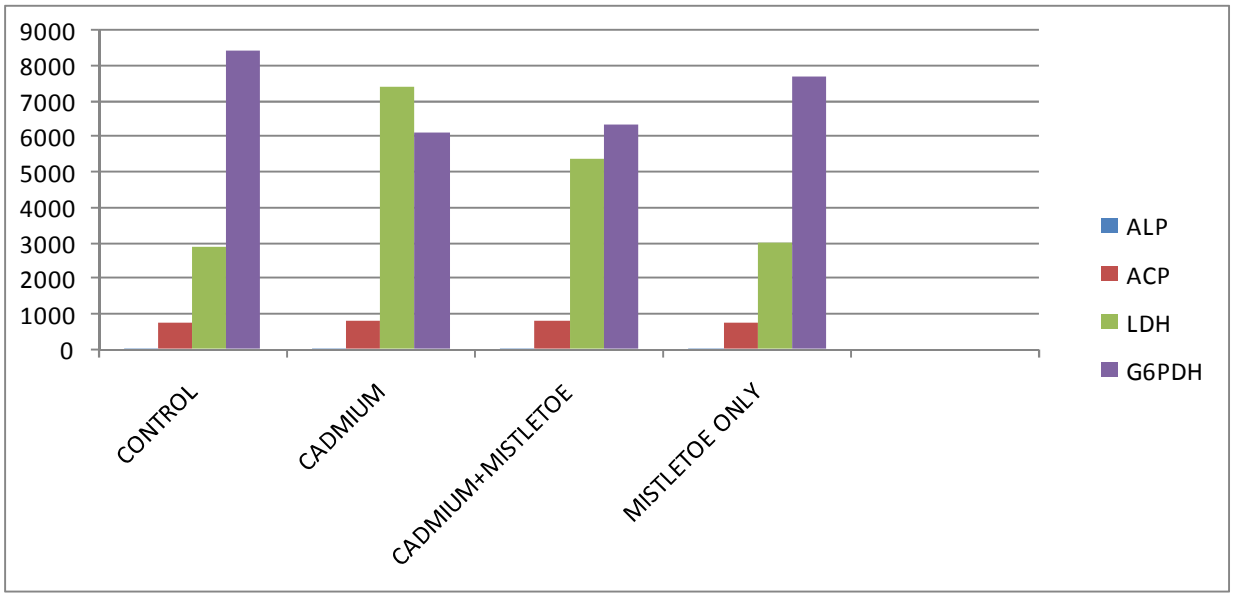

Figure 4: Level of activity of alkaline phosphatase, acid phosphatase, lactate dehydrogenase and glucose-6-phosphate dehydrogenase.

RESULTS AND DISCUSSION

Alkaline Phosphatase Analysis

Result of alkaline phosphatase assay (Table 4) indicates enzyme levels were highest in Group B rats, which received cadmium only (46±0.41µ/L). Enzymes activity was lowest in Group A rats (control), measuring 30±1.08µ/L. The enzymes levels in the prefrontal cortices of rats in Group C (cadmium+mistletoe) and Group D (mistletoe only) were in between the two levels at 41±0.82µ/L and 32±1.06µ/L respectively. Statistical analyses (Table 4) shows that the difference in activity of alkaline phosphatase between rats in Group C (cadmium+mistletoe) and those in control Group A is significant at P<0.05. A similar statistically significant difference is found between the enzyme's activities in the prefrontal cortices in Group B (cadmium only) rats and those of Group C (cadmium+mistletoe); (Table 4).

Acid Phosphatase Analysis

Result of acid phosphatase assay (Table 4) indicates enzyme levels were highest in Group B rats, which received cadmium only (820±2.35µ/L). Enzymes activity was lowest in Group A rats (control), measuring 753±1.06µ/L. The enzymes levels in the prefrontal cortices of rats in Group C (cadmium+mistletoe) and Group D (mistletoe only) were in between the two levels at 795±1.09µ/L and 756±1.08µ/L respectively. Statistical analyses (Table 4) shows that the difference in activity of acid phosphatase between rats in Group B (mistletoe only) and those in control Group A is significant at $P<0.05$. A similar statistically significant difference is found between the enzyme's activities in the prefrontal cortices in Group B (cadmium only) rats and those of Group C (cadmium+mistletoe); (Table 4).

Lactate dehydrogenase analysis

Result of lactate dehydrogenase assay (Table 4) indicates enzyme levels were highest in Group B rats, which received cadmium only (7441±1.22µ/L). Enzymes activity was lowest in Group A rats (control), measuring 2868±2.08 µ/L. The enzymes levels in the prefrontal cortices of rats in Group C (cadmium+mistletoe) and Group D (mistletoe only) were in between the two levels at 5373±1.08µ/L and 3026±0.95µ/L respectively. Statistical analyses (Table 4) shows that the difference in activity of lactate dehydrogenase between rats in Group C (cadmium+mistletoe) and those in control Group A is significant at $P<0.05$. A similar statistically significant difference is found between the enzyme's activities in the prefrontal cortices in Group B (cadmium only) rats and those of Group C (cadmium+mistletoe); (Table 4).

Glucose–6–phosphate dehydrogenase analysis

Result of glucose-6-phosphate dehydrogenase assay (Table 4) indicates enzyme levels were lowest in Group B rats, which received cadmium only (6116±0.73µ/L). Enzymes activity was highest in Group A rats (control), measuring 8428±0.81µ/L. The enzymes levels in the prefrontal cortices of rats in Group C (cadmium+mistletoe) and Group D (mistletoe only) were in between the two levels at 6350±0.82µ/L and 7648±0.01µ/L respectively. Statistical analyses (Table 4) shows that the difference in activity of glucose-6-phosphate dehydrogenase between rats in Group B (cadmium only) and those in control Group A is significant at $P<0.05$. A similar statistically significant difference is found between the enzyme's activities in the prefrontal cortices in Group B (cadmium only) rats and those of Group D (mistletoe only); (Table 4).

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 (Akerele, 1993).

The present study evaluated the possible ameliorative effects of mistletoe on the destructive effects of cadmium on the prefrontal cortex. The activities of alkaline phosphatase (ALP) was significantly high in Group B $p<0.05$. Groups A (30±1.08µ/L) and D (32±1.06µ/L) were similar and group C (41±0.82µ/L) were high but not as seen in Group B (46±0.41µ/L). The acid phosphatase (ACP) was significantly high in Group B $p<0.05$. Groups A (753±1.06µ/L) and D (756±1.08µ/L) were similar and group C (795±1.09µ/L) were high but not as seen in Group B (820±2.35µ/L).The activities of lactate dehydrogenase (LDH) were significantly high in Group B $p<0.05$. Groups A (2868±2.08µ/L) and D (3026±0.95µ/L) were almost similar and Group C (5373±1.08µ/L) were also high but not as seen in Group B (6116±0.73µ/L). The activities of glucose-6-phosphate dehydrogenase (G-6-PDH) were high in Groups A (8428±0.81µ/L) and D (7648±0.01µ/L). The activities were low in Groups B (6116±0.73µ/L) and C (6350±0.82µ/L) when compared to Group

(8428±0.81µ/L), but the difference in Group C (6350±0.82µ/L) was not as low as in Group B (6116±0.73µ/L).

Alkaline phosphatase is an enzyme present in almost all the animal cells. It involved in the uptake of phosphate and this process is called dephosphorylation and increase in the activities of these enzymes is a measure of tissue destruction (Nishimura *et al.*, 1992). As the name entails, it acts optimally at alkaline medium. From the study, the activity of this enzyme was highest in Group B animals. The activities of alkaline phosphatase in Groups A and D animals were almost similar. But the activities in this enzyme in Group C animals were more than Group D. This could explain that there was more dephosphrylation in cadmium induced animals. Also it showed that there was more cellular destruction in Group B animals than in Group C. The reduction in the activities of this enzyme might be due to the ameliorative effects of extract of mistletoe in tissue destruction. This could be explained in that there were flavonoids, lectins and phenolic compound present in mistletoe extract (Duong *et al.*, 2003).

Acid phosphatase is an enzyme present in almost all the animal cells. It involved in the uptake of phosphate and this process is called dephosphorylation and increase in the activities of these enzymes is a measure of tissue destruction (Nishimura *et al*., 1992). As the name entails, it acts optimally at Acid medium. From the study, the activity of this enzyme was highest in Group B animals. The activities of Acid phosphatase in Group A and D animals were almost similar. But the activities in this enzyme in Group C animals were more than Group D. This could explain that there was more dephosphrylation in cadmium induced animals. Also it showed that there was more cellular destruction in Group B animals than in Group C. The reduction in the activities of thisenzyme might be due also to the ameliorative effects of extract of mistletoe in tissue destruction.

Lactate dehydrogenase (LDH or LD) is an enzyme present in a wide variety of organisms, including plants and animals (Van Eerd, 1996). Lactate dehydrogenases exist in four distinct enzyme classes. Two of them are cytochrome c-dependent enzymes, each act on either D-lactate or L-lactate. The other two are NAD (P)-dependent enzymes, each act on either D-lactate or L-lactate (Light, 1972). Lactate dehydrogenase catalyzes the inter-conversion of pyruvate and lactate with concomitant inter-conversion of NADH and NAD^+. It converts pyruvate, the final product of glycolysis, to lactate when oxygen is absent or in short supply and it performs the reverse reaction during the Cori cycle in the liver (Joseph, 2002). At high concentrations of lactate, the enzyme exhibits feedback inhibition, and the rate of conversion of pyruvate to lactate is decreased. It also catalyzes the dehydrogenation of 2-Hydroxybutyrate, but it is a much poorer substrate than lactate (Dymo, 2002). If there is an increase in the level of lactate dehydrogenase beyond the normal level in the tissue, it undergoes degenerative changes in the cellular mechanism of action that may result to damage of cells or cell death, as noted in ischemia condition (Gallagher, 2004). From the study, the activity of this enzyme was highest in Group B animals. The activities of lactate dehydrogenase in Group A and D animals were almost similar. But the activities in this enzyme in Group C animals were less compared to Group B. This could explain that there were more degenerative changes that may result to damage of cells or cell death, as noted in ischaemia condition in cadmium induced animals Group B. The reduction in the activities of this enzyme might be due also to the ameliorative effects of extract of mistletoe on tissue destruction.

Glucose–6–phosphate dehydrogenase is an oxidative enzyme which liberates hydrogen and a substrate captures the hydrogen ion to form insoluble product (Cohen, 1976; Oakley, 1978). It is also an enzyme responsible for the initial deviation of glucose into the pentose phosphate pathway to form 6-phosphogluconate (Watkins, 1983). The pathway provides NADPH in the mitochondrial membrane of the neuronal cells for the conversion of oxidized to reduced glutathione which is essential for maintaining neuronal functions in the prefrontal cortex in the reduced state (Gale,

1985; Baron, 1989). The brain glucokinase has the highest affinity for glucose uptake (Oakly, 1978). For oxidation of glucose, or for conversion of carbohydrate to fat or protein, the glucose–6–phosphate can be converted in stages, via triose phosphates and phosphopyruvate, to pyruvate in the Embden–Meyerhof glycolytic pathway of oxidative phosphorylation (Baron, 1989). An alternative metabolic pathway for the oxidation of glucose, the hexose monophosphate shunt, which leads to the formation of NADPH (Watkins, 1983). Glucose–6-phosphate dehydrogenases are usually needed in a reduced form to release high energy–requiring ATP from the ADP and NADPH which are utilized by the cells (prefrontal cortex) during oxidative phosphorylation (Gale, 1985). From this study, the activity of this enzyme was highest in Group A animals while the activities of glucose-6-phosphate dehydrogenase in Groups B and C animals were almost similar. But the activities in this enzyme in Group D animals were more than Group C animals. This could be explained that there was more anabolic activities of G6PDH following G6PDH anabolic pathway to generate NADPH that eventually released ATP Group B animals has the least level of activity of G6PDH as compared to Groups A,C and D animals and this may due to damage caused by cadmium in the prefrontal cortex of Group B animals. The significant rise in activities of this enzyme in Group B animals compared to Group C showed that the effect there were flavonoids, lectins and phenolic compound present in mistletoe extract (Duong et al, 2003).

CONCLUSIONS

The results showed a variation in the enzyme histochemistry 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.

The varying data from the enzyme histochemistry analysis of the prefrontal cortex of the four groups of adult Wistar rats have a great evaluation showing that it is statistically significant. It is therefore reported that there is a enzyme histochemistry 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.

REFERENCES

[1]. Adeeyo O.A., Ajayi O.E., Salawu E.O., Omotoso O.D., (**2011**). Determination of LD50 of mistletoe and mango leaf extracts. Mec. J. Med Sci, 4:308-314.

[2]. Akerele O., (**1993**). Nature's medicinal bounty. World Health forum. 14:390-395.

[3]. Baron D.N., Whicher J.T., Lee K.E., (**1989**). A New Short textbook of Chemical Pathology 3rd Edn.

[4]. Cohen R.D., Wood H.F., (**1976**) Lactic Acidosis. Oxford Blackwell Scientific Publications.

[5]. Deeni Y.Y., and Sadiq N.M., (**2002**). Antimicrobial properies and phytochemical constituents of leaves of African mistletoe (Tapinanthus dodoneifolius (DC)Danser) (Loranthaceae): an ethnomedicinal plant of HausaLand, Northern. J. Ethnopharmacol. 83: 235-240.

[6]. DeYoung C. G., Hirsh J. B., Shane M. S., Papademetris X., Rajeevan N., Gray J.R., (**2010**). "Testing predictions from personality neuroscience". *Psychological Science.* 21(6): 820–828.

[7]. Duong Van Huyen J., and Delignat S., (**2003**). Comparative of the sensitivity of lymphoblastoid and transformed monocytic cell lines to the cytotoxiceffect of Viscum album extract of different origin. Chemotherapy 49:298-302.

[8]. Dymo O., Pratt E., Ho C., and Eisenberg D., (**2002**). The crystal structure of D-lactate dehydrogenase aperipheral membrane respiratory enzymes. Proc. Natl. Acad. Sci. 97(17): 9413-9418.
[9]. Frohne D., and Pfander H., (**1984**). Effect of Viscum album (mistletoe) extract on some body organs. Wolfe. Pub. London, 364-369.
[10]. Gale E., (**1985**) .The causes of hypoglycaemia. Br. J. Hosp Med.33: 159 – 62.
[11]. Galal-Gorchev H., (**1993**). Dietary intake, levels in food and estimated intakeof lead,cadmium and mercury Food Additive sand/Contaminants. 10:115-128.
[12]. Gallagher E., Deluxe O., (**2004**)., Cellular mechanism of action in relation to cell death. Med. B , 302-308.
[13]. Gupta A., Murthy R., Thakur S., Dubey M., and Chandra S., (**1993**). Neurochemical changes in developing rat brain after pre and postnatal cadmium exposure. Bull Environ.Contain Toxicol, 51:12-17.
[14]. Hoffman J.T., (**1989**). Control of western dwarf mistletoe with the plant growth regular. Northwest Research station. 89-102.
[15]. Ige S.D., Badmus J.A and Akinwale E.O., (**2009**). Allium cepa treats renal damage on cadmium induced. J. Nephr., 56:456-461.
[16]. Jarup L.B., Elinder C., Nordberg G., and Vahter M., (**1998**). Health effects of cadmium exposure review of literature and a risk estimate, Scandinavian Journal of VorkandEnvironmenlalHealth24. Supply.l±x52.
[17]. Jayaprakasha G.K., Singh R.P., and Sakarial K.K., (**2001**). Antioxidant activity of grape seed (Vitrus vinifera) extracts on peroxidation models in vitro. Food chem. 73:285-290.
[18]. Joseph J., Badnnath P., and Sahn S., (**2002**). Is albumin gradient of fluid to serum albumin ratio better than the pleural LDH in diagnostic of separation of pleural effusion. J,Pul. Med, 86-91.
[19]. Kornberg A., Liston C., and Carnuro J., (**1955**). Enzymology academic press, new York. 323.
[20]. Lauwerys R., Amery A., Bernard A., and Braux P (**1986**). Health effect of environmental exposure to cadmium objectives design and organization of the cadmium study. Environ. Health perspectives, 87,283-289.
[21]. Lide D.R., (**2000**). Magnetic susceptibility of the element and inorganic compounds. CRC handbook of chemistry and physic. 8Ed.
[22]. Light R., Macgreger M., Luchsinger P., and Ball W., (**1972**). Pleural effusions the diagnostic separation of transudites and exudates. Ann Interm. Med, 77(4) 507-513.
[23]. Lohr G.W., and Waller H.D., (**1974**). Glucose-6-phosphate dehydrogenase, method of enzymatic analysis. 3:636-640.
[24]. Lyu R., Levwi R., and Murray M., (**1998**). Are lectins of Viscum album interesting tools in treatment of disease. ZEKR Atrmungsorgane,166(3):247-256.
[25]. Makarem, A., (**1976**). Clinical Chemistry – Principles and Techniques 2nd Ed. Mc Laughlin M.J., and Singh B.R., (**1999**). Cadmium in soil and plants as a treat to human health. Academic publishers klluwer. 219-256.
[26]. Nishimura N., Nishimura H., Ghaffar A., and Tohyamu P., (**1992**). Localization of metallothionein in the brain of rat and mouse, J. Histochem cytochem. 40:309-315.
[27]. Oakley W.G., Pyke D.A., Taylor KW., (**1978**) Diabetes and Its management, 3rd Ed. Oxford: Blackwell Scientific Publications.
[28]. Obatomi D.K., Bikomo E.O., and Temple V.J., (**1994**). Antidiabetic properties of the african mistletoe in streptozotocin induced diabetic rabbits. J. Ethnopharm. 43:13 – 17.
[29]. Onay-Ucar R., Daniel V., and Elerk E., (**2006**). Immunomodulation with Viscum album andechinacea purpurea extracts. 10(3):27-33.

[30]. Reeves P., and Vanderpool R., (**1997**). Cadmium of men and women who report regular consumption of confectionary sunflower kernals containing a natural abundance of cadmium. Environ Health Perspective, 105: 1098-1104.
[31]. Salagapylak M., Pikila A., Klalka M., and Kowel T., (**2010**). The influence of intracerebral streptozotocin and cadmium on memory processes in mice exposed to transient cerebral oligemia. J. Toxicol. Environ. Health. 75(17-18):1159-1165.
[32]. Salawu E.O., Adeeyo O.A., and Ige S.D., (**2009**), the effect of cadmium on renal system of wiser rats. J. Med. 3(2)121-124.
[33]. Standring S., Borley N.R., Collin P., Crossman A.R., Gatzonlin M.A., Healy J.C., Newell R.L.M., and Wigley C.D., (**2008**). Gray's Anatomy (The anatomical Basis of Clinical Practice): Prefrontal cortex; 14 Ed., pg 308-352.
[34]. Van Eerd J.P.F.M., and Krentzer E.K.J., (**1996**)., klinische chemical voor Analisten deel. 138-139.
[35]. Watkins P.J., (**1983**). ABC of Diabetes. London. British Medical Journal. 2:14-17.
[36]. World Health Organization (**1989**). Evaluation of Certain Food Additives and Contaminants. Thirty-third Report of the Joint 10/WHO Expert Committee on Food Addrtives. WHO Technical Report Serie. 776. 28-33.
[37]. World health organization (**1989**). Evaluation of certain food additives and contaminants. Thirty-third repart of the joint/WHO expert committee.
[38]. Yiin S.J., Chem C.L., Sheu J.Y., Tseng V.C., Lin T.H., (**1999**). Cadmium-Induced renal lipid peroxidation in rats and protection by nium. J. Toxicoi. Iron Health A.Pubmed.23:57(6):403-13.