PRELIMINARY HISTOLOGICAL STUDIES ON THE EFFECT OF AQUEOUS FRUIT EXTRACT OF PHOENIX DACTYLIFERA L. (DATE PALM) ON LEAD ACETATE-INDUCED CEREBELLAR DAMAGES IN WISTAR RATS

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ABSTRACT
Aim: This study was to histologically assess the therapeutic effect of aqueous fruit extract of Phoenix dactylifera (AFEPD) on lead acetate-induced cerebellar damage in Wistar rats.

Methods: Twenty four rats were grouped into six (I–VI; n=4). Group I (control) received distilled water (1 ml/kg). Group II received lead acetate (LA, 120mg/kg) only. Groups III and IV received LA (120mg/kg) followed by AFEPD (1000mg/kg and 1500mg/kg, respectively). Groups V and VI received AFEPD (1000mg/kg and 1500mg/kg, respectively). All administrations were by oral route. Treatment lasted 28 days; LA was administered from day 1 to day 14, while AFEPD was administered from day 15 to day 28 of the experimental period. Therapeutic activity of AFEPD was assessed by histologic examination of the cerebellar cortex with H and E stain.

Results: Findings revealed neurodegenerative changes in the cerebellar cortex like perineuronal vacoulations and cytoplasmic shrinkage in molecular layer cells and Purkinje cells in LA-intoxicated group. The administration of AFEPD remarkably ameliorated LA–induced cerebellar damage dose-dependently. Normal cerebellar histoarchitecture was observed with administration of AFEPD only.

Conclusion: Results suggest that AFEPD has therapeutic potentials against lead acetate-induced cerebellar damage in Wistar rats.

Key words: Cerebellum, Lead acetate, Phoenix dactylifera

INTRODUCTION
Lead poisoning is a well-known public health risk especially in developing countries (Flora et al., 2012). Lead as a metal exists chemically in both organic (tetraethyl lead) and in the inorganic (lead acetate, lead chloride) forms in the environment (Shalan et al., 2005). Lead is highly toxic and can interrupt the body’s neurological, biological and cognitive functions (Elombah and HRW, 2012; Bauchi et al., 2016). The involvement of the nervous system in lead toxicity is well known (Martin et al., 1970; El-Neweshy and El-Sayed, 2011). In the CNS, symptoms of lead poisoning include dullness, forgetfulness, irritability, poor attention span, headache, fatigue, impotence, dizziness, and depression. Lead encephalopathy, a progressive and potentially fatal degeneration of the brain, is the most severe neurological effect of lead poisoning (ATSDR, 1999; Yun et al., 2011; Wagwas 2012). Cerebellar dysfunction may occur in association with exposure to a wide variety of toxins including heavy metals (such as mercury, lead, thallium, and manganese), drugs and solvents. These toxins may adversely affect the cerebellum directly or as part of a more generalized encephalopathy (Fredericks, 2011).
The cerebellum (little brain) is a region of the brain that plays an important role in motor control, but its movement-related functions are the most solidly established (Fine et al., 2002; Rebeer et al., 2013). The cerebellum does not initiate movement, but it contributes to coordination, precision, and accurate timing. Damage to the cerebellum produces disorders in fine movement, equilibrium, posture, and motor learning (Wolf et al., 2009). In folk medicine, plant extracts have a wide range of medicinal actions, and have been used to treat a variety of diseases. The biological activities of medicinal plants including their neuroprotective actions has become a popular area of investigation for scientists (Uddin et al., 2013; Bauchi et al., 2016). Phoenix dactylifera (date palm) is a member of the monocot family Arecaceae called “Nakhla” (Chandra et al., 1992). It is well cultivated and considered an important source of food in Middle East and North African countries. Dates are rich in certain nutrients and provide a good source of rapid energy due to their high carbohydrate content. The good nutritional value of dates is also based on their dietary fiber and on their essential minerals such as calcium, iron, magnesium, phosphorus, potassium, zinc, selenium and manganese (Sawaya et al., 1982; Mohamed and Al-Osabi, 2004; Al-shahib and Marshall, 1993). Studies have shown that that the aqueous extracts of the dates have potent antioxidants (Vayalil, 2002; Al-farshi et al., 2005). The antioxidant activity is attributed to the wide range of phenolic compound in dates including p-coumaric, ferulic, and sinapic acids, flavonoids and procyanidins (Regualu et al., 1987) and also to the presence of vitamin C (Allaith, 2007; Mrabet et al., 2008). The aim of this study was to histologically assess the therapeutic effect of aqueous fruit extract of Phoenix dactylifera (AFEPD) on lead acetate-induced cerebellar damage in Wistar rats.

MATERIALS AND METHODS

Experimental Animals
Twenty-four adult male Wistar rats (140 - 310g) were obtained from the Animal House of Faculty of Pharmaceutical Sciences, Ahmadu Bello University, Zaria, Kaduna, Nigeria and were housed in Animal House of Department of Human Anatomy, Faculty of Medicine, Ahmadu Bello University, Zaria and, acclimatized for a period of two weeks. The animals were housed under standard laboratory condition, light and dark cycles of 12 hours, and were provided with standard rodent pellet diet and water ad libitum. The rats were categorized into control and treatment groups. The treatment groups were administered, in addition to feed and water, AFEPD and/or lead acetate. The rats and their organs (brain) were weighed at the beginning, during and at the end of the study and organ (brain)/body weight ratio computed.

Plant Materials
Dried date palm (P. dactylifera) fruits were obtained from a local market in Zaria and authenticated in the Herbarium Unit of the Department of Biological Sciences, Faculty of Life Sciences, Ahmadu Bello University, Zaria with the voucher specimen number of 8017.

Extract Preparation
Preparation of P. dactylifera aqueous fruits extract was conducted in the Department of Pharmacognosy and Drug Development, Faculty of Pharmaceutical Sciences, Ahmadu Bello University, Zaria. The method of maceration (Agbon et al., 2013) for the preparation of aqueous P. dactylifera fruits extract was employed.

Drug
300g of lead acetate (LA), manufactured by BDH Chemicals Ltd Poole, England, was purchased and used as the neurotoxin in this study.

Experimental Procedure
The twenty-four rats were grouped in to six groups (I – VI; n=4). Group I served as control and received distilled (1 ml/kg). Group II received LA (120 mg/kg; 20% LD$_{50}$ (Sujatha et al., 2011)) only. Groups III and IV received LA (120 mg/kg) followed by AFEPD (1000 mg/kg and 1500 mg/kg, respectively). Groups V and VI received AFEPD (1000 mg/kg and 1500 mg/kg respectively). All administrations were by oral route and the treatment lasted for twenty-eight days; LA was administered from day 1 to day 14, while AFEPD was administered from day 15 to day 28 of the experimental period.

Animal Sacrifice and Collection of Samples
The rats were euthanized under chloroform anaesthesia and the organs (brain) harvested and fixed in Bouin’s fluid. The tissues were processed using the routine Haematoxylin and Eosin (H and E) stains for light microscopy.

Data Analysis
The data collected were analyzed using Sigma Stat 2.0 and the result expressed as mean ± SEM and presence of significant differences among means of the groups were determined using one-way ANOVA with Dunnett’s multiple comparison post hoc test for significance. Values were considered significant when p≤ 0.05.

RESULTS

Physical Observation
The rats in the control group showed normal physical activities, such as movement and playfulness, whereas rats in the treatment groups exhibited decreased activity especially in lead acetate–treated group. Differences were observed in the initial and final body weights of the rats. All treatment groups were observed to have increased in the body weights, except lead acetate (LA) only treated group which decreased significantly (p<0.05) in the body weight (Figure 1). Marked (p<0.05) decrease in the organ (brain) / body weight ratio (relative brain weight) in all the treated groups were observed, except in AFEPD (1000 mg/kg)-treated group when compared to the control (Table 1).

Histological Examination
Microscopic (histopathological) examination of tissue sections of the cerebellar cortex of the rats revealed the following: The cerebellar sections of rats in the control group showed normal histoarchitecture of the cerebellar cortex; the characteristic appearance of the three cortical layers: an outer molecular layer with distinct neurons and an inner granular layer. Sandwiched between these layers is a monolayer of flask-shaped Purkinje cells, the Purkinje cell layer (Plate 1). The cerebellar sections of rats treated with lead acetate revealed distortion in the histoarchitecture of the cerebellar cortex; cortical degenerative changes, such as satellitosis, perineuronal vacuolations and cytoplasmic shrinkage in the molecular layer, Purkinje cell layer showed perineuronal vacuolation and cytoplasmic shrinkage of Purkinje cells (Plate 2). The cerebellar sections of rats treated with lead acetate (120 mg/kg) followed by AFEPD (1000 mg/kg and 1500 mg/kg) revealed mild perineuronal vacuolation and cytoplasmic shrinkage of the Purkinje cells when compared to the severe histoarchitectural distortion observed in the lead acetate treated group (Plate 3 and Plate 4). The cerebellar sections of rats treated with AFEPD (1000 mg/kg and 1500 mg/kg) only showed normal histoarchitecture of the cerebellar cortex (Plate 5 and Plate 6).

Table 1: Brain/ body Weight Ratio of Wistar Rats Treated with Aqueous Fruit Extract of P. dactylifera against Lead acetate –induced Neurotoxicity

<table>
<thead>
<tr>
<th>Groups</th>
<th>Treatments</th>
<th>Organ (brain)/ body weight ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Distilled water (1 ml/kg)</td>
<td>0.252 ± 0.001</td>
</tr>
<tr>
<td>II</td>
<td>LA (120 mg/kg)</td>
<td>0.177 ± 0.001 ***</td>
</tr>
<tr>
<td>III</td>
<td>LA (120 mg/kg) + AFEPD (1000 mg/kg)</td>
<td>0.248 ± 0.001 *</td>
</tr>
<tr>
<td>IV</td>
<td>LA (120 mg/kg) + AFEPD (1500 mg/kg)</td>
<td>0.214 ± 0.001 ***</td>
</tr>
<tr>
<td>V</td>
<td>AFEPD (1000 mg/kg)</td>
<td>0.254 ± 0.001</td>
</tr>
<tr>
<td>VI</td>
<td>AFEPD (1500 mg/kg)</td>
<td>0.233 ± 0.001 **</td>
</tr>
</tbody>
</table>

n=4; Mean ± SEM. ANOVA, Dunnett’s multiple comparison Post hoc test; *= p < 0.05, **= p< 0.01, ***= p< 0.001 significant difference when compared with the control. AFEPD= Aqueous fruit extract of P. dactylifera, LA= lead acetate.
DISCUSSION

In this study, physical observation of body weight differences, relative organ (brain) weight and histopathological examination of cerebellar sections were employed to assess the therapeutic effect of aqueous fruit extract of P. dactylifera in Wistar rats. Decreased physical activity exhibited by LA-treated rats is suggestive of treatment-related toxicity. This is in agreement with reports on drug-related toxicity; altered physical activity manifesting as sluggishness and loss of appetite indicates drug-related toxicity (Toma et al., 2009; Agbon et al., 2014). Body weight changes serve as a sensitive indication of the general health status of animal (Salawu et al., 2009) and, used as an indicator of adverse effect of drugs and chemicals (Mikinda and Syce, 2007). The result of the study showed that exposure to LA significantly (p<0.05) decreased the body weight and relative brain weight of the rats when compared to the control. This significant decrease in body weight and relative brain weight could be attributed to the toxic effects of LA. This is in accordance with the findings of Wahab et al. (2010) and Haouas et al. (2014). It has been reported that lead toxicity causes malabsorption of nutrients and less efficient metabolic processes (Struzynska et al., 1997) which could be the result of observed decrease in body weight. Observed increased (p>0.05) body weights in AFEPD-treated groups could be related to the plant's nutritional composition and its relatively high caloric value (Agboola and
Adejumo, 2013; Mohamed et al., 2014; Shaba et al., 2015). Lead is known to cause damage in several tissues and possibly disturb the normal biochemical process. Mechanisms of lead-induced tissue injury include increased production of reactive oxygen species (ROS), and induced oxidative stress which results in DNA damage (Xu et al., 2008; Sharma et al., 2010). DNA alteration may occur either via caspase 3 activation or oxidative stress by generating the release of reactive oxygen species (ROS) such as superoxide radicals, hydrogen peroxide and hydroxyl radicals and lipid peroxidation (Xu et al., 2005; El-Nekeety et al., 2009). The brain is the most sensitive target of lead toxicity because it contains relatively low levels of enzymes that are capable of protecting it against oxidative stress (Goetz and Washburn, 1999; Waggas, 2012). In this present study, exposure of rats to LA revealed several histoarchitectural distortion in the cerebellar cortex manifesting as degenerative changes which are suggestive of neurotoxicity. Degenerative changes observed as, perineuronal vacuolations and cytoplasmic shrinkage in the Purkinje cell layer and molecular layer of the rats treated with LA when compared to the control group are indicative of LA related neurotoxicity. These findings agree with the reports of previous researchers on heavy metal induced nervous tissue damage (Fakunle et al., 2013; Ibegbu et al., 2013). Musa et al. (2012) and Farina et al. (2013) attributed many heavy metals such as lead, mercury, cadmium and other organic compounds to have the capacity to damage nervous system, and the most sensitive elements of the cerebellar cortex to these chemicals are the Purkinje cells in the Purkinje cell layer. In this study, LA possibly acted as a neurotoxicant to the cerebellar cortex, thus, distorting neuronal integrity. The degenerative effect of LA on the cortical layers of the cerebellum observed in this study may be responsible for the cerebellar degeneration. Mild distortion in the histoarchitecture of cerebellar cortex of rats treated with AFEPD (1000 mg/kg and 1500 mg/kg) after the administration of LA (120 mg/kg), such as perineuronal vacuolation and cytoplasmic shrinkage of the Purkinje cells when compared to the severe histoarchitectural distortion observed in the LA treated group, are indicative of AFEPD neuroprotective activity. Treatment with extract of P. dactylifera has been reported to attenuate oxidative stress induced cortical neuronal damage by bilateral common carotid artery occlusion (Pujari et al., 2011). Agbon et al. (2016) reported that administration of aqueous fruit extract of P. dactylifera showed preserved histoarchitecture of the cerebellar cortex parenchyma and cytoarchitectural preservation of neuronal cells Nissl substance in Wistar rats intoxicated with heavy metal. This suggest that AFEPD has ameliorative effect on LA induced neurotoxicity. Wan Ismail and MohdRadzi (2013) has reported marked decrease in neuronal damage in the form of shrinkage, atrophy and necrosis of neurons and increased levels of endogenous antioxidants in the brain of Wistar rats treated with fruit extract of P. dactylifera. Antioxidant effects have been implicated for ameliorative activity of P. dactylifera (Panahi et al., 2008; Kalantaripour et al., 2012). Rahmani et al. (2013) implicated the therapeutic effect of date fruits in the neutralization of reactive oxygen species to its anti-oxidant properties. Some of the reported antioxidants with ameliorativeactivity present in extracts of P. dactylifera are vitamin E, ascorbic acid and melatonin; a potent antioxidant and free radical scavenger with special tendency to brain (Vayalil, 2002; Al-farshi et al., 2005; Al-Qarawi et al., 2008). Phenolic compounds such as flavonoids with strong antioxidant properties, strong reactive oxygen species scavengers and metal ions chelating ability has been reported as one of the phytoconstituents of P. dactylifera (Biglari et al., 2008; Pujari et al., 2011). In this study, administration of AFEPD ameliorated the neurotoxic effect of LA in a dose-dependent manner. This is similar to the report of Agbon et al. (2016). In this study, normal histoarchitecture was observed in the cerebellar cortex of rats treated with AFEPD (1000 mg/kg and 1500 mg/kg) only. This implies that extract at these doses are relatively safe and beneficial to the brain by utilizing its antioxidant properties.

CONCLUSION

Findings of the present study suggest that administration of aqueous fruit extract of Phoenix dactylifera have ameliorative potentials on the histology of lead acetate-induced toxic effects in the cerebellum of Wistar rats. The ameliorative effect could be tied to the antioxidant properties of the plant extract.

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