European Journal of Medicinal Plants

21(2): 1-9, 2017; Article no.EJMP.37302
ISSN: 2231-0894, NLM ID: 101583475

Lead Acetate Induced Cerebral Tissue Damage; The Effect of *Phoenix dactylifera* Pits Extract

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Authors' contributions

This work was carried out in collaboration between both authors. Authors IMOK and IEO designed the study, performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript. Author IMOK managed the analyses of the study. Author IEO managed the literature searches. Both authors read and approved the final manuscript.

Article Information

DOI: 10.9734/EJMP/2017/37302
Editor(s):
(1) Ahmed Moussa, Pharmacognosy and Api-Phytotherapy Research Laboratory, Mostaganem University, Algeria.
(2) Marcello Iriti, Plant Biology and Pathology, Department of Agricultural and Environmental Sciences, Milan State University, Italy
Reviewers:
(1) Ioana Stanciu, University of Bucharest, Romania.
(2) Shi Lei, China Three Gorges University, China.
(3) Dinithi Peiris, University of Sri Jayewardenepura, Sri Lanka.
Complete Peer review History: http://www.sciencedomain.org/review-history/21817

Original Research Article

Received 10th October 2017
Accepted 4th November 2017
Published 9th November 2017

ABSTRACT

This study investigates effect of *Phoenix dactylifera pits* extract (PdPE) on Lead acetate induced cerebral tissue damage. Wistar rats of average weight 150 g were divided into seven groups of six animals each. GRPI animals received distilled water only, GRPII received 60 mg/kg Lead acetate 5 times a week for 3 weeks then distilled water only for ten days (Negative control), GRPIII and GRPIV (treatment groups) received 60 mg/kg Lead acetate 5 times a week for 3 weeks then treated with 150 mg/kg PdPE and 300 mg/kg PdPE respectively for 10 days, Group V and Group VI (protective groups) received 150 mg/kg PdPE and 300 mg/kg PdPE respectively for 10 days then 60 mg/kg Lead acetate 5 times a week for 3weeks, while GRPVII animals received 60 mg/kg Lead acetate 5 times a week for 3 weeks then treated with 25 mg/kg Dimercaptosuccinic acid (DMSA) alone 4 times a week then distilled water for 3 days.

Lead acetate induced cerebral tissue damage was evident from depleted reduced-glutathione and lipid peroxidation as shown by elevated malondialdehyde and nitrite concentration. Histological examination of the cerebral tissue showed congestion of the meningeal vessels and cellular

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infiltration. Malondialdehyde and nitrite were significantly reduced by 300 mg/kg PdPE (P<0.03). 300 mg/kg PdPE protective and treatment groups and 25 mg/kg DMSA ameliorates antioxidant depletion and showed significant protective effect against cerebral tissue damage. Memory assessment showed that 300 mg/kg PdPE and 25 mg/kg DMSA treatment significantly alleviate memory impairment induced by Lead acetate.
Results from the study indicate that PdPE has the ability to alleviate lead acetate induced cerebral tissue damage in rats. PdPE may exert its protective and therapeutic effect against lead-induced cerebral damage possibly through its antioxidant mechanisms and due to the presence of membrane protecting unsaturated fatty acids.

Keywords: Lead; lead-acetate (PbA); phoenix dactylifera pit extract (PdPE); cerebral tissue damage.

1. INTRODUCTION

Lead is one of the most studied heavy metals. This can be attributed to its use and misuse. This ubiquitous heavy metal have been known as major contaminants of the environment, airways and waterways, posing a great deal of health hazard to both humans, especially children and aquatic life. Studies have proposed that one possible mechanism of Lead (Pb) toxicity is the disturbance of pro-oxidant and antioxidant balance by generation of reactive oxygen species [1]. Disturbance of prooxidant and antioxidant balance can evoke oxidative damage of critical molecules such as proteins and macromolecules such as DNA and RNA.

Lead is also known to induce a broad range of impairment and dysfunctions in laboratory animals and humans, including central and peripheral nervous systems [2], haemopoietic system [3], cardiovascular system [4], kidney [5], liver [6], and male and female reproductive systems [7,8].

Studies have shown that exposure to lead have different adverse effect in the central nervous system. The pathophysiology of Lead in the CNS include impairments of cognitive functions, behavioral deficits and physical tissue damage, [9]. Symptoms following acute or chronic exposure to lead could appear immediately or may be delayed, examples include loss of memory and learning capabilities, behavioural aberrations, cognitive dysfunction e.t.c. More so, Lead also acts as a chemical stressor that alters homeostatic balance of both cellular and molecular mechanisms. Chemical stress often emanates initially at the anatomical site, for example, in the central nervous system where the cerebral tissue and the neurotransmitter systems which are crucial in modulating memory and learning are affected.

Phoenix dactylifera, Date fruit (*Phoenix dactylifera*) has become an important fruit in some countries as a source of nutrition and economics [10-16]. Date fruit consists of 73-79% carbohydrates, 14-18% total dietary fibers, 2.1-3.0% protein [17], and 2.0-3.2% fat [14], depending on the variety of the date fruit. The Food and Agriculture Organization of the United Nations reported that during 2010, the total world production of dates have exceeded 7million tons, meaning that approximately more than 1 million tons of date seeds were produced during that year.

Based on the phytochemical studies, date fruits contain anthocyanins, phenolics, sterols, carotenoids, procyanidins and flavonoids [18]. The antioxidant activity is recognized due to the wide range of phenolic compounds present these include p-coumaric, ferulic, and sinapic acids, flavonoids, and procyanidins [19,20]. Other study showed that palm date fruits constitute thirteen flavonoid glycosides of luteolin, quercetin, and apigenin at different stages of maturity, [21,22]. The most concentrated fatty acids were oleic acid (44.51 g/100 g), palmitic acid (23.05 g/100 g), linoleic acid (11.66 g/100 g) [23]. These natural compounds are known to function as free radical scavenger, antioxidant, antimutagenic, anti-inflammatory, hepatoprotective and nephroprotective agents [24].

The therapeutic effects of *Phoenix dactylifera* are attributed to its polyphenolic content [25]. Plant polyphenols have been found to possess a range of effects: estrogenic and anti-estrogenic activity, anti-proliferative activity, induction of cell cycle arrest and apoptosis, prevention of oxidation, regulation of the host immune system, anti-inflammatory activity, modulation of effect of cytochrome P450 enzymes involved in activation of pro-carcinogens, upregulation of genes producing anti-oxidant enzymes, and the ability to change cellular signaling [26].

Several studies and reviews have identified the abundant availability of anti-oxidant, especially polyphenols in date fruit as exceptional to that of other fruits, however, there are limited studies on its effect against environmental pollutants, especially lead induced oxidative stress in certain organs. Hence, present study investigates the Protective and Therapeutic effects of ethanolic extract of *Phoenix dactylifera* on Lead acetate induced cerebral tissue damage.

2. MATERIALS AND METHODS

2.1 Chemicals

Lead acetate (PbA) [$(C_2H_3O_2)2Pb{\cdot}3H_2O$] was obtained from the Department of Pharmacology and Therapeutics, University of Ibadan, Nigeria; meso 2,3 Dimercaptosuccinic acid was obtained from sigma chemical Co., Buffers, thiobarbituric acid; 5,5-dithiobis (2-nitrobenzoic acid), (DTNB), Griss reagent, e.t.c. All other chemicals were of analytical grade.

2.2 Animals

Wistar rats weighing 130-155 g were obtained from the central animal house of the faculty of Basic Medical Sciences, University of Ibadan. The rats were housed in transparent plastic cages, floored with soft a wood shaving that was changed three times in a week. The animals were acclimatized for 2weeks prior the study and were maintained under a 12 h light/dark cycle at 25°C ± 2°C), with free access to water and rat chow.

2.3 Preparation of Extracts

Date fruits (*Phoenix dactylifera*) were purchased from a local market in Ibadan, Oyo state, Nigeria. The seeds were carefully removed from the edible portion (pits or flesh) of the fruits. The Pits was air dried for 15 days, and then pounded using a mortar and pestle. 400 g of the powdered form was soaked in 800 ml of 70% ethanol and then covered with a piece of aluminum foil for 72 hrs. The mixture was filtered by a piece of double gauze and the filtrate was allowed to settle. The supernatant was evaporated using a rotatory evaporator. The 400 g of dried *Phoenix dactylifera* pits (PdP) yielded 43.09 g of crude extract (PdPE).

2.4 Dosage and Drug Preparation

Design: $1/10^{th}$ of Oral LD_{50} of Lead Acetate for Wistar rats (60 mg/kg) was administered orally via oral cannula [27,28].

DMSA was dissolved in distilled water and 25 mg/kg was administered 4times a week and was administered orally via oral cannula;

Phoenix dactylifera Pits Extract (PdPE) was dissolved in distilled water and was administered orally via oral cannula.

2.5 Memory Assessment

Rats were subjected to a three-arm Y maze for 5 mins with all three arms opened. The number and the sequence of arms entered were recorded. The dependent variables were activity defined as the number of arms entered, and percent alternation, calculated as the number of alternations (entries into three different arms consecutively) divided by the total possible alternations (i.e., the number of arms entered minus 2) and multiplied by 100, [29].

2.6 Grouping of Animals and Experimental Design

Group I: Distilled water only (Vehicle),
Group II: Received 60 mg/kg Lead Acetate once daily for 5 days for 3weeks then Distilled water for ten days (Negative Control),
Group III: Received 60 mg/kg Lead Acetate once daily for 5 days for 3weeks then treated with 150 mg/kg PdPE for 10 days (Treatment Group),
Group IV: Received 60 mg/kg Lead Acetate once daily for 5 days for 3 weeks then treated with 300 mg/kg PdPE for ten days (Treatment Group),
Group V: Received 150 mg/kg PdPE for 10 days then 60 mg/kg Lead Acetate once daily for 5 days for 3weeks (Protective Group),
Group VI: Received 300 mg/kg PdPE for 10days then 60 mg/kg Lead Acetate once daily for 5 days for 3weeks (Protective Group)
Group VII: Received 60 mg/kg Lead Acetate once daily for 5 days for 3 weeks then treated with 25 mg/kg DMSA alone once daily for 4 days then Distilled water for 3 days (Positive Control)

PdPE (*Phoenix dactylifera* Pits Extract); Lead Acetate (PbA).

2.7 Biochemical Assays

2.7.1 Reduced glutathione (GSH)

Concentration was determined spectrophotometrically by the method of Beutler

[30]. The method was based on the reduction of 5,5 dithiobis (2-nitrobenzoic acid) (DTNB) with GSH to produce a yellow compound. The reduced chromogen is directly proportional to GSH concentration and its absorbance was measured at 405 nm. GSH content was measured in µg/g tissue.

2.7.2 Determination of lipid peroxidation

Lipid peroxidation was estimated by measuring the level of malondialdehyde (MDA) in cerebral tissue. MDA level of the homogenate was determined spectrophotometrically using thiobarbituric acid reactive substances (TBARS) as described previously [31]. Thiobarbituric acid (TBA) reacts with MDA in acidic medium at temperature of 95°C for 30 min to form TBA reactive product. The absorbance of the resulting pink product was measured at 534 nm. MDA values were expressed as µg of MDA/g tissue.

2.7.3 Determination of nitrite level

Nitric oxide formation was measured in the cerebral homogenate by assaying nitrite spectrophotometrically using the Griss reagent (1% sulfanilamide in 5% phosphoric acid (sulfanilamide solution) and 0.1% N-1-naphthylenediamine dihydrochloride in distilled water (NED solution) [32].

2.7.4 Macroscopic examination

- Each animal was weighed every 5days interval before sacrifice
- Physical examination was carried out to identify any malformation
- Mortality rate was determined by the formula:

$$\frac{\text{Number of dead animals}}{\text{Total number of animals}} \times 100$$

2.7.5 Animal sacrifice and collection of samples

At the end of the experiment, the animals were anaesthetized with chloroform and then sacrificed by cervical dislocation. Blood samples were collected into plastic tubes via cardiac puncture. The blood was allowed to clot then centrifuged at 3000 rpm for 20 min for serum separation for enzymatic and biochemical parameters estimation.

2.7.6 Tissue specimens

The Brain was isolated, rinsed in ice-cold saline and were weighed. The macroscopical appearance of the Brain was also recorded.

2.8 Biochemical Measurements

The Cerebrum was carefully separated from the entire brain and was homogenized in Phosphate buffer (pH 7.4). The homogenate was then centrifuged at 3000 rpm for 15 min at 4°C, and the supernatant was collected for biochemical assays.

2.9 Histopathological Study

The Cerebrum of randomly selected animal from each group was removed and fixed in 10% formalin. Paraffin sections of 5 nm thick, were routinely stained with haematoxylin and eosin (H&E) [33] and assessed in a light microscope (Olympus 320).

2.10 Statistical Analysis

Data were evaluated with program of Graph pad prism version 5.0. Statistical analysis was done using the ANOVA and test for comparison of data in the control groups with the experimental groups. The results were expressed as mean ± S.E.M (standard error of means). $P < 0.05$ was considered as statistically significant.

3. RESULTS

3.1 Mortality Rate

33.33% Mortality rate was recorded in Group II on the 17th day of experiment.

4. DISCUSSION

Lead Acetate causes changes in the normal structure and function of the cerebrum. As shown from the study, 60mg/kg lead acetate induces cerebral tissue damage that was evident from elevated malondialdehyde, nitrite and depleted reduced glutathione.

Nineteen different flavonoid glycosides of luteolin, quercetin, and apigenin have been found to exist in *Phoenix dactylifera* in methylated and sulfated forms. Analysis of mass spectral data has suggested that sulfates are linked to these flavonol glycosides as opposed to phenolic hydroxyls, making *Phoenix dactylifera* the only fruit or vegetable known to contain flavonoid sulfates [34].

Table 1. Effect of *Phoenix dactylifera* Pits Extract (PdPE) and DMSA on Lead Acetate (PbA) induced changes in cerebral tissue reduced Glutathione (GSH), Malondialdehyde (MDA) and Nitrite in rats

	GROUPS						
Biochemical Parameters	**Vehicle**	**PbA only**	**PbA+150 mg/kg PdPE**	**PbA+300 mg/kg PdPE**	**150 mg/kg PdPE+ PbA**	**300 mg/kg PdPE+ PbA**	**PbA+25 mg/kg DMSA**
GSH (µg/g)	224.04±15.04	49.64±4.08	90.20±4.90*	143.81±9.72**	61.79±3.11	156.59±5.55**	129.61±5.99**
MDA (µg/g)	76.70±5.09	258.34±13.05	152.47±8.09*	102.63±5.04$^{**\#}$	131.01±5.11*	93.74±4.03$^{**\#}$	144.61±12.90*
Nitrite (µg/g)	69.14±3.77	227.61±11.07	163.55±7.91*	89.22±3.47$^{**\#}$	141.33±9.20*	83.44±5.12$^{**\#}$	149.23±5.53*

PbA- Lead Acetate: PdPE- Phoenix dactylifera Pits Extract: DMSA- 2,3 Dimercaptosuccinic acid; Result expressed as Mean±SEM: [(P<0.05) vs PbA only: **(P<0.03) vs PbA only; $^{\#}$(P<0.05) vs DMSA]*

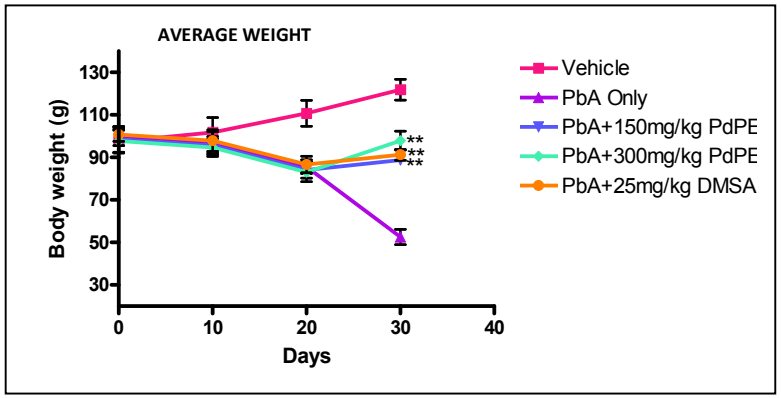

Fig. 1. Comparism of effect of *Phoenix dactylifera* Pits Extract (PdPE) and DMSA on Lead Acetate induced changes in Average weight of animals in Treatment groups. [*(P<0.05) vs PbA only: **(P<0.03) vs PbA only; $^{\#}$(P<0.05) vs DMSA]

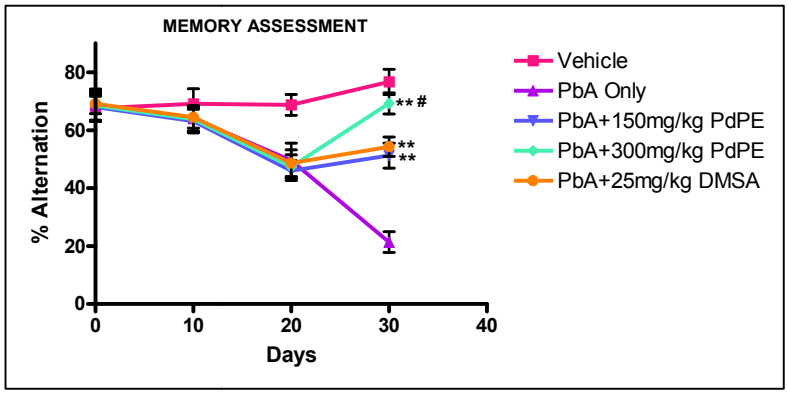

Fig. 2. Comparism of effect of *Phoenix dactylifera* Pits Extract (PdPE) and DMSA on Lead Acetate induced Memory impairment in animals in Treatment groups; [*(P<0.05) vs PbA only: **(P<0.03) vs PbA only; #(P<0.05) vs DMSA]

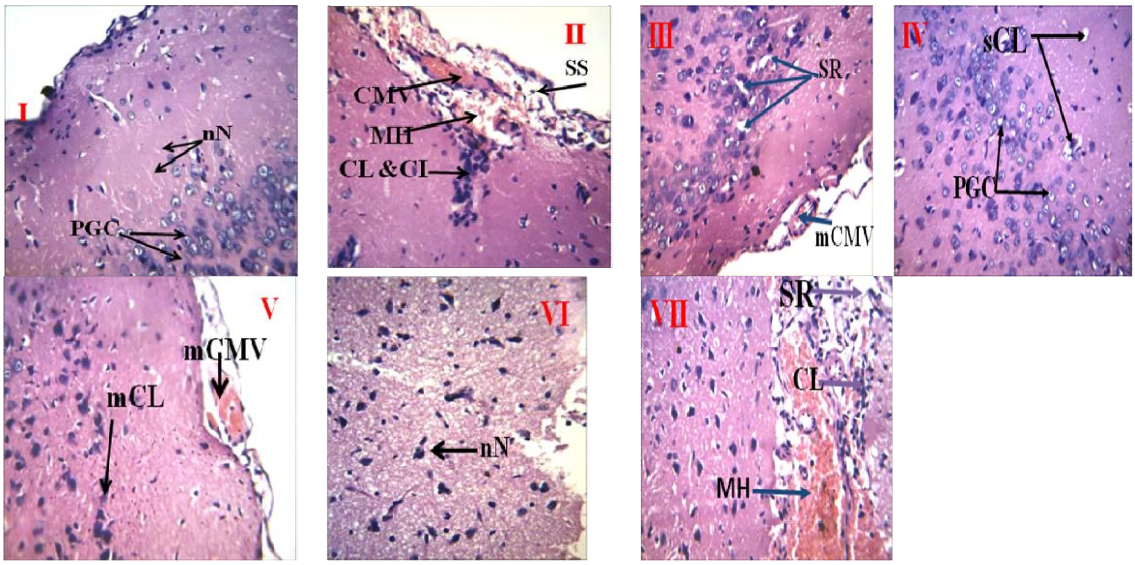

Fig. 3. Histology of the cerebral tissue of rats brains

nN- normal Nucleus; GC- Glial Cells; CL- Chromatolysis; mCL- Chromatolysis; CI-Cellular Infiltration; SS- Severe Spongiosis; SR- Spongiotic Regions; CMV- Congested Meningeal Vessel; mCMV- mild Congestion of Meningeal Vessels; MH- Meningeal Hemorrhage; PGC- Proliferating Glial Cells

GroupI-(Control) Cerebral photomicrograph showing normal nucleus and proliferating glial cells (Haematoxylin and Eosin stain, ×200), Group II- (Lead acetate only) Cerebral photomicrograph showing congested meningeal vessels, severe spongiosis, Chromatolysis and meningeal hemorrhage (Haematoxylin and Eosin stain, ×200), Group III-(Lead acetate +150 mg/kg PdPE) Cerebral photomicrograph showing spongiotic region and mild congestion of the meningeal vessels. Group IV- (Lead acetate +300 mg/kg PdPE) showing mild Chromatolysis and proliferating glial cells (Haematoxylin and Eosin stain, ×200). Group V- (150 mg/kg PdPE + Lead acetate) Cerebral photomicrograph showing mild congestion of the meningeal vessels and mild Chromatolysis (Haematoxylin and Eosin stain, ×200). Group VI- (300 mg/kg PdPE+ Lead acetate) Cerebral photomicrograph showing mild Chromatolysis and normal nucleus (Haematoxylin and Eosin stain, ×200). Group VII- (Lead acetate + 25 mg/kg DMSA) Cerebral photomicrograph showing mild meningeal hemorrhage spongiotic region and Chromatolysis (Haematoxylin and Eosin stain, ×200)

Phoenix dactylifera showed dose dependent protective and therapeutic effects. Biochemical parameters show that low dose 150 mg/kg PdPE had a significant effect *P*<0.05 while 300 mg/kg PdPE had *P*<0.03 against negative control groups that received lead acetate only. Memory impairment induced by lead acetate was significantly prevented and reduced by 300mg/kg PdPE than the conventional drug DMSA. This is in line with report that most conventional

chelating drugs do not have effect on already established damage or lesion in the central nervous system [35].

Lead alters certain membrane bound enzymes, which lead to oxidative stress as lead crosses the blood brain barrier readily. The impairment of memory due to exposure to lead can be attributed to the reduction of Acetylcholine and Acetylcholinesterase system as reported by [36,2,27].

The difference in the average weight of the brain tissue of animals in all groups was not statistically significant, however, **s**tructural and cellular damages observed in the histology photomicrograph showed that animals that received lead acetate only showed severe spongiosis, congestion of meningeal vessels and cellular infiltration. The degenerative and necrotic changes observed in the cerebral tissue represent ischemic changes resulting from congestion of the meningeal vessels. High dose 300 mg/kg PdPE in both protective and therapeutic groups protected and ameliorated cellular infiltration and congestion of meningeal vessels. Animals that received DMSA showed a moderate meningeal hemorrhage and spongiotic regions. The observation agrees with the reports of Federicks, 2011, [37] that many heavy metals and other organic compounds have capacity to damage nervous system and most sensitive elements of the cerebral cortex.

Results showed that lead acetate causes significant progressive decrease in body weights. Animals in the negative control group (lead acetate only) had reduction in body weight ($P<0.05$) when compared with all other groups. The anorectic effect exercised by lead is used to justify its involvement in the nerve transmission system (catecholaminergic, glutamatergic and serotonin) [38]. However, 300 mg/kg PdPE protective group showed a significant increase in body weight when compared with the positive control groups that received 25 mg/kg DMSA and low dose 150 mg/kg PdPE, ($P<0.05$).

Biochemical analysis of homogenized cerebral tissue showed a significant increase ($P<0.03$) in MDA and Nitrite in animals that received lead acetate alone as compared to all other groups. MDA and nitrite are clinical markers of oxidative stress, which occurs in lead exposure. MDA and nitrite were greatly reduced by 300 mg/kg PdPE. This is more significant $P<0.05$ than in animals that received DMSA ($P<0.05$), with efficacy of 300 mg/kg PdPE > 25 mg/kg DMSA > 150 mg/kg PdPE respectively.

Lead induces oxidative stress occurs on account of two different pathways operating simultaneously. First, the generation of reactive oxygen species, ROS and then the depletion of antioxidant. The level of reduced glutathione (GSH) was significantly reduced in animals that received lead acetate only as compared with other study groups ($P<0.05$). PdPE showed a significantly efficacious effect pertaining to preserving and or elevating the level of GSH ($P<0.05$). Studies on different parts and products of date fruit, that is, pits, seed and date oil, has shown that the fruit has the capacity to act as potent scavengers of reactive oxidative species. The antioxidant activity of the phenolic compounds in *Phoenix dactylifera* is as a result of the redox properties which play an important role in absorbing and neutralizing free radicals generated by lead acetate. *Phoenix dactylifera* also inhibit the lipid peroxidation cyclooxygenase enzymes COX-1 and COX2 [39].

Therefore, as shown by this study, *Phoenix dactylifera* has the ability protect the body at the cellular and tissue level when faced with lead induced oxidative damages.

5. CONCLUSION

Extracts that have specific establish phytochemicals may provide potential prevention of a number of health problems or diseases. The Protective and Therapeutic activities of *Phoenix dactylifera* Pit Extracts may be due to the presence of numerous antioxidants and phytochemicals that directly and indirectly scavenges reactive oxygen species, hence inhibiting oxidative damages induced by Lead (Pb).

Combination therapy involving Antioxidants of natural sources, chelating agent and supportive care when necessary is hereby recommended in Lead intoxication.

CONSENT

It is not applicable.

ETHICAL APPROVAL

All authors hereby declare that "Principles of laboratory animal care" (NIH publication No. 85-23, revised 1985) were followed, as well as

specific national laws where applicable. All experiments have been examined and approved by the appropriate ethics committee.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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