Physiological and Histopathological Effect of Argan (*Argania spinosa* L.) Seed Oil on Kidney Male Rats Exposed to Lead

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Abstract: The preset study has conducted to evaluate the possible protective effects of argan (*Argania spinosa* L.) seed oil on kidney toxicity induced by Lead (Pb) in male albino rats. Four groups of rats were used in these experiments. Rats of the first group were representing as control. The second group of rats was exposed to Pb (100 mg/kg) three times weekly. Rats of the third group was treated with argan seed oil (600mg/kg body weight/day) plus Pb. The fourth group was supplemented with argan seed oil at the same dose given to third treatment. After six weeks, the physiological and histopathological alterations were estimated. The kidney of rats exposed to Pb revealed some physiological and histological changes. The findings of this work indicated that argan seed oil slightly debilitated the physiological and histological alteration induced by lead. Furthermore, the result of the present study suggests that the antioxidant properties of argan seeds oil could be an attributed to the protective effect against toxicity induced by Pb.

Key words: Argan Seed Oil · Lead Toxicity · Kidney Histological · Physiological Changes

INTRODUCTION

Environmental pollution is one of the major problems of the world and it is increasing day by day due to urbanization and industrialization [1]. Environmental pollution is the pollution that gets into the ecosystem causing instability, disturbance and harm to physical systems and living organizations [2]. Human are exposed to various types of harmful environmental pollutants during their life stages due to use of toxic chemicals and some synthetic materials such as heavy metals compounds [3]. The global burden of disease linked to environmental factors that estimated by the World Health Organization more than 25%, including exposure to toxic chemicals that are increasingly had been used and many of them are introduced every year [4].

Heavy metals are natural group of minerals that has atomic density mainly for metals of the earth's crust more than 4 g/cm³ or 5 times or greater than water, so it can neither been degraded or destroyed [5]. They are a heterogeneous group of different elements in the chemical properties and biological functions, classified under the category of environmental pollutants because of the toxicity effects on living, organism, plants, animals and humans [6]. Heavy metals are serious due to tend to bioaccumulation which is known as an increase in the concentration of a chemical /toxicant in an organism over time compared with the normal concentration of the chemical in the environment [5]. There are toxic metallic elements even at relatively low levels of exposure because they have a relatively high density [7].

There are many heavy metals in our environment, lead (Pb), cadmium (Cd), chromium (Cr), copper (Cu) and other small amounts of which may be necessary for good health but are toxic when taken in large quantities so that can not metabolized by the body and accumulate in soft tissue [8]. The toxic affects that through intervention in the metabolism and mutations [7].

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Pb is a natural heavy metal, which was found as oxide or salts [9]. It had found in the earth, air, water, soil, dust, food and tissues of living organisms including human [10]. It has used widely in the industry and is a great environment health problem for all living things [11]. Pb found in pesticides, water pipes, in construction, bullets of gun, in petroleum refining, x-ray and atomic radiation protection [12]. Pb is used in the manufacture of storage batteries, the production of chemicals and paints and gasoline additives [10].

Pb poisoning had known since the Roman time, Pb uptake in the liver and then passes on the kidneys, where a small amount of it has excreted in the urine and the rest accumulates in the tissues of the body [13]. In children, the lower blood limit level of lead poisoning was 60 μg/dL in 1960s, but this value as reduction to 10 μg/dL in 1991. The US reported later through the Centers for Disease Control and Prevention, they no longer see that there in the proportion of Pb in the blood to be safe in children [14]. The toxicity of lead is due to the ability to induce the oxidation process through the generation of reactive oxygen species and reducing the efficiency of the antioxidant defense system resulting in damage to the organs [13, 15]. In living organisms, antioxidants are substances inhibit oxidative stress and work in different ways, such as catalytic removal of free radicals as scavengers of free radicals or in the form of proteins that minimize the availability of pro-oxidants such as metal ions [16]. The antioxidant enzymes such as catalase (CAT), superoxide dismutase (SOD) and glutathione peroxidase (GPx) they are working to reduce the toxicity of Pb [17]. Reactive oxygen species can mitigated the effects of the toxic by some chemical substances such as vitamin C, vitamin C, selenium (Se), N-acetyl cysteine dimercaptosuccinic acid, calcium disodium ethyldiaminetetra acetic acid and metatonin which have antioxidant properties [13].

In recent years, the World Health Organization (WHO) has identified some traditional medicine, which includes some herbal medicine that have treatment known hundreds of years before the modern medicine has developed and is still use now [18]. There is increasing interest from researchers in the study of the preventive effects of natural antioxidants against some toxins and finding natural compounds from medicinal and food plants has antioxidative properties that defend inside and outside the cell as lipid peroxides and oxygen radicals in response to oxidative stress [19]. Recently some studies have shown the efficacy of plants extracts to relieve and prevent the toxic effect of Pb, which has characterized as effective and retain good result when added to the diet, which also have a few side effects compared to chemical treatment [20]. Previous research has reported the positive effects of many of these herbs such as Rosemary [21], Green tea [22], Curry leaves [23] and others giants Pb toxicity.

Argan oil is extracted from kernel of the argan tree (*Argania spinosa* (L.) Skeel; Sapotaceae), which is one of endemic oldest forest tree in southwestern of Morocco and Algeria (Fig. 1(A-D)) [24, 25].

The chemical components of argan oil made it one of the highest quality plant seed oils that have a high nutritional and dietetic value [26].

The chemicals composition of argan oil has expressed in fatty acids including polyunsaturated fatty acids (PUFA), monounsaturared fatty acids (MUFA) and saturated fatty acids (SFA). Many other compounds in argan oil that are carotenes, tocopherols, phenols, triterpene alcohols, sterols, squalene and xanthophylls, these compounds make argan oil a rich source of antioxidants has an important role in organism [24, 27, 28]. The present study was carried out to assess the possible protective effects of argan seed oil on kidney toxicity induced by Lead (Pb) in male albino rats.

MATERIALS AND METHODS

The experimental animals male albino rats of the wistar strain (*Rattus norvegicus*), weighting 130-170g, were used in this research. Male albino rats were brought from the experimental animal Unit King Fahd Medical Research Central, King Abdul-Aziz University, Jeddah, Saudi Arabia. They were housed in standard plastic cage located in laboratory room with an ambient temperature of 21 ± 1°C and up to 12h of height daily. They were fed add libitum on normal commercial chow and had free access to water.

Forty rats have split into four experimental groups each consists of ten rats. Rat of group 1 served as the control and rat of group 2 were given lead acetate by oral daily. Rats of group 3 were orally supplemented with argan oil, also given lead acetate after 4 hours. The last, group 4 has given argan oil by oral daily.

Using the digital balance, individuals and total body weight of rats was determined at the beginning of the experimental and after 6 weeks.

After six weeks the rats were fasting for 12 hour, blood was collected from orbital venous plexus in non-heparinized tube, after centrifugation at 2500 rpm for 15 min the serum was separated immediately and until determination of albumin, total protein, creatinine, blood urea nitrogen and uric acid the samples were stored at 4°C [29].

Rats were dissected immediately after blood sampling and the tissues of the kidney were preserved in formalin (10%) after removal from the rats. The kidney tissues were prepared into sections and stained with hematoxylin and eosin according to method described by Dunn [30]. kidney sections were examined using light microscope (Olympus BX61- USA) connected to motorized controller unit (Olympus bx-ucb- USA) and photographed by a camera (Olympus DP72- USA) in the microscope unit at King Fahd Medical Research Center [31-34].

Statistical Analysis: Each data were analyzed using the Statistical Package for Social Sciences (SPSS for windows, version 21.0). All quantitative measurements were expressed as mean standard deviation (S.D.) of experimental animals. The experimental data has subjected to statistical analysis by conducting analysis of variance (ANOVA) to determine differences between the mean values of experimental groups. The significant of P-values less than 0.05 was considered.

RESULTS

The body weight of all expremintal groups after 6 weeks were showed different results (Graph 1). The values of body weight was changes in different treatments and apeard a moderate decline in rats exposed Pb plus argan compared to the control group and Pb group. Simple rise in the values of body weight in rats terated with Pb and that treated with argan oil compared to the control group.

Levels of uric acid, blood urea nitrogen and creatinine were represented in Table (1) and Graph (2). Significant increase in uric acid rate in rats exposed to Pb plus argan oil (17%) and compared with control group, also there is a slight rise in rats treated with lead only (3.8%). Regression in urea levels was significantly observed in rats exposed to oil (21%), Pb exposed plus oil (15.1%) and rats exposed Pb (11.5%) compared with control group. Increase in creatinine levels (13.4%) in the group of rats exposed to Pb plus oil and group of rats exposed to oil compared with control rats.
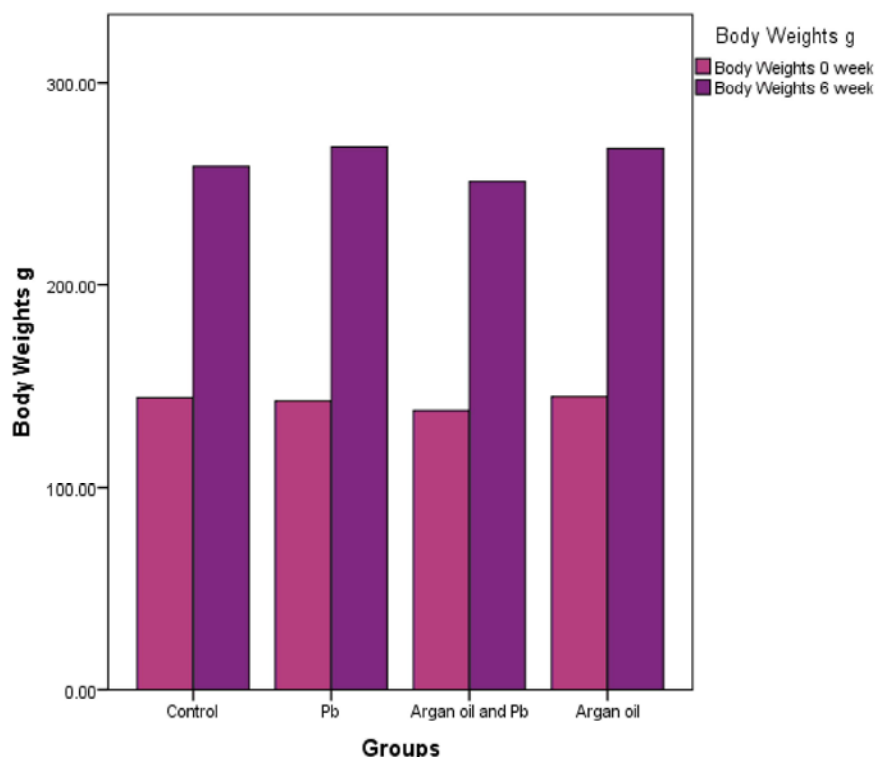
Graph 1. Comparison of body weight in different experimental groups.
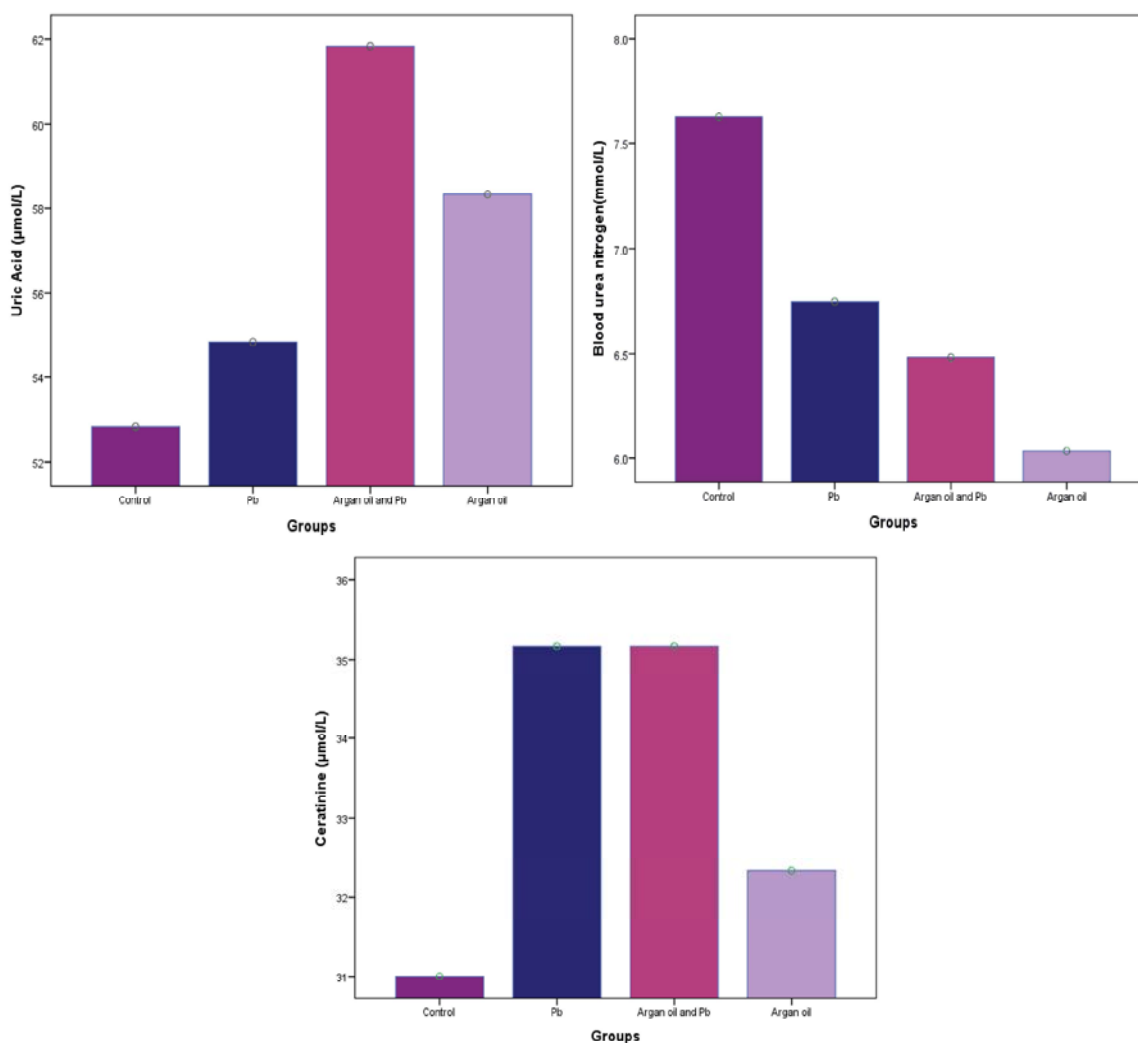
Graph 2: Comparison of uric acid, blood urea nitrogen and ceratinine of different experimental groups

Table 1: The serum levels of blood urea nitrogen, uric acid, blood urea nitrogen and creatinine in different experimental groups after 6 weeks.

Parameters	(Group1) Control	(Group2) Lead acetate	(Group3) Lead acetate + argan oil	(Group4) Argan oil
Uric acid (µmol/L) Significance	52.83±12.00	54.83±23.73[1]p =.996	61.83±11.56 [1]p =.735	58.33±8.73[1]p =.922
Blood urea nitrogen (mmol/L) Significance	7.63±1.30	6.75±1.14[1]p =.559	6.48±1.36[1]p =.338	6.03±0.68[1]p =.110
Creatinine (µmol/L) Significance	31.00±6.32	35.17±5.38[1]p =.520	35.17±5.38[1]p =.520	32.33±3.14[1]p =.970

Value are expressed as means± Std. Deviation, n=6. [1]p: Significance versus control group

Table 2: The serum levels of albumin and total protein after lead exposure and argan treatment in different studied group after 6 weeks

Parameters	(Group1) Control	(Group2) Lead acetate	(Group3) Lead acetate + argan oil	(Group4) Argan oil
Total protein (mmol/L) Significance	66.33±3.26	65.33±5.57[1]p =.988	62.83±5.34[1]p =.675	62.83±6.67[1]p =.675
Albumin(mmol/L) Significance	12.83±1.47	12±1.54[1]p =.793	11.67±1.75[1]p =.579	11.17±1.47[1]p =.283

Value are expressed as means± Std. Deviation, n=6. [1]p: Significance versus control group

Levels of total protein and albumin were represented in Table 2 and Graph 3. Slight reduction in total protein levels (3.5%) in rats exposed to Pb plus oil and rats exposed to oil compared with control group. Significant reduction in rat albumin levels, increase in the group of rats exposed to oil (13%), Pb plus oil (9.1%) and group of rats exposed to Pb (6.5%) compared with control rats.

Microscopic examination of kidney sections from control group appeeard normal glomerulus structure, double walled of the parietal epithelial cells surround the
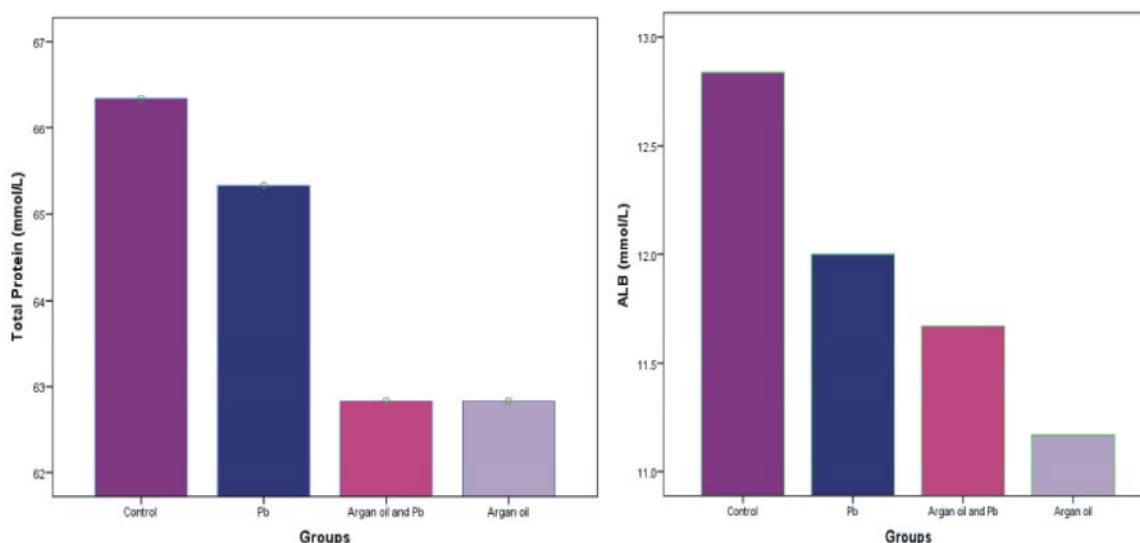
Graph 3: Comparison albumin and total protein in different studied group

bowman's capsule and bowman's space between the layer at natural description. The proximal tubules showed a distinctive border brush of microvilli. In the distal tubules have clear lumen and large and the cell lack of microvilli (Fig. 1 A).

The kidney sections from Pb group showed obvious changes in renal tubules include hyaline casts and dilation lumena in renal tubules, varying degrees of degeneration and the differences between proximal and distal tubule were not evident. There are changes observed in these samples deformation and degeneration in glomeruli structure and dilation and disintegration in the layers causes the expansion in bowman's capsules, the vascular pole were indistinct (Fig. 1 B).

The microscopic examination Pb plus argan oil group of kidney sections appeared mild positive reaction as compared with group 2. Less degeneration changes in renal tubules and dilation in space of bowman's capsules with congestion in glomerular capillaries (Fig. 1 C). In argan oil group the kidney, sections showed the glomerulus structure and renal tubules are close to being normal in comparison with control (Fig. 1 D).

DISCUSSION

The results of this study have shown the toxic effect of Pb on kidney, which has been shown changes of physiological parameters and tissue. Kidney is the first target in the toxicity of Pb because of their ability to expand and accumulate [35]. Previous studies have shown that oxidative stress is due to lead toxicity in terms of its

direct and indirect effect on the balance of antioxidants in cells [36]. Nwokocha *et al.* [37] showed that more accumulation with oral exposure in spleen, lungs and blood with values for kidney and stomach being significantly (p <0.05) higher in male wistar rats when compared with females.

Creatinine and blood urea nitrogen compounds resulting from protein metabolism in the normal kidneys filtered through the glomeruli into the urine. The increases of these substances in the present study refer to defect and damage in the kidneys. In normal conditions, uric acid is in a state of balance that has mainly excreted from the kidneys and when it increases its rate in the blood, it indicates a defect in kidney function [38]. The present study reveal that increase in level of uric acid and creatinine and decrease blood urea nitrogen in rats exposed to Pb than with control group which shows that Pb may lead to renal dysfunction.

On the contrary, the decrease in the level of albumin and total protein in rats exposed to Pb compared with control rats. The result was consistent with Abdou and Hassan [39] demonstrated that administration of lead acetate mitigate the levels of creatinine and urea and also decrease the levels of albumin and total protein which indicated the occurrence of kidney failure.

This result was also supported by a study of Abdel-Moneim *et al.* [40] to evaluate the effects of lead on kidney function and oxidative stress, the results observed low total protein level, revealed the harmful action of lead on the kidney tissue by augmented oxidative stress.
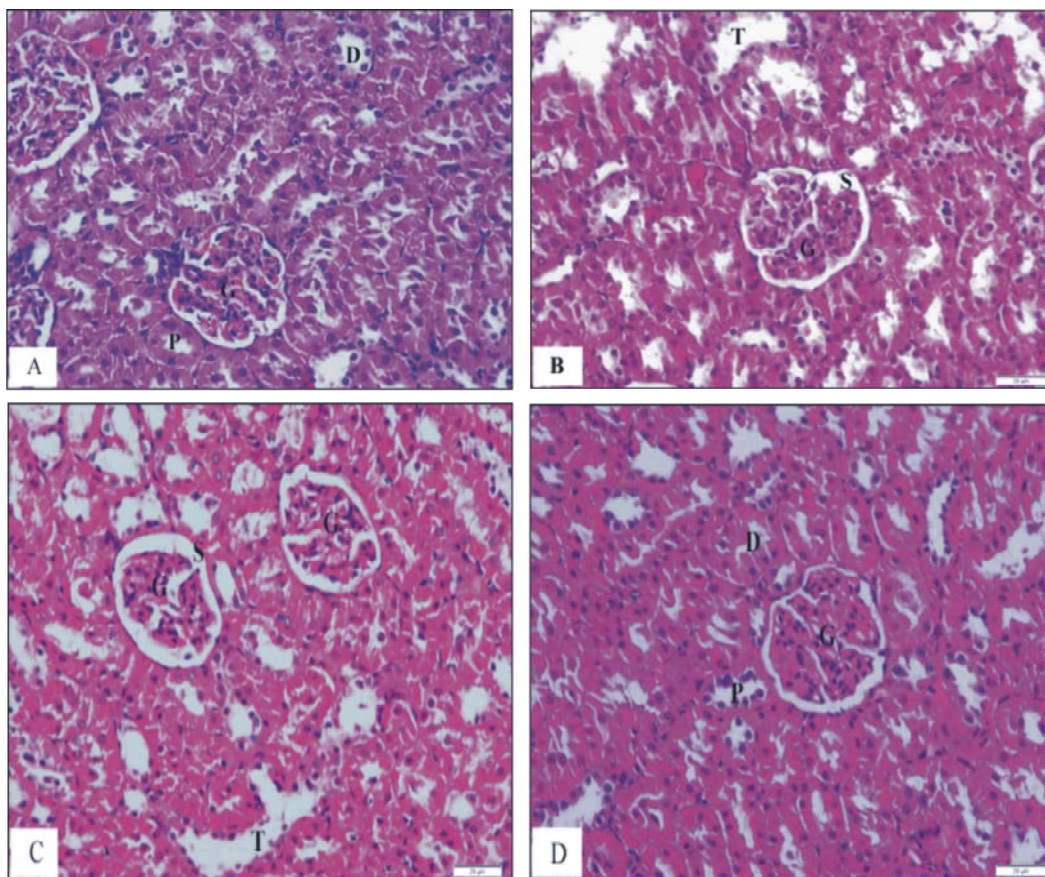
Fig. 1: (A-D) Photomicrograph of rat kidney with hematoxyline and eosin (x40)

    A: Photomicrograph of rats kidney of the control group showed normal glomeruli structure (G) and the proximal tubules (P) distal tubules (D) appear intact.

    B: Photomicrograph of rats kidney of the Pb group showed degeneration in glomeruli structure (G), expansion in bowman's capsules (S) and hyaline casts and dilation lumena in renal tubules (T).

    C: Photomicrograph of rat kidney of Pb group showed congestion capillary in glomerular (G) and degeneration changes in renal tubules (T).

    D: Photomicrograph of rat kidney of argan oil group showed normal glomerulus structure (G) and the proximal tubules (P) distal tubules (D).

Histological examination of both rats exposed to Pb in this research showed the degenerative changes of kidney structure, degeneration in glomerular and renal tubular alterations indicating the toxicity of Pb. Similar observations were reported by Dkhil *et al.* [41]. The harmful effect of lead acetate on renal tissue returned to excessive oxidation in the production of free radicals, which leads to cell injury and programmed cell death [42].

The present study observe that when rats were treated with Pb with argan oil, a slight decreased in albumin and protein levels in serum than it was of Pb group . In addition, a slight reduce levels of blood urea nitrogen, increase in uric acid level and also found that no significant differences in the levels of creatinine than it was in the group Pb. Moreover, histological examination showed a slight improvement in glomerular and renal tubular compared to Pb. The results proved that treatment with argan oil had used to reduce the toxic effect of Pb on renal tissue. Argan oil, which contains active ingredients that have, possesses an antioxidant activity in addition to induce free radical scavenging enzyme system [43]. In previous study proved that argan oil rich in tocopherols and other compounds such as unsaturated fatty acids and sterols, which has been beneficial effect on the blood pressure through the antioxidant properties at the level of vascular in chronically glucose-fed rats [44].

Argan oil reduced the metabolic effects of obesity, which induce the increase in plasma hydroperoxide, thiobarbituric acid-reacting substances and susceptibility of low-density lipoprotein [25]. A 2017 in argan oil studies showed that a protective effect against acrylamide toxicity in rats as appear by levels of hydroxydeoxyguanosine (8-OHdG), thiobarbituric acid reactive substances (TBARs). Protein carbonyl (PCO), glutathione (GSH) myeloperoxidase (MPO) levels, also concentrations of hematological parameters, the ratio of polychromatic erythrocytes (PCEs) and frequencies of micronucleus (MN) and megakaryocytic emperipolesis (ME) [45].

These results indicate that the oil of Argan may have potential therapeutic effects against the harmful effects of the kidney caused by lead. Recommendations include consideration and further study of these findings and increased interest in the study of argan oil in terms of its beneficial effect in oxidative stress.

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