Evaluation of Performance of Date Palm Pollen on Urea and Creatinine Levels in Adult Female Rats Exposed to Lead Acetate Intoxication

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Abstract
Background and objective: Exposure to lead continues to a serious public problem. The objective is to elucidate the effect of lead acetate exposure on serum Concentration of urea and creatinine in adult female rats.

Methods: Forty-eight adults female rats divided into four groups (12 rats/group). Group 1, (T1), given date palm pollen (150 mg/kg. B.W.) Group 2, (T2) given lead acetate (10mg/kg. B.W.), group 3, (T3) given lead acetate (10mg/kg. B.W.) and date palm pollen (150 mg/kg. B.W) for 6 weeks, Group 4(Control), given distilled water. Serum concentration of urea and creatinine was evaluated via ELISA method for all groups.

Results: The mean concentration of urea showed no significant difference between (T1) and (T3) while in T2 a significant (p < 0.05) increase in urea concentration compared to T1&T3. There was no significant difference within groups in urea concentration. Group (T1) exposed to DPP showed no significant decrease in serum creatinine on zero, 14, 28 day & 42 day post exposure respectively. Group (T2) exposed to lead acetate showed significant increase of creatinine concentration compared with control and (T1) group. Group (T3) showed significant decrease in creatinine levels compared with T2 group and these levels become closely to control group.

Histological changes indicated that DPP significantly ameliorate the toxic effects of lead acetate in glomeruli, collecting tubules associated with obvious decrease in inflammatory response.

Conclusion: DPP has the ability to counteract the toxic effect of lead acetate associated with improvement of renal histology and serum concentration of urea and creatinine.

Keywords: Date palm pollen, lead acetate, urea, creatinine

1. Introduction
Antioxidant activity is recognized due to the wide range of phenolic compounds present in dates including p-coumaric, ferulic, and sinapic acids, flavonoids, and procyanidins[1][2]. Palm date fruits constitutes thirteen flavonoid glycosides of luteolin, quercetin, and Phytochemical studies of Date palm pollen (DPP) showed the presence of estrone, α-amirin, triterpenoidal saponins, flavonoids and a crude gonadotrophic substance[3]also revealed the presence of estrone, estradiol and estriol, besides five flavonoids compounds.Antioxidants are chemicals that interact and deactivate the free radicals, therefore preventing them from causing harm[4]. The prevention of action of free radical is important step in the management of disease. Medicinal plant and their constituents play a vital and significant action to neutralize or inhibit the free radical by the use of antioxidant activity[5].

Date palms play a significant role in neutralization of free radical and finally suppress the various types of diseases development and progression. Earlier investigation found that Palme date has a potent ability to suppress free radicals[6]. Antioxidant components in the date (e.g. melatonin, vitamin E and ascorbic acid) were suggested to be the basis of the nephro protection[7]. The cerebroprotective effect of Phoenix dactylifera may be due to its antioxidant activity[8]. Palm pollen grain decrease creatinine
which treated with CCL\textsubscript{4}[9] Phoenix dactylifera has potential role to protect cellular damage caused by oxidative stress generated by free radicals production in body[10].

There are about 40 heavy metals which are capable of combining with wide variety of organic molecules and are potent enzyme inhibitors because of their interaction with ligands present in proteins and they inactivates the enzymes system of cell[11].

Lead after absorption is carried to various tissues of the body. In blood it causes basophilic stippling due to its inhibitory effect on α-Amino levulinate dehydratase enzyme which affects hemsynthesis[12][13]. Renal toxicity occurs in two forms, reversible usually seen after acute exposure of children to lead acetate and irreversible interstitial nephropathy is more commonly observed in long-term industrial lead exposure[12][13].

Lead toxicity produces histopathological changes in the renal proximal tubular epithelium which causes interstitial nephritis and often associated with hypertension; It accumulates in proximal convoluted tubules of renal cortex produces both morphological and biochemical evidences of toxicity.Although a lot of work has been undertaken to observe the effect of acute exposure of kidney to lead[14]. Environmental lead poisoning is an increasing health burden and chronic exposure to high levels of lead leads to adverse effect on renal function in both animals and humans[15]. Lead induced renal damage also occurs in the absence of acute intoxication so that occult lead nephropathy may not be recognized as such[15].

Chronic accumulation of lead in the body eventually leads to impairment in renal function[16]. Urea an creatinine are a waste product of amino acid metabolism they removed by kidney[17]. Oxidative stress appears to be involved in the development of renal toxicity. Environmental lead exposure cause significant pathological lesions on the renal systems of men and animals[18].

2. Material and Methods

Eighty-four adults female rats purchased from pharmaceutical and toxicological investigations department – Ministry of Health-Iraq were used in experiment. Rats weighing 250 – 300 gms, They were housed at animal house of College of Veterinary medicine, Diyala University, Iraq and fed on standard commercial rat diet, care was taken regarding maintenance of optimal light and temperature. They were given respective identification marks and kept in a separate wire screen cage. Date palm pollen was administered in a dose (150 mg/kg. B.W.) and lead acetate(10mg/kg. B.W.) [19].

The animals were divided at random into four groups comprising (12) rats each. Group 1, (T1), given date palm pollen (150 mg/kg. B.W.) Group 2, (T2) given lead acetate (10mg/kg. B.W.), Group 3, (T3) given lead acetate (10mg/kg.B.W.) and date palm pollen (150 mg/kg. B.W) for 6 weeks. Group 4(Control), given distilled water. Blood collected at (0, 14, 28 and 42) days respectively to determine Serum concentration of urea and creatinine via ELISA method for all groups according to (RAN-DOX®)[20], and Bio Maghreb®[21] instructions respectively.

2.1 Data analysis

Results obtained were analyzed using the statistical software, Statistical Package for Social Scientist (SPSS version 18.0) and Microsoft Office Excel 2007 for chart. Significant difference among means of the groups was determined using one way ANOVA with LSD post hoc test for significance. Paired t-test was also employed for the comparisons of means as appropriate. Values were considered significant when p<0.05.

3. Results

The mean concentration of urea showed no significant difference between (T1) and (T3) groups along all period of experiment while group T2 (lead acetate) caused a significant (p<0.05) increase in urea concentration compared with other groups. Also there were no significant differented within groups of experiment. In Table 2 animals of group (T1) exposed to DPP for 6 weeks showed no significant decrease in serum creatinine on day zero, 14 and 28 day of treatment period exposed the last period (42 day) while group (T2) exposed to lead acetate showed significant increase of creatinine concentration compared with control and (T1) group. On other hand DPP with lead acetate (group T 3) caused decrease of creatinine levels compared with T2 group and these levels become closely to control group. Histological Changes in kidney tissue elucidated in Figure 1.
Marah Salim Hammed / Evaluation of performance of Date Palm pollen on urea and creatinine levels

Table 1: Effect of date palm pollen, lead acetate and lead acetate plus date palm pollen on serum urea concentration (mg/dl) in adult female rats

| Group Time | Control Group | (T1) Group | (T2) Group | (T3) Group |
|------------|---------------|------------|------------|------------|
| Zero time  | 71.02±0.40 A  | 70.45±0.39 A | 72.20±0.28 a | 74.00±0.500 A |
| 14 day     | 75.0 ± 0.60 B | 76 ± 0.26 B | 85.25±0.30 A | 79.01±1.20 B |
| 28 day     | 74.15±1.42 B  | 70.21±0.40 B | 88.41±0.31 A | 74.18 ± 1.4 B |
| 42 day     | 72.20±0.30 B  | 68.10 ± 0.70 C | 88.10±0.60 A | 78.41±0.60 B |

L.S.D = 8.88
- (T1) given date palm pollen (150 mg/kg. B.W.), (T2) given lead acetate (10mg/kg. B.W.), (T3) given lead acetate (10mg/kg.B.W.) and date palm pollen (150 mg/kg. B.W).

Values are presented as means ± SE (n=5 rats/group). Capital letters denote significant differences between groups (P<0.05). Small litter denote significant difference within group (P<0.05).

Table 2: Effect of date palm pollen, lead acetate and lead acetate plus date palm pollen on serum creatinine concentration (mg/dl) in adult female rats

| Group Time | Control Group | (T1) Group | (T2) Group | (T3) Group |
|------------|---------------|------------|------------|------------|
| Zero time  | 1.20 ±0.21 A  | 0.84 ±0.08 A | 1.00 ± 0.07 A | 0.88 ± 0.04 A |
| 14 day     | 1.10 ±0.50 B  | 1.10 ±0.04 B | 1.80 ± 0.04 A | 1.02 ± 1.02 B |
| 28 day     | 0.86 ±0.10 B  | 0.62 ±1.20 B | 2.20 ±1.40 A | 0.84 ± 0.89 B |
| 42 day     | 1.20 ±0.20 B  | 0.72 ±0.10 C | 1.86 ±1.62 A | 1.60 ± 0.68 B |

L.S.D = 0.24
- (T1) given date palm pollen (150 mg/kg. B.W.). (T2) given lead acetate (10mg/kg. B.W.). (T3) given lead acetate (10mg/kg.B.W.) and date palm pollen (150 mg/kg. B.W.). Values are presented as means ± SE (n=5 rats/group). Capital letters denote significant differences between groups (P<0.05). Small litter denote significant difference within group (P<0.05).

Figure 1: Histological Changes in kidney Tissue
1-A: Represent T1 group (DPP group): Acute cellular swelling; characterized by vacuolation and sloughing of tubular lining epithelial cells, congestion of B.Vs with neutrophils in their lumen. In collecting tubules (in medulla); there is congestion and edema. Mononuclear cells aggregating in subepithelial layer of calyces also there are inflammatory cells in adipose tissue and fibrinous network in capsule.

1-B: Represent T2 group (lead acetate group): Atrophy of glomerular tuft with of bowman space, hemorrhage in capsule; infiltration of inflammatory cells in the wall of glomeruli. Proteins ions material with neutrophils in the lumen of a renal dilatation tubule; large vacuoles in the glomeruli with thickness of basement membrane due to fibrous connective tissue proliferation; aggregation of inflammatory cells (MNS) between tubules (in interstitial tissue) which showed sever enlargement lead to occlusion of their lumen. Hyaline casts seen in lumen of inflammatory cells in the lumen of collecting tubules.

1-C: Represent T3 group (lead acetate+DPP group): as in T2 but more severe or extensive lesions, also seen muscular hyper atrophy of blood vessels walls and there is acute cell swelling.

1-D: Represent control group normal histology for glomeruli and collecting tubules in control group administered distal water.

4. Discussion

The present study showed that DPP which contain flavonoid, ratin, carotenoids and vitamins worked as antioxidant[22][23][24], so that caused maintenance of urea concentration or decrease creatinine level especially at the last period of treatment period[6]. DPP as antioxidant lead to decrease the high elevation of urea and creatinine caused by lead acetate toxicity (group T3) thus lead acetate considered as heavy metal toxic material caused oxidative stress so the reactive oxygen species accumulation lead to damage of kidney tissues and dysfunction of this organ occurred leading to elevation of blood urea and creatinine[25]. The lead –protein complex forming the intra-nuclear inclusion body binds in a non-diffusible form, thereby reducing its toxic effects[26][27]. The mechanism may be relevant to man’s adaptation to his chronic body burden of lead[27]. Also DPP contained some antioxidants which play important role to reactive oxygen species scavenging[6] and play important role to maintain normal level of blood urea and creatinine[23].

The DPP cause significant decrease (P < 0.05) in creatinine in comparison to control group (Table 2). This may be attributed the ability of DPP to promote the filtration process and increase the efficacy of the both kidneys[28]. Acute lead poisoning has evidence of proximal tubular dysfunction manifested by aminoaciduria, glycosuria and hyperphosphaturia[29]. This tubular dysfunction is reversed to normal after treatment of lead poisoning by chelating agents[30]. In Conclusion, DPP has the ability to counteract the toxic effect of lead acetate associated with improvement of renal histology and serum concentration of urea and creatinine.

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