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Research Article

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Anti-inflammatory, immune-modulatory and antioxidant effects of date fruit (Phoenix dactylifera) extract in rats treated with $AlCl_3$.

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ABSTRACT

Date fruit (Phoenix dactylifera L) contains many macronutrients, minerals, vitamins and antioxidants which has been related to beneficial health properties. The objective of this study was to evaluate the potential efficacy of date fruit extract (DFE) against Al-induced toxicity in rat model. Male albino rats were divided into four groups of 8 rats : A control group, did not receive any treatment, the DFE group received date water extract (DFE) (500 mg kg-1 b. w) orally per day, the Al group: rats were supplemented with aluminum chloride ($AlCl_3$) added to the drinking water at a concentration of 53.5 mg/ l and the DFE-Al group, rats received DFE along with $AlCl_3$. The experimental duration lasted for six weeks. The data obtained indicate that Al administration results in inducing hematological alterations decline in the concentration of hemoglobin (Hb), RBC, Hct%, PCV, MCH, MCV and MCHC accompanied by a significant increase in WBC counts. Significant elevations in the serum inflammatory markers as C-reactive protein (CRP), interleukin-6 (IL-6), tumor necrosis factor-alpha (TNF-α) and the nuclear transcription factor (NF-κB) were observed in $AlCl_3$-treated rats. The results demonstrated, also, that Al promotes lipid peroxidation and decreases the level of antioxidants: superoxide dismutase (SOD) and catalase (CAT) activities and reduced glutathione (GSH) contents in serum. However, treatment of $AlCl_3$ group with DFE resulted in significant amelioration in the mentioned parameters as compared with $AlCl_3$ group. According to the results obtained in the present study, it could be concluded that date fruits have beneficial health properties through immunomodulatory, anti-inflammatory and antioxidant effects.
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Key words: *phoenix dactylifera, AlCl3, inflammatory markers, Lipid peroxidation, antioxidant enzymes*

INTRODUCTION

Aluminum (Al) is a common metal in the environment and one of the extremely numerous in the terrestrial crust. It is found in soil, in water and in the air. Its chemical and physical properties make it suitable for a multitude of uses. Aluminium-containing chemicals are widely used in medicine, food additives, and cosmetic products and are added to tap water in certain areas as a flocculating agent during the water refinement process (1). The increasing use in preparation and storage of food in aluminium utensils may increase one's exposure to aluminum, particularly when used with salty, acidic or alkaline foods (2). Furthermore, excessive consumption of food baked with aluminum foil may bring a serious health risk (3). Biological effects of aluminum (Al) are linked to the development of many

diseases. Some researchers have shown that $AlCl_3$ induced toxic effects on the brain, bone, immune, and hematopoietic system (4).

There is certainly growing evidence in the literature to use some plant extracts that have got a multitude of interesting pharmacological effects. Numerous studies focused on the health-promoting and antioxidant effects of dates. The date palm (*Phoenix dactylifera L.*), is considered the oldest fruit tree in the globe and also have been stated more than any other fruit-bearing plant in the Qur'an, is a sign often associated with Islam and Muslims. Prophet Muhammad (peace be upon him) is reported to acquire said that the best property is the date palm, that dates cure many disorders, and he advised Muslims to eat dates and tend the date palm (5). The dates, either fresh or dried, have high sugar content, low fats and protein contents, as well as iron and potassium. Furthermore, dates are considered a moderate supply of riboflavin, niacin, pyridoxal, folate, thiamin, retinol and ascorbic acid (6). Besides vitamins and mineral deposits, date fruits are abundant with phenolic and flavonoid compounds which possessing antioxidant activity. The phenolics have recently been isolated from *P. dactylifera* fruit extracts such as p caffeic, gallic, ferulic, p-coumaric, sinapic, chlorogenic acid as well as flavonoids, such as luteolin, quercetin and apigenin (7). Several research studies have proven the preventive effect of *P. dactylifera* against different environmental chemicals that may be toxic for some tissues in animal and human (8). Dates and their constituents are associated with a variety of important pharmacological effects in the human body, including antioxidant, anti-inflammatory (9), haemopoietic activity (10) and immunomodulatory effect (11).

Therefore, the current study has been designed to explore the immunomodulatory, anti-inflammatory and antioxidant effects of date fruit extract on rats treated with $AlCl_3$.

MATERIAL AND METHODS

Material:

Aluminum Chloride ($AlCl_3$) was purchased from Sigma, Chemical Company. Fresh fruit of Phoenix dactylifera (Agwa type) was purchased from a local market, Cairo, Egypt.

Preparation of aqueous extract of Phoenix *dactylifera*

Fruit flesh was extracted two times with distilled water (1/10 w/v) by grinding with a mortar and pestle. It was centrifuged at 4 ◦C for 20 min at 4000 g and the supernatant was collected. The aqueous date fruit extract was prepared daily and administrated to rats.

Experimental Animal and their management

Adult male albino rats (200-230 g) were used in the present research. They were acclimatized for one week over a commercial rat diet prior to experimentation. The rats were housed in plastic cages with wire screen tops. They were kept under sufficient ventilation with room temperature and relative humidity of 29 + 2ºC and 40 - 70%, respectively, with a 12 h natural light-dark cycle with free access to food and potable water ad libitum. All animal procedures were performed according to the Ethics Committee of the National Research Center for Radiation Research and Technology (NCRRT), Cairo, Egypt and in accordance with the advice for the proper care and use of laboratory animals.

Animals were randomly divided into four groups, each of 7 animals as follows.

Control group: animals served as the negative control group.

DFE group: animals received date fruit extract (DFE) 500 mg/kg orally by gavage (3).

$AlCl_3$ group: rats were supplemented with $AlCl_3$ added to the drinking water at a concentration of 53.5 mg/ l (12).

(4) Dates+ AlCl3 group: rats received date fruit extract (DFE) and AlCl3 simultaneously in the same manner as with groups 2 and 3.

METHODS

At the end of the experimental period (6 weeks), the animals were subjected to over night fasting and blood was collected though heart puncture after light anesthesia in two tubes, the first centrifuged for serum separation while the other accumulated into tubes containing EDTA and were immediately used for for complete blood count (CBC) analysis.

Biochemical analysis

Red blood cell (RBC), blood cell (WBC), blood hemoglobin (Hb), haematocrit (Hct), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) were carried out using a COULTER T890®.

Levels of tumor necrosis factor-alpha (TNF-α), interleukin-6 (IL-6), the nuclear transcription factor (NF-κB) and C-reactive protein (CRP) was determined by using diagnostic kits. The extent of lipid peroxidation was assayed by the measurement of TBARS according to Yoshioka et al. (13). SOD and CAT activities were determined according to Sun et al. (14) and Aebi (15), respectively. The content of GSH was determined according to Beutler et al. (16).

Statistical analysis

The statistical package for social sciences SPSS/PC computer program was used for statistical analysis of the results. Data were analyzed using one-way analysis of variance (ANOVA) followed by Newman–Keuls post hoc test for multiple comparisons. The data were expressed as mean ±SE. Differences were considered statistically significant at ($P < 0.05$).

RESULTS

Table 1 shows the hematological changes induced by $AlCl_3$ and the possible protective effect of date fruit extract (DFE). It was observed that Al treatment resulted in a significant ($P<0.05$) decline in the concentration of Hb, RBC, Hct%, PCV, MCH, MCV and MCHC accompanied by a significant increase in WBC counts. Whereas, administration of DFE significantly improved the hematological changes to near normal level.

Table (1): Effect of date fruit extract (DFE) administration to $AlCl_3$ intoxicated rats on some hematological parameters.

Parameters	Control	DFE	$AlCl_3$	DFE + $AlCl_3$
Hb (g/ dl)	15.39±1.02 [a]	15.62 ±1.07 [a]	10.35 ±0.57 [c]	13.92±0.47 [b]
Hct %	40.00 ±0.97 [a]	39.37±1.72 [a]	33.81±0.92 [c]	37.51±1.44 [b]
WBCs (103 mm_3)	7.91 ±0.42 [c]	7.88 ±0.59 [c]	10.01±0. 92 [a]	8.48±0.63 [b]
RBCs (106 mm_3)	8.81±0.39 [a]	9.02±0.43 [a]	6.69±0. 21 [c]	7.46 ±0.91 [a, b]
MCV(µg)	54.53 ±3.91 [a]	55.25 ±3.32 [a]	46.35±3.11 [c]	51.07±5.02 [b]
MCH (pg)	24.11±2.18 [b]	24.00 ±2.50 [b]	20.20±1.89 [c]	27.06±3.25 [a]
MCHC (g/dl)	35.90 ±2.29 [a]	36.68 ±1.49 [a]	30.31±.3.45 [c]	34.72±2.61 [b]

Data are expressed as mean± SE. of 7rats per group.

Values with different superscript in the same columns are significantly different at $P \leq 0.05$.

The results in Table (2) show the effect DFE on some serum inflammatory markers (CRP, IL-6, TNF-α and NF-κB) in $AlCl_3$-treated rats. The data obtained revealed that $AlCl_3$ produced a significant elevation ($P< 0.05$) in CRP, IL-6,

TNF-α and NF-*κ*B levels when compared with the control group. The incorporation of DFE along with $AlCl_3$ resulted in significant ($p<0.05$) amelioration of these diagnostic inflammatory markers.

Table 2. Effect of date fruit extract (DFE) on serum C-reactive protein (CRP), interleukin-6 (IL-6), tumor necrosis factor-alpha (TNF-α) and the nuclear transcription factor (NF-*κ*B) in rats treated with $AlCl_3$.

Animals Groups	C-reactive protein (CRP) (µg/ml)	IL-6 (µg/ml)	TNF-α (µg/ml)	NF-*κ*B (ng/ml)
Control	7.29±1.01 [c]	6.00±1.39 [c]	27.45±5.14 [b]	1.09±0.11[c]
DFE	6.67±0.91 [c]	5.88±1.05 [c]	21.00±6.07 [c,b]	1.15±0.13[c]
$AlCl_3$	13. 70±1.94 [a]	13.18±2.30 [a]	61.72±7.39 [a]	4.24±0. 35[a]
DFE + $AlCl_3$	9.41±1. 35 [b]	6.84±1.27 [b]	23.19±5.68 [c]	2.51±0.32[b]

Data are expressed as mean± SE. of 7 rats per group. Values with different superscript in the same columns are significantly different at $P \leq 0.05$

As seen in Table 3, $AlCl_3$ supplementation to rats induced significant increase of TBARs levels concomitant with a significant decrease in GSH content, SOD and CAT activities compared to their corresponding values in the control group. Supplementation of rats with DFE in continuous with $AlCl_3$ has significantly improved the oxidative stress.

Table 3. Effect of date fruit extract (DFE) on serum superoxide dismutase (SOD) and catalase (CAT) activities and reduced glutathione (GSH) and thiobarbituric acid reactive substances (TBARS) levels in rats treated with $AlCl_3$

Animals Groups	SOD (U /mol)	CAT (U/mol)	GSH (nmol/ml)	TBARS (nmol/ml)
Control	277.81±5.17 [a]	25.22±1.28 [a]	65.27±2.25 [a]	56.83±2.25[c]
DFE	278.28±6.25 [a]	25.58±1.44 [a]	67.61±1.94 [a]	54.42±1.65[c]
$AlCl_3$	245.01±5.21 [c]	17.91±1.24[c]	44.41±1.97 [c]	85.57±2.22[a]
DFE + $AlCl_3$	269.63±5.16 [b]	22.72±1.19 [b]	59.37±1.45 [b]	68.78±1.58[b]

Data are expressed as mean± SE. of 7 rats per group. Values with different superscript in the same columns are significantly different at $P \leq 0.05$

DISCUSSION

Todays, people's exposure to chemical substances such as metals is consistently on the rise more and more. These compounds have caused an excessive production of free radicals which, can be responsible for several cell alterations in the organism.

It absolutely was found significant decreases in Hb, RBCs and Hct among Al -treated rats. The present results are in coincide with Kalaiselvi et al. (17). The decrease in MCV, MCH and MCHC in rats administered $AlCl_3$ alone in the present study, refer to the sort of anemia (microcytic– hypochromic anemia). The reduction in Hb content might be credited to increased rate of destruction or reduction in the rate of creation of RBCs. This meaning was supported by the reduced levels of RBCs in the treated group. Vittori et al. (18) have reported that Al may disturb erythropoiesis through the combined effects on mature erythrocytes and cellular metabolism in late erythroid progenitors. Reductions in Hct, RBCs and Hb might be attributed to hyperactivity of bone marrow, bringing about

the production of RBCs with impaired integrity that easily destroyed in the circulation (19). The decrease in Hb could be not only due to decrease in RBCs count, but also to reduce biosynthesis of heme in the bone marrow (19). The white blood cells are the regulators of the immune system and the increase in WBCs count may be anticipated to generalized immune responses and a protective response to metal stress (20).

The results of the present study, evidently reveal that providing the animals with DFE found to be effective by improving the hematological alterations in comparison with a group that was administrated $AlCl_3$ only. This has been linked to constituents, of some mineral Mg, iron, calcium, phosphorus, potassium plus some nutritional vitamins as Vit C, A, B complex, thiamine and nicotinic acid (21). Onuh et al. (22) mentioned that both methanolic and aqueous fruit extract of *P. dactylifera* may have a stimulatory influence on the bone marrow for the haemopoietic activities as a consequence of the occurrence of alkaloids, flavonoids, steroids, tannins, estertepens, carbohydrates, vitamins and phenolic acids.

Inflammation is important physiologic defense mechanisms against various factors such as infection, burn, toxic chemicals, allergens and other stimuli. The unbalance inflammatory process shows an important role in the development and progression of various diseases. Reactive oxygen species (ROS) and pro inflammatory cytokines have been reported to play important roles in several aluminium-reducing processes (23). CRP (C-reactive protein) is an acute-phase protein, indicating inflammation and it is secreted by the liver. It truly is mostly synthesized and regulated in response to interleukin-6 (IL-6) or interleukin-1β (IL-1β) in hepatocytes (24). It has been suggested that accumulation of Al accompanied by the release of cytochrome c from mitochondria, which eventually causes an increase in the production of free radicals triggering oxidative stress by increasing oxidative damage to biomolecules and an increase in the production of proinflammatory cytokines (25). Further, it results in the increase in the gene expression of TNF-α, NF-κB and macrophage inflammatory protein-1 alpha (MIP-1α) (26). Additionally, systemic toxicity evoked by Al could be reflected by an increase in systemic inflammation variability, such as IL-6 and tumor necrosis factor- α (TNF- α) (27).

Date fruits play a significant role as anti-inflammatory and recent studies predict that the occurrence of phenolic compounds and flavonoids in date fruits contributed to the anti-inflammatory activity (28). Ajwa dates reduced the expressions of pro-inflammatory cytokines (IL-6, IL-10 and TNF-α) and apoptotic markers (caspase-3 and Bax) in injured Wistar rat heart tissues (29). The anti-inflammatory effect of dates could be linked to polyphenol compounds that act as antioxidants, which scavenge free radicals produced during the inflammatory process and preventing unwanted biochemical reactions.

The findings of the present study, exhibited that Al supplementation significantly ($p < 0.05$) increased the TBARs along with a concomitant decrease in the activity of SOD, CAT and the level of GSH compared with the control, declaring the prooxidant effect of Al. The observed decrease of these antioxidant enzymes during $AlCl_3$-induction is in accordance with that has been previously reported by Al-Olayan et al., (30). The enhanced TBARS suggests participation of free-radical induced oxidative cell injury in mediating the toxicity of Al. High lipid peroxidation is, at least in part, due to an inhibition or alteration in the activity of non-enzymatic and enzymatic components of the oxidative system. Glutathione plays a unique role in the cellular defense system against toxic chemicals of endogenous and exogenous origin, therefore, the exhaustion of GSH increases susceptibility to free radical evoked damage. Aluminum might impact the glutathione (GSH) synthesis by decreasing the activity of glutathione-synthase, thus leading to a reduced GSH levels (31). SOD and CAT constitute a mutually supportive team of enzymes. In the occurrence of inadequate CAT level to degrade H_2O_2, more H_2O_2 could be converted to toxic hydroxyl radicals that may contribute to oxidative stress. In the present study, the decline in the activities of SOD and CAT in serum of $AlCl_3$ rat group might be due to their inactivation caused by excess ROS production.

In our study, administration of DFE along with $AlCl_3$ has significantly reduced the levels of lipid peroxidation products and increased the activity of SOD, CAT and GSH content. The results are consistent with Attia et al., (32). This antioxidant effects of *P. dactylifera* may be anticipated to their total phenolic content, flavonoids, vitamins C, A and E and β- carotene (6, 7). Date fruit elevates the activity of superoxide dismutase and catalase enzymes, which suggest a potential mechanism whereby date fruit modulates enzymatic behavior, thus initiating a signalling cascade of the antioxidant defense system in an inflammatory situation(33).

CONCLUSION

According to the results obtained in the present study, it could be concluded that DFE, by enhancing antioxidant activities and decreasing lipid peroxidation, would protect from oxidative damage and preserve the integrity of tissue functions.

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