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POSSIBLE EFFECT OF DATE PALM FRUIT EXTRACT ON SOMEBIOCHEMICAL AND HEMATOLOGICAL PARAMETERS IN RATS INTOXICATED WITH METHOMYLINSECTICIDE

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ABSTRACT

The present work was designed to evaluate the protective role of date palm extract (DPE) as an antioxidant against toxicity induced with different doses; 1/5 LD_{50} and 1/10 LD_{50} of the methomyl. The changes in antioxidant parameters (SOD and CAT activities and GSH concentration) in addition to level of TBARS as an index of lipid peroxidation and NO concentration were investigated. In addition, the alteration in liver functions (ALAT, ASAT, ALP, TBIL,GGT, albumin, globulin and total proteins) was assessed. Also, some hematological parameters such as RBCs, WBCs count, lymphocyte, monocytes, neutrophils, platelets count, Hb concentration and HCT were measured. A patch of 42 male Wistar albino rats of average weight (125±5g) at the beginning of the experiment was divided into 7 main groups; ***I****: control group,* ***II****:DPE group, rats were treated orally with DPE at a dose (1 g/k/day)for 2 weeks,* ***III:*** *DPE group, rats were treated orally with DPE at a dose (1 g/k/day) for 4 weeks. Methomyl groups;* ***IV****: rats were intoxicated with a 1/5LD_{50} for 2 weeks* ***V****: rats were intoxicated with a 1/10$LD_{50}$for two weeks,* ***VI:*** *groups treated with DPE for two weeks as a protection before given DPE and methomyl1/5$LD_{50}$for two weeks,* ***VII:*** *groups treated with DPE for two weeks as a protection before given DPE and methomyl 1/10$LD_{50}$for two weeks. Each group contains 6 rats. The results showed a significant rise in TBARS, NO, ALAT, ASAT, ALP, GGT, and TBIL, while a significant reduction in some other parameters (CAT, SOD, GSH, Total protein, albumin).Significant decreases in some hematological parameters (RBCs, Hb, HCT and PLT, WBCs and lymphocytes) were reported. Also, the results showed a significant increase in neutrophils and eosinophils atin rats intoxicated with methomyl when compared to the control groups. The administration of the DPE ameliorated the deteriorative effects of the methomyl. In conclusion, the results obtained revealed that the administration of DPE had hepatoprotective effects against methomyl insulet in male Wistar albino rats by inhibiting oxidative stress through ROS scavenging activity and improvement of the biochemical markers.*

Keywords : Date-palm fruit extract, Methomyl, Antioxidants, Liver function test, Hematology.

1. INTRODUCTION

In the recent years, the use of insecticides in agriculture has been increased to enhance the food production by eradicating unwanted insects and controlling disease vectors. The widespread use of insecticidess carries more occupational exposure, to high levels of these compounds of agricultural and industrial workers as well as more contamination of food with insecticides residues (**Zeljezic and Garaj, 2001**). Methomylis one of the most common insecticidess which are used in the control of insects. It is used worldwide in agriculture and health programs. Besides its advantages in the agriculture, it causes several toxic effects (**Djeffal *et al*., 2015**). Animals and human exposure to methomyl during spraying of flies and ingestion of food contaminated (**Gil *et al*., 2013**). It is one of a class of chemicals called carbamates insecticide first registered in 1968 by the ***Environmental Protection Agency*** **(EPA)** as a restricted use insecticides and is used on a wide variety of crops. It is a cholinesterase inhibitor and is often most effective against pests that have developed a resistance to organophosphates **(Vanscoy *et al*., 2013).** The toxicity of methomyl and other insecticides is ascribed, at least in part, to the generation of reactive oxygen species (ROS), leading to **lipid peroxidation** (LPO) and oxidative stress.**(Halliwell *et al*.,1992; Rai and Sharma, 2007 and Heikal *et al*.,2014).**

Methomyl is a carbamate insecticide classified as a highly hazardous (**class 1A**) compound by the **Insecticide Resistance Action Committee** (IRAC) **2017.** The exposure to methomyl exerts neurodegenerative disorders, besides toxic actions on the liver, kidney, muscle, and eye. It is suspected to be carcinogens and mutagens with high mortality rates (**Lee *et al.*, 2011 and Hashish and Elgaml, 2016)**.

Date fruit (*Phoenix dactylifera L.*) is a good source of rapid energy, due to their high carbohydrate content (70 to 80%). The good nutritional value of dates is also based on the presence of vitamin C. Date fruit provides essential minerals such as calcium, iron, magnesium, phosphorus, potassium, zinc, selenium and manganese. The date fruit is listed in folk remedies for the treatment of various infectious diseases, cancer and has powerful antioxidant activity due to presences of flavonoid and phenolic compounds (**Mallhi *et al.*, 2014 and Tijani *et al.*, 2017)**.

Antioxidants are molecules that in low concentrations can prevent or delay the oxidation of an oxidizable substrate. Antioxidants are present in our body and exist in several foods. Also, antioxidants have a high affinity for free radicals and scavenge these molecules to protect our health. Compounds with antioxidant properties donate electrons to free radicals to reduce their reactivity and maintain the cellular pro-oxidant/antioxidant balance. **(Casas and Muriel, 2015).**

Aim of the work

The present study aimed to evaluate the ameliorative effects of date palm extract in reducing the hazards resulted from exposure of male albino rats to different doses (1/5 LD_{50} and 1/10 LD_{50}) of the methomyl.

MATERIALS AND METHODS

Experimental design

Forty-two male Wistar albino rats of about (125 ± 5 g B.W.) were handled in accordance with the criteria of the investigations and Ethics Committee of the Community Laws governing the use of experimental animals. The rats were placed in regular designed cages and maintained in conditions of good ventilation, normal temperatures, and humidity range. Six rats were placed into each cage. Food and water were provided to the animals*ad libtum*.

Rats distributed randomly into 7 groups; **Group I:** control rats. **Group II:** rats treated with date palm extract at a dose (1 g/k/day) via oral gavage directly into the stomach for 2 weeks. **Group III:** rats treated with date palm extractat a dose (1 g/k/day) for 4 weeks**. Group IV:** rats intoxicated with 1/5 LD_{50}methomyl (6.8 mg/kg) for 2 weeks**. Group V:** rats intoxicated with 1/10 LD_{50} methomyl (3.4 mg/kg) for 2 weeks. **Group VI:** rats pretreated with date palm extract at a dose (1 g/k/day) for 2 weeks (Protection) and treated with the high dose of methomyl and date palm extract for 2 weeks. **Group VII:** rats pretreated with date palm extractat a dose (1 g/k/day) for 2 weeks (Protection) and treated with the low dose of methomyl and date palm extract for 2 weeks.

The animals were observed daily for signs of toxicity. The body weights were recorded day after day during the period of the experiment.

Preparations of date palm extract (*Phoenix dactylifera L.*)

Dates palm (*Phoenix dactylifera L.*) fruits were washed with tap water, and the seeds were removed. The flesh of the fruits was left in distilled water (1:3 w/v) for 48 hours at 4°C **(Al-Qarawi *et al.*, 2005).** The whole solution was blended, then centrifuged at 4°C for 20 min at 4000 rpm. The supernatant was collected and stored at - 80°C till use. During the experiment, the aqueous date fruit extract was daily prepared and administrated to rats.

Dose calculation

The selected antioxidant dose was 1 g/kg/day from date-palm fruit extract in rats **(Sheikh *et al.*, 2014)**. The Food and Drug Administration Guidelines (FDA) recommended the standard serving size of dried

fruits for a human is (40 g/kg/day) **(Vinson *et al*., 2005).** In the present study, the serving size of dates was equivalent to 10 g extract (the flesh of 7 dates).The crudefruit extract human equivalent dose (HED) could be converted to albino rat a dose based on body surface area (BSA) according to the formula of the U.S. (FDA) **(Reagan *et al*., 2007)**.

Dose of methomyl

According to **Frederick, (2017)**,oral acute $LD_{50}$of methomyl for male rats equal 34 mg/kg. 1/5 LD_{50} equal (6.8 mg/kg B.W.) and 1/10 LD_{50} equal (3.4 mg/kg B.W.).

Collection and preparation of samples

At the end of the experiment, the blood samples were collected from each animal under diethyl ether anesthesia from retro-orbital venous plexus of eye puncture using blood capillary tubes. One part of blood was collected on Ethylene Diamine Tetra Acetic Acid (EDTA) for hematological parameters. Blood samples were collected without anti-coagulants and centrifuged at 4000 r.p.m for 10 minutes to harvest serum. The serum was frozen at -20 ºC until used. After sampling, animals were sacrificed and livers were, dissected out and washed with isotonic saline. The liver was homogenized inice-cold physiological saline (0.15mKCl). The homogenates were centrifuged in cooling centrifuge at 4000 rpm for 20 min. The supernatant was collected and stored at - 80°C till use.

Biochemical parameters

The liver tissue homogenates were used for the determination of hepatic thiobarbituric acid reactive substances (TBARS) **Satoh, (1978)**, and hepatic reduced glutathione (GSH) **Beutler *et al*., (1963).** In addition, the activities of superoxide dismutase (SOD) **Kakkar *et al*., (1984**), catalase (CAT) **Aebi, (1984),** andnitric oxide (NO) **Montgomery and Dymock (1961)**, were measured.

The serum levels of(ALAT) **Bergmeyer and Horden (1980)**, (ASAT) **Saris,(1987),** alkaline phosphatase (ALP) **Tietz, (1986)**, total protein (TP) **Weichselbaum, (1946)**, gamma-glutamyl transferase (GGT) **(Szasz *et al*., 1974)** albumin **Doumas *et al*., (1971)** and total bilirubin (TBIL) **Tokuda and Tanimoto, (1993)** were determined.

Hematological parameters

The total number of erythrocytes, leukocytes, differential leukocyte count, platelets count, hematocrit value % and hemoglobin concentration were determined by blood cell counter **(Sysmex XP 1300)**.

Statistical analysis

The statistical package for social sciences SPSS/PC computer program (version 20) was used for statistical analysis of the results. Data were analyzed using one-way analysis of variance (ANOVA). Differences were considered statistically significant at $P<0.05$. Data are summarized as a mean ± standard error.

RESULTS

Biochemical parameters in tissue and serum

The results in table (1) showed that the intoxicated groups with methomylat 6.8 mg/kg and3.4 mg/kg for two weeks had a significant elevation ($p<0.05$) in the hepatic thiobarbituric acid reactive substances (TBARS) and hepatic nitric oxide(NO) levels as compared with the corresponding values in the control or dates groups. In contrast, a significant decrease ($p<0.05$) in hepatic reduced glutathione (GSH), superoxide dismutase (SOD) & catalase (CAT) activities.

The pre-treated groups with DPE for two weeks plus 1/5 LD_{50} and 1/10 LD_{50} methomyl and DPE for two weeks, a significant decrease ($p<0.05$) in the hepatic TBARS, NO while a significant increase ($p<0.05$) in the hepatic GSH, SOD & CAT as compared with corresponding values of 1/5 LD_{50} and 1/10 LD_{50} methomyl groups for two weeks, wase reported (Table 1).

Data presented in table (2) revealed a significant increase ($p<0.05$) in serum

Table 1: The protective effect of date palm fruit extract on the oxidative stress markers and antioxidants in the liver tissue of rats intoxicated with methomyl insecticides.

Parameters Groups	TBARS (nmole/g tissue)	NO (µmol/g tissue)	SOD (U/mg tissue)	CAT (U/mg tissue)	GSH (mmol/g tissue)
Control	169.83[a] ± 17.78	50.51[a] ± 5.52	62.21[a] ± 3.60	1.50[a] ± 0.33	2.83[a] ± 0.27
D 2 W	170.00[a] ± 14.98	51.00[a] ± 4.42	60.11[a] ± 4.79	1.33[a] ± 0.14	2.61[a] ± 0.25
D 4 W	168.16[a] ± 14.62	51.81[a] ± 3.25	59.16[a] ± 3.94	1.43[a] ± 0.13	2.39[a] ± 0.34
1/5 LD_{50} M	257.33[b] ± 9.18	74.37[b] ± 2.35	27.21[b] ± 2.01	0.33[b] ± 0.10	0.85[b] ± 0.14
1/10LD_{50} M	254.50[b] ± 18.54	68.18[b] ± 3.98	28.61[b] ± 2.40	0.37[b] ± 0.07	1.00[b] ± 0.17
D+(1/5LD_{50} M + D)	190.00[a] ± 7.97	48.48[a] ± 4.81	55.88[a] ± 3.28	0.99[a] ± 0.23	2.12[a] ± 0.21
D+(1/10LD_{50} M + D)	181.83[a] ± 14.22	49.88[a] ± 4.97	57.68[a] ± 5.54	1.24[a] ± 0.12	2.30[a] ± 0.37

Note: Results are expressed as mean ± standard error. For each parameter, values not sharing common superscript letters are significant in different with each other at p<0.05; D: date palm extract; M: methomyl; W: weeks; TBARS: thiobarbituric acid reactive substances; NO: nitric oxide; SOD: superoxide dismutase; CAT: catalase; GSH: reduced glutathione; n= 6 value.

transaminases (ALAT&ASAT) alkaline phosphatase (ALP), gamma-glutamyl transferase (GGT) and total bilirubin (TBIL) level in rats intoxicated with 1/5 LD_{50} and 1/10 LD_{50} methomyl while a significant decrease ($p<0.05$) was recorded in serum total protein (TP) and albumin (ALB) when compared with the corresponding values in the control group.

The pre-treated groups with DPE for two weeks plus 1/5 LD_{50} and 1/10 LD_{50} methomyl and DPE for two weeks showed a significant decrease ($p<0.05$) in the serum ALAT, ASAT, ALP, GGT&TBIL level and a significant increase ($p<0.05$) in the serum TP and albumin as compared with 1/5 LD_{50} and 1/10 LD_{50} methomyl groups for two weeks.

Hematological parameters

Rats intoxicated with 1/5 LD_{50} and 1/10 LD_{50}methomyl for two weeks showed a significant decrease in RBCs, Hb, HCT, and PLT when compared with the control or dates groups. The results of WBCs and lymphocytes revealed a significant decrease while a significant increase in neutrophils and eosinophils was observed when compared with the control group. Monocytes showed a significant decrease in 1/5 LD_{50} group and insignificant change in 1/10LD_{50} group when compared with control or dates group. Basophils revealed insignificant change (Tables 3,4).

On the other hand groups pre-treated with DPE for two weeks plus 1/5 LD_{50} and 1/10 LD_{50} methomyl and DPE for two weeks revealed a significant increase in RBCs, Hb, HCT and PLT, and a significant increase in WBCs and observed enhancement in differential leucocytes when compared to the corresponding value of 1/5 LD_{50} and 1/10 LD_{50} methomyl groups for two weeks.

Table 2: The protective effect of date palm fruit extract on the liver functions in rats intoxicated with methomyl insecticides.

Parameters Groups	ALAT (U/L)	ASAT (U/L)	ALP (IU/L)	TBIL (mg/dl)	GGT (U/L)	TP (g/dl)	ALB (g/dl)
Control	36.11^{a} ± 4.27	90.06^{a} ± 8.15	112.15^{a} ± 8.17	0.12^{a} ± 0.04	2.20^{a} ± 0.51	6.64^{a} ± 0.20	4.92^{a} ± 0.18
D 2 W	33.48^{a} ± 4.10	88.38^{a} ± 11.20	111.70^{a} ± 11.55	0.10^{a} ± 0.03	2.06^{a} ± 0.49	6.49^{a} ± 0.14	4.71^{a} ± 0.21
D 4 W	32.08^{a} ± 2.75	87.58^{a} ± 8.06	113.06^{a} ± 7.96	0.08^{a} ± 0.02	1.80^{a} ± 0.60	6.30^{a} ± 0.25	4.69^{a} ± 0.22
1/5 LD_{50} M	79.90^{b} ± 6.46	154.66^{b} ± 6.22	218.13^{b} ± 20.33	0.76^{b} ± 0.20	5.56^{b} ± 0.59	4.81^{b} ± 0.41	2.12^{b} ± 0.19
1/10 LD_{50} M	67.63^{b} ± 5.13	143.90^{b} ± 8.84	216.61^{b} ± 30.84	$0.52^{b,c}$ ± 0.19	4.93^{b} ± 0.67	5.13^{b} ± 0.10	2.66^{b} ± 0.24
D+(1/5LD_{50} M + D)	34.63^{a} ± 4.27	87.51^{a} ± 7.12	115.05^{a} ± 10.01	$0.31^{a,c}$ ± 0.15	3.21^{a} ± 0.47	5.93^{a} ± 0.16	4.39^{a} ± 0.18
D+(1/10LD_{50} M+D)	30.56^{a} ± 3.68	77.71^{a} ± 12.64	112.08^{a} ± 6.02	0.13^{a} ± 0.02	2.11^{a} ± 0.61	6.14^{a} ± 0.31	4.60^{a} ± 0.21

Note: Results are expressed as mean ± standard error. For each parameter, values not sharing common superscript letters are significant in different with each other at p<0.05; D: date palm extract; M: methomyl; W: weeks; ALAT: alanine aminotransferase; ASAT: aspartate aminotransferase; ALP: alkaline phosphatase; TBIL: total bilirubin; GGT: gamma-glutamyltransferase; TP: total protein; ALB: albumin;n= 6 value.

Table 3: The protective effect of date palm fruit extract on some hematological parameters in rats intoxicated with methomyl insecticides.

Parameters Groups	RBCs count (10^6/mm^3)	Hb(g/dl)	HCT%	PLT(10^3/mm^3)
Control	6.23^{a} ± 0.43	$13.98^{a,e}$ ± 0.39	$41.76^{a,e}$ ± 1.46	722.83^{a} ± 32.90
D 2 W	$6.38^{a,d}$ ± 0.35	$14.05^{a,e}$ ± 0.32	$41.48^{a,e}$ ± 1.28	732.16^{a} ± 52.16
D 4 W	$6.71^{a,e}$ ± 0.19	$14.61^{a,b}$ ± 0.30	$44.26^{a,b}$ ± 1.59	766.16^{a} ± 19.87
1/5 LD_{50} M	3.26^{b} ± 0.18	8.73^{c} ± 0.48	27.38^{c} ± 1.62	496.66^{b} ± 41.45
1/10 LD_{50} M	3.91^{b} ± 0.33	10.16^{d} ± 0.25	31.51^{d} ± 1.29	501.16^{b} ± 29.84
D+(1/5LD_{50} M + D)	$6.58^{a,e}$ ± 0.24	$13.51^{e,f}$ ± 0.34	$40.01^{e,f}$ ± 1.08	714.66^{a} ± 37.07
D+(1/10LD_{50} M+D)	$7.16^{c,d,e}$ ± 0.24	14.76^{a} ± 0.36	44.31^{a} ± 0.98	736.16^{a} ± 41.07

Note: Results are expressed as mean ± standard error. For each parameter, values not sharing common superscript letters are significant in different with each other at p<0.05; D: date palm extract; M:methomyl;W:weeks; RBCs: Red Blood Corpuscles;Hb: Hemoglobin;HCT: Hematocrit; PLT :platelets count; n= 6 value.

Table 4: The protective effect of date palm fruit extract on the WBC sand differential count in rats intoxicated with methomyl insecticides.

Parameters Groups	WBCs count (10^3 /mm^3)	Lympho. %	Neutro. %	Mono.%	Eosino. %	Baso. %
Control	11.56^a ± 0.95	71.00^a ± 1.41	24.16^a ± 1.35	3.16^a ± 0.60	1.33^a ± 0.21	1.83^a ± 0.40
D 2 W	11.85^a ± 0.78	69.33^a ± 1.42	25.16^a ± 1.40	3.83^a ± 0.47	1.16^a ± 0.30	1.66^a ± 0.42
D 4 W	11.88^a ± 0.48	68.33^a ± 1.35	26.33^a ± 1.17	4.00^a ± 0.57	1.00^a ± 0.36	1.50^a ± 0.34
1/5 LD_{50} M	6.86^b ± 0.71	56.00^b ± 1.57	38.16^b ± 1.66	1.66^b ± 0.33	3.83^b ± 0.60	2.16^a ± 0.16
1/10 LD_{50} M	8.11^b ± 0.60	58.16^b ± 2.12	34.50^b ± 2.14	3.33^a ± 0.33	3.16^b ± 0.70	2.00^a ± 0.25
D+(1/5LD_{50} M + D)	11.75^a ± 0.68	69.16^a ± 1.32	26.50^a ± 0.61	3.00^a ± 0.36	1.66^a ± 0.21	1.33^a ± 0.33
D+(1/10LD_{50} M+D)	11.91^a ± 0.47	69.66^a ± 1.35	25.66^a ± 1.20	3.50^a ± 0.42	0.83^a ± 0.30	1.16^a ± 0.47

Note: Results are expressed as mean ± standard error. For each parameter, values not sharing common superscript letters are significant in different with each other at $p<0.05$; D: date palm extract; M:methomyl;W: weeks;WBCs:white blood cells;Lympho.:lymphocytes;Neutro.: neutrophils; Mono.: monocytes; Eosino.: eosinophils; Baso.:basophils;n= 6 value.

DISCUSSION

The results present in table (1) refer to a significant increase in TBARS and NO level this increase in TBARS and NO level may be due to an increase in free radicals as a result of intoxication with methomyl. The results are in agreement with **El-Missiry *et al.*, (2007) and Waret *et al.*, (2017).**TBARS is one of the important biochemical compounds used to indicate reactive oxygen species (ROS) generated from lipid peroxidation. In general, ROS which can be neutralized by a variety of antioxidants are generated by cellular metabolism. However, excessive ROS would damage various chemical and biological membranes and have been suggested as a cause of toxicity in several organs**.**

The antioxidant enzymes SOD, GSH and CAT as free radical scavengers have a role in the effects of oxidant molecules on tissues and active in the defense against oxidative cell damage. The results of liver CAT and SOD activities as well as GSH concentration showed a significant decrease in the group intoxicated with 1/5 LD_{50} and 1/10 LD_{50} methomyl for two weeks when compared to the control or dates group. This reduction of GSH appears to be a major factor that permits lipid peroxidation **(Santhosh *et al.*, 2013).** These results are in agreement with **Fatma *et al.*, (2013)** which studied effects induced by different time intervals of methomyl exposure on liver antioxidant defense system in mice results showed significantly decrease in the activity of antioxidant enzymes, CAT , SOD activities and GSH in mice liver. The decrease in the GSH in liver homogenate due to the elevation in lipid peroxidation is a consequence of depleted GSH stores, which are otherwise capable of moderating the levels of Lipid peroxidation LPO Therefore, reduced level of GSH enhances the toxic effect because GSH plays an important role in detoxification of ROS. The

observed decrease in SOD and CAT might be in response to increased oxidative stress. However, when a condition of oxidative stress strongly establishes, the defense capacities against ROS becomes insufficient, in turn, ROS also affects the antioxidant defense mechanisms, reduces the intracellular concentration of GSH and decreases the activity of SOD and CAT several studies reported that carbamate insecticidess inhibited the activities of antioxidant enzymes **Sameeh *et al.*, (2009) Abdel-Moneim *et al.*, (2010) and Sameeh *et al.*, (2017).**

On the other hand, groups pre-treated with DPE for two weeks plus 1/5 LD_{50} and 1/10 LD_{50}methomyl and DPE for two weeks showed significant increase in CAT, SOD and GSH when compared to the corresponding value of intoxicated rats with 1/5 LD_{50} and 1/10 LD_{50} methomyl for two weeks but the change was insignificant when compared with the control or dates group. Our results are parallel with the results reported by **Saafi *et al.*, (2011)** who demonstrated that pre-treatment with DPE restored the liver damage induced by dimethoate, as revealed by inhibition of hepatic lipid peroxidation and enhancement of SOD and CAT activities. These results suggested that DPP act as a potent antioxidant.

Hepatic enzymes (ALAT and ASAT) are markers for cellular damage. The results of this study of serum ALAT, ASAT, ALP, and GGT as well as TBIL enzymes activities in the groups intoxicated with 1/5 LD_{50} and 1/10 LD_{50} methomyl for two weeks showed a significant increase when compared to the corresponding value of the control or dates groups. These results are in agreement with **Zaahkouk *et al*. (2000)**; **Patil *et al.* (2008)**; **Djeffal *et al.*, (2015)**. **Hashish and Elgaml (2016)** who studied the protective effect of nicotinic acid against the acute toxic effects induced by methomyl in albino rats. The present results showed a significant increase in the activities of ALAT, ASAT, and ALP in the serum of treated rats suggesting that methomyl might cause critical injury to the liver. This increase may be indicative of initial cell injury occurring associated with methomyl toxicity. Also due to changing in membrane permeability and loss of the functional integrity of the cell membranes in the liver leading to cellular leakage with generalized release of these enzymes from the cell. The increased levels of serum enzymes indicate a hepatocytic damage or necrosis.

On the other hand ALAT, ASAT, ALP, and GGT as well as TBIL in the groups pre-treated with DPE for two weeks plus 1/5 LD_{50} and 1/10 LD_{50}methomyl and DPE for two weeks showed a significant decrease ($p<0.05$) when compared to the corresponding values of methomyl group. These results are in agreement with **Bastway *et al.*, (2008) and El Arem *et al.*, (2014)** who suggests that reduction in the serum enzymes activities by DPE may be due to by inhibition of hepatic lipid peroxidation or due to their phenolics and flavonoids contents.

An important function of the serum protein is the maintenance of the normal distribution of the body water by controlling the osmotic balance between the circulating blood and the cells (**Harper *et al*.,1977)**. Albumin values are associated with the function of hepatic cells **(Muriel *et al.*, 1992).** The results in the present work showed a significant decrease in serum total proteins and albumin in rats intoxicated with 1/5 LD_{50} and 1/10 LD_{50} methomyl for two weeks. The decrease in serum protein and albumin might be due to the hepatocellular damage induced an imbalance between the rate of protein synthesis and the rate of its degradation in the liver after methomyl intoxication. Also, it might be due to severe loss through the urine in severe kidney disease **(Hashish and Elgaml, 2016).** These results are in agreement with **Zaahkouk *et al*.,(2000); Sanagoudra and Bhat (2013).**

On the contrary, The levels of serum total proteins and albumin in groups pre-treated with DPE for two weeks plus 1/5 LD_{50} and 1/10 LD_{50}methomyl and DPE for two weeks showed a significant increase when compared to the corresponding values of methomyl group. These results are in agreements with **Abdelaziz**

and Ali (2014) who demonstrated that treatment of rats with aqueous DPE significantly improved the albumin and total protein after CCl_4 induced liver damage. Also with **Okwuosa *et al.*, (2014)** who demonstrated that treatment of rats with aqueous and methanolic DPE improved the thioacetamid-induced liver damage represented by alterations in liver function parameters. Flavonoids have been reported to exert membrane stabilizing action. It is, therefore, likely that the flavonoids present in *P.dactylifera* extract could be responsible for the membrane stabilizing property. The significant reduction in mean serum albumin level of the thioacetamide group is as a result of thioacetamide-induced cellular toxicity which affected the synthetic capacity of the liver. Interestingly, date palm extract treatment preserved the synthetic capacity of the liver. Also, the results are inagreements with **Said *et al.*,(2008); Abdelrahman *et al.*,(2012) and Hussein *et al.*, (2015).**

Rats intoxicated with 1/5 LD_{50} and 1/10 LD_{50}methomyl for two weeks showed a significant decrease in RBCs, Hb, HCT and PLT when compared with the control or dates groups. The sereductions throughout the experimentation in RBCs, Hb, and HCT levels reflect acute exposure to methomyl which may induce normocytic normochromic anemia or could be attributed to the ability of the methomyl to cause acute extravascular hemolysis or it might be due to its ability to cause oxidative stress. It was thought that these changes were due to an increased rate of breakdown of red cells and/or the toxic effect of methomyl on bone marrow. The erythrocyte membrane was reported to be highly vulnerable in an oxidative stress condition because it contains high amounts of lipid, iron and is bathed in serum that has low antioxidant properties. Also, the reduction in Hb content may be due to an increased rate of breakdown of red cells and/or reduction in the rate of formation of RBCs. The results of WBCs and lymphocytes showed a significant decrease while a significant increase in neutrophils and eosinophils was observed when compared with the control group. Monocytes significant decrease in 1/5 LD_{50} and insignificant change in 1/10LD_{50} when compared with control or dates group. Basophils revealed in significant change. Erythropenia in rats treated with methomyl may arise due to depression of erythropoiesis the observed leukopenia found in treated rats suggest that the immune response of rats was suppressed. These results are in agreement with **Zaahkouk *et al.*,(2000)**; **Garg*et al.*, (2008)**; **Mossa and Abbassy (2012) and Hashish and Elgaml (2016).**

On the other hand groups pre-treated with DPE for two weeks plus 1/5 LD_{50} and 1/10 LD_{50}methomyl and DPE for two weeks revealed a significant increase in RBCs, Hb, Hct and Plt, also showed a significant decrease in WBCs and observed enhancement in differential leucocytes when compared to the corresponding value of 1/5 LD_{50} and 1/10 LD_{50} methomyl groups for two weeks. Significant increase observed in Hb and Hctvalue in group pre-treated with DPE for two weeks plus 1/10 LD_{50}methomyl and DPE for two weekswhen compared with group pre-treated with DPE for two weeks plus 1/5 LD_{50}methomyl and DPE for two weeks. **Wahab *et al.*, (2010)** showed elevation in hematocrit, RBCs, WBCs, hemoglobin concentration, lymphocyte and monocyte count; and reduction in neutrophil count after treatment rats with ethanolic DPE against hematotoxicity induced by lead he found also that.

DPE besides having different pharmacological activities, also have hemopoietic activity, results of this study reveald **Onuh *et al.*, (2012)**that level of RBCs, Hb, and PLT, count increased after administration of both extracts of aqueous and methanolic DPE. Total and differential count of WBCs did not differ significantly from the control group These results are in agreement with **Said *et al.*, (2008) and Ufelle *et al.*, (2016)** who showed that the effect of seed extract fractions of *Phoenix dactylifera* on Wistar rats after myelo-suppression on day 15, the myelo-suppressed and normal groups

revealed dose and time-dependent significant increase in Hb, HCT, RBCs and total WBCs.

CONCLUSION

The remarkable amelioration of using date as a natural antioxidant in rats intoxicated with methomyl accompanied with significant improvements in biochemical and hematological parameters postulated a considerable protective role of dates against induction of oxidative stress and biochemical impairments observed after toxic exposure.

RECOMMENDATION

Date palm extract could have a potential benefit *via* protection of normal cells and tissues during toxic exposure. This study recommends the daily intake of 7 dates, as said by Prophet Mohamed –Peace Be Upon Him- which have potent antioxidant and hepatoprotective properties. Protection programs, including educational ones, on the appropriate use of insecticidess to minimize population exposures, as well as preventive health monitoring, are needed principally in developing countries.

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الملخص العربى

تم تصميم هذه التجربة لتقييم الدور الوقائى لمستخلص التمر لما يحتويه من مضادات للأكسدة ضد الجرذان المسممة بجرعات مختلفة (٦,٨ ملجم / كجم ، ٣,٤ ملجم / كجم) من الجرعة نصف المميتة من مادة الميثوميل (كمادة سامة). تسبب السم فى تغييرات فى إنزيم السوبراوكسيد ديسميوتيز(SOD) وكذلك إنزيم الكتاليز(CAT) وتركيز الجلوتاثايون المختزل (GSH)، بالإضافة الى مستوى تركيز المواد المتفاعلة مع حمض الثيوبربتيوريك (TBARS) التى تعد بمثابة مؤشر لأكسدة الدهون وأيضا تركيز اكسيد النيتريك (NO). وبالإضافة إلى ذلك تغير فى وظائف الكبد (الأنزيمات الناقلة لمجموعة الأمين، الألبيومين، البروتين الكلى، إنزيم الفوسفات القلوى (ALP)، الصفراء الكلية(TBIL) وناقلة البيبتيد جاما جلوتاميل(GGT) و بعض قياسات الدم المتمثلة فى عدد كرات الدم الحمراء ، وعدد خلايا الدم البيضاء WBCs، اللمفاويات ، الوحيدة ، المتعادلات ، عدد الصفائح الدموية ، تركيز الهيموجلوبين Hb و الهيماتوكريتHCT .

تمت هذه الدراسة بإستخدام إثنين و أربعين (٤٢) من ذكور الجرذان البيضاء ويتراوح الوزن بين ١٢٥ ± ٥ جرام ، وتم توزيع الحيوانات فى ٧ مجموعات وفقاً للمعالجة ومتطلبات التجربة،كل مجموعة تحتوى على ستة حيوانات في أقفاص منفصلة..

وهذه المجموعات هى:

١- المجموعة الأولى:المجموعة الضابطة تغذية عادية لمدة أربعة أسابيع.

٢-المجموعة الثانية: مجموعة الجرذان المجرعة بمستخلص التمرلمدة أسبوعين تم إعطاء مستخلص التمر عن طريق الفم (١ جم / كجم/ يوم).

٣- المجموعة الثالثة : مجموعة الجرذان المجرعة بمستخلص التمرلمدة أربعة أسابيع تم إعطاء مستخلص التمر عن طريق الفم (١ جم / كجم/ يوم).

٤- المجموعة الرابعة : مجموعة الجرذان التى تم إعطائها جرعة مقدارها (٦,٨ ملجم / كجم) من الميثوميل لمدة أسبوعين.

٥- المجموعة الخامسة : مجموعة الجرذان التى تم إعطائها جرعة مقدارها (٣,٤ ملجم / كجم) من الميثوميل لمدة أسبوعين.

٦- المجموعة السادسة : مجموعة الجرذان التى تم إعطائها جرعة وقائيه من التمر لمدة أسبوعين مقدارها(١ جم / كجم/ يوم) ثم إعطيت الميثوميل بجرعة مقدارها(٦,٨ ملجم / كجم)مع التمرلمدة أسبوعين اخرين.

٧- المجموعة السابعة : مجموعة الجرذان التى تم إعطائها جرعة وقائية من التمر لمدة أسبوعين مقدارها(١ جم / كجم/ يوم) ثم إعطيت الميثوميل بجرعة مقدارها(٣,٤ ملجم / كجم)مع التمرلمدة أسبوعين اخرين.

لخصت نتائج هذه الدراسة على النحو التالى:

أظهرت النتائج الحالية للقياسات الدموية و البيوكيميائية زيادة ملحوظة في (TBARS، NO، ALAT، ASAT، ALP، GT، TBIL) ، بعد التسمم بالجرعة(٦,٨ ملجم / كجم ، ٣,٤ ملجم / كجم) من الميثوميل لمدة أسبوعين مقارنة بالمجموعة الضابطة.

على العكس من ذلك ، أظهرت الدراسة نقص معنوى فيإنزيم الكتاليز(CAT) و السوبر أوكسيد ديسميوتيز(SOD) وتركيز الجلوتاثايون المختزل(GSH)، البروتين الكلى، الألبيومين) بعد التسمم بالجرعة (٦,٨ ملجم / كجم ، ٣,٤ ملجم / كجم) من الميثوميللمدة أسبوعين مقارنة بالمجموعة الضابطة.

وأظهرت القياسات الدموية إنخفاض معنوى في بعض القياسات (كرات الدم الحمراء(R.B.Cs)،الهيموجلوبين (Hb) ، الهيماتوكريت (HCT)، الصفائح الدموية (PLT)، خلايا الدم البيضاء (WBCs)والخلايا الليمفاوية. بعد التسمم بالجرعة(٦,٨ ملجم / كجم ، ٣,٤ ملجم / كجم) من الميثوميل لمدة أسبوعين مقارنة بالمجموعة الضابطة.

كما وضحت الدراسة أيضاً أن الجرذان المعالجة مسبقاً بمستخلص التمر بجرعة (١ جم / كجم يوم) لمدة أسبوعين بالإضافة إلى الجرعة(٦,٨ ملجم / كجم ، ٣,٤ ملجم / كجم) من الميثوميل مع مستخلص التمر بجرعة (١ جم / كجم / يوم) لمدة أسبوعين اظهرت زيادة معنوية فى عدد كرات الدم الحمراء ، تركيز الهيموجلوبين ، الهيماتوكريت، عدد الصفائح الدموية خلايا الدم البيضاء مقارنة مع المجموعة المسممة بجرعة (٦,٨ ملجم / كجم ، ٣,٤ ملجم / كجم) من الميثوميل.بالإضافة إلى ذلك ،حدث تحسن ملحوظ فى القياسات البيوكيميائية للكبد وإيضا تحسن فى المواد المضادة للأكسدةانزيم الكتاليز(CAT) و السوبر أوكسيد ديسميوتيز(SOD) وتركيز الجلوتاثايون المختزل (GSH).