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RESEARCH ARTICLE

Protective effects of date palm extract as natural antioxidants on hepatotoxicity induced by *Cerastes cerastes* venom in albino rats.

***Mohamed A. Bashandy[1], Ali Abd-El-Aal[2], Diaa F. Ibrahim[1], Mahmoud A. El-Sharkawy[1]**

1. Zoology Department, Faculty of Science, Al-Azhar University, Cairo, Egypt.
2. Zoology Department, Faculty of Science, Ain-shams University, Cairo, Egypt.

msharkawy2011@gmail.com

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Corresponding Author

Mohamed A. Bashandy.

Abstract

The present study was designed to determine the possible protective effects of date palm extract against *Cerastes cerastes* venom induced oxidative stress and biochemical changes in liver of albino rats. A patch of 104 male Wister albino rats averaged weights (130±10g) at the beginning of the experiment were divided into 6 main groups according to the treatment and requirements of the experiment. Control rats group, DPE group treated orally with DPE at dose 1.2 mg/kg body weight, venoms groups: Rats injected with a single dose of *Cerastes cerastes* for one day, this group divided into three subgroups injected by a single dose of 1/4 LD_{50}, 1/2 LD_{50} and LD_{50} for one day also for two weeks groups, venoms treated with DPE groups treated orally with DPE for two weeks before /and after injecting of a single dose of venom at dose 1/4 LD_{50}, 1/2 LD_{50} and LD_{50} for one day. Each group contains 6 rats except venom groups of LD_{50} were 8 rats and six rats were sacrificed after the 1st day, 2nd and 4th week from each group of treatment.

The results showed a significant raise of some hepatic parameters (ALAT, ASAT, ALP, TG, CHO and LDL-C), while a significant reduction in some other parameters (Total protein, albumin and HDL-C), on the 1st day and 2nd weeks in rats injected intraperitoneal with *Cerastes cerastes* venom as compared to the control groups. The administration of the DPE ameliorated the side effects of the poisonous changes of *Cerastes cerastes* venom.

In conclusion, according to the results obtained revealed that the administration of DPE had hepatoprotective effects against envenomation with *Cerastes cerastes* venom in male Wister albino rats by inhibiting oxidative stress through ROS scavenger and improvement of the biochemical markers.

Introduction:-

Venomous animal bites are a significant health problem for rural populations in many parts of the world **(Warrell, 1992).** Venom of snakes consists of a complex mixture of many substances, such as toxins, enzymes, growth factors, activators and inhibitors with a wide spectrum of biological activities and non-enzymatic proteins with pro and anticoagulant activities, causing hemorrhage, hypovolemic shock, thrombosis and tissue damage of vital organs **(White, 2005; Al-Sadoon *et al.*, 2013; Cherifi and Laraba-Djebari, 2013 and Berling and Isbister, 2015).**

Viperidae toxicity is including cytotoxicity, haemotoxicity and neurotoxicity **(Masood, 2012 and WHO, 2012).** Viper venoms are slowly absorbed and reach maximum serum levels within 6-24 hours **(Warrell, 1995).**

The most common venoms snake bite was of the horned viper, *Cerastes cerastes* **(Al-Sadoon, 2015),** It contains different enzymes displaying proteolytic activity and causes many toxicities **(Warrell, 2004).** Also **Boumaiza *et al,* (2016)** suggested that significant physiopathological changes in liver, heart and skin. Enzyme in *Cerastes cerastes* such as, phospholipases A_2 (PLA_2) has been associated with multiple toxicities including lung toxicity **(Uma *et al.,* 2000),** nephrotoxicity **(de Castro *et al.,* 2004),** cardiotoxicity **(Cher *et al.,* 2005),** neurotoxicity and hepatotoxicity **(Mukherjee and Maity, 1998).**

The lethal effects of snake venom were largely attributed to its active ingredients as phospholipase A_2 (PLA_2). Phospholipid hydrolysis by PLA_2 releases arachidonic acid whose metabolism results in the formation of potentially toxic ROS and lipid peroxides **(Adibhatla *et al.,* 2003 and Abdel-Rahman *et al.,* 2013).**

The rise in the activities of liver enzymes indicate the damage of liver, heart and other organs brought about by the venom **(Al-Sadoon and Fahim, 2012 and Al-Quraishy, *et al.,* 2014). Salman (2009)** reported that intraperitoneal injection of the venom of *Echis coloratus* induced a significant increase in plasma cholesterol level in rabbits.

Antioxidants play an important role in inhibiting and scavenging radicals, thus providing protection to infection and degenerative diseases (**Reddy, *et al.,* 2010)**. Nutritional antioxidants are important in controlling and improving the harmful effects of oxidative stress, High eating of fruits with high antioxidant content contributes to reduced risk of oxidative stress-mediated diseases such as liver disease and cancer **(Lindquist, *et al.,* 2000).**

Date palm (*Phoenix dactylifera*), is a good source of energy, vitamins, and a group of elements like phosphorus, iron, potassium and calcium **(El-Gazzar *et al*., 2009).** Dates contain several vitamins including a small amount of vitamin C, and vitamins B1 (thiamine), B2 (riboflavin), nicotinic acid (niacin) and vitamin A **(Al-Shahib and Marshall, 1993)** Many researchers have also documented the antioxidant property of dates **(Mohamed and Al-Okbi, 2004; Allaith and Abdul, 2005 and Al-Qarawi *et al*., 2008).**

Dates are considered to be one of the most significant commercial crops and also have been documented in Holy Quran and modern scientific literature. Earlier studies have shown that constituents of dates act as potent antioxidant, anti-tumour as well as anti-inflammatory, provide a suitable alternative therapy in various diseases cure **(Rahmani, *et al.,* 2014).**

Also, date palm extract (DPE) has anticancer **(Ishurd *et al*., 2004)** and antiviral **(Vayalil, 2002),** antimutagenic properties **(Hasan *et al,*. 2010)** and immunomodulatory benefits to health but also has diverse medicinal values, including antihyperlipidemic, gastroprotective, hepatoprotective, and nephroprotective properties **(Tang *et al.,* 2013).**

Date palm could be considered as a functional protective food for liver toxicity (**Abdu. 2011**). Aqueous DPE have strong antioxidant activity it's attributed to the wide range of phenolic compounds in dates including p-coumaric, ferulic and sinapic acids, flavonoids and procyanidins **(Kelm, *et al.,* 2003; Al-Farsi, *et al.,* 2005).**

Date palm characteristic contents of a high percentage of carbohydrates, dietary fibers, fats, proteins, vitamins and minerals gave it the superiority over other types of dates **(Al-Shahib and Marshall, 2003 and Abdu, 2011).** Melatonin is found to be capable of protector of DNA **(LopezBurillo *et al.,* 2003),** protein and lipids in cellular membranes **(Cuzzocrea and Reiter, 2001).** It also acts as an antagonist and suppresses of a number of endogenous and exogenous free radicals generated during the cellular process **(Zang *et al.,* 1998 and Guo *et al.,* 2003).**

Abdelrahman *et al.* (2012) Studied pretreated dogs with DPE found that significantly decrease the levels of liver enzymes at 6 and 14 days post CCL_4 injection. Palm date syrup showed significant hepatoprotective activity in carbon tetrachloride (CCl_4) induced hepatotoxicity among New Zealand rabbits **(Mallhi *et al.,* 2014); El Arem *et al.* (2014)** demonstrated a hepatoprotective effect of date palm fruit extract on oxidative damage induced by dimethoate, trichloroacetic, and carbon tetrachloride (CCl_4).

Aim of the work:-

The present study aimed to evaluate the ameliorative effects of date palm extract in reducing the hazards resulted from venom injection.

Materials and Methods:-

Experimental Design:-

One hundred and four male Wister albino rats (*Rattus norvegicus*) of about 130 ± 10 g body weight were conducted in accordance with the criteria of the investigations and Ethics Committee of the Community Laws governing the use of experimental animals. The rats 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 *adlibitum* to the animals.

The rats were distributed into 6 main groups and 16 subgroups according to the treatment and requirements of the experiment as the following: **Group I:** Control rats **Group II:** treated orally with DPE at dose 1.2 mg/kg body weight **Group III:** Rats injected with a single dose of *Cerastes cerastes* for one day, this group divided into three subgroup injected by a single dose of 1/4 LD_{50}, 1/2 LD_{50} and LD_{50} for one day **Group IV:** Rats injected with a single dose of *Cerastes cerastes* for two weeks, this group divided into three subgroup injected by a single dose of 1/4 LD_{50}, 1/2 LD_{50} and LD_{50} for two weeks **Group V:** treated orally with DPE for two weeks and injected by a single dose of venom at dose 1/4 LD_{50}, 1/2 LD_{50} and LD_{50} for one day **Group VI:** treated orally with DPE for two weeks and injected by a single dose of venom at dose 1/4 LD_{50}, 1/2 LD_{50} and LD_{50} for one day and completely treated by DPE for two weeks.

The animals were observed daily for signs of toxicity. The body weights were recorded weekly during the period of the experiment. Six rats from each group were sacrificed on the 1st and 15th days for control and treatments.

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 extract was prepared by removing the nuclei of the fruit and grounded into fine Powder. The flesh of the fruits was left in distilled water (1:3) for 48 hours in 4°C **(Al-Qarawi *et al*., 2005).** The whole solution was ground, then centrifuged at 4°C for 20 min at 4000 rpm. The supernatant was collected and stored at - 80°C till use **(Vayalil, 2002).**

Dose calculation:-

The method described by **Sutken *et al*., (2007)** the dose level of 1.2 mg/kg/day of DPE is equivalent to 7 DP for each person per day, the human date fruit recommended antioxidant dose was 35g/day. Rat dose (mg/kg) = 1.2 g/kg body weight, according to studies of **Al-Qarawi *et al*., (2004).**

Determination of the half lethal dose (LD50):-

The approximate median lethal dose (LD_{50}) of the crude venom (*Cerastes cerastes*) was calculated according to the method described by **Meier and Theakston (1986).** The Lethal Dose (LD_{50}) of venom of *Cerastes cerastes* viper equal 3.8 mg/kg.

Induction of hepatotoxicity:-

Hepatotoxicity was induced by intraperitoneal injection of *Cerastes cerastes* venom at different doses (1/4 LD_{50}, 1/2 LD_{50} and LD_{50}).

Sample preparation:-

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.

Biochemical parameters:-

The serum levels of transaminases, alkaline phosphatase, total protein and albumin were estimated using kits from Egyptian Company for biotechnology spectrum, Egypt. The concentrations of transaminases (ALAT and ASAT) were determined using the method of **Reitman and Frankel (1975)**. Serum ALP was determined according to the method described by **Tietz *et al.*, (1983)**. Serum total protein was determined according to the method described by **Gornal *et al.* (1949)**. Serum albumin was determined according to the method described by **Doumas *et al.* (1971)**. Lipid profile was estimated using kits from Egyptian Company for biotechnology spectrum, Egypt. The concentrations of triglycerides were determined according to the method described by **MGowan *et al.* (1983).** Serum cholesterol level was determined according to the method described by **Tietz (1976).** Serum HDL-C was determined according to the method of **Burstein *et al.* (1970)**. The LDL-C uses estimated according to the formula of **Wieland and Seidel (1982).**

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). The data were expressed as mean ± S.E. Differences were considered statistically significant at $P < 0.05$.

Results:-

The present results showed thatrats injected with a single dose of *Cerastes cerastes* venoms at 1/4 LD_{50}, 1/2 LD_{50} and LD_{50} exhibited biochemical disorders established by significant increase ($p \leq 0.05$) in some hepatic parameters (ALAT, ASAT and ALP) post-injection with a single dose of venom for one day as well as for two weeks groups as compared to the control group. On the other hand, insignificant differences with recorded in DPE groups when compared to control groups. Also, the present study demonstrated that the rats treated with DPE at a dose level of 1.2 mg/kg/day before/ and after injection with a single dose of venom at doses 1/4 LD_{50}, 1/2 LD_{50} and LD_{50} showed significantly decrease ($P \leq 0.05$) in liver function enzymes activities (ALAT, ASAT and ALP) when compared to venom groups. As shown in Table and figure (1, 2 and 3).

Serum total protein and albumin level in rats injected with a single dose of venom for one day as well as for two weeks groups recorded a significant decrease ($p < 0.05$) as compared to the control group. On contrast insignificant differences with recorded in DPE when compared to control groups. Rats treated with DPE at a dose level of 1.2 mg/kg/day before and /or after injection with a single dose of venom at doses 1/4 LD_{50}, 1/2 LD_{50} and LD_{50} observed a significant increase amelioration ($p < 0.05$) when compared with venom for two and four weeks. On the other hand, the rats treated with DPE for four week at a dose level of 1.2 mg/kg/day before and after injection with a single dose of venom at doses 1/4 LD_{50}, 1/2 LD_{50} and LD_{50} showed insignificant change in albumin when compared to control groups. As shown in Table and figure (4 and 5).

Resulted data which found in table and figure (6, 7, 8 and 10) showed a significantly increase ($p < 0.05$) in lipid profile (triglycerides, cholesterol and LDL-C) level, also a significantly decrease ($p < 0.05$) in HDL-C level in in rats injection with a single dose of venom for one day as well as for two weeks groups as compared to the control group. On the other hand, insignificant differences with recorded in DPE groups when compared to control groups. Rats treated with DPE at a dose level of 1.2 mg/kg/day before and /or after injection with a single dose of venom at doses 1/4 LD_{50}, 1/2 LD_{50} and LD_{50}, observed a significant increase for (triglycerides, cholesterol and LDL-C) level and increase for HDL-C level ($p < 0.05$) when compared with venom for two and four weeks.

Table (1): Effect of pre-and post-treatment of date palm extract on serum alanine aminotransferase (ALAT) activity (U/ml) of rats injected with *cerastes cerastes* venom.

Groups	Non venom groups				Venom groups											
	Control		Date		Venom groups without date palm						Venom groups with date palm					
					Venom for 1 day			Venom for 2week			Date 2w +venom			Date2w+venom + Date 2w		
Sub Gp.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Dose	Saline		1.2g/k/day		1/4 LD50	1/2 LD50	LD50	1/4 LD50	1/2 LD50	LD50	1/4 LD50	1/2 LD50	LD50	1/4 LD50	1/2 LD50	LD50
Period	2W	4W	2W	4W	1 Day			2W			2W			4W		
Mean ± SE	45.63^{a} ± 0.56	45.75^{a} ± 0.71	45.79^{a} ± 1.12	45.39^{a} ± 0.57	104.92^{b} ± 1.19	108.06^{c} ± 0.46	115.66^{d} ± 0.98	99.8^{e} ± 0.68	102.46^{f} ± 0.85	110.33^{c} ± 0.87	77.23^{g} ± 0.58	85.32^{h} ± 1.2	88.03^{i} ± 1.68	46.02^{a} ± 0.39	46.8^{a} ± 0.56	48.28^{a} ± 0.6
%			0.35	0.25	59.29	62.43	70.03	54.17	56.83	64.69	31.6	39.69	42.39	0.28	1.1	2.5

Each value represents mean of 6 records ± S.E, Except LD_{50} groups are 4 records, Means with dissimilar superscript letter are significantly different at ($p<0.05$), W:Weeks, Venom: single dose, % Percent of changes from control values.

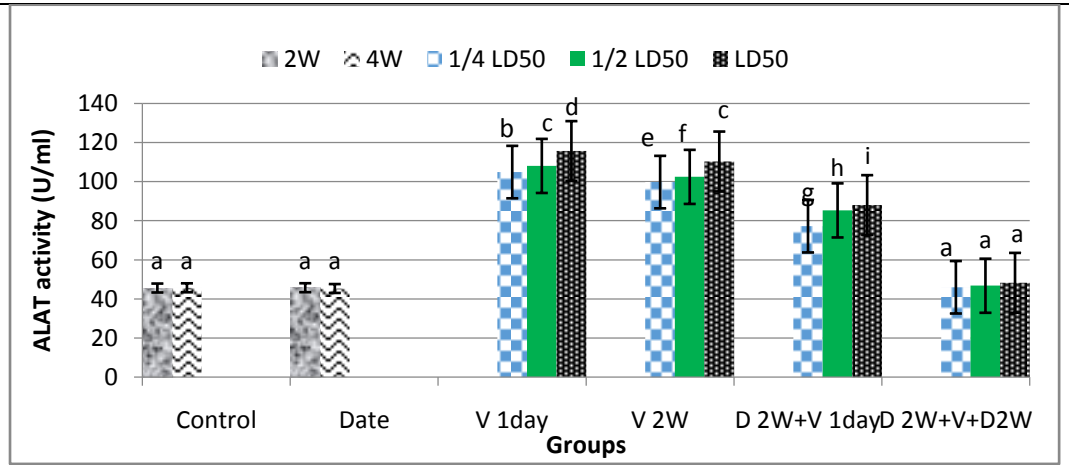

Fig (1): serum alanine aminotransferase (ALAT) activity (U/ml) in different animal groups.

Table (2): Effect of pre-and post-treatment of date palm extract on serum aspartate aminotransferase (ASAT) activity (U/ml) of rats injected with *cerastes cerastes* venom.

Groups	Non venom groups				Venom groups											
	Control		Date		Venom groups without date palm						Venom groups with date palm					
					Venom for 1 day			Venom for 2week			Date 2w +venom			Date2w+venom + Date 2w		
Sub Gp.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Dose	Saline		1.2g/k/day		1/4 LD_{50}	1/2 LD_{50}	LD_{50}	1/4 LD_{50}	1/2 LD_{50}	LD_{50}	1/4 LD_{50}	1/2 LD_{50}	LD_{50}	1/4 LD_{50}	1/2 LD_{50}	LD_{50}
Period	2W	4W	2W	4W	1 Day			2W			2W			4W		
Mean ± SE	119.3^{a} ± 0.7	119.3^{a} ± 0.46	119.4^{a} ± 0.63	119.6^{a} ± 0.3	213.77^{b} ± 1.46	226.54^{c} ± 0.62	237.53^{d} ± 1.08	201.63^{e} ± 1.11	228.72^{c} ± 0.93	226.48^{c} ± 0.54	160.75^{f} ± 0.85	162.03^{f} ± 0.55	166.95^{g} ± 0.46	121.15^{a} ± 0.74	122.28^{a} ± 0.99	124.53^{a} ± 1.05
%			0.27	0.47	94.6	107.37	118.36	82.47	109.55	107.3	41.58	42.87	47.78	1.98	3.12	5.36

Each value represents mean of 6 records ± S.E, Except LD_{50} groups are 4 records, Means with dissimilar superscript letter are significantly different at ($p<0.05$), W:Weeks, Venom: single dose, % Percent of changes from control values.

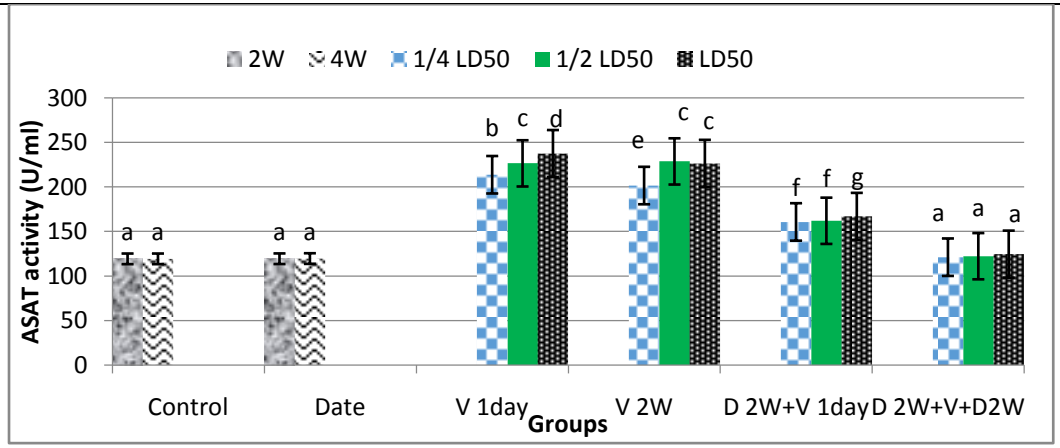

Fig (2): Serum aspartate aminotransferase (ASAT) activity (U/ml) in different animal groups

Table (3): Effect of pre-and post-treatment of date palm extract on serum alkaline phosphatase (ALP) activity of rats injected with *cerastes cerastes* venom.

Groups	Non venom groups				Venom groups											
	Control		Date		Venom groups without date palm						Venom groups with date palm					
					Venom for 1 day			Venom for 2week			Date 2w +venom			Date2w+venom + Date 2w		
Sub Gp.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Dose	Saline		1.2g/k/day		1/4 LD_{50}	1/2 LD_{50}	LD_{50}	1/4 LD_{50}	1/2 LD_{50}	LD_{50}	1/4 LD_{50}	1/2 LD_{50}	LD_{50}	1/4 LD_{50}	1/2 LD_{50}	LD_{50}
Period	2W	4W	2W	4W	1 Day			2W			2W			4W		
Mean ± SE	116.82^{a} ± 1.08	117^{a} ± 1.28	117.15^{a} ± 0.8	117.23^{a} ± 0.59	263.33^{b} ± 0.83	265.64^{b} ± 0.66	277.67^{c} ± 1.06	223.3^{d} ± 0.81	220.88^{d} ± 0.77	219.91^{d} ± 0.62	201.5^{e} ± 3	209.52^{f} ± 1.97	213.33^{f} ± 0.83	151.77^{g} ± 2.33	160.5^{h} ± 2.51	167.55^{i} ± 2.17
%			0.33	0.42	146.52	148.82	160.85	106.48	104.06	103.1	84.68	92.7	96.51	34.95	43.68	50.73

Each value represents mean of 6 records ± S.E, Except LD_{50} groups are 4 records, Means with dissimilar superscript letter are significantly different at ($p<0.05$), W:Weeks, Venom: single dose, % Percent of changes from control values.

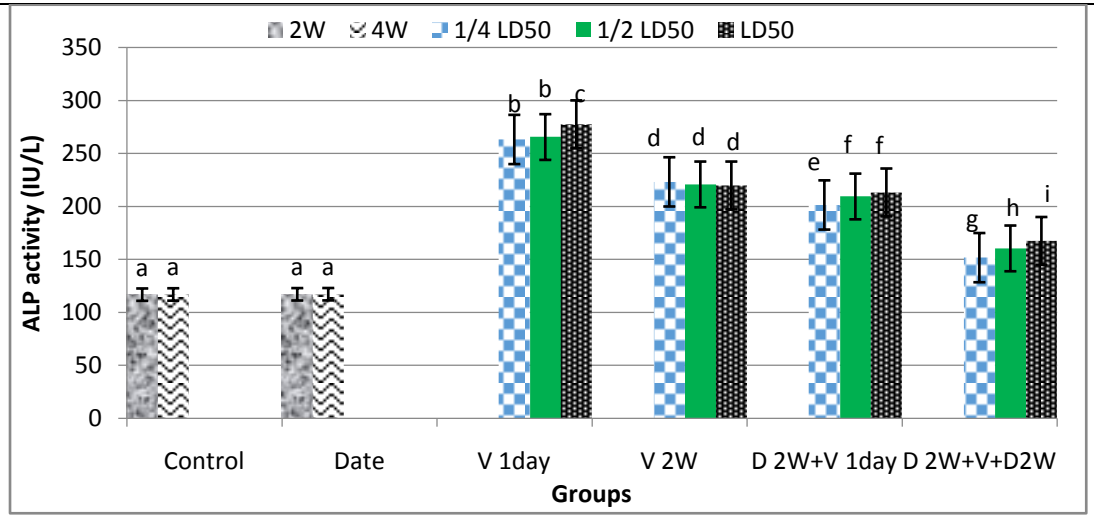

Fig (3): serum alkaline phosphatase (ALP) activity (IU/L) in different animal groups.

Table (4): Effect of pre-and post-treatment of date palm extract on serum total protein concentration (g/dl) of rats injected with *cerastes cerastes* venom.

Groups	Non venom groups				Venom groups											
	Control		Date		Venom groups without date palm						Venom groups with date palm					
					Venom for 1 day			Venom for 2week			Date 2w +venom			Date2w+venom + Date 2w		
Sub Gp.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Dose	Saline		1.2g/k/day		1/4 LD_{50}	1/2 LD_{50}	LD_{50}	1/4 LD_{50}	1/2 LD_{50}	LD_{50}	1/4 LD_{50}	1/2 LD_{50}	LD_{50}	1/4 LD_{50}	1/2 LD_{50}	LD_{50}
Period	2W	4W	2W	4W	1 Day			2W			2W			4W		
Mean ± SE	6.64^{a} ± 0.11	6.61^{a} ± 0.11	6.61^{a} ± 0.11	6.65^{a} ± 0.12	5.2^{b} ± 0.12	5.14^{c} ± 0.11	4.96^{d} ± 0.12	5.69^{e} ± 0.12	$5.54^{b,f}$ ± 0.11	5.27^{c} ± 0.12	5.96^{g} ± 0.11	5.75^{g} ± 0.11	5.89^{f} ± 0.12	6.31^{h} ± 0.11	$6.2^{h,i}$ ± 0.11	$6.06^{g,I}$ ± 0.12
%			0.03	0.02	-1.44	-1.5	-1.68	-0.95	-1.1	-1.36	-0.68	-0.89	-0.75	-0.29	-0.41	-0.55

Each value represents mean of 6 records ± S.E, Except LD_{50} groups are 4 records, Means with dissimilar superscript letter are significantly different at ($p<0.05$), W:Weeks, Venom: single dose, % Percent of changes from control values.

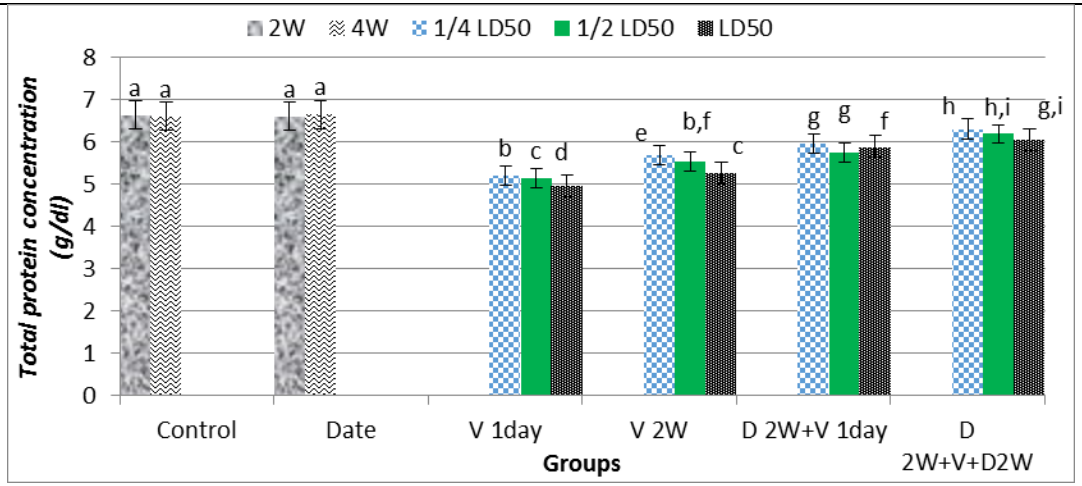

Fig (4): Serum total protein concentration (g/dl) in different animal groups.

Table (5): Effect of pre-and post-treatment of date palm extract on serum albumin concentration (g/dl) of rats injected with *cerastes cerastes* venom.

Groups	Non venom groups				Venom groups											
	Control		Date		Venom groups without date palm						Venom groups with date palm					
					Venom for 1 day			Venom for 2week			Date 2w +venom			Date2w+venom + Date 2w		
Sub Gp.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Dose	Saline		1.2g/k/day		1/4 LD_{50}	1/2 LD_{50}	LD_{50}	1/4 LD_{50}	1/2 LD_{50}	LD_{50}	1/4 LD_{50}	1/2 LD_{50}	LD_{50}	1/4 LD_{50}	1/2 LD_{50}	LD_{50}
Period	2W	4W	2W	4W	1 Day			2W			2W			4W		
Mean ± SE	4.42^{a} ± 0.03	4.43^{a} ± 0.3	4.41^{a} ± 0.3	4.43^{a} ± 0.3	3.36^{b} ± 0.05	3.27^{b} ± 0.017	3.5^{c} ± 0.025	3.88^{d} ± 0.082	3.68^{e} ± 0.063	3.7^{e} ± 0.07	4.01^{g} ± 0.04	$3.93^{d,g}$ ± 0.02	$3.93^{d,g}$ ± 0.05	4.3^{a} ± 0.03	4.31^{a} ± 0.03	4.21^{a} ± 0.04
%			-0.07	-0.02	-1.06	-1.15	-0.92	-0.55	-0.74	-0.71	-0.41	-0.49	-0.48	-0.12	-0.11	-0.21

Each value represents mean of 6 records ± S.E, Except LD_{50} groups are 4 records, Means with dissimilar superscript letter are significantly different at ($p<0.05$), W:Weeks, Venom: single dose, % Percent of changes from control values.

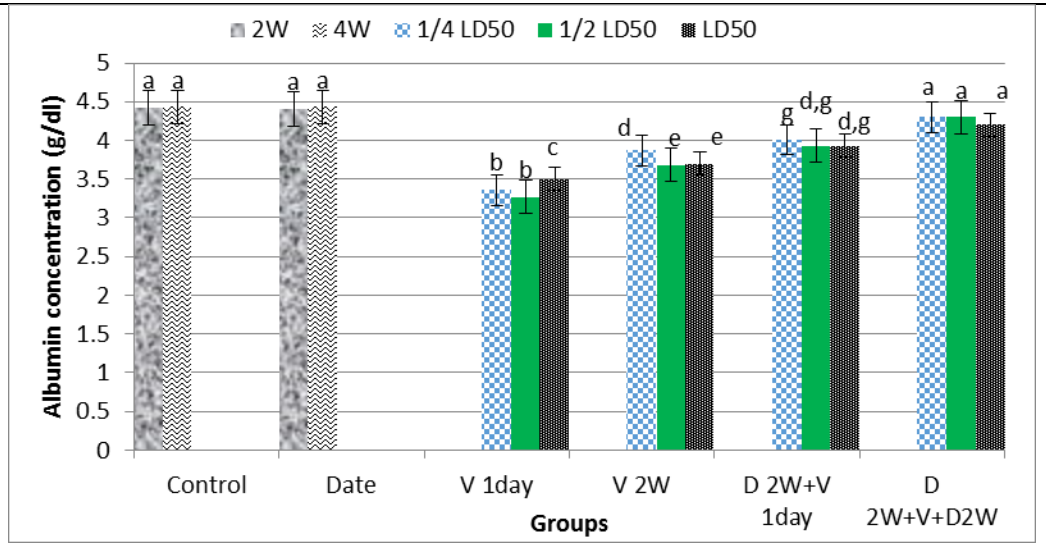

Fig (5): serum albumin concentration (g/dl) in different animal groups.

Table (6): Effect of pre-and post-treatment of date palm extract on serum Triglycerides (TG) concentration (g/dl) of rats injected with *cerastes cerastes* venom.

Groups	Non venom groups				Venom groups											
	Control		Date		Venom groups without date palm						Venom groups with date palm					
					Venom for 1 day			Venom for 2week			Date 2w +venom			Date2w+venom + Date 2w		
Sub Gp.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Dose	Saline		1.2g/k/day		1/4 LD_{50}	1/2 LD_{50}	LD_{50}	1/4 LD_{50}	1/2 LD_{50}	LD_{50}	1/4 LD_{50}	1/2 LD_{50}	LD_{50}	1/4 LD_{50}	1/2 LD_{50}	LD_{50}
Period	2W	4W	2W	4W	1 Day			2W			2W			4W		
Mean ± SE	114.55^{a} ± 0.36	114.5^{a} ± 0.58	114.52^{a} ± 0.25	114.45^{a} ± 0.3	164.32^{b} ± 1.12	147.97^{c} ± 1.48	183.5^{d} ± 1.48	143.67^{e} ± 0.79	147.11^{f} ± 1.31	155.6^{g} ± 1.03	129.85^{h} ± 0.75	138.22^{i} ± 0.62	139.68^{i} ± 1.28	$118^{j,k}$ ± 0.37	$119.35^{j,k}$ ± 0.33	121.32^{k} ± 0.4
%			0.03	0.1	49.77	60.42	68.95	29.12	32.56	41.04	15.3	23.67	25.13	3.45	4.8	6.77

Each value represents mean of 6 records ± S.E, Except LD_{50} groups are 4 records, Means with dissimilar superscript letter are significantly different at ($p<0.05$), W:Weeks, Venom: single dose, % Percent of changes from control values.

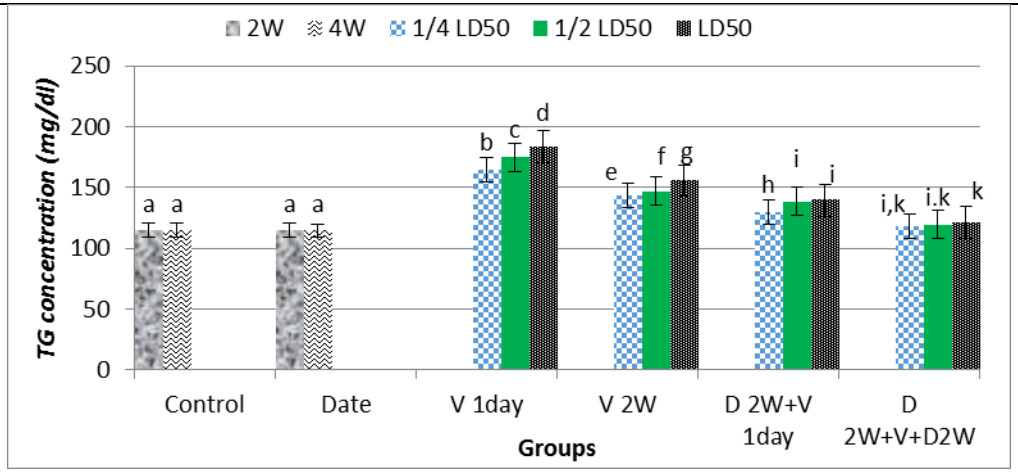

Fig (6): serum triglycerides (TG) concentration (mg/dl) in different animal groups.

Table (7): Effect of pre-and post-treatment of date palm extract on serum cholesterol concentration (mg/dl) of rats injected with *cerastes cerastes* venom.

Groups	Non venom groups				Venom groups											
	Control		Date		Venom groups without date palm						Venom groups with date palm					
					Venom for 1 day			Venom for 2week			Date 2w +venom			Date2w+venom + Date 2w		
Sub Gp.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Dose	Saline		1.2g/k/day		1/4 LD_{50}	1/2 LD_{50}	LD_{50}	1/4 LD_{50}	1/2 LD_{50}	LD_{50}	1/4 LD_{50}	1/2 LD_{50}	LD_{50}	1/4 LD_{50}	1/2 LD_{50}	LD_{50}
Period	2W	4W	2W	4W	1 Day			2W			2W			4W		
Mean ± SE	114.14^{a} ± 0.54	114.06^{a} ± 0.48	114.36^{a} ± 0.54	114.3^{a} ± 0.86	203.26^{b} ± 1.13	214.96^{c} ± 0.67	218.77^{d} ± 0.72	178.88^{e} ± 0.86	184.72^{f} ± 0.97	195.43^{g} ± 0.85	137.23^{h} ± 1.17	143.88^{i} ± 0.73	158.05^{j} ± 1.24	120.36^{k} ± 0.69	123.78^{l} ± 0.44	125.06^{l} ± 1.85
%			0.21	0.16	89.12	100.82	104.63	64.74	70.57	81.29	23.09	29.73	43.9	6.21	9.64	10.92

Each value represents mean of 6 records ± S.E, Except LD_{50} groups are 4 records, Means with dissimilar superscript letter are significantly different at ($p<0.05$), W:Weeks, Venom: single dose, % Percent of changes from control values.

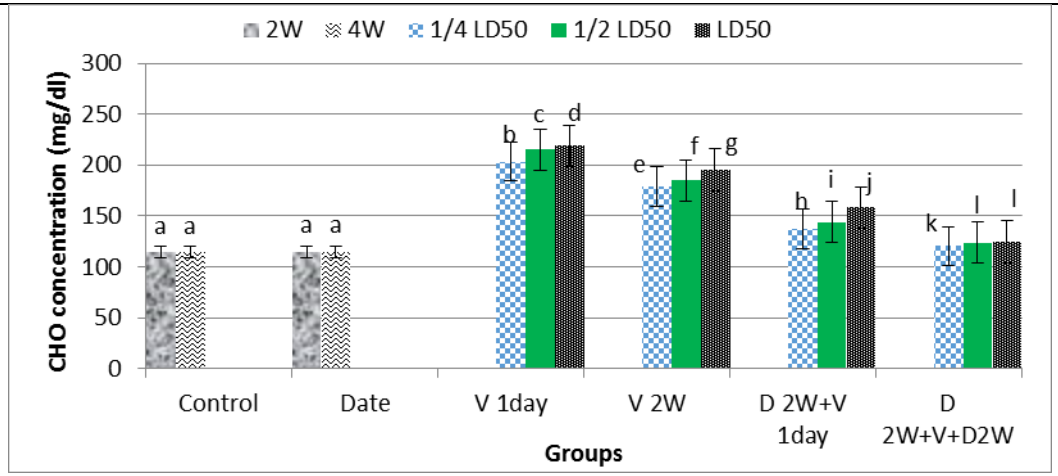

Fig (7): serum cholesterol (CHO) concentration (mg/dl) in different animal groups.

Table (8): Effect of pre-and post-treatment of date palm extract on serum low-density lipoprotein cholesterol (LDL-C) concentration (mg/dl) of rats injected with *cerastes cerastes* venom.

Groups	Non venom groups				Venom groups											
	Control		Date		Venom groups without date palm						Venom groups with date palm					
					Venom for 1 day			Venom for 2week			Date 2w +venom			Date2w+venom + Date 2w		
Sub Gp.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Dose	Saline		1.2g/k/day		1/4 LD_{50}	1/2 LD_{50}	LD_{50}	1/4 LD_{50}	1/2 LD_{50}	LD_{50}	1/4 LD_{50}	1/2 LD_{50}	LD_{50}	1/4 LD_{50}	1/2 LD_{50}	LD_{50}
Period	2W	4W	2W	4W	1 Day			2W			2W			4W		
Mean ± SE	27.26^{a} ± 0.78	27.11^{a} ± 0.39	27.03^{a} ± 0.47	27.08^{a} ± 0.68	145.06^{b} ± 1.09	152.97^{c} ± 1.71	158.57^{d} ± 1.25	124.48^{e} ± 1.99	130.9^{f} ± 1.84	136.06^{g} ± 2.31	69.76^{h} ± 1.01	77.23^{i} ± 1.03	93.11^{j} ± 1.78	32.62^{k} ± 0.82	46.18^{l} ± 0.65	52.04^{m} ± 1.27
%			0.22	0.18	117.8	125.71	131.31	97.22	103.64	108.81	42.5	49.97	65.85	5.36	18.92	24.78

Each value represents mean of 6 records ± S.E, Except LD_{50} groups are 4 records, Means with dissimilar superscript letter are significantly different at ($p<0.05$), W:Weeks, Venom: single dose, % Percent of changes from control values.

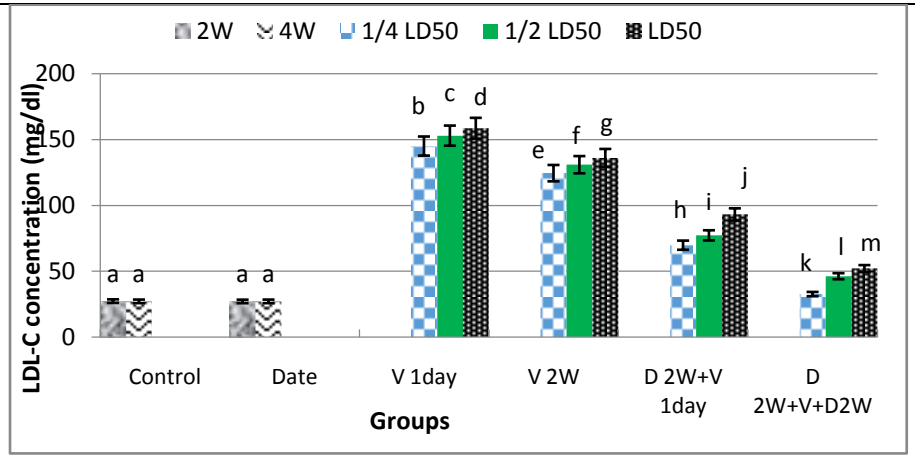

Fig (8): Serum low-density lipoprotein cholesterol (LDL-C) concentration (mg/dl) in different animal groups.

Table (9): Effect of pre-and post-treatment of date palm extract on serum high-density lipoprotein cholesterol (HDL-C) concentration (mg/dl) of rats injected with *cerastes cerastes* venom.

Groups	Non venom groups				Venom groups											
	Control		Date		Venom groups without date palm						Venom groups with date palm					
					Venom for 1 day			Venom for 2week			Date 2w +venom			Date2w+venom + Date 2w		
Sub Gp.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Dose	Saline		1.2g/k/day		1/4 LD_{50}	1/2 LD_{50}	LD_{50}	1/4 LD_{50}	1/2 LD_{50}	LD_{50}	1/4 LD_{50}	1/2 LD_{50}	LD_{50}	1/4 LD_{50}	1/2 LD_{50}	LD_{50}
Period	2W	4W	2W	4W	1 Day			2W			2W			4W		
Mean ± SE	67^{a} ± 0.58	67^{a} ± 0.58	67.33^{a} ± 0.67	67.25^{a} ± 0.81	$25.33^{b,d}$ ± 1.12	$27^{b,d}$ ± 1.05	23.5^{b} ± 0.65	$25.67^{b,d}$ ± 1.5	24.4^{b} ± 1.21	28.25^{d} ± 3.09	41.5^{e} ± 0.92	$39^{e,f}$ ± 0.58	37^{f} ± 1.08	56.17^{g} ± 0.83	50.67^{h} ± 1.43	45.5^{i} ± 2.25
%			0.33	0.25	-41.67	-40	-43.5	-41.33	-42.6	-38.75	-25.5	-28	-30	-10.83	-16.33	-21.5

Each value represents mean of 6 records ± S.E, Except LD_{50} groups are 4 records, Means with dissimilar superscript letter are significantly different at ($p<0.05$), W:Weeks, Venom: single dose, % Percent of changes from control values.

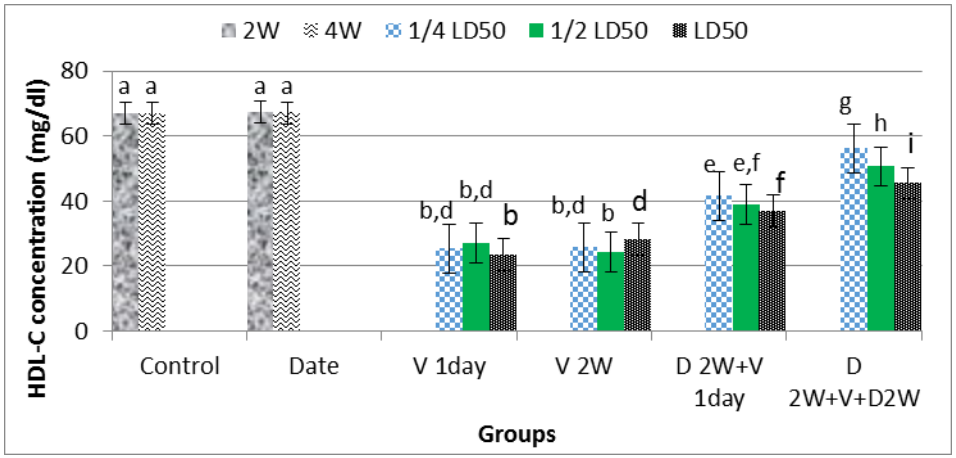

Fig (9): Serum high-density lipoprotein cholesterol (HDL-C) concentration (mg/dl) in different animal groups.

Discussion:-

Liver enzymes (ALAT and ASAT) are markers for cellular damage, although ALAT is more specific to detect liver injury the liver than is ASAT.

The increases of ALAT, ASAT and ALP enzyme activities in rats injected with a single dose of venom (*Cerastes cerastes*) at dose of 1/4 LD_{50}, 1/2 LD_{50} and LD_{50} for one day as well as two weeks showed significant increase ($p<0.05$) when compared to the corresponding values in control group. This increase could be attributed to venom induced oxidative stress on the liver cells and distortion of the architecture pattern of the hepatocytes by the interaction of released free radicals with cell membrane and this may be indicated to necrosis or hepatocellular injury; These results are in agreement with **(Al-Jammaz 2001; Al-Sadoon and Fahim, 2012 and Al-Sadoon *et al.*, 2013).**

Otherwise their activities in the groups pre/or post-treated with DPE and injected with a single dose of venom (*Cerastes cerastes*) at dose of 1/4 LD_{50}, 1/2 LD_{50} and LD_{50} for two weeks as well as four weeks showed significant decrease ($p<0.05$) when compared to the corresponding values in venom groups. Also showed insignificant change in the rats treated with DPE for four week plus a single dose of venom at doses 1/4 LD_{50}, 1/2 LD_{50} and LD_{50}, showed insignificant change in liver function enzymes activities (ALAT and ASAT) when compared to control groups. This reduction in the serum enzymes activities by DPE may be due to reduction in the lipid peroxidation of hepatocellular membrane induced by the venom. While, it is may be due to the accelerated regeneration/ repairing of damaged hepatocytes.

El-Gazzar *et al.*, (2009) suggested that aqueous DPE can be ameliorated hepatotoxicity induced by CCl_4, also **Araak and Abdulhussein (2012)** who demonstrated that treatment of rabbits with date palm pollen aqueous suspension decreased serum levels of ALT, AST and ALP induced by administration of CCl_4.

DPE had been found to contain high amount of melatonin and vitamin E **(Al-Qarawi *et al.*, 2005)**, and this may reduce the hydroxyl and superoxide anion radicals **(Vayalil, 2002).** The decrease in ALAT, ASAT and ALP activities may improve the liver activity and this in agreement with other research worked on CCL4,Trichloracetic acid and Ochrotoxin A, **El-Mougy *et al.*, 1991; Zang *et al.*, 1998; Cuzzocrea and Reiter, 2001; Al Qarawi, *et al.*, 2004; Al-Farsi *et al.*, 2005; Bastway *et al.*, 2008; Rock *et al.*, 2009; Saafi *et al.*, 2010; Abd el-rahman *et al.*, 2012; Azadbakht *et al.*, 2012; Ragab *et al.*, 2013; Sangi *et al.*, 2014 and Sheikh *et al.*, 2014.**

The results of serum total protein and albumin concentrations in the groups injection with a single dose of venom (*Cerastes cerastes*) at dose of 1/4 LD_{50}, 1/2 LD_{50} and LD_{50} for one day as well as two weeks showed a significant decrease ($p<0.05$) when compared with the corresponding values in control group. This decrease may be due to inhibition of oxidative phosphorylation which leads to decrease in protein synthesis, increase in catabolic processes and reduction of protein absorption. As well as the albumin levels in hepatic were found to be decreased in envenomed rats.

Meier and Stocker 1991 and **March *et al.*, 1997** described reduction of total protein and albumin in envenomated rats was observed by the increasing in vascular permeability and hemorrhages in vital organs, and this is agreement with **(Al-Sadoon, 1991; Abdul- Nabi *et al.*, 1997; Fahim 1998; Al-Jammaz *et al.*, 1998 and 1999; Al-Sadoon and Fahim, 2012).** Also, this is in accordance with the results of **Salman, (2009)** and **(2014)** who showed the levels of serum albumin, globulin and total protein were significantly decreased in the envenomated guinea pigs by viper. Also, the level of total protein indicated a significant decrease in the envenomated male albino rats after injected with *cerastes viper* snake venom **(Al Jammaz, *et al.*, 2002).** The results also are in agreements with the finding of **abdou *et al.* (2015)** who indicated that plasma total protein which is an indicator for hepatic function showed a significant decrease in male albino mice injected interaprotenial with a single dose.

Otherwise their activities in the groups pre/and post treated with DPE and injection with a single dose of venom (*Cerastes cerastes*) at dose of 1/4 LD_{50}, 1/2 LD_{50} and LD_{50} for two weeks as well as four weeks revealed significant increase ($p<0.05$) in serum albumin, globulin and total protein when compared to the corresponding values in venom group. On the other hand, the rats treated with DPE for four weeks plus injection with a single dose of venom at doses 1/4 LD_{50}, 1/2 LD_{50} and LD_{50}, showed insignificant change in albumin when compared to control groups.

Okwuosa *et al.* (2014) demonstrated that treatment of rats with aqueous DPE and methanolic DPE, respectively, improves thioacetamide-induced liver damage represented by alterations in liver function parameter. Dates seeds significantly increased serum level of albumin **(Paranthaman *et al.*, 2013).** While, the present results are this

disagreement with the findings of **Orabi and Shawky, 2014** reporting that Date seed causes a significant decrease in total protein and ALAT when compared with the control groups**.**

The results of the serum total cholesterol, triglycerides and low-density lipoprotein cholesterol concentrations showed significant increase as well as a significant decrease of high-density lipoprotein cholesterol concentration in the groups injection with a single dose of venom (*Cerastes cerastes*) at dose of 1/4 LD_{50}, 1/2 LD_{50} and LD_{50} for one day and for two weeks when compared to the corresponding values in control group. The results may be due to the hepatocytes damage rendering them unable to phosphorylate the increasing amounts of fatty acids, hence leading to fatty liver and alteration of cell membranes of tissues. Coronary heart disease is strongly related to decrease in the concentrations of HDL-cholesterol and increase in LDL-cholesterol **(Salah and Al-Maiman, 2005). Al-Jammaz (2001) and Salman (2009)** observed that the envenomated rats and guinea pigs showed an increase in serum cholesterol levels and triglycerides.

Results decreasing protein synthesis mainly, low density lipoprotein (LDL) that is responsible for carrying capacity of triglycerides from the liver to extra hepatic tissues, thus lipids tends to accumulate inside the hepatocytes giving the characteristics of fatty liver or steatosis (**Clawson *et al,* 1987**).

Al-Sadoon *et al*., 2013 suggested that the increases in serum total cholesterol, triglycerides and low-density lipoprotein cholesterol concentrations in envenomated guinea pig could be due to the hepatocytes damage rendering them unable to phosphorylate the increasing amounts of fatty acids, hence leading to fatty liver and alteration of cell membranes of tissues and this is in agreement with the finding of **Al-Jammaz, (2002); Lewis and Gutmann, (2004); Salman, (2009) and Al- Sadoon and Fahim, (2012).** And disagreement with **Abd El-Aal and Ezzat (1997)** found that a sub-lethal dose of *Naja haje* venom decreased serum total cholesterol in rabbits.

On the other hand, those levels in the groups of pre-or post-treated with DPE and injected with a single dose of venom (*Cerastes cerastes*) in TG,CHO and LDL-C at dose of 1/4 LD_{50}, 1/2 LD_{50} and LD_{50} for two weeks revealed significant decrease when compared to the corresponding values in venom groups. while, the groups of pre-or post-treated with DPE and injection with a single dose of venom (*Cerastes cerastes*) at dose of 1/4 LD_{50}, 1/2 LD_{50} and LD_{50} for four weeks revealed a very high significant decrease as compared to the corresponding values in venom groups.

A reduction in serum levels of triglyceride may be due to decreased lipogenesis and increased lipolytic activity by activation of the hormone-sensitive lipase or lipogenic enzymes **(Pari and Venkateswaran, 2004)**. Also, the present results are in agreement with **Borochov-Neori *et al.* 2013** who used ethanol and acetone extracts of DPE to inhibited LDL oxidation, and most extracts also stimulated total cholesterol removal from macrophages. Also, acetone extracts exhibited a significantly higher anti-atherogenic potency for most varieties. Our data are in agreement with **Abdelaziz and Ali, (2014) and Sheikh *et al.* (2014)** who found that treatment of rats with DP seeds and DPE, respectively, reduced the occurrence of liver injuries caused by CCl_4 intoxication.

Our data coincide with **Abdelaziz and Ali (2014)** and **Sheikh *et al.* (2014)** who found that treatment of rats with DP seeds and DPE, respectively, reduced the occurrence of liver injuries caused by CCl_4 intoxication.

Salah and Al-Maiman (2005) reported that feeding date seed flour to rats reduced the plasma triglycerides, total cholesterol and low density lipoprotein**,** and this in agreement with **(El-Mougy *et al.*, 1991; Panahi and Asadi, 2009 and Mard *et al.* 2010).**

Conclusion:-

The dates (DPE) showed improvement in all biochemical parameters of lives human after injection of venom at different doses.

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