Research Article **ISSN: 2349 – 7114**

Asian Journal of Research in Pharmaceutical Sciences and Biotechnology

Journal home page: www.ajrpsb.com

https://doi.org/10.36673/AJRPSB.2020.v08.i01.A04

ANTI- HYPERCHOLESTEROLEMIA ACTIVITY OF ESSENTIAL OIL EXTRACTED FROM DATE PALM SEED IN MALE ALBINO MICE

.Maraia F. Elmhdwi[1], Wejdan K. Almesmari[1], Hayam A. Elawamy*[2], Naema M. El Aali[1]

[1]Department of Chemistry (Biochemistry), Fac. of Science, Benghazi University., Libya.

[2]Department of Medical Laboratory, College of Medical Technology, Benghazi, Libya.

ABSTRACT

Medicinal plants keep on giving significant therapeutic agents, both in traditional and modern medicine. Traditional medicines are earning significant and being concentrated to locate the premise of their helpful activities. Date palm "The princess of the plant kingdom", symbolize one of the most significant plant families with regarding human use. Essential oil extracted from the seed of date palm possesses a variety of pharmaceutical activities. This study investigate the effect of hypolipidemic in these oils by studying the curative effect in male albino mice for two weeks. Mice fed on high fat diet exhibit significant an increase in lipid profile and significant depletion in high-density lipoprotein (HDL) level and antioxidant enzymes, the study revealed that the essential oil extracted from date seed was able to reduce the serum lipid and improvement in level of antioxidant enzymes and HDL. The result compared with gemfibrozil (lopid), a standard orally effective hypolipidemic drug.

KEYWORDS

Hyperlipidemia, Curative, Seed of date palm, Lipid Profile and Liver Function.

Author for Correspondence:

Hayam A. Elawamy,
Department of Medical Laboratory,
College of Medical Technology, Benghazi, Libya.

Email: hayam.abdalla@gmail.com

INTRODUCTION

Anti-oxidants and free radicals assume a double role as both beneficial and toxic complex since they may be either hurtful or beneficial to the body. Normal cell metabolism also generated or from extrinsic sources (radiation, pollution, medication, cigarette smoke)[1]. Free radicals characterized as retrograde chemical type having a solitary unpaired electron in an external orbit, this unstable configuration generate energy, which released through reactions with neighboring molecules, such

as lipids, carbohydrates, nucleic acids and proteins. Most of free radicals that harm biological systems are oxygen-free radicals, and these are more mostly known as "reactive oxygen species" (ROS)[2]. The formation and removal of free radicals balanced in a normal cell. However, with more creating of free radicals or when value of antioxidants diminished, the cell enters a state called as ''oxidative stress''. This state if prolonging can cause cell damage and death. Oxidative stress plays a major role in the development of chronic and degenerative diseases such as cancer, arthritis, aging, autoimmune disorders, and cardiovascular and neurodegenerative diseases[3]. Cardiovascular diseases (CVDs) are considered to be the leading cause of death all over the world, and it is anticipated that the number of death due to CVDs may augment by the year 2020[4]. Atherosclerosis (also known as Arteriosclerotic Vascular Disease or ASVD) is a condition in which an artery wall thickens as a result of a build-up of fatty materials such as cholesterol[5]. Hyperlipidemia is a condition in which an increase in lipid content of the body that probably includes the high-rise in triglycerides and cholesterol levels[6]. Low-density lipoproteins (LDL cholesterol) is considered as the bad cholesterol because it participates in plaque formation that clogs arteries and leads to atherosclerosis which may further precipitate heart attack, stroke or peripheral artery disease. It carries cholesterol from the liver to muscles, tissues and heart for deposition. Thus when present in high levels in the blood stream than necessary increases the risk of development and progression of CVD.

The human body has several mechanisms to neutralize oxidative stress by generating antioxidants, which normally generated in the body or externally provided through food and/or supplements. Antioxidants are those molecules that are present at low level and significantly delays or prevent oxidation of the oxidizable substrate[7]. Exogenous and endogenous antioxidants are effective as free radical scavengers by donating its electrons to ROS and thereby neutralize adverse effects of the latter[8]. Thus, they can enhance the immune defense and lower the risk of cancer and degenerative diseases[9]. Herbal medicines are widely used all over the world. They often perceived as being natural and therefore harmless. Many herbal remedies individually or in combination with different formulations such as leaf, powder, pastes, decoction, infusion, etc. Recommended to treat various diseases. Many, if not most of medicinal plants contain flavonoids; such compounds have been associated with several beneficial effects such as antioxidant which consider to be a fundamental property important for life[10]. One of the most important medicinal herbs is Dates palm (*Phoenix dactylifera L.*) which is well documented worldwide possess several highly beneficial properties were investigated for their antibiotic activity against pathogenic bacteria by using three types of seed extraction. The seed of the date palm had a substantial amount of oil that needed to be describe for constituent components, biological activities and stability. Seed contained large quantities of fiber and possibly, resistant starch that may have potential health benefits[11]. The aim of this study was to extract the Essential Oil from *Phoenix dactylifera L* (Date palm) seed and to investigate the hypolipidemic effect of this oil on experimental animals.

MATERIAL AND METHODS

Materials

Plant material

The seed of Date palm of good quality supplied from a local market, Benghazi, Libya 2017, (Figure No.1).

Chemicals

Commercial kits to evaluate antioxidant enzymes were from Biodignostic Company, liver function tests carried out in Benghazi medical center.

Gemfibrozil (Lopid)

Lopid is one of the yield products, which considered as a standard hypolipidemic agent. It supplied from Libyan International Pharma Company.

Experimental animals

Male albino mice weighing between 25-35gm, used in this study. The animals fed with standard diet and water.

Methods

Extraction of Essential Oil

The essential oil from the powdered seed of Date palm (100g) extracted with light petroleum ether (40-60°C) in a soxhlet apparatus for about 4hrs and rotary vacuum evaporator removed the solvent. In dark air-tight containers the oil samples were stored at 7°C after drying over anhydrous sodium sulfate.

Determination of LD_{50}

A total Number of 25 male albino mice used in this study to determine the lethal dose of Essential Oil extracted from Date palm seed. In each experiment were used five mice, divided into five groups were given different amounts of the oil "50, 75, 100, 125, 150µl" oral. The animals monitored for 24 hrs for mortality. The number of animals survived at a specific dose (S) and the number of those died at that dose (D) was determined[12]. The percent mortality calculated for each dose group as in the following:

% Mortality= D/(S+D) X 100 is Calculated lethal doses for half the number depending on the equation[13]. LD_{50}=Biggest dose - $\sum a \times b / n$

a: The difference between the doses in two consecutive terms.

b: The average number of dead animals for two consecutive terms.

n: number of animals in each group.

As: a = Differences between potions. b = Average number of animals killed between the first and second group n = Average number of animals per group LD50 = Biggest dose – Σ (a X b) / n.

Induction of hyperlipidemia

Hyperlipidemia induced in male Swiss Albino mice by using cholesterol/cholic acid mixture (3:1) and mixed with the synthetic diet in a dose calculated on the basis that each mouse was received (0.5g) of this mixture/kg b.w daily for Three weeks. Ten% saturated fat used in the diet instead of the corn oil. In addition, 50% of sucrose (a source of carbohydrate) used in the composition of the diet in order to accelerate the incidence of hyperlipidemia.

Experimental design

Mice provided from the animal house in faculty of medicine and acclimatized for Two weeks in the animal house under normal conditions. Animals allowed free access of water and fed on a standard synthetic diet according to N.A.C.L.A.R, 2004[14]. Fifteen mice (15) were fed on the standard synthetic diet (S.D) and served as negative control (-ve) "Group 1". The other mice subjected to the induction of experimental hyperlipidemia for Three weeks as described before. The hyperlipidemic mice (45 mice) divided randomly into equal three sub-groups (15 mice each) as follows:

Group 2

Mice served as hyperlipidemic animals (+ve).

Group 3

Mice was daily-received Essential Oil at a dose of 75µl (oral+ SD).

Group 4

Mice was daily-received 0.2mg/kg b.w. of Lopid as a standard hypolipidemic agent (oral + S.D).

The dose administered to the animals calculated according to the recommended therapeutic human dose and converted to the mice according to[15].

Biochemical analysis

Blood samples collected before (zero time), and after four weeks of experimental diets to estimate S. TG, TC, LDL, HDL, and VLDL cholesterol and antioxidant enzymes from retro-orbital veins by micro-hematocrit blood tube into the corner of the eye socket underneath the eyeball[16].

Serum and plasma samples carefully separated and stored at -20°C for later use in different biochemical analysis. All of biochemical analysis carried out in biochemistry laboratory at Benghazi medical center while the antioxidant enzymes tested in biochemistry laboratory at Benghazi University.

Statistical analysis

Resulting data were represented as mean ± SD. Statistical data was analysed by T test; between control vs all treated groups. A probability level of less than 5% ($p<0.05$) was considered significant.

RESULTS

Effects of essential oil on serum lipid profile in induced hyperlipidemic mice after 4weeks of treatment. All the plasma lipids parameters were significantly increased in induced hyperlipidemic mice (positive group), when compared to normal control (NC) values. The concentration of TC, HDL, LDL, VLDL and TG was significantly increased by 200, 158, 26.8 and 134%, respectively, but the level of HDL-cholesterol was decreased by (15.2%) as illustrated in Table No.1. Essential oil had a significant decrease in lipid profile levels as compared with positive group. 138, 93, 20.8 and 104%, respectively at a dose of 75µL of essential oil for two weeks, significantly decreased TC, LDL, VLDL and TG. It also decreased by the same dose of the volatile oil by 25.5, 41.3, 31.4 and 29.4%, while the concentration of HDL-chol was increased by 24.2% at a dose of 75µL of essential Oil. Effects of essential oil on liver function tests (LFT) in stimulate hyperlipidemic mice after 4 weeks of treatment. Liver function tests in hyperlipidemic mice displayed a mild to moderate increase in serum alanine aminotransferase (67.0%), aspartate aminotransferase (64.2%), G-glutamyltransferase (26.5%), alkaline phosphatase (64.0%), in comparison with normal control group and significantly decrease in the serum total protein TP by (5.32%).

Sequential changes in serum ALT, AST, G-GT, ALP and TP summarized in Table No.2 respectively; in addition, Groups 3, which ingested 75µL essential oil with hyperlipidemic diet for four weeks, serum ALT, AST, G-GT, ALP 46.0, 56.6, 17.4 and 55.7 were lowered, respectively by the essential oil. While the serum TP present no significant difference as compared with the positive group as showed in Table No.3.

Essential oil effect on antioxidant enzymes in induced hyperlipidemic mice after four weeks of treatment. The average value of superoxide dismutase (SOD), peroxidase (GPx), glutathione reductase (GR) and Catalase (CAT) significantly decreased in hyperlipidemic group by 5.42, 8.7, 17.4 and 32.0%, respectively. As shown in Table No.3, the co-administration of essential oil at dose of 75µL showed a significant increase in plasma SOD, GR, GPX, and catalase by 6.13, 14.1, 27.8 and 38.1% respectively as compared with the positive group. The mice was fed with hyperlipidemic diet present significant increase in the plasma levels of malondialdehyde (MDA) (50.3%) when compared with normal group, however the administration of essential oil decrease the MDA level by 36.0%.

DISCUSSION

There is a wealth of scientific data coming from *in vitro* studies or from different animal models, supporting the validity of the oxidative hypothesis of atherosclerosis which states that the oxidative modifications of lipoproteins is a pivotal event in the evolution of atherosclerotic plaques. A corollary of this hypothesis is that antioxidant enzymes should therefore prevent LDL oxidation and protect against the development of atherosclerosis[17].

In hypercholesterolemia, one of the mechanisms that may be activate and might hinder coronary vascular function is a shift in scavenging activity and redox status, a state known as increased oxidative stress. Numerous studies show that a close relationship exists between high blood cholesterol and atherosclerosis; it has been suggest that this relationship may be dependent on enhanced oxidative stress[18]. Mice fed on hypercholesterolemia diet developed hypercholesterolemia mark by significant increase in serum triglycerides (T.G), total cholesterol (T. Chol), low-density lipoprotein cholesterol (LDL-C), and decrease in high-density lipoprotein cholesterol (HDL-C) compared with normal control mice.

The effect of oil from date seed extract induced hyperlipidemia was due to chemical composition of oil from date seed extract contain. Generally, oil from date seed extract at the dose had to resemble potential effect or Lopid (as standard hypolipidemic agent).

Phoenix dactylifera L. tree and its products used traditionally in treatment of many pathological conditions[19]. Palm date fruit is composed of a

fleshy pericarp and seed[20]. Date seed represents about 15% of the total weight of the date fruit[21]. The fleshy fruit part is delicious and highly nutritious[22] and can be consumed in any of the three major stages of maturity[19]. The extracts of palm date fruits and seed showed strong antioxidant properties in many *in vitro* studies[19,23] and in vivo studies[24,25]. Recently, El Arem *et al*. demonstrated the beneficial effect of aqueous date palm fruit extract (ADE) against oxidative stress and hepatotoxicity induced by trichloroacetic acid (TCA)[26]. The studies of Saafi-Ben Salah and El Aremelucidate the salutary effect for palm date extracts against nephrotoxicity caused by dichloroacetic acid and dimethoate respectively[27,26]. In our study lipoprotein, results were a significant reduction in the levels of total cholesterol and triglycerides indicated after the administration of the dose of the oil from date seed extracts when compared with the corresponding hyperlipidemic control group at the same time intervals. It is clear that the oil from date seed extract contain a powerful hypolipidemic material. The effect oil from seed to decrease the lipid fraction levels was partially due to caffeic acid and gallic acid has the highest concentration[26]. The hypolipidemic effect of oil may be due to the presence of active potent agents such as quercitin and p-coumaric acid[28]. Found that quercitin and p-coumaric acid a pungent principle increase the secretion of bile as one of its effects on digestive tract functions and thus is protectant suggested that The date seed of stimulate the conversion of cholesterol to bile acids, an important pathway of elimination of cholesterol from the body. Furthermore, another constituent present in the date seed, namely [caffeic acid and gallic acid], was shown to inhibit cholesterol biosynthesis in homogenate rat liver[28]. In line with an agreement with El Arem study attributed the nephroprotective effect of aqueous extracts of palm date against the renal damage induced by dichloroacetic acid to its richness in antioxidant compounds such as ferulic, caffeic and p-coumaric acids. It could be concluded that the hypolipidemic effect of oil from seed may be due to the presence of active potent agents quercitin and p-coumaric acid[26]. Similar finding report in Amani study[28] who found that quercitin and p-coumaric acid increase the secretion of bile as one of its effects on digestive tract functions and thus is protectant suggested that the date seed of stimulate the conversion of cholesterol to bile acids, an important pathway of elimination of cholesterol from the body. Furthermore, another constituent present in the date seed, namely [caffeic acid and gallic acid], was shown to inhibit cholesterol biosynthesis in homogenate rat liver[28]. In line with agreement with El Aremattributed the nephroprotective effect of aqueous extracts of palm date against the renal damage induced by dichloroacetic acid to its richness in antioxidant compounds such as ferulic, caffeic and p-coumaric acids[26].

However, the decrease in lipid fraction levels could be due to the antioxidant properties[29]. The authors it found that, date seed caused decreased in the levels of cholesterol, phospholipids and triglyceride in the tissues and serum.

The elevations indicated of the dose of oil from seed on HDL-Cholesterol level when compared with the (+ve) control group seemed to be runs parallel with the protective role of HDL-Cholesterol against induced atherosclerosis or development of CHD. The role of HDL-cholesterol in this case is to scavenge the superoxide formation[30].

High-density lipoprotein inversely related to total body cholesterol. The mechanism of action may include transport of cholesterol back to the liver, the only organ that can catabolize and excrete quantitatively significant amounts of cholesterol[31]. HDL changes the equalization of non-esterified cholesterol among plasma and cells by expanding its utilization in the lecithin/ cholesterol acyltransferase system to form cholesterol-ester which back into the cell more less slowly[32].

The reductions indicated in the level of serum ALT. AST, ALP, LDH after the administration of the oil from seed, may suggest that the antioxidant properties. Similar findings also reported by who found that rats receiving paracetamol in combination with α-tocopherol reduction in serum

ALT, AST and ALP levels compared with the receiving paracetamol only. In fact, date seed constituents exhibit higher antioxidant activity than α- tocopherol[29].

Table No.1: Results for the determination of lethal dose LD50 of the fixed oil extracted from seed powder of Dates after oral ingestion in male albino mice (n = 5)

S.No	Dose(µl)	(a)	Number of mice			(b)	(a×b)	% mortality
			Total	Survived	Died			
1	50	0	5	5	0	0	0	0%
2	75	25	5	5	0	0	0	0%
3	100	25	5	4	1	0.5	12.5	20%
4	125	25	5	2	3	2	50	60%
5	150	25	5	1	4	3.5	87.5	80%

a: The difference between the doses in two consecutive terms.
b: the average number of dead animals for two consecutive terms.
LD_{50} = 120µl

Table No.2: Effect of different treatments on lipid profile during the induction of hypercholesterolemia for 4weeks in male albino mice

Parameters	S. T. cholesterol (mg/dl)		S.HDL. (mg/dl)		S. LDL (mg/dl)		S. VLDL (mg/dl)		S. Triglyceride (mg/dl)	
	Zero	4-weeks	zero	4-weeks	Zero	4-weeks	zero	4-weeks	Zero	4-weeks
Control	89 ± 4.11	89.3 ± 1.58†	32.6 ± 1.08	32.7 ± 2.00†	45 ± 2.11	45.1 ± 2.44†	11.4 ± 0.87	11.2 ± 0.47†	57 ± 1.47	56 ± 0.01†
Positive control	180 ± 5.31	200 ± 4.39†	16.5 ± 2.34	15.2 ± 1.53†	137.1 ± 2.04	158 ± 2.90*	26.4 ± 1.11	26.8 ± 1.89†	132 ± 1.69	134 ± 2. 20†
Essential Oil (75µl)	178 ± 4.15	138 ± 3.22*	16.6 ± 1.69	24.2 ±1.23**	134.8 ± 3.77	93 ± 3.88**	26.6 ± 1.27	20.8 ± 1.30*	133 ± 3.00	104 ± 2.01 *
Lopid (0.2 mg/kg b.w)	182 ± 4.90	120 ± 2.69*	15.8 ± 1.09	27.1 ± 2.03***	139.8 ± 4.05	75.3 ± 4.00***	26.4 ± 1.90	17.6 ± 1.00**	132.2 ± 3.77	88 ± 2.33**

Table No.3: Effect of different treatments on liver function parameters during the induction of hypercholesterolemia for 4weeks in male albino mice

Parameters	ALT(u/ml)		AST(u/ml)		GGT ((U/L)		T. Protein ((g/dl)		ALP ((IU/l)	
	Zero	4-weeks	zero	4-weeks	Zero	4-weeks	Zero	4-weeks	Zero	4-weeks
Control	30.6 ± 1.88	32.7 ± 3.04†	52.3 ± 2.80	52.7 ± 3.67†	11.4 ± 1.34	12.0 ± 1.67†	6.1 ± 1.22	5.92 ± 0.48†	34.9 ± 4.20	34.5 ± 4.11†
Positive control	57.4 ± 2.12	67.0 ± 2.55†	63.7 ± 4.01	64.2 ± 2.75†	22.7 ± 1.06	26.5 ± 2.01†	5.83 ± 0.23	5.32 ± 0.50†	59.7 ± 5.03	64.0 ± 4.32†
Essential Oil (75 µl)	59.2 ± 3.45	46.0 ± 1.79*	60.2 ± 2.68	56.6 ± 3.99*	24.4 ± 2.77	17.4 ± 2.00 *	5.42 ± 0.59	5.88 ± 1.02†	60 ± 5.00	55.7 ± 2.21*
Lopid (0.2 mg/kg b.w)	58.3 ± 3.06	42.7 ± 2.89**	62.6 ± 4.11	52.2 ± 2.60*	25.2 ± 2.40	15.8 ± 1.92 **	5.66 ± 0.36	5.93 ± 0.89*	63.1 ± 4.71	52.2 ± 2.17*

Table No.4: Effect of different treatments on antioxidant enzymes during the induction of hypercholesterolemia for 4 weeks in male albino rats

Parameters	P. SOD (u/mol)		P. MDA (n mol/ml)		P. GR (u/l)		P. GP_X (mu/ml)		P. CAT (u/l)	
	zero	4-weeks	zero	4-weeks	Zero	4-weeks	zero	4-weeks	Zero	4-weeks
Control	7.59 ± 0.41	7.60 ± 0.18†	13.4 ± 1.59	13.7 ± 2.10†	23.5 ± 2.11	22.8 ± 1.01†	32.5 ± 1.36	32.1 ± 1.17†	57.0 ± 2.33	58.3 ± 4.01†
Positive control	5.37 ± 0.28	5.42 ± 0.29†	46.1 ± 2.89	50.3 ± 3.06†	9.5 ± 1.11	8.7 ± 1.03†	18.7 ± 1.66	17.4 ± 1.54†	35.3 ± 2.04	32.0 ± 2.04†
Essential Oil (75 µl)	5.09 ± 0.38	6.13 ± 0.06*	45.9 ± 3.92	36.0 ± 2.42 *	9.00 ± 0.04	14.1 ± 0.63**	19.5 ± 2.07	27.8 ± 2.11**	34.0 ± 1.69	38.1 ± 1.33*
Lopid (0.2 mg/kg b.w)	5.12 ± 0.10	6.49 ± 0.28*	46.3 ± 3.00	30.2 ± 2.16*	8.90 ± 1.20	15.9 ± 0.77*	21.2 ± 1.25	28.4 ± 0.90**	34.3 ± 1.88	43.0 ± 1.30*

† Non - significant difference from the corresponding control at $P > 0.1$; *Significant difference at $P < 0.05$; * *highly sig. difference at $P < 0.01$; ***Very highly sig. difference at $P < 0.001$; ↓ Decrease; ↑ Increase; [a] compared with control group; [b]compared with positive group.

Figure No.1: Date palm seed

Figure No.2: The oil of Date seed

CONCLUSION

In conclusion, we found that the essential oil extracted from Date seed could decrease lipid profile and oxidative stress in hyperlipidemic conditions, which produced by administration of high lipidemic diet. Based on the evidence from biochemical result, our results suggested that the hypolipidemic activity of essential oil can be attributed to presence of several chemical (antioxidant) constituents-suppressing lipid peroxidation and modulating antioxidant enzymes. In general, to use these oils safely prophylactic and curative agents, more studies should be carried out to know all the active/inactive components and their action mechanism, another types of experimental animals for a long period in order to assessment the biological activity of these herbs also their side effects.

ACKNOWLEDGMENT

The authors wish to express their sincere gratitude to Department of Medical Laboratory, College of Medical Technology, Benghazi, Libya for providing necessary facilities to carry out this research work.

CONFLICT OF INTEREST

There is no conflict of interest.

BIBLIOGRAPHY

1. Pham-Huy L A, He H, Pham-Huy, Chuong. Free Radicals, Antioxidants in Disease and Health Lien, *Int J Bio Sci,* 4(2), 2008, 89-96.
2. Rahman M S, Kasapis S, Al-Kharusi N S, Al-Marhubi I M, Khan A J. Composition characterisation and thermal transition of date pits powders, *J. Food Eng,* 80(1), 2007, 1-10.
3. Vibha Rani Umesh Yadav. Free radicals in human health and disease, *Free Radicals in Human Health and Disease,* 1st Edition, 2015, 430.
4. Jorgensen, T. *et al*. Population-level changes to promote cardiovascular health, *Eur. J. Prev. Cardiol,* 20(3), 2013, 409-421.
5. Kakadiya J. Causes, symptoms, pathophysiology and diagnosis of atherosclerosis - a review, *Pharmacologyonline,* 3, 2009, 420-442.
6. Braamskamp M J A M, Wijburg F A, Wiegman A. Drug therapy of hypercholesterolaemia in children and adolescents, *Drugs,* 72, 2012, 759-772.
7. Kohen R, Nyska A. Oxidation of biological systems: oxidative stress phenomena, antioxidants, redox reactions, and methods for their quantification, *Toxicol. Pathol,* 30(6), 2002, 620-650.
8. Kunwar A and Priyadarsini K. Free radicals, oxidative stress and importance of antioxidants in human health, *J. Med. Allied Sci,* 1(2), 2011, 53-60.
9. Sevanian A and Ursini F. Lipid peroxidation in membranes and low-density lipoproteins: Similarities and differences, *Free Radic. Biol. Med,* 29(3-4), 2000, 306-311.
10. Halliwell B, Gutteridge J M C. The importance of free radicals and catalytic metal ions in human diseases, *Mol. Aspects Med,* 8(2), 1985, 89-193.
11. Saleh F R. Antibacterial activity of seed of iraqi dates, *J.Bio.Innov,* 5(2), 2016, 313-318.
12. Al-Ali A, Alkhawajah A A, Randhawa M A, Shaikh N A. Oral and intraperitoneal LD50 of thymoquinone, an active principle of Nigella sativa, in mice and rats, *J. Ayub Med. Coll. Abbottabad,* 20(2), 2008, 25-27.
13. Rashed Y D, Ahmed N M B. Effectiveness of matricaria chmomilla flow extracts with bacillus subtilis, pseudomonas fluorescens, in the biological criteria of the blood of laboratory male rats, *Plant Arch,* 19, 2019, 549-554.
14. Tan B. Guidelines on the Care and Use of Animals for Scientific Purposes, *Naional Advis. Comm. Lab. Anim,* 2004, 8-11.
15. Okerholm R A, Keeley F J, Peterson F E, Glazko A J. The metabolism of gemfibrozil, *Proc. R. Soc. Med,* 69(2), 1976, 11-14.

16. Hoff J. Methods of Blood Collection in the Mouse, *Lab Ani. (NY),* 29(10), 2000, 47-53.
17. Dutta K, Bishayi B. Escherichia coli lipopolysaccharide administration alters antioxidant profile during hypercholesterolemia, *Indian J. Clin. Biochem,* 24(2), 2009, 179-183.
18. Green C O, Wheatley A O B, McGrowder D A, Dilworth L L, Asemota H N. Modulation of antioxidant enzymes activities and lipid peroxidation products in diet-induced hypercholesterolemic rats fed ortanique peel polymethoxylated flavones extract, *J. Appl. Biomed,* 10(2), 2011, 91-101.
19. Vayalil P K. Date fruits (Phoenix dactylifera Linn): an emerging medicinal food, *Crit. Rev. Food Sci. Nutr,* 52(3), 2012, 249-271.
20. Ahmed M B, Hasona N A S, Selemain H A H. Protective effects of extract from dates (Phoenix Dactylifera L.) and ascorbic acid on thioacetamide-induced hepatotoxicity in rats, *Iran. J. Phar. Res,* 7(3), 2008, 193-201.
21. Hussein A S, Alhadrami G A, Khalil Y H. The use of dates and date pits in broiler starter and Finisher Diets, *Bioresource Technology,* 66(3), 1998, 219-223.
22. Vinson J A, Zubik L, Bose P, Samman N and Proch J. Dried fruits: excellent *in vitro* and in vivo antioxidants, *J. Am. Coll. Nutr,* 24(1), 2005, 44-50.
23. Al-Farsi M, Alasalvar C, Morris A, Baron M, Shahidi F. Comparison of antioxidant activity, anthocyanins, carotenoids, and phenolics of three native fresh and sun-dried date (Phoenix dactylifera L.) varieties grown in Oman, *J. Agr. Fo Ch,* 53(19), 2005, 7592-7599.
24. Rajab A R, Elkablawy M A, Sheik B Y. Antioxidant and Tissue-Protective Studies on Ajwa Extract : Dates from Al Madinah Almonwarah, Saudia Arabia, *Egypt. J. For. Sci. Appl. Toxicol,* 3(1), 2012, 201-223.
25. Saafi E B, *et al.* Protective effect of date palm fruit extract (Phoenix dactylifera L.) on dimethoate induced-oxidative stress in rat liver, *Exp. Toxicol. Pathol,* 63(5), 2011, 433-441.
26. El Arem, A. *et al.* Nephroprotective effect of date fruit extract against dichloroacetic acid exposure in adult rats, *Food Chem. Toxicol,* 65, 2014, 177-184.
27. Saafi-Ben Salah E B, *et al.* Antioxidant-rich date palm fruit extract inhibits oxidative stress and nephrotoxicity induced by dimethoate in rat, *J. Physiol. Biochem,* 68(1), 2012, 47-58.
28. El-Mousalamy A M, Hussein A A M, Mahmoud S A, Abdelaziz A, Shaker G. Aqueous and methanolic extracts of palm date seed and fruits (phoenix dactylifera) protects against diabetic nephropathy in type II diabetic rats, *Bioch. Phy,* 5(2), 2016, 205.
29. Trabzuni D M, Ahmed S E B, Abu-Tarboush H M. Chemical Composition, Minerals and Antioxidants of the Heart of Date Palm from Three Saudi Cultivars, *Food Nutr. Sci,* 5(14), 2014, 1379-1386.
30. Maraia F. Elmhdwi, Salem, Gaber El Tumi. Biochemical studies on the effect of methanolic extract of Arbutus pavarii "leaves" as an antioxidant in treatment of hypercholesterolemia in albino rats, *International Journal of Chemistry and Pharmaceutical Sciences,* 2(8), 2014, 1046-1052.
31. Ducharme N, Radhamma R. Hyperlipidemia in the Elderly, *Clin. Geriatr. Med,* 24(3), 2008, 471-487.
32. Prasanna G S, Purnima B C A. Protective effect of leaf extract of trichilia connaroides on hypercholesterolemia induced oxidative stress, *International Journal of Pharmacology,* 7(1), 2011, 106-112.

Please cite this article in press as: Hayam A. Elawamy *et al.* Anti- hypercholesterolemia activity of essential oil extracted from date palm seed in Male Albino Mice, *Asian Journal of Research in Pharmaceutical Sciences and Biotechnology*, 8(1), 2020, 31-39.