Effect of pine fruits on some biological changes in hypercholesterolemia rats induced

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Abstract

This study aimed to determine chemical composition, antioxidant contents, fatty acids of pine nut fruits and also, the effect of pine powder with different ratio (3, 6 and 9%) on biochemical changes of hypercholesterolemic rats. Biological evaluation of growth performance, Lipid profile, serum protein, blood sugars, liver and kidney functions were also studied. The obtained results indicated that pine nut fruits are considered as a good source of oils, crude fibers, ash, protein, soluble carbohydrates, total energy polyphenols and flavonoids. Besides, it is a rich source of unsaturated fatty acids mainly, linoleic acid ($C_{18:2}$) which represent more than half amount of total fatty acids. Biological evaluations showed that pine nut was significantly decreased ($P \leq 0.05$) of body weight, total lipids, triglycerides, LDL, HDL, VLDL cholesterol of serum hypercholesterolemic rats as compared with positive group of rats Also, it caused significant decrease ($P \leq 0.05$) in liver enzyme (AST, ALT and ALP) and kidney parameters (urea, uric acid and creatinine, i.e. improved liver and kidney functions as compared with positive control group. Meanwhile, both total protein and albumin were increased.

INTRODUCTION

Pine nuts are eaten raw or roasted; they are included as ingredients in a variety of traditional dishes, such as breads, cakes, sauces, candies, as well as vegetable and meat dishes. Pine nuts are a good source of nutrients **(Savage, 2001)**. In particular, nuts include plant protein, unsaturated fatty acids, dietary fiber, plant strolls, phytochemicals and micronutrients like tocopherols **(Kris-Etherton *et al*., 2001)**.

The pine nut (Pinus koraiensis Sieb. et Zucc) as an abundant plant resource has the capacity of anti-fatigue, anti-aging, anti-radiation etc. **(Xie *et al*., 2016)**. It contains essential and relatively complete amino acids, and fatty acids **(Noa *et al*., 2015)**. Pine nuts are easy to obtain with a reasonable price, therefore it has many culinary uses in their own right, especially the

oil was extracted and used widely. A report optimized the conditions of inositols extraction from pine nuts **(Ruiz-Aceituno *et al*., 2014)**.

Pine nuts (*Pinus pinea*, L.) have been used in European cooking for a long time as they are highly valued as ingredients in pesto, sauces and as a garnish in desserts. The main species consumed in Europe are *Pinus pinea, P.koraiensis, P. sibirica* and *P.gerardiana*. As local production in Europe is costly and not sufficient to meet current demand, most commercial products are imported from China, Korea and Pakistan where the climate is suitable for efficient production. It is estimated that 36,080 tonnes of pine nut kernels were produced globally in 2011. China and the Russian Federation are the largest producers contributing 55% and 14%, respectively, to the world production. In 2010, New Zealand imported 22 tonnes of pine nut kernels for domestic consumption **(Food and Agriculture Organization Statistical Database**, **2011)**.

European Food Safety Authority (EFSA), (2009) published the list of functional health claims received from the European Commission for assessment. Of the claims, two are specific for nuts: Eligible nuts for both claims include almond, hazelnut, pecan, pistachio, walnut, and peanut. These health claims are of great importance for the status of tree nuts and peanut.

Recently, numerous epidemiological and clinical studies have provided evidence that frequent nut consumption is associated with favourable plasma lipid profiles **(Ternus *et al*., 2009)** and reduced risk of CHD **(Kris-Etherton *et al*., 2008)**, type-2 diabetes (**Jiang *et al*., 2002 and Jenkins *et al*., 2008**), and cancer **(Davis *et al*., 2008)**. In addition, although nuts are a high-fat and energy-dense food and are therefore a potential threat for weight gain, controlled clinical trials have reveal little or no weight change with inclusion of various types of nuts in the diet over 1-6 months **(Rajaram and Sabaté, 2006 and Mattes *et al*., 2008)**.

In vitro research demonstrated that PNLA is a potent dual against for co-activation of free fatty acid receptors 1 and 4, which could enhance glucose-dependent insulin secretion and insulin sensitivity. PNO increased the activity of antioxidant protective enzymes in serum and decreased the concentration of malondialdehyde, an indicator of lipid peroxidation. Thus, PNLA and its parent PNO could be of potential benefit to human health, although this requires further investigation **(Kayin *et al*., 2016)**.

Some of the reported health benefits derived from nut and seed consumption are control of body weight and blood pressure, reduction of coronary heart disease, and reduction of levels of blood cholesterol and triacylglycerols. In addition, nuts and edible seeds provide antioxidant, anti-microbial, anti-inflammatory, anti-mutagenic, anti-cancer, anti-diabetic and glucoregulatory (**Kendall, *et al*., 2010; Gentile *et al*., 2012)** properties.

MATERIALS AND METHODS

Materials:

Pine nuts *(Pinus Koraiensis)* were purchased from locally hyper marketing center, Cairo, Egypt.

Animals: Adult Male Sprague – Dawely rats were obtained from the Ophthalmology, Medical Analysis Department, Animal House Department, Giza, Egypt.

Methods:

Analytical methods:

Chemical composition of pine nuts, moisture, protein, oils, ash, crude fibers, carbohydrates and soluble carbohydrates and were determined according to the method described by **AOAC, (2000)**. Results calculated as g/100g fresh weight. Total calories were calculated by multiplying 1.0g protein or carbohydrate by 4.0 and 1.0g fat by 9.0. Results calculated as K.cal/100g sample Extraction of antioxidant contents of pine nut powder as described by **Batista et al., (2011)**. Total phenols (mg galic acid/g) and flavonoids catechin equivalents (CE)/g were determined as the method of **Batista et al., (2011)**. Also, DPPH radical assay (%) was determined according to the method described by **Hanato et al., (1988)**. Fatty acids % were separated and identified by Gas Liquid Chromatography (GLC) as described by **Stahle (1967)**.

Biological investigation:

Diets:

Basal diet (g/100g) consisted of 12.5% protein (casin), 10% corn oil, 4.0% salt mixture, 1.0% vitamin mixture, 5% cellulose, 0.2% choline chloride and 67.5 corn starch as described by **AIN, (1993)**. Salt mixture was

prepared as described by **Hegested, (1941)**, meanwile, vitamin mixture was prepared as described by **Campblle, (1961)**.

Experimental design:

Thirty mal albino rats, Sprague Dawley strain weighting (150 ± 0.5). All rats were fed on basal diet for one week to adapted. Rats were divided into two main groups, the first group (6 rats) fed on basal diet as a negative control. The second group (24 rats) was fed on hypercholesterolemic diet for 4 weeks (basal diet containing 1.0% cholesterol + 10% sheep tail fat + 0.25% colic acid), according to **Abdel Maksoud et al., (1996)**, then divided into four subgroups as follows as: the first was fed on hypercholesteerolemic diet as a positive control, the other three subgroup of rats were fed on hypercholesterolemic containing 3, 6 and 9% pine nut powder. At the end of experimental (4 weeks), blood samples of rats were collected according to **Drury and Wallington, (1980)**.

Biological evaluations:

Body weight gain (g) and feed efficiency ratio of hypercholesterolemic rats were estimated as the method of **Chapman et al., (1959**).

Serum Lipids (mg/dL) of triglycerides according **Fassati and Prencipe, (1982)**, total lipids as the method of **Blank et al., (1967)** and triglycrides according to **Alian, (1974)**. Serum HDL cholesterol (mg/dL) was determined as the method of **Lopez, (1977)**, Serum LDL and VLDL cholesterol (mg/dL) were determined according to **Lee and Nieman, (1996)**. Meanwhile, alanine transaminase activity (AST) μ/L were carried out as the method of **Tietz, (1976)**; Asparatate transferase activity (AST) μ/L was determined as the method of **Henery (1974) and yound, (1975)**, alkaline phosphate (ALP) μ/L was determined according to **Moss, (1982)**.

Serum Urea (mg/da) was determined as the method of **Petton and Crouch, (1977)**; uric acid (mg/dL) was determined as **While et al., (1970)**; creatinine (mg/dL) was determined according to **Henery, (1974)**.

Statistical analysis of the obtained data were calculated by SPSS Version 10 Software **SPSS, (1998)**. Results expressed as means ± standard deviations (SD). Values of $P \leq 0.05$ were significant difference.

RESULTS AND DISCUSSION

1- Chemical composition of pine nuts

Table (1) shows chemical composition of fresh pine nut fruits (g/100g on fresh weight basis). The obtained results illustrated that total protein was 13.45%, ash 2.52%, crude fiber 8.6%, oil 55.38% moisture content 3.57%, total carbohydrates 25.08% and total soluble carbohydrate 16.48%. These results are in agreement with those found by **Savage, (2001); Cevdet and Iclal, (2003)** United States Department of Agriculture **USAD**, **(2016)**. The chemical composition of pine nuts showed variation among species depending on both climate conditions and geographical area **(Zadernowski et al., 2009)**. It is obvious from the results that total energy of pine nut fruits was 652.4 k.cal /100g on fresh weight basis). This results are in agreement with those noticed by **Mattes et al., (2008)** who reported that nuts are a highly energy. In addition, **Venkatachalam and Sathe, (2006)** mentioned that oils of the most nuts are the main constituents and has a rich source of plant oil.

Table (1): Chemical composition of pine nut fruits

* Moisture	* Protein	* Oil content	* Crude fibers	* Ash	* Total carbohydrates	* Total soluble sugars	** Total energy
3.57	13.45	55.38	8.6	2.52	25.08	16.48	652.4

* g/100g on dry weight basis ** total calories (k.cal/100g).

2- Antioxidant contents

Total phenols, flavonoids and DPPH radical scavenging activity of pine nut fruits are illustrated in table (2). The obtained data showed that total phenols was 0.95 (gallic acid equivalent /g, and total flavonoids 1.91 mg/g as quercetin equivalent /g). These results are in agreement with those noticed by **Shahidi and Naczk, (2004); Tripoli *et al*., (2005) and Dogan *et al*., (2010)**. They reported that tree nuts contain several types of antioxidants **(Sang et al., 2002; Wu *et al*., 2004 and Blomhoff *et al*., 2006)**.

It is evident from the results that DPPH radical scavenging activity was 89.17%. The same trend of results are in agreement with those obtained by several studies **(Durmaz *et al*., 2010; Chandrasekara and Shahidi, 2011)**

who reported that microconstituents of free nut oils exhibited good antioxidant activity. Moreover, tree nuts contain phenolics which has stronger antioxidant activities **(Shahidi and Naczk, 2004; Tripoli et al., 2005 and Dogan et al., 2010)**.

Generally, it could concluded that pine nut fruits is considered as a good source of natural antioxidants such as phenols and flavonoids. Moreover, it has an antioxidant activity.

Table (2): Antioxidant contents and DPP radical scavenging activity of pine nuts.

*Total phenols mg/g	**Total flavonolids mg/g	DPPH %
0.95	1.91	89.17

* mg/g as gallic acid ** mg/g as quercetin

3- Fatty acids of pine nut

Data in table (3) illustrates fatty acid composition of oils in white pine nut fruit powder. Results indicated that there are 12 fatty acids could be identified by Gas liquied Chromatography (GLC) analysis. These fatty acids are six saturated fatty acids; myristic ($C_{14:0}$), palmatic ($C_{16:0}$), margaric ($C_{17:0}$), stearic ($C_{18:0}$), arachidonic ($C_{20:0}$), and behenic ($C_{22:0}$) and six unsaturated fatty acids (UFAs), palmateoleic ($C_{16:1}$), ($C_{17:0}$), oleic ($C_{18:1}$), linoleic ($C_{18:2}$), linolenic ($C_{18:3}$),and gadoleic ($C_{20:1}$).

It is evident from the results that, the predominant fatty acids were linoleic ($C_{18:2}$) 51.81% and oleic ($C_{18:1}$) 38.62% of the total fatty acids these results showed that pine nut fruit is a rich source of unsaturated fatty acids. On the other hand, palmatic acids ($C_{16:0}$) 5.09% and was the predominant of saturated fatty acids (SFs). Moreover, total unsaturated fatty acids was 91.97 %, meanwhile, total saturated fatty acids was 7.35%. These results are in agreement with those obtained by **Zadernowski et al., (2009) and USAD, (2016).** In addition**, Destalittas et al., (2010)** reported that linoleic and oleic acid in species of pine nuts are the major fatty acids.

Table (3): Fatty acids composition of pine nuts.

Saturated fatty acids (RAP%)			Un saturated fatty acids (PAR%)		
Myristic	$C_{14:0}$	0.03	Palmetoleic	$C_{16:1}$	0.06
Palmatic acid	$C_{16:0}$	5.09		$C_{17:1}$	0.04
Margaric	$C_{17:0}$	0.04	Oleic	$C_{18:1}$	38.62
Stearic acid	$C_{18:0}$	2.0	Linoleic	$C_{18:2}$	51.81

Arachedonic	$C_{20:0}$	0.50	Linoleic	$C_{18:3}$	0.35
Behenic	$C_{10:0}$	0.14	Gadoleic	$C_{20:1}$	0.79
Total saturated F.A.		7.35	Total unsaturated F.A.		91.67

(RAP%) Relative area percent

4- Biological parameters

Body weight

Table (4) illustrates the influence of white pine fruit powder on growth performance as body weight gain (BWG), food intake (FI) and feed efficiency ratio (FER) of hypocholesterolemic rats. The obtained results showed that body weight gain of positive control group was significantly increased ($p\leq 0.05$) than negative control group. BWG of positive control was recorded 189.8±3.19, meanwhile negative control was 185.5±3.23g. On the other hand, rats fed on experimental diet containing pine nut with the ratio 3, 6 and 9% were recorded 185.8±3.28, 184.2±2.97 and 183.6±3.08g, respectively. There were no significant difference ($p\leq0.05$) between negative control and groups of rats fed on diet supplemented by pine nut powder.

It is worthy to mention that, body weight gain of rats which fed on diet containing pine nut powder was decreased. This decrease may be attributed that pine nut has a rich source of crude fibers as shown in the previously results in table (1). Diets supplemented by various types of nuts controlled clinical trials have reveal little or no weight changes **(Rajaram and Stbate 2006 and Mattes et al., 2008)**. **Park et al., (2013)** they found that mice fed on high fat diet containing PNO reduced adipose tissue deposition.

It is obvious from the results that food intake of positive control (hypocholesterolimic rats was recorded 17.8± 2.04g, then decreased to 16.4±1.74, 14.6±1.53 and 14.8±1.77g for rats fed on diet supplemented by 3, 6 and 9%, respectively. There was significant difference ($P\leq0.05$) between negative and positive control group of rats.

On the other hand, feed efficiency ratio was recorded 2.36±0.19 of negative control and decreased to 1.98±0.11 in positive control (hypocholesterolemic rats). There were significant decrease ($P\leq0.05$) in FER of positive than negative control group of rats. Hypocholesterolimic rats which fed on diet supplemented by 3, 6 and 9% pine nut powder were recorded 1.97±0.16, 1.98 ± 0.21 and 2.02 ± 0.17, respectively.

Table (4): Effect of white pine nuts powder on growth performance of hypercholesterolemic rats.

Parameter / Groups	Initial body weight (g)	Final body weight(g)	Food intake (g)	Body weight gain (g)	Feed Efficiency ratio (FER)
control (–Ve)	$152.3^{a} \pm 3.25$	$185.5^{b} \pm 3.23$	$14.1^{c} \pm 1.54$	$33.2^{b} \pm 1.87$	$2.36^{a} \pm 0.19$
control (+Ve)	$154.6^{a} \pm 3.49$	$189.8^{a} \pm 3.19$	$17.8^{a} \pm 2.04$	$35.2^{a} \pm 1.93$	$1.98^{c} \pm 0.11$

Hyp. rats with 3%white pine	153.5 ^{a} ± 3.28	185.8^{b} ± 3.28	16.4^{b} ± 1.74	32.3^{b} ± 1.67	1.97^{b} ± 0.16
Hyp. rats with 6%white pine	155.3 ^{a} ± 3.41	184.2^{b} ± 2.97	14.6^{b} ± 1.53	28.9^{c} ± 1.85	1.98^{b} ± 0.21
Hyp. rats with 9%white pine	153.7 ^{a} ± 3.57	183.6^{b} ± 3.08	14.8^{b} ± 1.77	29.9^{b} ± 1.79	2.02^{a} ± 0.17
L.SD ($p \leq 0.05$)	3.48	3.27	1.89	1.75	0.18

Each value in a column followed by the same letter are not significantly at ($P \leq 0.05$).

5- Lipid profile

Table (4) illustrates the influence of white pine nut fruits powder on lipid profile of total lipids (TL), total cholesterol (TC) and total triglyceride (TG) (mg/dL) on hypercholesterolemic rats. Total lipids values of negative and positive control group of rats were 195.32±3.32 and 354.44±5.62 mg/dL, respectively. Meanwhile, total lipids values of hypercholesterolemic rats fed on diet supplemented by 3, 6 and 9% pine nut powder were 274±3.95, 270.41±3.85 and 246.06±4.02 mg/dL, respectively. Results showed that high significant difference ($p \leq 0.05$) of total lipids between positive and negative control group of rats. Also, there are significant differences of total lipids within group of rats fed on different percentage of pine nut powder.

Moreover, total lipids of hypercholesterolemic rats was decreased due to the addition pine nut powder to the diet. The higher percentage of pine nuts, the lower values of total lipids of rats was observed.

On the other hand, total cholesterol values of negative and positive control group were 94.07±2.61 and 194.43 ± 3.19 mg/dL, respectively. Total cholesterol values of hypercholesterolemic rats fed on diet supplemented by 3, 6 and 9% pine nut powder were 152.58 ± 3.05, 134.2 ± 2.84 and 121.8±3.03, respectively.

Results revealed that high significant difference ($p \leq 0.05$) of total cholesterol between negative and positive control and also within group of rats fed on pine nut powder. Total cholesterol of hpercholestertemic rats was decreased as the addition of pine nut powder increased. This results are in agreement with those obtained by **Ramadan and Morsel, (2002)**. They reported that nuts are rich source of linoleic acids which lowering serum cholesterol due to metabolism and produces the hormone-like prostaglandins.

It is evident from the results that total triglyceride values of negative and positive control group of rats were 85.61±2.85 and 170.02 ± 4.02 mg/dL, respectively. Total triglyceride values of hypercholesterolemic rats fed on diet supplemented by 3, 6 and 9% pine nut powder were 112.99±3.14, 114.56±3.86 and 92.74±3.59 mg/dL, respectively. It is worthy to mention that total triglyceride was decreased as the level of pine nut powder increased. It is obvious from the results that total lipids, total cholesterol and total triglyceride values of hypercholesterolemic rats were decreased as the addition of pine nuts powder increased. This decrease may be attributed that pine nut is a good source of polyphenols and flavonoids as illustrated in the previously results in table (5). Several studies showed that nut oils is a rich source of antioxidant contents and also have an antioxidant activity **(Durmaz et al., 2010; Chandrasekara**

and Shahidi, 2011). In addition, nuts also contained tochopherols, phytosterols and pinoleic acid **(Zadernowski et al, 2009)**. On the other hand, nut polyphenols enhanced the antioxidant potential in plasma and lowered total cholesterol. **Kaviarasan et al, (2005)** showed that a significant increase in the levels of total cholesterol (TC), triglycerides (TG), lipid peroxidation, glycoprotein components and glucose in hypercholesterolemic patient with/ without diabetic.

Table (5): Effect of white pine nuts powder on TC, TG and TL of hypercholesterolemic rats.

Lipid profile / Groups	Total cholesterol (TC) mg/dL	Total Lipids (TL) mg/dL	Total triglyceride (TG) mg/dL
control (–Ve)	94.07^{e} ±2.61	195.32^{d}± 3.32	85.61^{d} ± 2.85
control (+Ve)	194.43^{a} ± 3.19	345.44^{a} ± 5.62	170.02^{a} ± 4.02
Hyp. rats with 3%white pine	152.58^{b} ±3.05	274.32^{b} ± 3.95	112.99^{b} ± 3.14
Hyp. rats with 6%white pine	134.20^{c} ± 2.84	270.41^{bc} ±3.85	114.56^{b} ± 3.86
Hyp. rats with 9%white pine	121.80^{d} ± 3.03	246.06^{c} ± 4.02	92.74^{c} ± 3.59
L.SD ($p \leq 0.05$)	**2.89**	**4.15**	**3.96**

Each value in a column followed by the same letter are not significantly at ($P \leq 0.05$).

6- Lipid profile

Table (6) shows the effect of white pine nuts powder on high density lipoproteins (HDL), Low density lipoproteins (LDL) and very low density lipoprotein (VLDL) (mg/dL) of hypercholesterolemic rats. Results indicated that LDL values of negative and positive control group of rats were 28.85±2.17 and 109.06±4.19 mg/dL, respectively. While, LDL values of hypercholesterolemic rats fed on diet containing 3, 6 and 9% pine nut powder were 84.11±2.54, 65.58 ± 2.66 and 56.36±2.38 mg/dL, respectively. Positive control group showed highly significant increase ($p \leq 0.05$) as compared to either negative group or rats fed on diet supplemented by pine nut powder. Results indicated that LDL values of hypercholesterolemic rats were decreased as the addition level of pine nut increased. Moreover, supplementing with 9% pine nut powder showed that the best results than the other levels of 3 and 6%. Such decrease may be attributed that pine nut is a rich source of antioxidant contents such as phenols and flavonoids as shown in the previously results in table (1). Also, pine nut is a rich source in polyunsatrurated. Fatty acids, especially linoleic acid which caused a decreased in LDL cholesterol **(Destaillates et al., 2010)**. In addition, nut polyphenols enhanced that antioxidant potential in plasma and lowered LDL-cholesterol **(Durak et al., 1999)**.

Various polyphenols have the ability to protect LDL from oxidation. However, a very small proportion of plasma polyphenols are associated with the LDL fraction after consumption of nutritional dose of these compounds **(Jimenez et al., 2004)**. Also,

flavonoids as an antioxidants may inhibit the oxidation of LDL cholesterol and reduced platelet aggregation or reduced ischemic damage **(Buring et al., 2003)**.

It is evident from the results that HDL values of negative and positive control group of rats were 48.10±1.46 and 42.17±2.19 mg/dL, respectively. While, HDL values of hypercholesterolemic rats which fed on diet containing 3, 6 and 9% of pine nut were 46.28±2.07; 45.71±1.47 and 46.89±2.49, respectively.

Results showed that there are significant difference ($P \leq 0.05$) among all group of rats in LDL-colesterol.

Also, very low density lipoprotein VLDL showed that same trend of results in LDLC. Very Low density lipoprotein cholesterol values of negative and positive control were 17.12 and 34.0 mg/dL, respectively. While, VLDL values of hypercholesterolemic rats fed on diet containing 3, 6 and 9% pine nut powder were 22.60; 22.91 and 18.55 mg/dL, respectively. There are significant increased ($P \leq 0.05$) in positive than negative control group. VLDL cholesterol values of hypercholesterolemic rats was decreased as the level of pine nut increased.

Clinical and epidemiological studies revealed that nut consumption is associated with flavourable plasma lipid profiles **(Griel and Kris-Etherton, 2006 and Ternus et al., 2009)** and also reduced chronic heart disease **(John and Shahidi, 2010)**.

Table (6): Effect of white pine nuts powder on HDL, LDL and VLDL of hypercholesterolemic rats.

Lipid profile / Groups	High density lipoproteins (HDL) mg/dL	Low Density lipoproteins (LDL) mg/dL	Very Low Density lipoproteins (VLDL)mg/dL
control (–Ve)	$48.10^{a} \pm 1.46$	$28.85^{e} \pm 2.17$	17.12
control (+Ve)	$42.17^{c} \pm 2.19$	$109.06^{a} \pm 4.19$	34.0
Hyp. rats with 3%white pine	$46.28^{a} \pm 2.07$	$84.11^{b} \pm 2.54$	22.60
Hyp. rats with 6%white pine	$45.71^{b} \pm 1.47$	$65.58^{c} \pm 2.66$	22.91
Hyp. rats with 9%white pine	$46.89^{a} \pm 2.49$	$56.36^{d} \pm 2.38$	18.55
L.SD ($p \leq 0.05$)	**2.05**	**3.16**	-

Each value in a column followed by the same letter are not significantly at ($P \leq 0.05$).

7- Liver functions

Table (7) shows the influence of pine nut powder on liver functions of aspartate aminotransferase (AST), alanine aminotransferase (ALT) of hypercholesterolemic rats. Results showed that AST values of negative and positive control group of rats were 63.42±1.94 and 98.52±4.26 µ/L, respectively. Meanwhile, AST values of

hypercholesterolemic rats which fed on diet supplemented by 3, 6 and 9% pine nut powder were 79.76±3.05, 76.82±2.86 and 72.39±2.79 µ/L, respectively.

AST of positive group control showed highly significant increase p≤0.05 than negative control group of rats. Beside, ALT enzyme activity showed the same trend of AST results. On the other hand, ALT values of negative and positive control group were 23.53±1.86 and 49.84±3.15 µ/L, respectively. Diets supplemented by 3, 6 and 9% pine were decreased ALT enzyme activity in serum rats to 31.96±2.69, 28.29±2.74 and 24.56±2.55 µ/L, respectively, the higher level of pine nut, the lower value of ALT was observed.

It is obvious from the results that ALP values of negative and positive control group of rats were 70.13±2.74 and 102.35±3.86 µ/L, respectively. Meanwhile, ALP values of hypercholesterolemic rats which fed on diet containing 3, 6 and 9% pine nuts were 91.53±3.06, 89.67±3.07 and 102.35±3.86 µ/L, respectively. On the other hand, ALP values of hypercholesterolemic rats which fed on diet containing 3, 6 and 9% were 91.53±3.06, 89.67±3.07 and 87.42±2.86 µ/L, respectively.

It is evident from the results that positive group was highly significant (P≤0.05) than negative group. It is worthy to mention that diets containing pine nut powder showed significant decrease (P≤0.05) in AST, ALT and ALP values of serum rats. Diet contained 9% pine nut showed the best results of liver enzymes and improved liver functions.

Such decrease in liver enzymes of serum rats as the result of the addition of pine to the diets of rats may be attributed that pine nut is a good source of antioxidant contents as shown in the previously results in table (1). Also, several studies proved that pinenut is considered as a good source of antioxidant content **(Blomhoff et al., 2006 and Dogan et al, 2010)**.

Table (7): Effect of white pine nuts powder on AST, ALT and ALP of hypercholesterolemic rats.

Liver functions / Groups	Aspartate aminotransferase (AST) (IU/L)	Alanine Aminotransferase (ALT) (IU/L)	Alkaline Phosphatase (ALP) (IU/L)
control (–Ve)	$63.43^{d} \pm 1.94$	$23.53^{d} \pm 1.86$	$70.13^{d} \pm 2.74$
control (+Ve)	$98.52^{a} \pm 4.26$	$49.84^{a} \pm 3.15$	$102.35^{a} \pm 3.86$
Hyp. rats with 3%white pine	$79.76^{b} \pm 3.05$	$31.96^{b} \pm 2.69$	$91.53^{b} \pm 3.06$
Hyp. rats with 6%white pine	$76.82^{bc} \pm 2.86$	$28.29^{c} \pm 2.74$	$89.67^{bc} \pm 3.07$
Hyp. rats with 9%white pine	$72.39^{c} \pm 2.79$	$24.56^{d} \pm 2.55$	$87.42^{c} \pm 2.86$
L.SD (p≤0.05)	**2.75**	**2.19**	**3.19**

Each value in a column followed by the same letter are not significantly at (P≤0.05).

Biological antioxidants included enzymatic antioxidants a such as superoxide dismutase SDD, catalase, glutathione peroxidase (GPx) and nonenzymatic antioxidants such as

antioxidant enzyme factors, oxidative enzyme inhibitors reactive oxygen nitrogen species (ROs/ RNS) scavengers and transition metal cheaters a dietary antioxidant can scavenge ROS/RNS ratio to stop radical chain reactions or inhibit the reactive oxidants **(Huang et al., 2005)**.

8- Kidney functions

Table (8) shows the influence of white pine fruits powder on kidney functions of urea, uric acid and creatinine (mg/dL) of hypercholesterolemic rats. Urea is formed as the end of protein metabolism. Results indicated that serum urea content values of negative and positive control group of rats were 17.52±2.07 and 24.39±2.54 (mg/dL), respectively. Meanwhile, serum urea of hyperccholesterolimic rats fed on diet supplemented by 3,6 and 9% pine nut powder were 21.18±1.76, 20.79±1.85 and 20.53±1.97 (mg/dL), respectively. There are significant increase ($P \leq 0.05$) in positive than negative control group of rats. Also, hypercholesterolimic rats showed significant increase as ($p \leq 0.05$) compared with negative control. Serum urea of rats fed on diet containing pine nut powder showed significant decrease ($P \leq 0.05$) as compared with positive control group of rats. On the other hand, serum uric acid values of negative and positive control group were 31.86±2.15 and 52.62±3.21 (mg/dL), respectively. Serum uric acid of hypercholesterolemic rats fed on diet containing 3,6 and 9% pine nut powder were 41.04±3.17, 38.02±2.15 and 31.86±2.12 (mg/dL) respectively. Results indicated that there are significant increase ($P \leq 0.05$) in positive than negative control. Also, there are significant decrease ($P \leq 0.05$) in serum urea in hypercholesterolemic rats fed on pine nut powder as compared with positive control group.

It is obvious from the results that serum creatinine values of negative and positive control group were 0. 63±0.10, 1.83±0.13, respectively. Meanwhile, serum creatinine values of hypercholesterolemic rats were 0.87±0.11, 0.70±0.13 and 0.16 and 0.65±0.15 (mg/dL) for rats fed on diet supplemented by pine nut powder with the percentage 3,6 and 9%, respectively. The results indicated that there are significant difference ($P \leq 0.05$) among all groups of rats. Serum creatinine of hypercholesterolimic was significant decreased as the addition levels of pine nut powder increased. Such decrease in serum urea, uric acid and creatinine due to the addition of pine nut powder may be attributed that pine is a rich source of phenols and flavonoids as shown in the previously results in table (1). Polyphenols improved serum concentration of both urea and creatinine, as well as increased the activity of superoxide dismutase in the kidney **(El-Nashar, 2007)**. Moreover, flavonoids lowered plasma, urea and cratinine levels **(Van Hoorn et al., 2006)**.

9- Serum proteins

Table (9) illustrates the effect of pine nut fruits on total protein (TP), albumin (AL) and bilirubin (Bil) of hypocholesterolemic rats. The obtained results showed that total protein values of negative and positive control group of rats were 10.96±1.36 and 7.54±0.79 mg/dL, respectively. Total protein of hypocholesterolimic rats was significantly decreased ($P \leq 0.05$) as compared with negative group. Moreover, diet

containing pine nuts with the levels of 3, 6 and 9% increased total protein in serum rats to 9.29±0.98, 9.83±0.85 and 10.42±1.02 mg/dL, respectively.

Table (8): Effect of white pine nuts powder on urea, uric and creatinine of hypercholesterolemic rats.

Kidney functions / Groups	Uric (mg/dl)	Urea (mg/dl)	Creatinine (mg/dl)
control (–Ve)	$31.86^{c} \pm 2.15$	$17.52^{c} \pm 2.07$	$0.63^{b} \pm 0.10$
control (+Ve)	$52.62^{a} \pm 3.21$	$24.39^{a} \pm 2.54$	$1.38^{a} \pm 0.13$
Hyp. rats with 3%white pine	$41.04^{b} \pm 3.17$	$21.18^{b} \pm 1.76$	$0.87^{b} \pm 0.11$
Hyp. rats with 6%white pine	$38.02^{bc} \pm 2.15$	$20.79^{b} \pm 1.85$	$0.70^{b} \pm 0.13$
Hyp. rats with 9%white pine	$31.86^{c} \pm 2.12$	$20.53^{b} \pm 1.97$	$0.65^{b} \pm 0.15$
L.SD ($p \leq 0.05$)	**3.18**	**2.01**	**0.134**

Each value in a column followed by the same letter are not significantly at ($P \leq 0.05$).

On the other hand, albumin values of negative and positive group of rats were 5.98±0.35 and 3.75±0.29 mg/dL, respectively. Meanwhile, alsbumin, values of rats fed on 3, 6 and 9% pine nut were 4.56±0.41, 4.92±0.38 and 5.82±0.36 mg/dL, respectively. It is worthy to mention that albumin value was increase as the level of pine nut increased.

It is evident from the results that Bilirubin (Bil) values of negative and positive group of rats were 1.01±0.23 and 2.18±0.34 mg/dL, respectively. Bilirubin of hypercholesterolemic rats (Positive control) was significantly increased than negative control group of rats. Meanwhile, pine nut powder showed significant increase in bilirubin of serum rats as compared with positive control. It was found that total proteins and albumin of hypercholesterolemic rats showed that there were significant difference ($p \leq 0.05$) with in treatments.

It is worthy to mentioned that both total serum protein and albumin were increased as the level of pine nut increased. Such increase may be due to that pine nuts are a good source of phenols and flavonoids as shown in the previously results in Table (9).

Table (9): Effect of white pine nuts powder on total protein (TP), albumin (AL) and bilirubin (Bil)of hypercholesterolemic rats.

Parameters / Groups	Total protein (TP) mg /dl	Albumin (AL) mg /dl	Bilirubin (Bil) mg /dl
control (–Ve)	$10.96^{a} \pm 1.36$	$5.98^{a} \pm 0.35$	$1.01^{c} \pm 0.23$
control (+Ve)	$7.54^{c} \pm 0.79$	$3.75^{d} \pm 0.29$	$2.18^{a} \pm 0.34$
Hyp. rats with 3%white pine	$9.29^{b} \pm 0.98$	$4.56^{c} \pm 0.41$	$1.56^{b} \pm 0.21$

Hyp. rats with 6%white pine	$9.83^{ab} \pm 0.85$	$4.92^{b} \pm 0.38$	$1.37^{bc} \pm 0.19$
Hyp. rats with 9%white pine	$10.42^{a} \pm 1.02$	$5.82^{a} \pm 0.36$	$1.18^{c} \pm 0.21$
L.SD ($p \leq 0.05$)	**1.08**	**0.34**	**0.26**

Each value in a column followed by the same letter are not significantly at ($P \leq 0.05$).

Finally, it could be concluded that pine nut fruit caused an increase in either serum total protein or albumin, as well as improved the level of blood proteins.

REFERENCES

Abdel-Maksoud, A.M.; Noor, E.F. and Abd-El-Galil, A.M. (1996): Study of protective and curative effects of *nigella sativa* on serum lipid pattern of rats fed on hyperlipidemic diet. Egyptian J. Nutrition, 11(1):65-85.

AIN., (1993). American Institute of Nutrition (AIN), Purified diet for laboratory rodent. Final Report. ADHOC Writing Diet. J. Nutr., 123: 939 – 1951.

Alian, C.C. (1974). Cholesterol enzymatic colorimetric method. J. Clin. Chem, 20: 470.

AOAC., (2000). Official Method of Analysis of the Association of the Analytical chemists 17th published by the Association of Official Analytical Chemists. POBox 540. Benjamin Franklin Station Washington D.C. 20044.

Batista, C; Barros, L; Carvalho, AM and Ferrira, I.C. (2011). Nutritional and nutraceutical potential of rape (*Brassica napus* L. vor *napus*) and "tronchuda" cabbage (*Brassica oleraceae* L. var. *Costata*) inflorescences. Food & Chemical Toxicology, 49; 1208-1214.

Blank, M.L.; Schmit , J. A. and Privett O.S. (1964): Quantitative analysis of lipids by thin-layer chromatography. American Oil Chemists' Society (AOCS). https://doi.org/10.1007/BF02654817.

Blomhoff, R.; Carlsen, M.H.; Andersen, L.F. and Jacobs, D. R. (2006). Health benefits of nuts: potential role of antioxidants. Br. J. Nutr., 96 (2): 52S–60S.

Buring, J.E.; Liu, S.; Gaziano, J.M. and Sesso, H.D. (2003): Flavonoid intake and the risk of cardiovascular disease in women, Am. J. Clin. Nutr., (77): 1400-1408.

Cevdet, N. and Iclal D. (2003). Chemical composition and nutritive value of *Pinus pinea*, L. seeds. J. Food Chemistry 86: 365–368.

Chandrasekara, N. and Shahidi, F. (2011). Oxidative stability of cashew oils from raw and roasted nuts. J. Am. Oil Chem. Soc. 88: 1197–1202.

Chapman, D.G.; Castilla, R. and Cambell, J.A. (1959). Evaluation of protein in foods. LA. Method for determination of protein efficiency ratio. Can. J. Biochem physiol., 37: 679 – 686.

Davis, P.A.; Jenab, M.; Heuvel, J.P.; Furlong, T. and Taylor S. (2008). Tree nut and peanut consumption in relation to chronic and metabolic diseases including allergy. J. Nutr., 138 (1):1757S–1762S.

Destaillats, F.; Cruz-Hernandez, C.; Giuffrida, F. and Dionisi, F. (2010). Identification of the botanical origin of pine nuts found in food products by gas−liquid chromatography analysis of fatty acid profile. Journal of Agricultural and Food Chemistry, 58(4): 2082–2087.

Dogan, S.; Diken, M.E. and Dogan, M. (2010): Antioxidant, phenolic and protein contents ol' some medicinal plants. J. Medicinal Plants Res., 4: 2566 - 2573.

Drury, R.A. and Wallington, E.A. (1980): Carletons Histological Technique. 5th Ed. Oxford Univ., 96: 101.

Durak, I.; Koksal, I.; Kacmaz, M.; Buyukkocak, S.; Cimen, B.M. and Ozturk, S.H. (1999). Hazelnut supplementation enhances plasma antioxidant potential and lowers plasma cholesterol levels. Clinica Chimica Acta., 284(1): 113–115.

Durmaz, G.; Karabulut, I.; Topcu, A.; Asilturk, M. and Kutlu, T. (2010). Roasting-related changes in oxidative stability and antioxidant capacity of apricot kernel oil. J. Am. Oil Chem. Soc. 87: 401–409.

El-Nashar, N.G. (2007): Development of Primary Liver Cell Culture from Fish as a Valuable Tool in Nutrition and Biotechnology Research, Ph.D. Thesis, Faculty of Home Economics, Minoufia University.

European Food Safety Authority (EFSA) (2009). The Official List of 4.185 Functional Health Claims to be Assessed Under Article 13 of EC Regulation on Nutrition and Health Claims. EFSA, Parma, Italy.

Fassati, P. and Prencipe, L. (1982). Triglyceride enzymatic colorimatric method. J. Clin. Chem., 28: 2077.

Food and Agriculture Organization Statistical Database (2011). FAOSTAT database: Rome, Italy.

Gentile, C.; Allegra, M.; Angileri, F.; Pintaudi, A.M.; Livrea, M.A. and Tesoriere, L. (2012). Polymeric proanthocyanidins from Sicilian pistachio (*Pistacia vera*, L.) nut extract inhibit lipopolysaccharide-induced inflammatory response in RAW 264.7 cells. European Journal of Nutrition, 51: 353–363.

Griel, A.E. and Kris-Etherton P.M.(2006). Tree nuts and the lipid profile: A review of clinical studies. Br. J. Nutr., 96 (2): 68S–78S.

Hanato, T; Magawa, H.; Yasuhara, T. and Okuda, T. (1988). Two new flavonoids and other constituents in licorice root: their relative a stringency and radical scavenging effects. J. Chem. Pharm. Bull. 36: 2090-2097.

Hegested, D.M.; Mills, R.C. and Hart, E.B. (1941). Choline in the nutrition of chicks. J. Biol. Chem., (138): 459 – 470.

Henery, R.J. (1974). Clinical Chemist: Principles and Techniques. 2nd, Edition, Hagerstoun (MD), Harcer, ROW, p. 882.

Huang, D.J.; Ou, B.X. and Prior, R.L. (2005): The chemistry behind antioxidant capacity assays. Journal of Agricultural and Food Chemistry, 53: 1841-1856.

Jiang, R.; Manson, J.E.; Stampfer, M.J.; Liu, S.; Willett, W.C. and Hu F.B. (2002). Nut and peanut butter consumption and risk of type 2 diabetes in women. J. Am. Med. Assoc., 288: 2554–2560.

Jimenez, L.; Remesy, C.; Morand, C.; Dealbert, A. and Manach, C. (2004): Polyphenols: food sources and bioavailability. Am. J. Clin. Nutr., (79):727-747.

John, J.A. and Shahidi, F. (2010). Phenolic compounds and antioxidant activity of Brazil nut (*Bertholletia excelsa*). Journal of Functional Foods, 2: 196–209.

Kaviarasan, K.; Argunan, M.M. and Pugalendi, K.V. (2005): Lipid profile, oxidant –antioxidant status and glycoprotein components in heyperlipidemic patients with / without diabetes. J. Clin. Chem. Acta., 362(1-2), 49-56.

Kayin, X.; Elizabeth, A.M. and Philip C.C. (2016). A review of the potential health benefits of pine nut oil and its characteristic fatty acid pinolenic acid. Journal of Functional Foods, 23:464 - 473.

Kendall, C.W.; Josse, A.R.; Esfahani, A. and Jenkins, D.J. (2010). Nuts, metabolic syndrome and diabetes. British Journal of Nutrition, 104: 465–473.

Kris-Etherton, P.M.; Hu, F.B.; Ros, E. and Sabaté J. (2008). The role of tree nuts and peanuts in the prevention of coronary heart disease: Multiple potential mechanisms. J. Nutr., 138 (1): 1746S–1751S.

Kris-Etherton, P.M.; Zhao, G.; Binkoski, A.E.; Coval, S.M.; and Etherton, T.D. (2001). The effects of nuts on coronary heart disease risk. Nutrition Reviews, 59(4): 103–111.

Lee, R. and Nieman, D. (1996). Nutritional Assessment. 2nd Ed., Mosby, Missouri, USA, pp. 591 – 594.

Lopez, M.F. (1977). HDL cholesterol colorimetric method. J. Clin. Chem., 23: 882.

Mattes, R.D.; Kris-Etherton, P.M. and Foster G.D. (2008). Impact of peanuts and tree nuts on body weight and healthy weight loss in adults. J. Nutr., 138 (1): 1741S–1745S.

Mattes, R.D.; Kris-Etherton, P.M. and Foster G.D. (2008). Impact of peanuts and tree nuts on body weight and healthy weight loss in adults. J. Nutr., 138 (1): 1741S–1745S.

Moss, D.W. (1982). Alkaline Phosphates Isoenzymes, Clin. Chem., 28:2007-2016.

Noa, D.S.; Zhao, T.T.; Kim, Y.; Yoon, M.R.; Lee, J.S.; and Kim, I.H. (2015). Preparation of highly purified pinolenic acid from pine nut oil using a combination of enzymatic esterification and urea complication. Food Chemistry, 170: 386-393.

Park, S.; Lim, Y.; Shin, S.; and Han, S.N. (2013). Impact of Korean pine nut oil on weight gain and immune responses in high fat diet-induced obese mice. J. Nutrition Research and Practice, 7(5): 352–358.

Petton, C.J. and Crouch, S.R. (1977). Enzymatic Determination of Urea. J. of Anal. Chem., 49:464-469.

Rajaram, S. and Sabaté J. (2006). Nuts, body weight and insulin resistance. Br. J. Nutr., 96 (2): 79S–86S.

Ramadan, M.F. and Morsel, J.F. (2002). Neutral lipids classes of black cumin (*Nigella sativa*, L.) seed oils. European Food Research Technology, 214: 202–206.

Ruiz-Aceituno, L.; Rodríguez-Sanchez, S.; Sanz, J.; Sanz, M.L. and Ramos, L. (2014). Optimization of pressurized liquid extraction of inositols from pine nuts (*Pinus pinea*, L.). J. Food Chemistry, 153: 450-456.

Savage, G.P. (2001). Chemical composition of wallnuts (*Juglans regia*, L.) grown in New Zealand. Plants Foods for Human Nutrition, 56:75–82.

SPSS., (1998). Statistical Package for Social Science Computer Software. Ver. SPSS Company, London.

Stahle, E., (1967): Thin Layer Chromatography. A laboratory handbook. Ed, springer Verloag Berline, Properties pp35, Heidel Berg, New York.

Ternus, M.E.; Lapsley, K. and Geiger C.J. (2009). Health benefits of tree nuts. In: Tree Nuts: Composition, Phytochemicals, and Health Effects. Eds. C. Alasalvar, F. Shahidi, CRC Press, Taylor & Francis Group, Boca Raton, FL (USA), pp 37–64.

Ternus, M.E.; Lapsley, K. and Geiger C.J. (2009). Health benefits of tree nuts. In: Tree Nuts: Composition, Phytochemicals, and Health Effects. Eds. C. Alasalvar, F. Shahidi, CRC Press, Taylor & Francis Group, Boca Raton, FL (USA), pp 37–64.

Tietz, N.W. (1976). Fundamental of clinical Chemistry. Philadelphia, W.B.Saunders, P. 243.

USDA,United States Department of Agriculture (2016). National Nutrient Database For Standard Reference Release 28; United States Department of Agriculture: Washington, DC, USA.

Van Hoorn, D. E.; Nijveldt, R. J. and Boelens, P.G. (2006): Effects of preoperative flavonoid supplementation on different organ functions in rats. J. Parenter Enteral Nutr., 30(4):302-8.

Venkatachalam, M. and Sathe, S.K. (2006). Chemical composition of selected edible nut seeds. J. Agric. Food Chem. 54: 4705–4714.

While, B.A., Erickson, M.M. and Steven, S.A. (1970). Chemistry for Medical Theologies TS.3 Rd Ed., C.V. Mosby Company Saint Louis, USA, P. 662.

Xie, K.; Miles, E.A. and Calder, P.C. (2016). A review of the potential health benefits of pine nut oil and its characteristic fatty acid pinolenic acid. Journal of Functional Foods, 23:464-473.

Yound, D.S. (1975). Determination of GOT. J. Clin. Chem., 21: 1.

Zadernowski, R.; Naczk, M. and Czaplicki, S. (2009). Chemical composition of Pinus Siberia nut oils. Eur. J. Lipid Sci. Technol., 111: 698–704.

Zadernowski, R.; Naczk, M. and Czaplicki, S. (2009). Chemical composition of Pinus Siberia nut oils. Eur. J. Lipid Sci. Technol., 111: 698–704.

Campbell, J.A. (1961): Methodology of Protein Evaluation RAG Nutr., Document R. 10 Led., 37. WHO; June Meeting New York.

Jenkins, D.J.; Hu, F.B.; Tapsell, L.C.; Josse, A.R. and Kendall C.W. (2008). Possible benefit of nuts in type 2 diabetes. J. Nutr., 138 (1): 1752S–1756S.

Rajaram, S. and Sabaté J. (2006). Nuts, body weight and insulin resistance. Br. J. Nutr., 96 (2): 79S–86S.

تاثير ثمار الصنوبر على بعض التغيرات البيولوجية في الفئران المصابة بارتفاع كولسترول الدم

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الهدف من البحث تقدير كل من التركيب الكيميائي، ومضادات الأكسدة والأحماض الدهنية لفاكهة الصنوبر وكذلك دراسة مدى تأثير التغذية بنسب مختلفة من الصنوبر المجفف (3، 6، 9%) على التغيرات البيولوجية في الفئران المصابة بارتفاع الكولسيترول. وكانت النتائج المتحصل عليها ما يلي يعتبر الصنوبر مصدر جيد للزيت والألياف والبروتين والكربوهيدرات الذائبة، الفينولات، الفلافونوبدات كما يعتبر مصدر غني بالأحماض الدهنية الغير مشبعة وخاصة حمض اللبنوليك (ك18: 2) والذي يمثل حوالي نصف كمية الأحماض الدهنية الكلية. بالنسبة للتغيرات البيولوجية فقد لوحظ أن الوجبات المدعمة بالصنوبر أدت إلى إنخفاض معنوي عند مستوى معنوية 0.05 في كل من وزن الجسم، الدهون الثلاثية، الليبيدات الكلية، الكوليسترول، LDL، VLDL في الفئران المصابة بالكوليستيرول بالمقارنة بالمجموعة الضابطة الموجبة. لوحظ أيضاً أن الصنوبر أدى إلى حدوث إنخفاض معنوي (عند مستوى 0.05) في نشاط إنزيمات الكبد وكذلك اليوريا، الكرباتينين، حمض اليوريك في الفئران المصابة بالكوليستيرول بالمقارنة بالمجموعة الضابطة بينما لوحظ زيادة في نسب كل من بروتينات وألبيومين سيرم الفئران.