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Hanaa F. El Mehiry

Home Economics Dept., Faculty of Specific Education, Mansoura University, Egypt.

Rehab Ibrahim Tag Al Deen

Home Economics dept., Faculty of Specific Education, Zagazig University, Egypt.

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البريد الإلكتروني للمجلة E-mail JSROSE@foe.zu.edu.eg

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Abstract:

Pumpkin (*Cucurbitae Pepo L.*) seed oil (PSO) is suggested as an antioxidant, as well as reproductive effect, whereas zinc claimed as fertility supplement. Current work aims to assess the possible effect of PSO combined with zinc on thyroids, testosterone hormones and oxidative damage against zinc deficiency in male rats. Thirty-six rats weight ranged 70-80g classified into four nine rats, were used for the research that lasted for six weeks. Group I, the control group, received fed on basal diet with sufficient zinc and water ad libitum. Group II, received zinc-deficient diet (3ppm), group III was received zinc-deficient diet and zinc sulphate supplemented (3+81 ppm) and group IV received the same of group III treated with 200 mg PSO/kg/b.wt/rats. The results showed an excellent quality of PSO with high content of polyunsaturated fatty acids, and phenolic phytochemicals. Results showed statistically significant increase in serum levels of triodothyronine hormone (T3), testosterone, antioxidant microsomal enzymes in liver and testis in the test PSO compared with the zinc-deficient rats. The results also revealed statistically significant decrease in the serum levels of lipid profile except HDL-C in groups ZD + Zn and ZD + Zn + PSO when compared with zinc deficient group (ZD). The increase in zinc levels in blood and liver and testis in dose dependent as there were consistent increment in groups ZD + Zn and ZD + Zn + PSO after which the levels increased with adding PSO to zinc supplemented in zinc-deficient rats. In conclusion, PSO has some significant positive effects on reproductive functions in male rats in particular those

suffering from zinc deficient. All of the effects could be potentially attributed the high bioactive compound content fatty acids, menieral, antioxidant activity, testis and liver by PSO.

Key words: Linolenic acid, antioxidant, testosterone, testis, phytochemicals.

Interduction

Zinc represents an essential trace element for the growth of human and other animals. It has a unique and extensive role in biological processes and work as essential cofactor for more 200 metallo-enzymes **(Seyedmajidi *et al.*, 2014).** Zinc has a key role in normal testicular development, spermatogenesis and sperm motility in the body **(Khan *et al.*, 2014).** The mean reason of zinc deficiency is largely related to inadequate intake, malabsorption or deficient metabolic disorders of zinc **(Abo Raya *et al.*, 2016).** Deficiency of zinc reduces testosterone concentrations, alters thyroid metabolism and hepatic steroid, and modulate sex steroid hormone receptor levels, thereby participating to the pathogenesis of male reproductive dysfunction **(Rajeswari and Swaminathan, 2015).** Deficiency of zinc was first documented in Egyptian subjects with growth retardation **(Karaca *et al.*, 2007).** Recently oral zinc supplementation is considered to be the most effective and efficiency strategy for combating long term deficiency of nutritional zinc **(Bakheet *et al.*, 2015).**

Consumption of foods rich in natural antioxidants has been reported as being protective some various types of diseases **(Kirbaslar *et al.*, 2012).** Therefore, dietary natural supplements, consisting of phytochemicals such unsaturated fatty acids (USFA's) and vitamins which could be used to effectively prevent body cells from the risk of oxidative stress and to protect human body health in general **(Sies *et al.*, 2005).** Pumpkin (*Cucurbita Pepo L.*) has been used as a medicine to treat a variety of diseases throughout the world since ancient times **(Adams *et al.*, 2011).** It has been used as a medicine to treat a variety of diseases throughout the world since ancient times. Pumpkin seeds are a rich natural source of (USFA's), proteins, phytosterols, phytochemicals, sterols, vitamins and fibers and rich in trace elements, such zinc and selenium, also seeds known to have anti-atherogenic and hepatoprotective activities, useful for reproductive health, immunomodulation and therapeutic advantage over a

wide range of disease conditions **(Gossell-Williams *et al.*, 2006; Fruhwirth and Hermetter, 2007; Stevenson *et al.*, 2007; Makni *et al.*, 2008 and Abou Seif, 2014).** Being excellent source of (USFA's) particularly oleic and linoleic acids and tocopherols, which have shown high oxidative stability (**Abdel-Rahman, 2006 and Stevenson *et al.*, 2007).** Pumpkin seed oil is recommended as a healthy addition to human diet and for food and industrial applications, related to potential suitability of the oil **(Akwaowo *et al.*, 2000; Kreft, 2002; Paulauskiene *et al.*, 2005; Jariene *et al.*, 2007 and Mohammadi *et al.*, 2013).** In addition, the functional activity of oil in wounds healing was reported in diabetic rats (**Bardaa *et al.*, 2016).** Therefore, pumpkin oil is proposed for both the prevention and treatment of reproductive dysfunctions in male experimental animals **(Tuberoso *et al.*, 2007; Oyeyemi *et al.*, 2008 and Gülçin 2010).** Thus, the present work aimed to assess the potential protective effects of PSO against zinc-deficient diet induced reproductive and thyroid hormone dysfunctions in male rats.

Material and Methods

Materials:

Pumpkin seed oil (*Cucurbita Pepo* L.):

Was obtained from Agriculture Research Center, Giza, Egypt.

Experimental animals:

Thirty six male albino rats of Sprague Dawley strain weighing (70-80 g) purchased from Laboratory of Animal Colony, Helwan, Egypt. Rats were maintained under controlled hygienic conditions.

Methods

Chemical analysis:

Pumpkin in seed were analyzed for the moisture, protein, fat, ash, fiber and total carbohydrates contents such as described in **AOAC, (2000).**

Determination of fatty acid*:*

The fatty acid profile of ethanolic extract of pumpkin seed oil was determined according to ISO 5508 (1990) and ISO 5509 (2000) by gas chromatography (GC) as described by **(Nath, 1996).**

Preliminary phytochemical screening of pumpkin seeds oil:

Detection of tannins and resins:

Tannins and resins were detected in the plant sample according to the method of **(El-Badrawy, 1996).**

Detection of saponins:

Saponins substances were detected in different crude oil under investigation according to the method of **(Trease, 1961).**

Detection of terpenes:

Terpenes substances were detected in different crude oil under investigation according to the method of **(Finar, 1968).**

Detection of flavonoids:

Flavonoids substances were detected in oil of different samples using the method of **(Geissman, 1962).**

Detection of carbohydrates and glycosides:

Carbohydrates and glycosides were treated by Molish test according to the method of **(Blabaa *et al.*, 1976).**

Experimental design:

Thirty six weanling male albino rats weight ranged 70-80 g were equally assigned at random dietary treatment for six weeks. The rats were housed in plastic cages located in the Animal House of Colony, Helwan, Egypt, under the same managerial condition and to prevent contamination, the cage and all equipments used for feeding and watering were soaked in 1% EDTA and rinsed with deionized water before using. Feed and deionized water were available *ad libitum.* All rats procedures are in accordance with the recommendations for the proper care and use of laboratory rats stated by the Canadian Council on Animal Care **(CCAC, 1993).** The rats were randomly divided into four groups (9 rats) per each**.**

Group I: (Control group)

Fed zinc adequate diet contains 79 mg ZnSO4/ kg diet equal 35mg/kg.

Group II: (Zinc-deficient (ZD) group)

Zinc deficiency was induced in the rats of this group by given zinc-deficient diet (Zn=3ppm)/kg diet

Group III: (ZD + Zn group)

Fed on the same Zn- deficient diet as group (II) for four weeks, afterwards 200 mg ZnSO4 (equal 81 mg/kg zinc) was added to each Kg diet (contained 3+81 ppm Zn) for two consecutive weeks. Composition of the diet was given in Table A.

Group IV: (ZD + Zn + PSO group)

Rats were given the zinc deficient diet and received supplemented ZnSO4 (84 ppm) plus pumpkin seed oil at a dose of 200 mg/kg/b. wt/ intraperitoneally /day.

Plasma and tissue preparation

Sample collection:

At the end of the 6 th week of the treatment, animals were fasted overnight before sampling days. Rats were anaesthetized by intraperitoneal injection of sodium thiopental (40 mg/kg), afterwards all rats were bled by head decapitation. Trunk blood was collected from each animal without anticoagulant in clean dry tubes frozen under-20^{o}C until assayed. Under the same condition, liver and testis were carefully excised from each rat immediately immersed in a cold physiological saline solution (0.9% NaCl), quickly freezes in liquid nitrogen and stored at -40^{o}C.

Table (A): Composition of zinc-deficient diet (3 mg/kg) of group (II) as g /kg diet

Ingredient	g/kg diet
Casein	200
starch	497
Cellulose	30
corn oil	50

Salt mixture	47
Vitamin mixture	20
Sucrose	100

* The composition of both salt and vitamin mixture were exactly formulated as recommended by **Dura Trave *et al.* (1986).**
* ZnSO4 was incorporated in the salt mixture of diet of control group (Zn sufficient diet) in account of 79 mg/kg to provide about 35 ppm Zn/kg diet.

Biochemical determination:

Serum was diluted 1:3 with deionized water to measure the level of zinc. Portions of the freeze dried organs were weighted and ashed in a muffle furnace at 450^{o}c. residue from the ash was dissolved in concentrated HCl and then diluted with deionized water as required to determine their content of zinc. Concentrations of zinc in serum and tissues were analyzed by atomic absorption spectrophotometer using a Perkin Elmer atomic absorption spectrophotometer 305B.

The concentration of serum total cholesterol (T.C), triglycerides (TG), high density lipoprotein (HDL) and low density lipoprotein (LDL) were determined by the methods of **(Roeschlau *et al.*, 1974 and Fossati and Prencipel 1982),** respectively.

Hormonal assays:

Serum total thyroxine (T4), triodothyronine (T3), and serum testosterone were assayed by **(Larsen 1972, Schuurs and Van Weeman 1977 and Maruyama 1987)** respectively.

Antioxidant activity analysis:

Parts of liver were also quickly excised on ice-cold plates to evaluate the levels of glutathione (GSH), glutathione peroxidase (GPX) and Alcohol dehydrogenase (ADH) according to **(Tietze, 1969, Ursini *et al.*, 1985 and Burnett and Felder 1978)** respectively.

Statistical analysis:

Results are expressed as the mean ±SD. Data were statistically analyzed for variance "ANOVA" test at ($P < 0.05$) according to **(Snedecor and Cochran 1967)** using SPSS statistical software, version 13.0.

Results

Proximate composition of pumpkin seeds:

Table 1 shows the results of proximate analysis of pumpkin seeds. Pumpkin seed contains 32.95, 30. 93, 11.78 and 3.92% of protein, fat, ash and crude fiber contents respectively on dry weight basis. As determined in this study, mineral composition indicated that pumpkin seeds contained remarkable levels of mineral. The predominant minerals element among minerals analyzed in the seeds the Zn, Ca, Fe and Co which recorded 13.86, 10.09, 4.46 and 3.23 mg/ 100g respectively. The obtained results indicate that pumpkin seeds relatively contain excellent amounts of protein, fat, fiber and minerals which consider a major cause of their quality and functionality.

Table (1): Proximate composition % dry basis of pumpkin seeds

Constituents	Value
Moisture (g/100g)	8.40
Protein (g/100g)	32.95
Fat (g/100g)	30.93
Ash(g/100g)	11.78
Crude fiber(g/100g)	3.92
Total carbohydrates	12.02
Zn (mg/100g)	13.86
Ca (mg/100g)	10.09
Fe (mg/100g)	4.46
Co (mg/100g)	3.23

Values are expressed as mean of three triplicates.

Fatty acid composition of pumpkin seeds oil :

The results according to the chromatography profile of fatty acid composition in PSO are summarized in Table 2. The important identified fatty acids were: oleic, linoleic and palmitic. The main fatty acids in pumpkin oil seeds are oleic acid (Omega 9 monounsaturated fatty acid) and linoleic acid (Omega 6 polyunsaturated essential fatty acid) which account in total for about 48.01 and 33.14%. Results revealed significant levels of polyunsaturated fatty acids which qualified as essential fatty acids. PSO are particularly rich in monounsaturated oleic acid and polyunsaturated linoleic acid. In addition linolenic acid and palmitic acid, the precursor of unsaturated fatty acids is present at a remarkable percentage of about 0.14 and 11.07%. Likewise, palmitoleic acid, archidic acid behenic acid, stearic acid, myristic acid and gadoleic acid which detected 0.40% 0.28%. This data confirms the good nutritional quality with functional properties of pumpkin seeds oil as our body is unable to synthesize unsaturated fatty acids.

Table (2): Fatty acid composition of pumpkin seeds oil

Component	Value
Oleic acid	48.01
Linoleic acid	33.14
Linolenic acid	0.41
Palmitic acids	11.07
Palmitoleic acids	0.40
Arachidic acid	0.28
Behenic acid	1.11
Stearic acid	5.03
Myristic acid	0.24
gadoleic acid	0.31

Preliminary phytochemical screening of pumpkin seeds oil:

The phytochemical screening of tannins, resins, saponins, carbohydrates terpenes and flavonoids in PSO were detected and recorded in Table 3. Pumpkin seeds oil are a rich source of phenolic phytochemicals having high antioxidant activity.

Table (3): Preliminary phytochemical screening of pumpkin seeds oil.

pumpkin seeds oil	Tannins	Resins	Saponins	Carbohydrates	Terpenes	Flavonoids
	+	+	+	+	+	+

+ Present - Absent

Feed intake, body weight and FRE in zinc-deficient rats:

Feed intake, body weight and FRE of the animals subjected to the different treatments are shown in Table 4. Body weights and food efficiency ratio (FER) of normal control, ZD + Zn and ZD + Zn + PSO treated group rats increased progressively throughout the study. Zinc deficiency resulted in a significant ($p < 0.05$) decrease in the BWG after six weeks, when compared to normal control rats. However, no significant difference was observed in the mean FI between different treatment groups. PSO treatment the zinc deficient rats tended to improve the body weight growth even, better than normal control group in comparison to zinc-deficient (ZD) rats.

Table (4): Effect of PSO treatment on feed intake (FI), body weight (BWG) and food efficiency ratio (FRE) in zinc-deficient rats

Groups / Parameters	Control	ZD	ZD + Zn	ZD + Zn + PSO
Feed intake (FI) (g/d)	15.32± 2.14 [a]	13.55±2.55 [b]	15.45±2.42[a]	15.40±2.33[a]
Body weight (BWG) (g)	114.77± 8.11 [a]	65.89± 6.11 [b]	101.14±9.13[a]	117.33±8.0[a]
FER	0.125± 0.01[a]	0.082± 0.03 [b]	0.111±0.02[a]	0.115±0.04[a]

Values are expressed as means ± SD. Values with the same letters indicate of raw and nonsignificantly difference of P< 0.05

Zinc blood concentration:

Figure 1 shows zinc levels in blood of different experimental groups expressed as µmol/L in serum. Zinc-deficient rats (ZD), showed a highly significant (P < 0.05) reduction in serum blood zinc concentrations in comparison with normal group (6.09±0.08 versus 11.24±0.12 µmol/L) (P < 0.05). However, zinc supplemented plus PSO administration to the zinc-deficient rats helped in raising zinc concentration as compared to zinc-deficient group (ZD). However, no significant difference was detected between PSO group and normal control rats in zinc concentration.

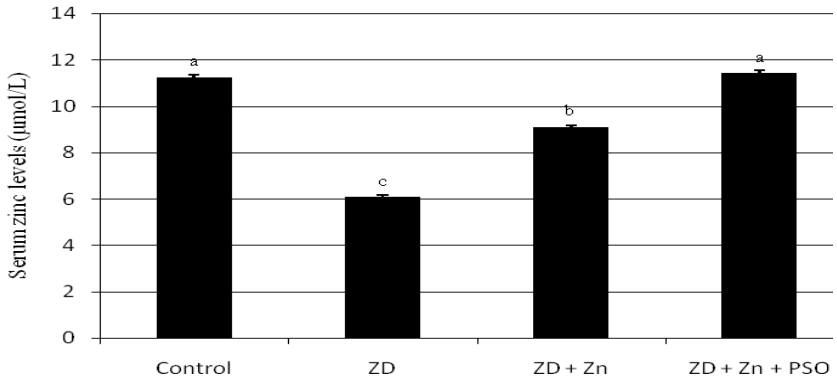

Fig. (1): Effect of PSO treatment on serum zinc levels in zinc-deficient rats, Values are expressed as means ± SD. Values with the same letters indicate of raw and nonsignificantly difference of P< 0.05

Lipid profile of zinc-deficient rats:

The results on the lipid profile of normal group and zinc-deficient rats are shown in Table 5. A highly significant (p <0.05) increase in total cholesterol level was recorded in zinc-deficient (ZD) rats group, when comparing with normal group. Conversely, a high significant decrease in total cholesterol level was observed in treated group with PSO when compared with zinc-deficient (ZD) group. Serum triglycerides levels were also elevated in the zinc-deficient (ZD) group when compared to normal control rats group (75.83 and 60.78 mg/dl). There was a

significant decrease on T.C, TG and LDL-c levels of treated group that administrated PSO (200 mg/kg/ b.w/rats/day) when compared with zinc-deficient rats ($p<0.05$). But there is an increase in level of HDL-c of treated group of PSO even better than normal control when compared to zinc-deficient rats (ZD) significance ($p<0.05$).

Table (5): Effect of pumpkin seed oil treatment on lipid profile in zinc-deficient rats.

Groups / Parameters	Control	ZD	ZD + Zn	ZD + Zn + PSO
T.C (mg/dl)	55.38 ± 0.28^{d}	71.54 ± 0.89^{a}	66.06 ± 0.63^{b}	60.46 ± 0.39^{c}
TG (mg/dl)	60.78 ± 0.38^{d}	75.83 ± 0.87^{a}	70.14 ± 0.64^{b}	64.52 ± 0.50^{c}
HDL (mg/dl)	16.75 ± 0.13^{b}	16.03 ± 0.11^{b}	17.21 ± 0.14^{a}	17.71 ± 0.15^{a}
LDL (mg/dl)	26.42 ± 0.18^{d}	40.38 ± 0.36^{a}	34.78 ± 0.29^{b}	29.86 ± 0.24^{c}

Values are expressed as means ± SD. Values with the same letters indicate of raw and nonsignificantly difference of $P< 0.05$

Total Cholesterol, TG: Triglycerides, HDL: High Density Lipoprotein, LDL: Low Density Lipoprotein.

Effect of PSO treatment on serum testosterone, T3 and T4 hormones in zinc-deficient rats:

As shown in Figures (2, 3 and 4) serum testosterone, triiodothyronine (T3) and thyroxin (T4) levels. Zinc-deficient (ZD) group showed significant ($p<0.05$) decrease in T3 and testosterone hormones while PSO group showed significantly increase in T3 and testosterone hormones ($p<0.05$) compared to zinc-deficient (ZD) group. The level of T4 hormone showed no significant difference between different treated groups. But PSO and zinc supplemented reference group showed normal values of these hormones at ($P< 0.05$) compared to zinc-deficient (ZD) group.

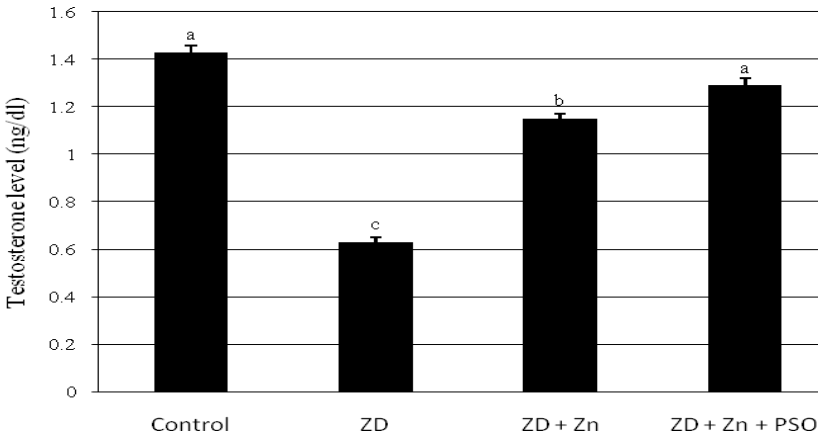

Fig. (2): Effect of PSO treatment on serum testosterone in zinc-deficient rats, Values are expressed as means ± SD. Values with the same letters indicate of raw and nonsignificantly difference of P< 0.05

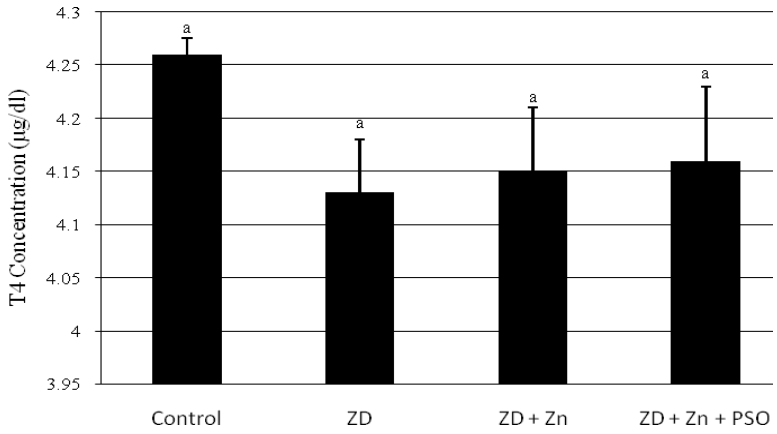

Fig. (3): Effect of PSO treatment on serum thyroxine hormones in zinc-deficient rats, Values are expressed as means ± SD. Values with the same letters indicate of raw and nonsignificantly difference of P< 0.05

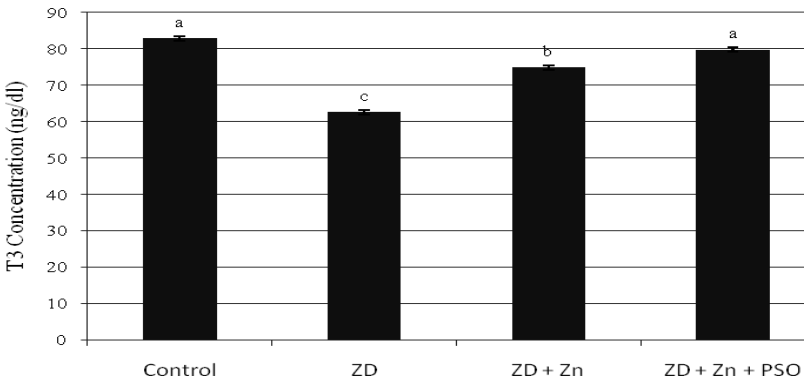

Fig. (4): Effect of PSO treatment on serum triiodothyronine hormones in zinc-deficient rats. Values are expressed as means ± SD. Values with the same letters indicate of raw and nonsignificantly difference of P< 0.05.

Zn, GSH, GPX and ADH concentration in liver:

The data presented in Table 6 showed that Zn concentration in liver tissue was significantly decreased (p<0.05) in ZD (zinc-deficient) rats as compared to control group. However zinc levels got elevated to within normal levels in ZD + Zn + PSO group in which PSO was administrated along with zinc treatment. A significant (p< 0.05) inhibition in the levels of GSH and GPX activities were detected following zinc deficiency in normal group. ADH levels were significantly declined (p<0.05) in ZD (zinc-deficient) rats as compared to control group. Both Zn supply and PSO to ZD rats only partially reversed this change. In fact, ADH concentration in the ZD + Zn + PSO group was lower than in the supply ZD + Zn group (p<0.05) but still significantly (p<0.05) lower than zinc-deficient (ZD) rats.

Table 6: Effect of PSO treatment on liver Zn, GSH, GPX and ADH

Groups / Parameters	Control	ZD	ZD + Zn	ZD + Zn + PSO
Zn (µmol/100 g)	6.67±0.08 [a]	4.99±0.06[d]	5.89±0.07[c]	6.17±0.08[b]
GSH (mg/g tissue)	20. 56±0.17[a]	14.62±0.09 d	17.38±0.13 c	19.02±0.14 b
GPX (µmol/min/g protein)	94.96±0.82 a	69.51±0.56 d	80.01±0.67 c	89.24±0.77 b
ADH (nmol/NADH oxidized/min/mg /protein)	35.01±0.23 d	52.43±0.41 a	47.28±0.37 b	39.89±0.28 c

Values are expressed as means ± SD. Values with the same letters indicate of raw and nonsignificantly difference of P< 0.05. GSH: Glutathione, GPX: Glutathione Peroxidase, ADH: Alcohol dehydrogenase.

Zn, GSH, GPX and ADH in testis:

Table 7 shows the alterations occurring in Zn, GSH, GPX and ADH levels in different groups of rats. The zinc concentration is significantly lower in zinc-deficient (ZD) rats compared to normal and treated with supplemented zinc and pumpkin seed oil rats. The treatment zinc-deficient rats with PSO had a very high significant influence on zinc concentration, GSH level and GPX activity ($p<0.05$) comparing with zinc-deficient rats (ZD). ADH activity was increased in the zinc-deficient (ZD) group compared to that in both of control group and treated with PSO group ($P < 0.05$). Induction of zinc deficiency led to a reduction of GSH level in the testis tissues of the zinc-deficient (ZD). However treatment with PSO at a dose of 200 mg/kg b.wt. increased the Zn, GSH and GPX levels by 38.89%, 29.88% and 37.24% respectively, which were statically significant ($P<0.05$).

Table (7): Effect of PSO treatment on testis zinc and microsomal enzymes activities in zinc-deficient rats.

Groups / Parameters	Control	ZD	ZD + Zn	ZD + Zn + PSO
Zn (µmol/100 g)	0.80 ± 0.02^{a}	0.54 ± 0.03^{b}	0.76 ± 0.02^{b}	0.75 ± 0.04^{b}
GSH (mg/g tissue)	3.77 ± 0.06^{a}	1.84 ± 0.03^{d}	2.31 ± 0.03^{bc}	3.05 ± 0.04^{b}
GPX (µmol/min /g protein)	10.02 ± 0.0^{a}	6.82 ± 0.06^{d}	8.07 ± 0.07^{bc}	9.36 ± 0.08^{b}
ADH (nmol/NADHoxidized/min/mg/protein)	7.59 ± 0.06^{c}	11.58 ± 0.09^{a}	9.29 ± 0.08^{b}	8.12 ± 0.07^{bc}

Values are expressed as means ± SD. Values with the same letters indicate of raw and nonsignificantly difference of $P< 0.05$. ZD: Zinc-deficient, PSO: Pumpkin Seed Oil, GSH: Glutathione, GPX: Glutathione Peroxidase, ADH: Alcohol dehydrogenase.

Discussion

Several decades ago, medicinal plants therapy, mainly using natural antioxidants, represents a reasonable therapeutic approach for the protection and treatment of different deficiencies related to the role of oxidative stress in contributing to initiation and progression of tissues damage. It exerts their protective effects by scavenging free radicals and improving antioxidant defense system **(Li *et al.*, 2015).** PSO is a natural product rich in many antioxidant and nutritional components, as indicated the data of the present study, it relatively contain excellent amounts of protein, fiber, minerals, unsaturated fatty acids and phenolic phytochemicals which consider a major cause of their quality and functionality such results in parallel with the finding of **(Hashemi 2013).**

Although mean body weights of the groups at the beginning of the study were not different, it has been reported that ZD (zinc-deficient) group had a significant weight loss at the end of the study. It can be said that the weight loss observed in ZD group was an expected result, as it was reported in several studies that zinc deficiency in the diet caused weight loss **(Seyedmajidi *et al.*, 2014).** Besides, it is a widely accepted view that the most obvious parameter of zinc deficiency is a reduction in body weight (**Sunar *et al.*, 2009**). The weight loss that observed in the zinc-deficient group is consistent with the reports to the effect that zinc deficiency in animals associated with weight loss, poor food efficiency ratio and delays in growth **(Xiaogang Yu *et al.*, 2016).**

In the male reproductive system, zinc is essential for optimal performance and output. Zinc in seminal fluid assist to stabilize the cell membrane and nuclear chromatin of spermatozoa **(Egwurugwu *et al.*, 2013 and (Khan *et al.*, 2014).** Furthermore **Bakheet *et al.*, (2015)** demonstrated that zinc supplementation caused a decrease in plasma lipid peroxides, so adequate zinc intake could play an important role in the prevention and/or modification of different diseases **(Sutyarso *et al.*, 2016).**

Pumpkin seeds oil zinc supplements in this study has important role to increase of zinc concentration**.** Pumpkin seeds oil can prevent changes in plasma lipid, which linked with being rich in linoleic acid, an essential unsaturated fatty acid. The protective effect is related to abundant linoleic acid and oleic acid, these oils were good for reducing serum cholesterol,

triacylglycerides and LDL and increase HDL levels which associated with reducing risk of heart attack **(Gossell-Williams *et al.*, 2006 , Kim *et al.*, 2012, Kırbaşlar *et al.*, 2012 and Al-Masri *et al.*, 2015).).**

The significant alterations in testosterone levels were agreed with **(Al-Attar 2011 and Al-Masri, 2015).** The results showed statistically significant increase in serum levels of testosterone in the test group ZD + Zn + PSO that ingested 84 ppm of zinc plus pumpkin oil when compared with the ZD (zinc-deficient) group. The observed increase in testosterone levels in treated group ZD + Zn was less when compared with ZD + Zn + PSO group that was given PSO dose as natural zinc supplementation. This result agrees with the works of **(Egwurugwu *et al.*, 2013).** Zinc supplementation by PSO activity secretion and testosterone which can lead to raise efficiency of spermatogenic machinery and increased account of germ cells in the seminiferouse tubules **(Dissanayke *et al.*, 2009 and Abdella *et al,* 2011).** Also in a parallel with **(Hashemi, 2013 and Ramah *et al.*, 2015)** they reported that pumpkin seed can used as antioxidant, which have rich source of zinc and phytochemical compounds, which promote prostate health by increasing serum testosterone, thus improving reproductive functions.

Oral administration of PSO in this study has important role in increase zinc concentration, and consequently effect on thyroid hormone metabolism **(Christy and Stella 2007and Zearah *et al.*, 2016).** In addition, zinc as co-enzyme factor for many enzymes involved in metabolism may have key role for sensitizing the liver and testis tissues of the body to thyroid hormone (**Kilic 2007).** Zinc is also a cofactor for iodothyronine iodinase enzyme , the enzyme which convert T4 hormone to T3 **(El-Sisy *et al.*, 2008)** as well as zinc may play a role in thyroid hormone metabolism in patients with low levels of T3 hormone and may control the conversion of T4 to T3 in human **(Suleyman *et al.*, 2007).** Furthermore, the obtained data may be explained by activities of PSO in zinc tissues concentration and thyroids function. These activities were reflected by the increase of serum testosterone, T3 and T4 levels **(Al-Masri 2015).** In other studies were observed the deficiency in zinc element was linked with reducing free thyroxin hormone levels in serum by approximately 30 % when compared with zinc sufficient **(Marreiro *et al.*, 2008, Akang et al., 2010 and Elfiky *et al.*, 2012).**

The current work provided clear evidence that PSO supplementation caused a significant increase on serum antioxidant enzymes of zinc-deficient rats. Several studies demonstrated that zinc deficiency caused to increase free radical production or increase oxidative damage in both vitro and vivo **(Ho and Ames, 2002)**. Zinc-deficient (ZD) group showed significant decrease in testis Zn, GSH and GPX and significant increase in ADH ($p<0.05$) while PSO group showed significant increase in testis Zn, GSH and GPX at ($p<0.05$) and normal values of ADH. Similar observation was documented by **(Binoy et al., 2016)** they reported a decline in ADH activity which associated with zinc deficiency. Administration of PSO with combination of Zn to experimental rats showed best results as it showed normal antioxidant enzymes as, it showed significant increase in testis Zn, GSH and GPX significant decrease in ADH compared to zinc-deficient (ZD) group as recorded in Table 5 & 6. The results in this study was similar to that observed by **(Gossell-Williams *et al.*, 2006 and Tsai *et al.*, 2006)** they attributed the beneficial activity to the high content of phenloic phytochemicals which present in the PSO. In addition **(Rendon-Ramirez *et al.*, 2007, Van Hoed *et al.*, 2009 and Kim *et al.*, 2012)** who reported that PSO contain high levels of tocopherol which act as an antioxidant agent. Furthermore **(Aghaei *et al.*, 2013 and Mohammadi *et al.*, 2013)** indicated that the protective action and improvability effects of PSO are possibly related to its high amount of polyunsaturated fatty acids, antioxidant and free radical scavenging ability which causes to increase antioxidant enzymes levels on rats **(Ryan *et al.*, 2007; Derouiche *et al.*, 2013 and Ramah *et al.*, 2015).**

Conclusion

This study has revealed that oil from pumpkin seeds extracted by cold pressure is an important source of many healthy components such as polyunsaturated fatty acids, phenolic phytochemicals in PSO makes it an excellent source of bioactive components which may provide potential protection against zinc deficiency. In fact, these findings revealed also that zinc deficiency in rats treated with PSO was better than reference supplemented zinc groups by the means of above data. Further researches with purified constituents are recommended to better understanding of the complete mechanism of PSO in thyroid, testosterone, serum zinc levels in

(blood, liver and testis) and oxidative enzymes. Besides, additional studies of the phytochemicals components of the obtained PSO would contribute to accomplish other nutritional and medicinal approaches.

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تأثير زيت بذور القرع العسلي على نقص الزنك المستحث لخلل الوظائف التناسلية في ذكور الفئران

هناء فاروق المهيري
قسم الاقتصاد المنزلى– كلية التربية النوعية – جامعة المنصورة– مصر

رحاب إبراهيم تاج الدين
قسم الاقتصاد المنزلي– كلية التربية النوعية– جامعة الزقازيق– مصر

الملخص

يعزي استخدام الزيت المأخوذ من بذور القرع العسلى إلى العديد من الخصائص البيولوجية الهامه ومنها كمضاد للأكسدة وكذلك لتأثيره على الوظائف التناسلية، في حين يزعم أن الزنك يستخدم كمكمل للخصوبة. وعلى الرغم من ذلك فان الدراسات العلمية عن آثار زيت القرع العسلى بالإضافة إلى الزنك لا تزال محدودة للغاية. لذلك يهدف البحث الحالى لتقييم التأثير المحتمل لزيت بذور القرع العسلى الى جانب الزنك على هرمونات الغدد الدرقية و هرمون التستوستيرون والضرر التأكسدي ضد نقص الزنك في ذكور الفئران البيضاء. تم استخدم ستة وثلاثون من الفئران تراوحت اوزنها بين 70– 80 جم، وقسمت إلى أربعة مجموعات كلا منها 9 فئران، واستمرت التجربة لمدة ستة أسابيع. وقد تلقت المجموعة الضابطة، طعام الفئران العادي مع الزنك الكافي و المجموعة الثانية تلقت النظام الغذائي الناقص فى الزنك (3 جزء فى المليون) و المجموعة الثالثة تلقت نفس غذاء المجموعة الثانية بالاضافة الى مكمل كبريتات الزنك (3 + 81 جزء في المليون) وقد تلقت المجموعة الرابعة نفس المجموعة الثالثة بالإضافة إلى 200 ملجم من زيت بذور القرع العسلى/ كجم/ وزن الجسم. وفى نهاية التجربة تم جمع عينات الدم وتقدير هرمون التستوستيرون، تراي ايدوثيرونين، الثيروكسين، وكذلك صورة دهون الدم (الكوليسترول الكلي، الجليسريدات الكلية، ومستويات الكوليسترول منخفض الكثافة والكوليسترول مرتفع الكثافة). وقد تم تحليل الزنك والانزيمات المضادة للاكسدة في الكبد والخصية. وأظهرت النتائج أن زيت بذور القرع العسلى ذات جودة عالية مع وجود نسب ملحوظة من الأحماض الدهنية غير المشبعة والمركبات

الفعالة النسبة من هذه المواد الفعالة كانت لها علاقة مباشرة مع فعالية الزيت ونقص الزنك وذلك من متوسطات الدراسة التى تم اجرائها. وأظهرت النتائج زيادة ملحوظة في مستويات السيرم من هرمون تراودثيرونين، وهرمون التستوستيرون، ودرجة نشاط الانزيمات المضادة للأكسدة في الكبد والخصية في المجموعة المعالجة بزيت القرع العسلى بالمقارنة مع مجموعة الفئران التي تعاني من نقص الزنك. كما أظهرت النتائج انخفاضا ملحوظا في مستويات السيرم من اقسام الدهون المختلفى باستثناء الكوليسترول مرتفع الكثافة في المجموعات المعالجة بمكمل الزنك ومجموعة زيت القرع العسلي بالمقارنة مع مجموعة نقص الزنك. كما سجلت زيادة معنوية فى مستويات الزنك في كل من الدم والكبد والخصية باستخدام الجرعة المستخدمة في المجموعة المعالجة بمكمل الزنك والمجموعة المعالجة بزيت القرع العسلى كما ارتفعت تلك المستويات باضافة زيت القرع العسلى مع مكمل الزنك في الفئران التى تعانى من نقص الزنك. وخلصت الدراسة إلى أن إضافة زيت بذور القرع العسلي إلى الوجبات الغذائية كان له أثر واضح فى تحسن الوظائف التناسلية لذكور الفئران خاصة التى تعانى من نقص الزنك. ربما يرجع ذلك إلى محتواه العالى من المركبات النشطة حيويا وكذلك فعله المضاد للأكسدة.