

EFFECT OF PUMPKIN OIL AND VITAMIN E ON LEAD INDUCED TESTICULAR TOXICITY IN MALE RATS

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ABSTRACT

Lead poisoning has been recognized as a major public health risk particularly in developing countries. So the principal aim of this study was occurred to evaluate the protective effect of pumpkin oil and vitamin E on lead induced testicular toxicity in male rats. Forty Sprague Dawley strain rats weighting $165\pm 5g$ were divided into five groups (8 rats each). Groups 1 and 2 acted as negative -ve and positive control +ve, while groups 3, 4 and 5 were administered pumpkin oil, vitamin-E and pumpkin oil plus vitamin E respectively, throughout the study period of the experiment (45 days). After 21 days of the beginning of the experiment, the negative control group -ve was injected with normal saline (1cc) intraperitoneally daily, but control +ve, pumpkin oil and vitamin E groups received lead chloride at a dose 12 mg/kg intraperitoneally daily for twenty four days. Results revealed that lead evoked prominent testicular toxicity, deterioration of sperm characters, and disruption of serum gonad/ pituitary hormone levels luteinizing hormone (LH), follicle stimulating hormone (FSH) and (testosterone). Further, lead markedly raised testicular oxidative-stress (lipid-peroxides), while reduced activity of the antioxidant-enzymes, superoxide dismutase (SOD), glutathione peroxidase (GPX) and catalase. These changes were also accompanied by a significant body weight gain along with a rise in food efficiency ratio (FER). Treatment with either or both of vitamin-E and pumpkin-seed oil appreciably ameliorated the Pb-induced anomalies as showed a significantly increased in body weight gain, weigh gain% and FER. Also it showed significant improvement of sperm characters, a significant increase in serum LH, FSH and testosterone hormones and increase in antioxidant enzymes. Combined treatment with both showed a superior testicular protection than their individual responses. These results were agreement with that demonstrated by (Kutlubay *et al.*, 2006 and Jamilah 2013) who reported that the improvement of reproductive efficiency of male rats by vitamin E is attributed to its powerful antioxidant property. It is concluded that pumpkin oil plus vitamin-E administration could reduce the testicular toxicity of lead in male rats. The adverse effects due to its antioxidant properties

Key words: Lead, vitamin-E, pumpkin oil, testicular toxicity, rats.

INTRODUCTION

The humans are exposed to various harmful types of environmental contaminants at different stages of their life span. This is due to the use of toxic chemicals or xenobiotic substances or by certain synthetic compounds such as heavy metallic compounds. The physiological influence of metals on the organisms of human and animals is conditioned by the nature of metal, type of compounds and their amount (Ezzati *et al.*, 2002 and Danielyan, 2010).

Lead is a heavy soft metal, found at homes in many forms. Soil contamination may be responsible for the presence of lead in many herbs. Human get exposed to lead by inhalation or ingestion of contaminated food and water. Lead has wide range of toxic biochemical and histological effects where it deposits in many organs such as kidney, ovary, liver, brain, blood and endocrine system (Saroyan and Zen 1994 and Madiha *et al.*, 2008).

Pumpkin seed oil has gained wide acceptance not only as edible oil but as a nutraceutical, too. Pumpkin is rich in β -carotene and V.E, which had powerful

antioxidant and profound protective actions against tumour. There was increasing interest in the role of antioxidant vitamins like β -carotene in neutralizing free radicals and overtly aggressive oxygen species (Abd El-Ghany *et al.*, 2009).

The environmental lead on the male reproductive system has been a major area of concern for several years by which the testicular spermatogenesis and spermatozoa within the epididymis are the major targets for lead action to produce toxicity on reproduction (Sharma and Garu 2011). Lead toxicity increase of reactive oxygen species (ROS) and can enter the tight junctions that form the inter sertoli barrier, damaging the epithelium, with a decrease in its height due to germ cell loss thus increasing the tubular lumen (Chowdhury 2009). It is known that vitamins (special Vitamin E) are essential to maintain normal metabolic processes and homeostasis within the body. Vitamins are ideal antioxidants to increase tissue protection from oxidative stress due to their easy, effective and safe dietary administration in a large range of concentrations (Janisch *et al.*, 2005). Vitamin E refers to a group of eight fat

soluble compounds that include both tocopherol and tocotrienols. Vitamin E has antioxidant function and other functions include enzymatic activities, gene expression, and neurological function a (Songthaveesin *et al.*, 2004).

Many studies have shown that pumpkin belonging to the family Cucurbitaceae is a perennial plant and is consumed traditionally in a variety of foods such as fresh or cooked vegetables, as well as also stored frozen or canned. Pumpkins (*Cucurbita Occidentalis*) is widely cultivated and consumed in tropical Africa because of high nutritive value of the leaves and seeds. Pumpkins are highly nutritious and seeds contain 13% of oil (Okoli and Nyanayo 1988, and Christian 2006)

The aim of this study is to test for potential effects of pumpkin oil and vitamin E either alone or in combination on lead testicular toxicity in adult male rats.

MATERIALS AND METHODS

Materials: Lead chloride and vitamin E was purchased from El-Gomhoria Company in (Cairo, Egypt) while pumpkin oil was obtained from Arab Company for Pharmaceutical and Medicinal Plants, MEPACO in Riyadh. Vitamin E and pumpkin oil were given to rats at a dose of 50 IU/kg body weight and 400 mg/kg body weight intraperitoneally daily, respectively all over period of the experiment. Forty Sprague Dawley strain rats weighing 165±5g were obtained from animals centre in Medicine Collage of King Saudi University in Riyadh. They were maintained in the animal house under controlled conditions (12hrs light/12hrs dark, at temperature 22 ±1°C) and given the standard diet and water ad lib. The standard diet prepared according to (NRC 1995).

Methods: Experimental rats were divided Randomly into five groups (8 rats each). Groups 1 and 2 act as control –ve and control +ve that received standard diet while group 3,4 and 5 administered pumpkin oil, vitamin E and pumpkin oil plus vitamin E, respectively throughout the study period of the experiment (45 days). After 21 days of the beginning of the experiment, control negative (–ve) group received injection normal saline 1 cc intraperitoneally daily but control positive (+ve) , pumpkin oil, vitamin E and pumpkin oil plus vitamin E groups received lead chloride at dose 12 mg/kg intraperitoneally daily for twenty four days. Daily food intake and the weekly body weight gain were recorded. Food efficiency ratio (FER) was calculated (Philave *et al.*, 2012) At the end of the experiment (45 days), the rats were sacrificed to obtain blood samples and their epididymis and testis was removed immediately. Sperm count, morphological abnormalities, viability and motility were determined according to (Rezvanfar *et al.*, 2008, and Kumar *et al.*, 2006) respectively.

Serum luteinizing hormone (LH), follicle stimulating hormone (FSH) and testosterone hormone were estimated according to (Bee and Kah 2003), using ELISA and RIA respectively. Testis superoxide dismutase (SOD), glutathione peroxidase (GPX), catalase and lipid peroxide (LPX) were estimated according the methods described by (Wigand *et al.*, 2009) using Spectrophotometer

Statistical analysis: Each data in the table was presented as average of replicates ± SD. The data was subjected to statistical analysis by conducting analysis of variance (ANOVA) using SPSS software package (version 11). The significant difference of means was compared using least significance difference according to (Artimage and Berry 1987).

RESULTS AND DISCUSSION

In comparing to control –ve group, control +ve group had highly significantly decreased in body weight gain, weight gain % and FER at $p < 0.001$ while other groups which treated with pumpkin oil and pumpkin oil plus vitamin E had normal values of body weight gain, weight gain % and food intake and significantly increased FER ($p < 0.01$), while rat group which treated with vitamin E had normal values of body weight gain, weight gain % and food intake and significantly decreased FER ($p < 0.01$).

In comparing with control +ve group, pumpkin oil, vitamin E and pumpkin oil plus vitamin E had significantly increased in body weight gain, weight gain % and FER as illustrated in table 1.

The results are in line with Nehru and Kanwar (2004) who showed that the final body weight in groups treated with high limits of lead was significantly lower than the groups treated with permissible limits of lead.

The improvement of nutritional results was related to the effect of administration vitamin E and pumpkin oil. Vitamin E is ideal antioxidant to increase tissue protection from oxidative stress. It may due to chelate lead from the tissues along with restoring the pro/anti oxidant balance (Flora *et al.*, 2012) Vitamin E has antioxidant function and the other functions such as enzymatic activities, gene expression and neurological function (Songthaveesin *et al.*, 2004)

Pumpkin seed oil can prevent change in plasma lipid and blood pressure. Pumpkin seed oil is rich in linoleic acid, an essential fatty acid. It is due to this abundant linoleic acid and oleic acid, this oil is good for reducing serum cholesterol and LDL and increase HDL levels. The extracted protein from this plant has an essential role in decreasing the harmful effects of protein malnutrition (Okoli and Nyanayo 1988, and Christian 2006)

Data in table 2 showed that control +ve group were significantly decreased in motility, count and viability of sperm and significantly increased in sperm abnormality at $P < 0.001$ while pumpkin oil and vitamin E groups showed significantly decreased in motility, count ($p < 0.05$) and viability of sperm ($p < 0.01$) and non significantly increased in sperm abnormality at ($p > 0.05$) compared to control -ve group. Pumpkin plus vitamin E group showed normal values of epididymal sperm cell characters.

The obtained results are in line with Chowdhury (2009) who reported that the potential toxicity of lead caused alterations in sperm morphology, count, motility, as well as hormones. The major function of testes is spermatogenesis and hormone synthesis to produce spermatozoa. So when the testicular tissue is damaged by the toxic effects of lead, the process of spermatogenesis would be impaired and sperm production rate will also reduced (Brennan and Capel 2004, and Garcia *et al.*, 2010). Lead induced apparent damage and reduction in the number, changes in shape and size of developing seminiferous tubules. Oral exposure of lead acetate changed the arrangement and shape of spermatogonial cells and reduced the number of sertoli cells. It also diminished the development of Leydig cells (Saxena *et al.*, 1987). Lead could disturb mitosis of spermatogenic cells and cause alterations in the proliferation of Sertoli cells so the decreased in the sperm count within testes of adult offspring, and subsequently reduction of epididymal sperm count were observed (Corpas *et al.*, 1995). The lead induced reactive oxygen species (ROS) have involved in lipid oxidation, in particular, membrane lipids that are required to give the plasma membrane fluidity, which is essential for sperm motility, and structural integrity, and ultimately, for sperm viability. Moreover, it can damage the germ cells and alter the gene in germ cells that lead to varieties of decline in sperm count and sperm motility (Baumber *et al.*, 2000, Acharya *et al.*, 2003 and Teijon *et al.*, 2006). As an antioxidant, vitamin E acts as a peroxy radical scavenger, preventing the propagation of free radicals in tissues, by reacting with them to form a tocopherol radical, which will be reduced by a hydrogen donor and thus return to its reduced state. As it is fat-soluble, it is incorporated into cell membranes, which protects them from oxidative damage. There is a significant improvement in sperm motility and viability following vitamin E treatment in vitro (Azzi 2007 and Atkinson 2007).

Control +ve group showed significant decrease in LH, FSH and testosterone hormones ($p < 0.001$) while pumpkin oil group showed significantly decreased in LH ($p < 0.05$) and FSH hormones ($p < 0.01$) and none significantly decreased in testosterone hormone at ($p > 0.05$) compared to control -ve group. Vitamin E group showed significantly decreased in LH, FSH and testosterone hormones at ($p < 0.05$) and ($p < 0.01$) but

pumpkin oil plus vitamin E group showed normal values of these hormones at $P > 0.05$ compared to control -ve group. Administration of pumpkin oil or vitamin E or pumpkin oil plus vitamin E showed significantly increased in LH, FSH and testosterone hormones compared to control +ve group as shown in table 3. It is may due to the responsible for improving semen quality and quantity. Moreover, administration of pumpkin oil and V.E attributed to their antioxidant activity (Bairy and Rao, 2010)

The significant alterations in testosterone, LH and FSH levels were agreed with (Al-Attar 2011, and Muthu and Krishnamoorthy 2012). LH and FSH activity depends on both the quantity of these hormones and the number of specific receptors in the testis. It has been shown that Leydig cells of the testis are responsible for then biosynthesis and secretion of androgens, critical for developmental and reproductive function in the male. Testosterone production is directed by LH. However, FSH affects sertoli's cells, in that it triggers the formation of a spermatogonial cells testosterone binding protein. Oral exposure of lead acetate changed the arrangement and shape of and reduced the number of sertoli cells. It also diminished the development of Leydig cells (Wilhelm *et al.*, 2007 and Garu *et al.*, 2011). Also, the obtained results may be explained by activities of vitamin E and pumpkin oil. These activities were reflected by the increase of serum testosterone, LH and FSH levels. Vitamin E is an important antioxidant, residing mainly in cell membranes. It is thought to interrupt the chain reactions involved in lipid peroxidation and to scavenge ROS generated during oxidative stress. Vitamin E is believed to be the primary component of the antioxidant system of the spermatozoa and is one of the major membrane protect ants against ROS and lipid peroxidation attack (Hsu and Guo 2002)

The pumpkin extracts had high content of total phenolics and antioxidant activity coupled to moderate to high alpha-glucosidase and anti giotensin converting enzyme inhibitory activities and has the potential to reduce hyper glycemia-induced pathogenesis and also associated complication linked to cellular oxidation stress (Kwon *et al.*, 2007). The action mechanism of pumpkin seed oil is well known by its inhibition on 5- α -reductase which converts testosterone into dihedrontestosterone (Tsai *et al.*, 2006).

Control +ve group showed significant decrease in testes SOD, GPX and catalase and significant increase in LPX ($p < 0.001$) while pumpkin oil group showed significant decrease in testes SOD at ($p < 0.05$) and normal values of GPX, catalase and LPX but vitamin E group showed only significant decrease in GPX ($p < 0.05$) and normal values of SOD, catalase and LPX. Administration of pumpkin plus vitamin E to experimental rats showed best results as it showed normal antioxidant enzymes compared to control -ve group. Administration of

pumpkin oil or vitamin E alone or combination of them to experimental rats showed significant increase in testes SOD, GPX and catalase and significant decrease in LPX compared to control +ve group as recorded in table 4. The results in this study was similar to observed by (Burk, R.2002) who reported that lead induced oxidative stress and reproductive toxicity in male rats

Alterations in antioxidant homeostasis, resulting from lead toxicity, can cause increased free-radical generation, lipid per oxidation, and, consequently, oxidant stress. Lead alters the activity of antioxidant enzymes like superoxide dismutase, catalase, and glutathione peroxidase and glucose 6-phosphate dehydrogenase and antioxidant molecules like GSH in animals, and human beings (Gurer and Ercal, 2000). It has been claimed that vitamin-E is the most important lipid soluble antioxidant, and that it protects cell membranes from oxidation by reacting with lipid radicals produced in the lipid per oxidation chain reaction (Rendon-Ramirez *et al.*, 2007). Vitamin E, a chain breaking antioxidant, not only scavenges oxygen radicals from the membrane but also intercepts pyroxy and

alkoxyl radicals which are generated during the conversion of lipid hydro peroxides that fuel the per oxidative chain reaction thereby preventing this damaging process from propagating through plasma membrane (Brigelius-Flohé 2009 and Nadia *et al.*,2013). The treatment of rats prior or during lead toxicity with pumpkin oil and vitamin E resulted in an increase of antioxidant enzymes and decrease in lipid per oxidation was agreement with (Kashif *et al.*,2004). Vitamin E present in pumpkin prevents oxidative damage to cell by preventing the oxidation of unsaturated fatty acids in cell membrane (Kim *et al.*, 2012). Studies show that Pumpkin oil can benefit the treatment of benign prostate hyperplasia. This is due to the high content of - sitosterol content present in it (Gossell-Williams *et al.*, 2006 and Tsai *et al.*, 2006). Pumpkin oil has been previously shown to contain high levels of tocopherol which render it antioxidant activity and thus may be capable of reducing lipid peroxidation. J and act as an antioxidant (Van Hoed *et al.*, 2009).

Table (1). Mean values± SE of body weight gain, feed intake and FER of the experimental rat groups

Groups	Weight gain (g)	Weight gain %	Food intake (g/w)	FER
Control –ve	75.71± 2.2 ^{ab}	45.88±0.76 ^{ab}	18.77±0.4 ^a	0.089±0.00 ^b
Control +ve	51.61±1.84 ^{c***}	30.72±0.75 ^{c***}	17.21±0.46 ^a	0.066±0.00 ^{d***}
Pumpkin oil	77.41±1.29 ^a	46.35±3.13 ^a	18.55±0.433 ^a	0.092±0.00 ^{a**}
Vitamin E	63.61±2.18 ^b	38.08±1.12 ^b	17.88±0.48 ^a	0.079±0.00 ^{c**}
Pumpkin oil + vitamin E	80.14±2.49 ^a	48.27±1.5 ^a	18.44±0.43a	0.096±0.00 ^{a**}

Significant with control (-ve) group *p<0.05**p<0.01***p<0.001

Values with the same letters in a column indicate non-significant difference (P>0.05) and vice versa

Table (2). Mean values± SE of epididymal sperm cell characters of the experimental rat groups

Groups	Motility %	Count (10 ⁶ /epididymis)	Viability %	Abnormality %
Control –ve	93.10±3.25 ^a	70.99-0.76 ^a	85.32±0.59 ^a	4.03±0.142 ^b
Control +ve	49.50±1.84 ^{c***}	48.21±0.42 ^{c**}	40.71±0.05 ^{a***}	8.71±0.05 ^{a***}
Pumpkin oil	75.14±2.63 ^{b*}	65.11±0.99 ^{b*}	63.19±1.134 ^{b**}	5.31±0.05 ^b
Vitamin E	76.30± 2.8 ^{b*}	62.45±0.75 ^{b*}	61.31±1.06 ^{b**}	5.61±0.10 ^b
Pumpkin oil + vitamin E	88.80 ±3.09 ^{ab}	69.15±0.59 ^a	80.61±1.29 ^a	4.20±0.10 ^b

Significant with control (-ve) group *P<0.05** P<0.01 *** P<0.001

Values with the same letters in column indicate non-significant difference (P>0.05) and vice versa

Table (3). Mean values± SE of LH, FSH and testosterone of the experimental rat groups

Groups	LH(ng/ml)	FSH(ng/ml)	Testosterone(ng/ml)
Control –ve	1.21±0.08 ^a	8.10±0.42 ^a	6.35±0.26 ^a
Control +ve	0.44±0.00 ^{d***}	3.41±0.15 ^{c***}	2.55±0.09 ^{d***}
Pumpkin oil	0.98±0.03 ^{b*}	5.96±0.27 ^{b**}	5.14±0.185 ^{abc}
Vitamin E	0.88±0.04 ^{bc*}	5.67±0.33 ^{b**}	4.88±0.15 ^{c*}
Pumpkin oil+ vitamin E	1.01±0.09 ^{ab}	7.15±0.37 ^a	6.11±0.27 ^{ab}

Significant with control (-ve) group *P<0.05 ** P<0.01*** P<0.001

Values with the same letters in column indicate non-significant difference (P<0.05) and vice versa

Table (4). Mean values± SE of LH, FSH and testosterone of the experimental rat groups

Groups	SOD(μ /mg protein)	GPX(n mol)	Catalase(μ /mg protein)	LPX(n mol)
Control –ve	7.99±0.41 ^a	4.14±0.14 ^a	8.60±0.15 ^{bc}	3.41±0.157 ^{bc}
Control +ve	2.53±0.03 ^{c***}	1.20±0.03 ^{c***}	4.77±0.17 ^{c***}	9.80±0.47 ^{a**}
Pumpkin oil	5.90±0.13 ^{b*}	3.26±0.11 ^{ab}	6.64±0.40 ^{ab}	4.68±0.25 ^b
Vitamin E	5.99±0.16 ^{ab}	3.08±0.075 ^{b*}	6.75±0.407 ^{ab}	4.51±0.24 ^b
Pumpkin oil + vitamin E	6.31±0.4 ^a	3.89±0.11 ^a	7.14±0.43 ^a	3.81±0.21b ^c

Significant with control (-ve) group *P<0.05 ** P<0.01*** P<0.001

Values with the same letters in column indicate non- significant difference (P<0.05) and vice versa

Conclusion: Both pumpkin oil and vitamin E minimize adverse effects of lead induced testicular toxicity in adult male rats due to its antioxidant properties.

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