



Effect of pectin from date (*Phoenixd actylifera*) upon lead acetate induced reproductive toxicity in male rats

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ABSTRACT

Lead is a ubiquitous heavy metal and its toxicity remains an important public health issue, especially infertility, is causing oxidative burst in the exposed individuals leading to testes damage. In previous work, we reported that ingestion of pectin extract from date (*Phoenix Dactylifera*) may decrease the toxic effects of this metal. Then, we evaluated the potential detoxifying effect of pectin after oral exposure of male rats to Pb. To achieve our goal, the young rats received 350mg/Kg of lead acetate by gavage for 30 days of lead acetate, the treatment with pectin of date at 3% started after one month of intoxication. This study was designed to associated reproductive disorder (Sperm Quality) and biochemical parameters were analyzed in the testes: alkaline phosphatase, lipid peroxidation and Catalase. Administration of pectin after intoxication with lead acetate ameliorated in the weight of both the testes. Moreover, pectin improved sperm viability and decrease of sperm abnormalities in rats treated compared with the intoxicated rats. Therefore, pectin provided a significant protection to thiobarbituric acid reactive substances level in testes, while cause a decrease in catalase and phosphatase alkaline levels. The results show that pectin has beneficial effects which could be able to antagonize lead toxicity.

Keywords: *Phoenix dactylifera*; pectin; lead acetate; testes, sperm quality, peroxidation.

INTRODUCTION

A substantial body of evidence has accumulated in recent years that consistently indicate various adverse effects of environmental contaminants on human health. Decreasing trend of male fertility has been correlated to the exposure to environmental contaminants^[1]. Lead is one of the most widespread contaminants among the myriad of xenobiotic^[2]. Lead affects each and every organ and system in the body^[3]. It was considered as a major environmental toxic; the animals may be exposed to low concentrations of Pb via contaminated feed, water, and feed additives^[4].

A lot of recent studies have demonstrated effects of Pb exposure on reproductive system in terms of sperm counts and sperm quality, along with other changes in male reproductive health, including congenital malformations and testicular cancer in humans^[5, 6, 7]. The impact of lead on reproductive functions has been studied on the laboratory animals, which provoked some chromosomal aberrations^[8, 9], reduction in the spermatogenesis, fertility and induces alterations in testes^[10, 11]. The signs of such disorders are similar to those of antioxidant deficiency. Therefore, the patients are usually treated with additional administration of non-enzymatic antioxidant^[12]. But, the more reasonable method is the use of chelate therapy with the purpose of elimination of toxicants. Dietary pectin can bind heavy metals and toxic metabolites^[13].

Date fruit (*Phoenix dactylifera L*) is an important food source rich in nutriment^[14] and carbohydrates^[15]. Moreover, date fruit has been used as a source of active components as soluble fiber like pectin substances^[16]. Pectin is

complex polysaccharides present in all plant primary cell walls. That, it contain 1,4-linked α -D-galactosyluronic acid (GalpA) residues^[17]. The supplementation of pectin has been proved to increase the lead excretion through urine and fecal way^[18, 19]. At present study, we investigated the effects of pectin extract of date « Var *Delglabeida* » upon reproductive system in male rats exposed to lead acetate.

EXPERIMENTAL SECTION

2-1-Plant Material:

Fruits of *P. dactylifera* (*Arecaceae*) were collected in 2013 from palm grove situated in the Bechar region (Algeria) at the « Tamr stage » which is characterized by dry dates allowing easy preservation. After denutting, the date fleshes were dried and ground to obtain a powder of date.

2-2-Extraction of Pectin^[20]:

Dried date flesh powder (100g) was blended with 600ml distilled water. Then, the blend was acidified using 53% nitric acid and pH was maintained at 1.5. The acidified mixture of blended flesh powder was then heated at 95° C for around 60min. After this treatment, the mixture was centrifuged and the supernatant was separated from insoluble residue. The pectin solutions previously adjusted to pH 3.5 with 10 M NaOH were precipitated with an equal volume of ethanol (96%). After filtration, the ethanol insoluble pectin was washed twice with 70% ethanol to further remove any remaining impurity. Finally, precipitate was kept for drying at 40°C in hot air oven and percentage yield was found to be around 6%. It was then stored in desiccators until further use.

2-3-Animals:

Thirty male *albino* rats weighing 100 ± 5 g were obtained and housed in Experimental Bio-Toxicology, Bio-Depollution and Phyto-Remediation Laboratory, Department of Biology, University of Oran (Algeria). The experiment was approved by the ethical committee. Rats were housed in stainless steel, wired cages in groups of 6 per cage and kept in an isolated room at a controlled temperature of 20°C to 22°C, 55±5% humidity and lights were maintained on an artificial 12-hour light-dark cycle. Animals were first adapted to the facility for 1 week and provided with water and standard feed *ad libitum*.

2-4- Experimental design and sacrifice:

In the first experiment after the adaptation period, 30 rats were randomly divided into 2 groups of rats. These groups included the control group (12 rats) and group that received Lead Acetate solution (“LA”= 24 rats). All groups were fed the standard diet. The control group received daily distilled water by gavage. Animals of other group were given daily lead acetate solution at dose of 350 mg/Kg by gastric gavage. After one month of the experiment and overnight fasting, 6 rats of each group (group Control « C1 »=6 rats, group given lead acetate solution « LA1 »=6 rats) were anesthetized with chloral solution (3 mL/kg bw). Blood was collected from the abdominal aorta into dry tubes, and plasma was prepared by low-speed centrifugation (3000g for 20 min, 4°C). Testes was removed immediately, rinsed with cold saline, and weighed. Average tissue ratio was determined according to the formula:

$$\text{Average tissue ratio of paired testes} = 100 \times \frac{\text{Weight of paired testes in grams}}{\text{Weight of body in grams}}$$

In the second experiment, after sacrifice of 12 rats of first period, the rest of rats of the control group received daily distilled water by gavage (C2=6 rats), then, the second group were given lead acetate solution for one month divided into 2 groups, the first group, 6 rats received daily distilled water by gavage (LA 2 =6 rats), the second group received orally pectin solution at 3% (LA+P=6 rats). Four weeks later, the rats were killed, and testes was removed, weighed, rinsed, and prepared for analysis as described before.

2-5-Biochemical estimation:

Testes were homogenized (10%) in cold KCl buffer (1, 15%; pH 7, 2). The homogenate was centrifuged at 10.000×g for 10 minutes at 4°C to obtained post-mitochondrial supernatant which was used for the quantification of ALP. Enzymatic activities (alkaline phosphatase) (ALP) was determined calorimetrically using commercial chemical kit (Kit Chronolab).

2-6-Tissues lipid peroxidation

As marker of the lipid peroxidation, Thiobarbituric acid reactive substance (TBARS) concentrations in tissues was assessed by the complex formed between malondialdehyde and Thiobarbituric acid (TBA)^[21]. Briefly, the testes (1g) were homogenized with 9 mL of KCl (1.15%). The homogenate (100 μ L) was mixed with 0.1 mL of sodium

dodecylsulfate (8.1%), 750 μL of acetic acid (20%), and 750 μL of TBA reagent (0.8%). The reaction mixture was heated at 95°C for 60 minutes. After heating, the tubes were cooled, and 2mL of n-butanol-pyridine (15:1) was added. After mixing and centrifugation at 4000g for 10 minutes, the upper phase was taken for measurement at 532 nm.

2-7-Enzymatic antioxidant defense

Catalase catalyzes the decomposition of hydrogen peroxide to water and oxygen. Catalase activity was assayed in tissues by measuring the rate of hydrogen peroxide (H_2O_2) decomposition according to the method described by [22]. Briefly, 250 μL of homogenate (100 mg of tissue in 0.9 mL KCl), 250 μL H_2O_2 (30 mmol in phosphate-buffered saline 50 mmol/L), and 250 μL of phosphate-buffered saline were added. The contents were shaken and incubated for 5 minutes, and then Potassium Dichromate solution (5%) was added. The absorbance was measured at 620nm.

2-8- Sperm Analysis and Evaluation:

The estimate of sperm viability (live/dead ratio) of testis was calculated by the method of [23] and expressed as percentage viability. The percentage of abnormal sperm was scored in 10 to 20 speared fields [24].

2-9-Statistical analysis

Data were expressed as means \pm SEM for six rats per group. Differences between control and treatment groups were analyzed using Student's "t" test. A value of $p < 0, 05$ was considered significant.

RESULTS

3-1-Effect of pectin on Body weight gain and testis relative weight

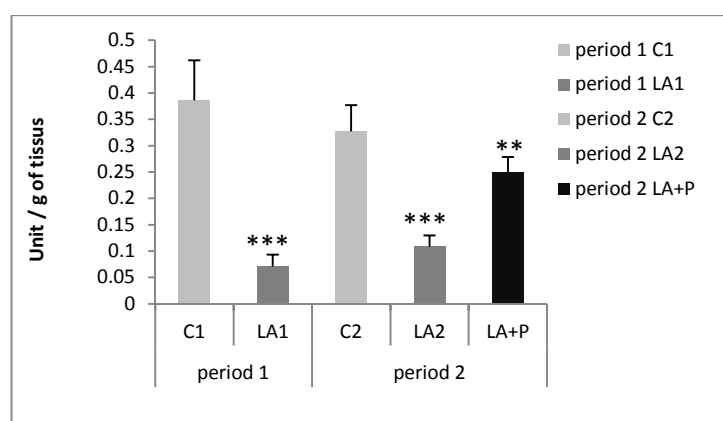
Administration of lead acetate showed a significant decrease ($p < 0.001$) in body weight gain of rats at day 30 and 60. The decrease percentage is 39, 49% and 19.65% successive when compared with control, whereas the pectin of date reduced the loss body weight in rats (-11.59%) (Table1).

Table (1): Effect of Pb and Pectin on body dot gain and testes, relative weight of tested rats

	Body weight gain (g)	Testis relative weight
Group C 1	80.01 \pm 1.39	1.61 \pm 0.12
Group LA 1	60.41 \pm 8.25***	1.23 \pm 0.04***
Group C 2	195.03 \pm 7.47	1.26 \pm 0.05
Group LA 2	156.7 \pm 15.6***	1.06 \pm 0.05***
Group LA+ P	172.41 \pm 10.88***	1.22 \pm 0.09

Values are means \pm SE of 6 rats per group. *** $p < 0.001$.

Testicular organ weight was decreased ($p < 0.001$) remarkably after lead administration (Table 1), whereas in the treated group with pectin, the decrease percentage is 3.17% when compared with control group.



Values are means \pm SE of 6 rats per group. ** $p < 0.01$ and *** $p < 0.001$.

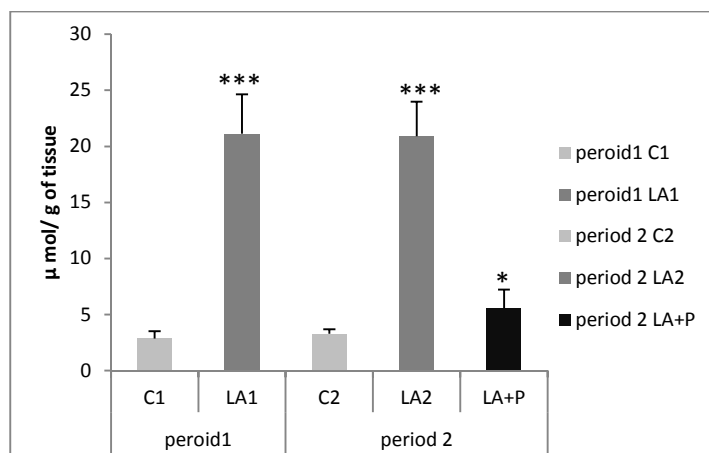
Figure 1 : Effect of lead and oral administration of pectin on ALP of the tested groups

3-2-Effect of pectin on testicular PAL concentration:

The results of Figure 1 showed that lead administration produced a high significant decrease ($p < 0.001$) the ALP Level at day 30 and 60 (-54.17% and -52.38% respectively), in contrary, pectin of date decreased the level of ALP in rats (+41,30% compared to intoxicated group).

3-3-Effect of pectin on testicular Lipid Peroxidation Products (LPP):

The results on lipid peroxidation products for testes were presented in **Figure 2**. The LPP showed a very high significant increase ($p < 0.001$) in their levels up to group LA 1 and LA2 when compared to their respective controls (+86.53%, +84.72% respectively). Pectin of date treatment decreased significantly lipid peroxidation in testis (-73.35%) compared to intoxicated group(LA2).

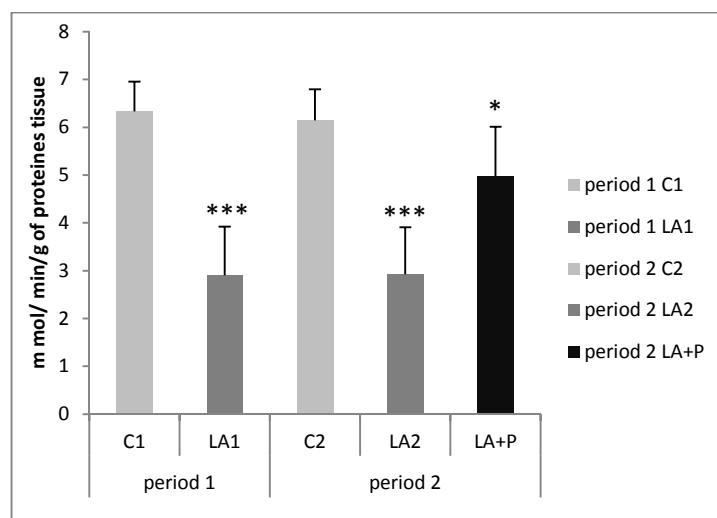


Values are means \pm SE of 6 rats per group. * $p < 0.05$ and *** < 0.001 .

Figure 2: Testis lipid peroxidation in tested rats

3-4-Effect of pectin on testicular catalase activities :

The effect of treatments on testicular CAT activity is shown in **Figure 3**. There was a high significant decrease ($P < 0.001$) in testicular activity of CAT in the intoxicated groups(LA1 and LA2) while compared to the control (C1 and C2) groups, the decrease percentage is 54.17% and 52.38% respectively. When, CAT activity was increased in testis (+41.30%) by pectin treatment compared to intoxicated group.



Values are means \pm SE of 6 rats per group. * $p < 0.05$ and *** < 0.001 .

Figure 3 : Effect of lead and pectin on catalase activity of tested rats

3-5-Effect of pectin on Sperm Quality:

Table 2: Sperm Quality in male rats treated with lead acetate and pectin

Groups	C1	LA1	C2	LA2	LA+P
Sperme Viabilité	78.73 \pm 5.16	50.67 \pm 3.59**	80.45 \pm 4.94	59.35 \pm 5.71**	71.82 \pm 4.64*
Sperme Anormalité Ratio %	14.51 \pm 1,98	37,65 \pm 3,87*	15.83 \pm 2.29	34.72 \pm 4.30*	27,29 \pm 3.54

Values are means \pm SE of 6 rats per group. * $p < 0.05$ and ** < 0.01 .

Sperm viability and abnormal sperm are reported in Table (3). It can be seen that sperm viability was significant decrease in group LA1(-35.64%) and group LA2 (-26.22%) as compared with the control groups, C1 and C2

respectively. when total sperm abnormalities were analyzed, the LA 1(+ 61.46%) and LA2 (+54.40%) groups have significantly the highest level of abnormalities as compared with control groups. On the other hand, treatment with pectin of date in combination with lead acetate significantly alleviated the decline in sperm viability (-10.72%), and decreased the percent of abnormal sperm compared to LA2 untreated group (+41.99%), and this means that pectin minimized the toxicity of acetate of lead.

DISCUSSION

Many industrial chemicals are known to have a negative impact on human reproduction^[25], particularly occupational and environmental exposures to heavy metals such as lead^[26], can affect male reproductive functions including sperm counts^[27]; motility and morphology^[28]; spermatogenesis^[29]. Many studies on reproductive system of male animals have documented lead as a toxicant for testicular tissue and functions^[30] such as significant reductions in the number of spermatozoa within the epididymis in mice and halted spermatogenesis in rats^[31].

Our results revealed that lead induced a higher significant decrease in the body weight gain. This is in accordance to^[32, 33, 34], who reported that mean body weight of the animals treated with LA was significantly lower than that of the other groups, many others studies have suggested that the decrease of weight according to dose administrated and the time of exposition and appeared for feed intake in tested rat^[35].

A number of mediators were involved into the regulation of ingested behavior, among them serotonin and dopamine, which have a dominant role in the control of the satiety. Indeed, lead interference with a number nervous pathways and cerebral structure which involved in the process of control of satiety and hunger, which justified anorexic effect of lead in rats.^[36]

Nevertheless, heavy metals are known to have direct effects on body weight of animals. In accord to this data they have concluded that low dose of lead induced the same effects^[37]. Furthermore, Male rats exposed to lead acetate showed a significant decrease in the weight of both the testes, this effect could result of high vulnerability of testes towards various toxic^[38]. Another study^[39] has demonstrated that the decrease of weight testes results at loss of germinal cells.

Treatment with pectin at 3% extract from date (P) for one month increased significantly the body weight gain, these results agreed with results obtained by^[40] who studied the effect of HEP and LEP in rats intoxicated by lead. These data are also consistent with those of^[41] who reported the beneficial effect of pectin extract from carrot on lead toxicity.

The heavy metals, especially lead, are able to produce reactive oxygen species (ROS) that result in DNA damage, depletion of cell antioxidant defense systems and lipid peroxidation (TBARS) in tissue and blood^[42]. Our current investigations showed significantly increased concentration of LPP in testis after an exposure to lead acetate. Our results are in agreement with results obtained by several others^[43]. Increased contents of tissues thiobarbituric acid reactive substances accompanied by altered antioxidant defense systems were confirmed by many other studies, which have pointed to either elevated lipid peroxidation or decreased intrinsic antioxidant defense in various tissues of lead-exposed animals^[44, 45]. Furthermore,^[46] measured lead concentrations in various brain areas as well as the rate of lipid peroxidation. He concluded that the increase in the rate of lipid peroxidation followed a pattern similar to that of lead concentrations in different regions of the brain.

At the same time, the study shows that tissues TBARS concentrations were significantly lower in the group treated with pectin compared with untreated rats. These results showed that pectin extract might protect the tissues against the cytotoxic action and oxidative stress of lead. Furthermore, the reduction in lipid peroxidation could be due to the increased of antioxidant status. One has to remember that the contribution of antioxidants to the overall therapeutic properties of medicinal plants used for prevention of oxidative stress related disorders is still disputed^[47, 48]. These results agreed with results obtained by^[49] who showed that pectin treatment promoted the decrease in lead content in the liver, reduction in lipid peroxidation, and recovery of parameters of lipid metabolism.

Anterior study^[50] stated that application of native pectin as well as oligogalacturonic acids increased lead elimination through blood and organs. And addition of rhamnogalacturonic parts of pectin into the lead-enriched diet in rats contributed to slow absorption of lead in rats and enhanced lead excretion with feces during the period of the experiment. Moreover, The use of the pectin substances in animals preliminary exposed to high doses of lead acetate contributed to fast elimination of the metal from the organs, in particular, the femur^[51]. In contrary to this, lead chelated by dRGII in fruits and vegetables and fruit juice is thus mostly unavailable for intestinal absorption. However, the addition of dRGII after chronic lead exposure in rats does not help Pb detoxification. Additionally,

they found that MEDETOPECT (made on apple pectin) is effective agent for prophylaxis of lead incorporation in industrial conditions^[52]. It is favorable for the chelation in the gastrointestinal tract ^[53]. In some cases, orally administered pectin contributed to increased retention of heavy metal in tissues^[54], in blood serum levels and in 24-hour urine collection, of children between the ages of 5 and 12 years ^[55].

Many studies illustrated the relationship between the degree of methylation of pectin and metal binding, proved that pectin exerts high metal binding activity regarding bivalent metal ions and ^[56]confirm that carboxylic acid groups are active participants in Pb binding by citrus pectin. The largest amount of Pb(II) ions were bound by pectin with the low degree of esterification^[57].

In the present study, catalase activity was significantly decreased after lead exposure. This diminution of enzyme levels both in testis can be attributed to the inhibition of Hem biosynthesis ^[58], In contrary, ^[59] reported that lead intoxication caused an increase in the catalase activity. In the other hand, *pectin of date* treatment increased catalase activities in testis of rats; this increase might constitute a protection against superoxide anion elevation. Because SOD catalyzes the decomposition of superoxide radicals to hydrogen peroxide (H₂O₂). The H₂O₂ produced by SOD is excreted as H₂O based on the activity catalase ^[60].

Our data suggest that lead produced a high significant increase the ALP level at day 30 and 60 compared with the control group, ours results were in accordance with study^[61]who reported that increased activity of testicular, epididymis and pituitary alkaline phosphatase in lead treated rats reflects tissues degeneration, which may likely be an indicative of lytic activity. In addition, treatment with pectin of date increased the level of ALP in rats, which may be attributed to the ability of pectin to chelate the acetate of lead.

Acetate of lead induced significantly decrease in sperm viability (%), with increase in dead and abnormal sperm count as compared to both intoxicated group and pectin treated group, this means that orally administered of pectin minimized the toxicity of lead. Moreover, the observed decrease in sperm viability could be attributed in part to the concomitant reduction in testosterone production following lead treatment^[62].

Previous studies showed that, sexual behavior of male rats was suppressed after ingestion of lead acetate. Necrosis of spermatocytes/ spermatids was observed in the testes of rat exposed to lead ^[63]. They suggest that lead significantly reduce epididymis and testicular sperm counts including daily sperm production that may be owed to the toxic effect of lead to testicular histological structure. Others showed that the intensification of lipid peroxidation caused by lead may affect the composition of cytoplasmic, mitochondrial and acrosomes membranes. It is known that on the way from caput to cauda epididymis the sperm membranes become richer in unsaturated fatty acids and therefore they show tendency to instability. In promotes acrosomes reaction but at the same time the membranes become more prone to prooxidative damage^[64].

In conclusion, many chelating agents are currently used to manage lead toxicity. At the same time, the most common, however, are non-specific and have some adverse effects in humans such as induction of misbalance of essential microelements. Pectin had the ability to chelate to lead and subsequently works as active natural compound to discharge lead contamination.

REFERENCES

- [1] B Saradha, PP Mathur *toxicology and pharmacology* Volume 21, Issue 1, January **2006**, Pages 34–41
- [2] A Graca, J Ramalho-Santos, M de Lourdes Pereira. *Asian J Androl* **2004**; 6: 237-241.
- [3] DY Yu, ZR Xu, XG Yang. *Asian-Aust. J. Anim. Sci* **2005**; Vol 18, No. 12 : 1775-1779.
- [4] EI FA, F Ghorbel, M Smaoul, F Makni-Ayadi, AKammoun. *GynécolObstétFertil* **2000**; 1:51-59.
- [5] P Apostoli, P Kiss, S Porru, J.P Bonde, M Vanhourne. *Occup. Environ. Med* **1998**; 55: 364–374.
- [6] M Sallmen, ML Lindbohm, M Nurminen. *Epidemiology* **2000**; 11:148-152.
- [7] N Pant, G Upadhyay, S Pandey, N Mathur, DK Saxena, SP Srivastava. *ReprodToxicol* **2003**; 17:447-450.
- [8] J García-Lestón, JMéndez, EPásaro, BLaffon. *Environment International* **2010**; 36: 623–636.
- [9] M Ahmed, M.K.J Siddiqui. *Clinica Chimica Acta* **2007**; 383: 57–64.
- [10] H Liu, R Niu, I Wang, Y He, I Wang. *Research report Fluoride* **2008** ; 41: 184-191.
- [11] AEI-Shafai, N Zohdy, KH El-Mulla, M Hassan, N Morad. *Food and Chemical Toxicol* **2011**; 49: 734-743.
- [12] C Ross, AMorriss, M Khairy, Y Khalaf, P Braude, A Coomarasamy, et al. *Reproductive Biomedicine Online* **2010**; 20: 711–723.
- [13] M Khotimchenko, I Sergushchenko, Y Khotimchenko. *Environmental Toxicology and Pharmacology* **2004**; 17: 67–71.
- [14] M Al-Farsi, CY Lee. *Critical Reviews in Food Science and Nutrition* **2008**; 48: 877–887.

- [15] AEI-Shafai, N Zohdy, KH El-Mulla, M Hassan, N Morad. *Food and Chemical Toxicol* **2011** ; 49: 734-743.
- [16] MS Baliga, BRV Baliga, SM Kandathil, HP Bhat, PK Vayalil. *Food Research International* **2011**; 44: 1812–1822.
- [17] LR Brent, AO Malcolm, M Debra. *Phytochemistry* **2001**; 57: 929–967.
- [18] VA Ostapenko, AI Tepyakov, AS Prokopovich, TI Chegerova. *MeditsinaTruda I PromyshlennayaEkologiya* **2001**; 5: 44-47.
- [19] M Tahiri ,J Tressol, T Doco, Y Rayssiguier, C Coudray. *British Journal of Nutrition* **2002**; 1(87): 47-54.
- [20] C Borchani, S Besbes, C Blecker, M Masmoudi, R Baati, H Attia. *African Journal of Biotechnology* **2010**; 9, (26)4096-4105.
- [21] H Ohkawa, N Ohishi, K Yagi. *Anal Biochem* **1979**; 85: 351-8.
- [22] HU Bergmeyer. Catalase. In *Methods of Enzymatic Analysis*, second ed. Verlag Chemie, Weinheim, Germany **1974**, pp. 673-684
- [23] MR Prasad, NJ Chinoy, KM Kadam. *Fertil Steril* **1972**; 23 (3): 186-90.
- [24] P Talbot, RS Chacon. *J expZool* **1981**; 21 (2): 201-8.
- [25] S Tas, R Lauwerys, D Lison. *Crit Rev Toxicol* **1996**; 26: 261-307.
- [26] M Vigeih, K Yokoyama, F Ramezanzadeh, M Dahaghin, T Sakai, Y Morita, et al. *Environ Res* **2006**; 100:268-275.
- [27] SE Chia, CN Ong, ST Lee, FH Tsakok. *Arch Androl* **1992**; 29: 177-183.
- [28] JD Meeker, MG Rossano, B Protas, MP Diamond, E Puscheck, D Daly, N Paneth, JJ Wirth. *Environ Health Persp* **2008**; 116:1473-1479.
- [29] S Telisman, B Colak, APizent, J Jurasovic, P Cvitkovic. *Environ res* **2007**; 105: 256-266.
- [30] PC Hsu, MY Liu, CC Hsu, LY Chen, YL Guo. *Toxicology* **1998**; 128: 169-179.
- [31] N Batra, B Nehru, MP Bansal. *J ApplToxicol* **2001**; 21: 507-512.
- [32] JK Kang ,D Sul, JK Kang, S Nam, H Kim, E Lee. *Toxicol. Sci* **2004**; 82: 228–236.
- [33] AA Berrahal, ANehdi, N Hajjaji, N Gharbi, S El-Fazâa. *C. R. Biology* **2007**; 330:581–588.
- [34] AA El-Nekeety, El-Kady AA, MS Soliman, NS Hassan, M AAbdel-Wahhab.. *Food and Chemical Toxicology* **2009**; 47: 2209–2215.
- [35] M Duane. Jr Smith., W Howard. B Mielke, James. Heneghan. *Archives of Environmental Contamination and Toxicology* **2008**; 55, (3), pp 518-528
- [36] MW Schwartz, SC Woods, DJR Porte, RJ Seeley, DG Baskin. *Nature* **2000**; 404: 661-671.
- [37] M Prasenjit, S Mahua, CS Parames. *Reproductive Toxicology* **2008**; 26:282-291.
- [38] L Wang, P Xun, Y Zhao, X Wang, L Qian, F Chen. *Toxicol Environ Health A* **2008**; 71 (7): 454-63.
- [39] NM Biswas, P Ghosh. *Kathmandu University Medical Journal*. **2004**; 2 (1): 43-46.
- [40] DM El-Nahal. *The Journal of American Science*, **2010**; 6(12) : 541-554.
- [41] O Ouldali, A Aoues, B Meddah, MSlimani, A Nicolas, O Kharoubi. *International Journal of Green Pharmacy* **2011**; 5: 126-130.
- [42] L Patrick,. *Altern. Med. Rev* **2006** ; 11: 114–127.
- [43] XJin, LLing-jun, WChen, WXiao-feng, FWen-yu, XLi-hong. *Food and Chemical Toxicology* **2008**; 46:1488–1494.
- [44] MMishra, URacharya. *Journal of Trace Elements in Medicine and Biology* **2004**; 18: 173–178.
- [45] MGShalan, MSMostaza, MMHassouna, SEEl-Nabi, AEI-Refaie. *Toxicology* **2005**; 206:1-15.
- [46] SShafiq-ur-Rehman, S Rehman, OChandra, MAbdulla. *Biometals* **1995**; 8:275–279.
- [47] RJulio, IRMarissa, GManuel, YSandra, MSara, F GGustavo. *Food and Chemical Toxicology* **2006**; 44: 1114–1122.
- [48] LDjuna, SAgoes, AAgus, KSoedjajadi. *Pharmaceutical, Biological and Chemical Sciences* **2014**; 5(4): 282-294.
- [49] MKhotimchenko, EAKolenchenko. *Experimental Biology and Medicine* **2007**; 1: 60-62.
- [50] GDongowski, EWalzel, BOzierensk, CStark, JKroll, ALorenz. (1999). Effects of pectin and oligogalacturonic acids on excretion and incorporation of lead in subchronic lead exposed rats. In: RHartemink, editor. Non-digestible oligosaccharides: healthy food for the colon. Wageningen: Graduate School VLAG; p. 151.
- [51] ISSerguschenko, VVKovalev, VEBednyak, YSAKhotimchenko. *J. Mar. Biol* **2004**; 30:83-85.
- [52] VA Ostapenko, AITepyakov, ASProkopovich, TIChegerova. *PromyshlennayaEkologiya* **2001**; 5: 44-47.
- [53] VSKushneva, IGKoltunova. *PromyshlennayaEkologiya* **1997**; 7: 27-31.
- [54] HERose, JQuarterman. *Environ. Res* **1987**; 42: 166-175.
- [55] ZYZhao, LLiang, XFan, ZYu, ATHotchkiss, BJWilk, IEliaz. *Alternative therapies in health and medicine***2008**; 4(14): 34-38.
- [56] BAnkit, SSilke. *Separation and Purification Technology* **2008** ; 63: 577–581.
- [57] MKhotimchenko, VKovalev, YKhotimchenko. *Journal of Hazardous Materials* **2007**; 149: 693–699.
- [58] SJSFlora, MMegha, AMehta. *J Med Res* **2008**; 128: 501-523.
- [59] NAit Hamadouche, MSlimani, BMerad-Boudia, CZaoui. *American Journal of Scientific* **2009**; 3: 38-50.

- [60] DYYu, ZRXu, XGYang. *Asian-Aust. J. Anim. Sci* **2005**; Vol 18, No. 12: 1775-1779.
[61] JKuladip, JSubarna, KSPrabht. *Reproductive Biology and Endocrinology* **2006**; 4(9): 1-13.
[62] AMRAfify, HSEI-Beltagi. *Asian Pacific Journal of Tropical Biomedicine* **2011**; S226-S231.