WORLD JOURNAL OF PHARMACY AND PHARMACEUTICAL SCIENCES
SJIF Impact Factor 6.647
Volume 6, Issue 12, 30-42 Research Article ISSN 2278 – 4357

THE PROTECTIVE EFFECT OF FLAXSEED OIL SUPPLEMENTED WITH HIGH SOURCE OF BRANCHED CHAIN AMINO ACIDS AGAINST THE RATS TESTICULAR TOXICITY INDUCED BY LEAD ACETATE

Ahmed-Farid O. A.[1*] and Mona A. Hassan[2]

[1]Physiology Department, National Organization for Drug Control and Research (NODCAR), Giza 12553, Egypt.

[2]Food evaluation and food Science Department, National organization for Drug Control and Research.

Article Received on
27 Sept. 2017,
Revised on 16 October 2017,
Accepted on 05 Nov. 2017
DOI: 10.20959/wjpps201712-10496

***Corresponding Author**
Dr Ahmed-Farid O. A.
Physiology Department, National Organization for Drug Control and Research (NODCAR), Giza 12553, Egypt.

ABSTRACT

The study was performed to study the effect of whey protein against lead acetate induced toxicity in rats. The forty-eight male albino rats were divided to six group 8 rats for each. The 1st group served as a control. The 2nd injected with 20mg/kg b.w of lead acetate. The 3rd injected with lead acetate plus glutathione (50 mg/kg b.w). The 4th injected with lead acetate plus a mixture of flaxseed oil and whey protein (0.5ml/kg b.w plus 20 mg/kg b.w respectively). The experiment lasted for 90 days. Revealed data showed that the lead acetate increase testes reductive stress markers, decrease cell energy, decrease endogenous antioxidant enzymes and decrease serum testosterone level. On the other hand, another treatment ameliorates all negative alteration. Obtained data concluded that treatments are a natural source of antioxidant that decreases oxidative stress markers and improves male reproductive performance.

KEYWORDS: Flaxseed oil, whey protein, lead acetate, Glutathione, Reductive stress.

INTRODUCTION

Nowadays, it is essential need to find appropriate approach to prevent or reduce the harmful effect of the heavy metals by using chelating agents or complexions.[1] The available

literatures proved that lead acetate (Pb) elicits toxic pathological changes in the testes, leading to its atrophy.[2] Lead is considered as one of the most hazards of environmental pollutants that has been detected in air, water, soil and consequently in food. Pb and heavy metals are known to induce over production of reactive oxygen species (ROS) and increase lipid peroxidation, decrease the saturated fatty acids and increase the unsaturated fatty acid contents of membranes.[3]

Exposure to lead decrease male reproductive health testes function and semen quality. Direct toxic effects on sperm and gonads have been observed in animal tests. Furthermore, lead exposure has been linked with chromosomal aberrations. Exposure may cause a partial replacement of zinc which is essential for sperm head chromatin stabilization. Failure of or delay in sperm chromatin decondensation may lead to decreased fertility or different kinds of DNA damage in the fertilization process. Indirect mechanism by imbalances between HPT hormonal axis induced by lead exposure, particularly by inappropriate LH level and changes the steroid negative feedback loop.[4]

Glutathione recently conceder reference antioxidant drug that may be due to formation of strong complexes with lead.[5] Several studies have been completed on the effectiveness of glutathione to people with cystic fibrosis, tumor cells that may act to protect cancerous cells by conferring resistance to chemotherapeutic drugs.[6, 7]

Flaxseed oil considered an excellent source of antioxidant which contains linolenic acid, Omega-3 fatty acids, especially Docosahexaenoic acid (DHA). The vertebrates cannot synthesized omega-3 and omega-6 series of polyunsaturated fatty acids (PUFAs) and hence must be provided by the diet, either in the form of short chain 18 carbon derivatives found in plants or in the form of long chain 20-22 carbon containing 2-6 double bonds derivatives found in animal tissues.[8] Therefore the omega-3 rich food must be supplemented into their feed to fulfil the animal requirement.

Whey includes proteins, lactose, vitamins, minerals, and traces of fat. Whey proteins consist of different major proteins, including β-lactoglobulin, α-lactalbumin, glycomacropeptide (GMP), proteose peptone 3, immunoglobulins, and bovine serum albumin. In addition, whey proteins contain lactoferrin (LF), lactoperoxidase, natural growth factor, and other minor proteins.[9] It has been claimed that whey proteins can exert many different biological activities.

According the preceding view, the aim of the present was to investigate the impact of natural antioxidant of flaxseed oil containing whey protein against the toxicity induced by lead acetate on testicular function, reductive stress, testis cell energy, and enzymatic defense System and sexual hormonal profile.

MATERIALS AND METHODS

Materials

Flaxseed oil obtained from National Center for Research. Unit of oil extraction, GSH obtained from glutathione enhancer capsules from NOVA/Sigma Pharmathiotical Company and lead acetate obtained from Loba Chemie, India.

Chemicals: All chemicals used were analytical grade.

In-vitro study

Stability of flaxseed oil

Determination of stability by Rancimate 679 (metrohm) Ltd. CH-9100Herisau, Switzerland) and used for the determination of oxidative stability of flaxseed oil as described by.[10]

DPPH scavenging activity

Determination of DPPH scavenging activity according to.[11]

In–vivo study

Animals

Adult male albino rats weighting about 250 ± 20 g were used in these experiment animals were kept under normal laboratory conditions in the animal house of National organization of drug control and research for one week before the start of the experiment rats were allowed to feed and water on uniformity diet.

Experimental design

After an acclimatization period of one week, six equal group of forty eight adult male albino rats 8 rats of each. The 1st group injected with 1ml of distilled water p.o served as a control. The 2nd group injected with 20mg/kg b.w of lead acetate p.o. The 3rd group injected with lead acetate plus glutathione (50 mg/kg b.w). The 4th group injected with lead acetate plus flaxseed oil and whey protein (0.5ml/kg b.w plus 20 mg/kg b.w respectively). The experiment lasted for 90 days and all treatments were treated parallel as a prophylactic method.

Collection of blood samples

Blood samples were withdrawn from the retro-orbital vein of each animal, under light anesthesia by diethyl ether, according to the method described by.[12] Blood was allowed to coagulate and then centrifuged at 3000 rpm for 15 min. to obtained serum for testosterone level.

Preparation of testes samples

Immediately after blood sampling, animals were sacrificed by cervical dislocation and the test tissues were rapidly removed, washed in ice-cooled saline, plotted dray and weighed. A weighed part of each test was homogenized, using a homogenizer (Medical instruments, MPW-120, Poland), with ice-cooled saline (0.9%NaCl) to prepare 10% w/v homogenate. The homogenate was then centrifuged at 4000 rpm for 5 min. The aliquot was used for the assessment of malondialdehyde (MDA), reduced glutathione (GSH), oxidized glutathione (GSSG), nitric oxide (NO), ATP, ADP and AMP, superoxide dismutase(SOD) and catalase (CAT).

Table (1): Methods and kits used to quantify the different biochemical analyses of blood and liver homogenate.

Parameters	Method	Company	Reference
Testosterone (ng/ml)	ELISA	Fortrees Diagnostic Limited, United Kingdom and north Ireland.	
MDA (nmol/g tissue)	HPLC	Standard of 1, 1, 3, 3 tetraethoxypropane (Sigma)	[13]
GSH & GSSG (µmol/g tissue)	HPLC	GSH and GSSG (Sigma)	[14]
NO (µmol/g tissue)	HPLC	Standard of nitite and nitrate (Sigma).	[15]
SOD (U/g tissue)	Colorimetric	Against pyrogallol (Sigma)	[16]
CAT (U/g tissue)	Colorimetric	Against H_2O_2	[17]
ATP, ADP, AMP (µg/g tissue)	HPLC	Sigma	[18]

Histopathological examination

Samples were taken from the testes of rats in different groups and fixed in 10% neutral buffered formalin for twenty four hours. Washing was done in tap water then serial dilutions of alcohol (methyl, ethyl and absolute ethyl) were used for dehydration. Specimens were cleared in xylene and embedded in paraffin at 56 degree in hot air oven for twenty four hours. Paraffin bees wax tissue blocks were prepared for sectioning at 4 microns by slidge microtome. The obtained tissue sections were collected on glass slides, deparaffinized and

stained by hematoxylin and eosin stains[19] for histopathological examination through the electric light microscope.

Statistical analysis

Statistical analysis of the obtained data was performed using the general linear model (GLM) Produced by Statistical Analysis Systems Institute (SAS, 2004). Significant differences among means were evaluated using Duncan's Multiple Range Test. The following linear model was applied: $Yij = \mu + \alpha i + \xi ij$

Yij= Observation measured.

μ = Overall mean.

αi= Effect of treatment.

ξIj = Experimental error assumed to be randomly distributed ($\sigma 2 = 0$).

RESULTS

Stability of flaxseed oil incorporated with whey proteins

The oxidative stability, or storage life until development of rancidity, is an important factor in Processing of oils. Therefore the aim of this study is to investigate the effect of whey proteins as antioxidant agent against the rancidity of flaxseed oil. In order to achieve this purpose, rancimate method has been carried out for the measurement the activity of whey proteins blended with flaxseed oil against rancidity.

It can be noticed from Table (2) that the combination of whey proteins with flaxseed oil caused an Increase of stability period up to 4 hours in comparison with the control (oil) 3.13 hours. The obtained data are agreement with that obtained by[20] who mentioned that obtained the role of milk protein fraction as antioxidant against lipid peroxidation. This finding indicate that the role of milk proteins fractions namely whey proteins against the rancidity of fatty foods. Prophylactic effect of flaxseed oil blended with whey proteins on liver functions. In the present study thirty six male albino rats were subjected to feeding experiment for four successive weeks.

Table 2: oxidative stability of flaxseed oil with whey proteins by rancimate at 100 Co.

Substances	Induction period / h
Flaxseed oil	3.13
Whey protein + flaxseed oil	4.01

Antioxidant activities of whey protein and flaxseed oil

The antioxidant activities of whey protein, flaxseed oil, the mixture of whey protein and flaxseed oil were determined by DPPH method and the obtained results were presented in Table (3), Fig (1). Data showed that the free-radical scavenging capacity which had the highest inhibition of free radical values which were approximately in similar results for whey protein and flaxseed oil in rang of 83.3 to 97.1 and 87.7 to 93.5 for whey protein and flaxseed oil respectively. The lowest value was 83.3 for whey protein. On the other hand it can be seen that the free-radical scavenging capacity % of the supplemented flaxseed oil with whey protein were ranged between 86.2 and 94.2 at concentration20 µg /L and 120 µg /L respectively. This finding may be due to whey protein and flaxseed oil content of antioxidant active ingredient. Also, the obtained data proved that whey protein is a natural antioxidant can be used for a modulator and protect the flaxseed oil against lipid oxidation.

Table 3: DPPH scavenging effect (% of inhibition).

Concentrations (µg/ml)	DPPH scavenging effect (% of inhibition)		
	Whey protein	Flaxseed oil	Whey protein+ Flaxseed oil
20 µg/ml	83.3	87.7	86.2
40 µg/ml	86.2	89.1	87.7
80 µg/ml	89.1	90.6	91.3
100 µg/ml	94.9	92.0	92.8
120 µg/ml	97.1	93.5	94.2

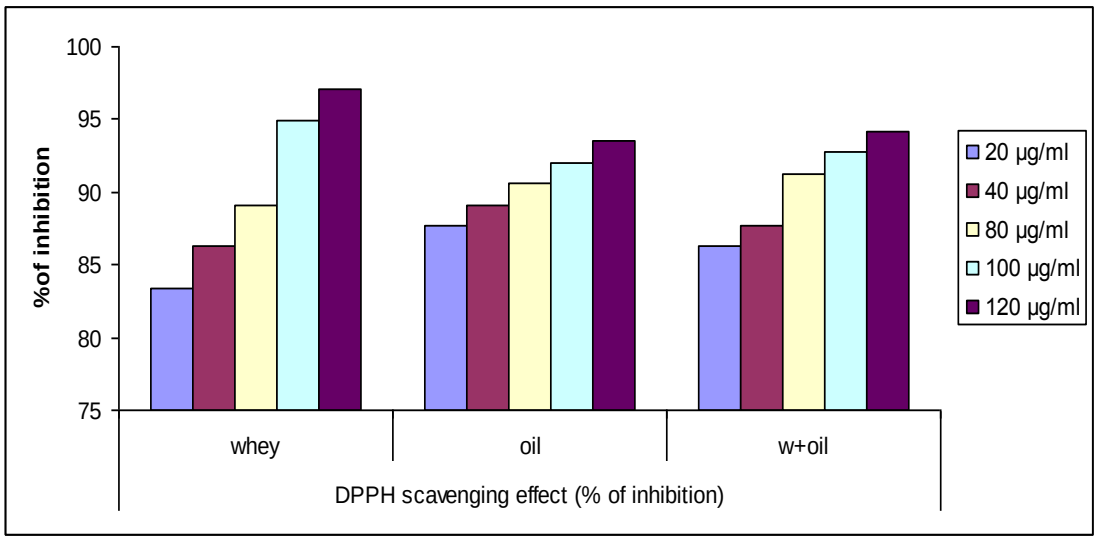


Fig (1): DPPH scavenging effect (% of inhibition)

It is well known that lead acetate elicits toxic pathological changes in the testes[21] when disorders are occurred in human health, it is important to bring such levels under control.

This is done more safety with supplemented dietary to increase the consumption of functional nutraceuticals diet.

Table (4): protective effect of whey protein and flex seed oil on sexual hormonal profile and testes oxidative stress markers against the toxicity induced by lead acetate in rats.

Parameters		Groups			
		Control	Lead	Lead + GSH	Lead + FO + WP
Serum	Testosterone (pg/ml)	30.8 ± 0.91	12.7 ± 0.42a	23.5 ± 0.77ab	27.4 ± 0.79b
Testes tissue	MDA (nmol/g)	20.9 ± 0.61	41.3 ± 1.35a	33.4 ± 0.96ab	34.8 ± 1.08a
	NO (µmol/g)	2.60 ± 0.09	4.50 ± 0.14a	3.6 ± 0.120ab	3.14 ± 0.10a
	GSH (µmol/g)	34.5 ± 0.99	20.9 ± 0.64a	30.3 ± 0.95ab	28.1 ± 0.86a
	GSSG (µmol/g)	1.20 ± 0.04	1.84 ± 0.05a	1.65 ± 0.05ab	1.47 ± 0.05b
	SOD (U/g)	237 ± 7.77	101 ± 3.04a	205 ± 5.95ab	188 ± 5.97ab
	CAT (U/g)	39.7 ± 1.3	21.1 ± 0.69a	27.3 ± 0.82ab	23.8 ± 0.74ab
	ATP (µg/g)	99.8 ± 3.26	66.3 ± 2.18a	81.4 ± 2.40ab	91.2 ± 2.96b
	ADP(µg/g)	46.0 ± 1.39	52.1 ± 1.62a	32.6 ± 0.99ab	29.5 ± 0.89ab
	AMP (µg/g)	34.9 ± 1.16	45.2 ± 1.33a	41.6 ± 1.28ab	39.8 ± 1.30b

- Data are expressed as Mean ± S.E. for 6-rats/group.
- a significant from control group with one way ANOVA at $P < 0.05$.
- b significant from lead group with one way ANOVA at $P < 0.05$.

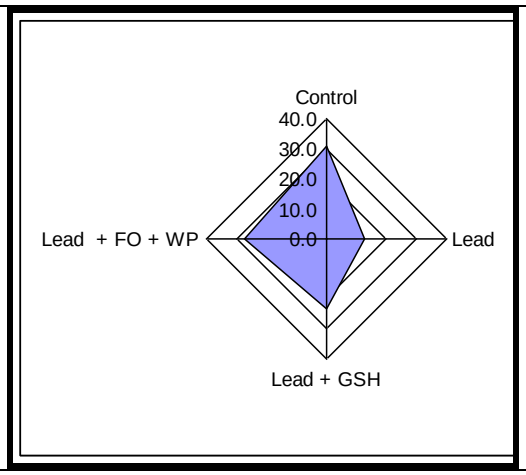


Fig (3). Radar chart showed force of tighten between different treatments for testosterone concentration. This chart concluded that control introduce maximum tight then lead + FO + WP then lead + GSH and the last is lead only.

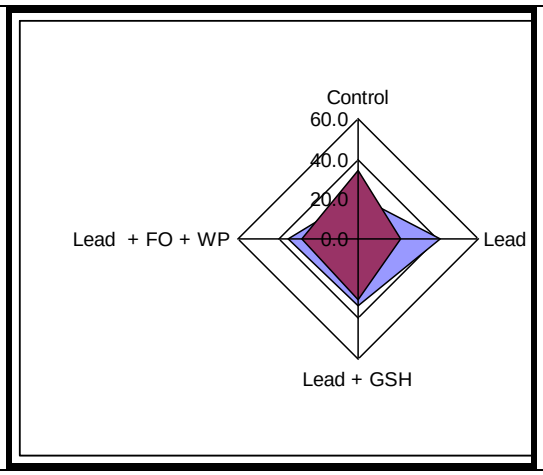


Fig (4). Radar chart showed force of tighten between different treatments for MDA (blue) & GSH (red) concentration. This chart concluded that control introduce maximum tight then lead + FO + WP then lead + GSH and the last is lead only for MDA and its reversed of sequences for GSH with the same behavioral.

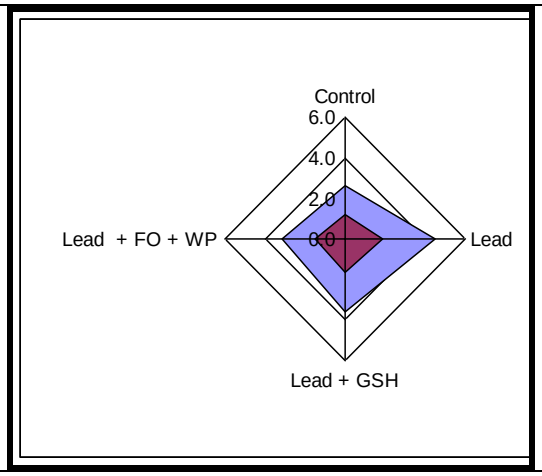


Fig (5). Radar chart showed force of tighten between different treatments for NO (blue) & GSSG (red) concentration. This chart concluded that lead introduce maximum tight then lead + GSH then lead + FO + WP and the last is control only.

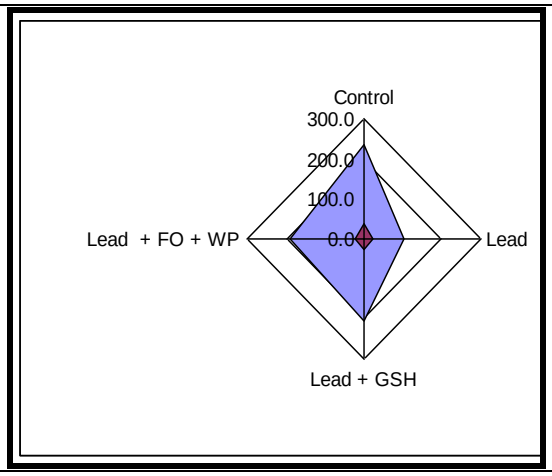


Fig (6). Radar chart showed force of tighten between different treatments for SOD (blue) & CAT (red) concentration. This chart concluded that control introduce maximum tight then lead + FO + WP then lead + GSH and the last is lead only.

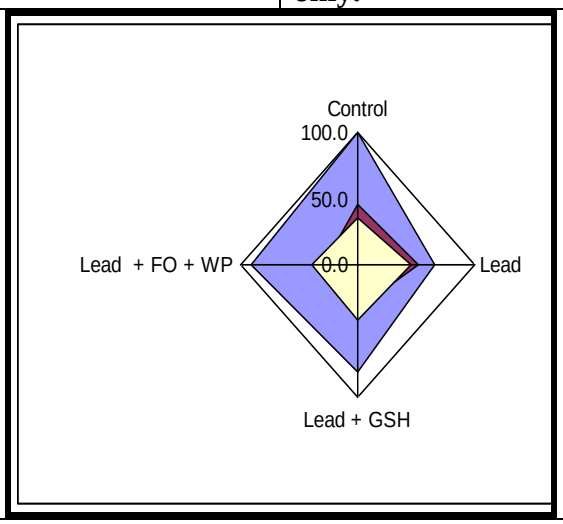


Fig (4). Radar chart showed force of tighten between different treatments for ATP (blue) & ADP (red) & AMP (yellow) concentration. This chart concluded that control introduce maximum tight then lead + FO + WP then lead + GSH and the last is lead only for ATP and its reversed of sequences for ADP and AMP.

Results in Table (4) showed the pathological alteration of lead acetate in rats at the end of experiment in comparing with control group. In this regard lead acetate group decrease testosterone, GSH, SOD, CAT and ATP concentration meanwhile, lead increase the level of MDA, NO, GSSG, ADP and AMP. In contrast glutathione and flaxseed oil containing whey protein ameliorate all pathological alteration.

Histopathological studies

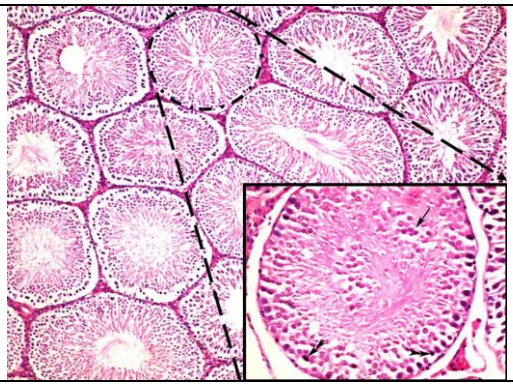


Fig (2). Testes in control group showed normal histological structure of somniferous tubules with complete spermatogenesis series and the basement membrane is lined with germinal epithelial cells from which spermatozoa are developed. H & E X 100 & 400

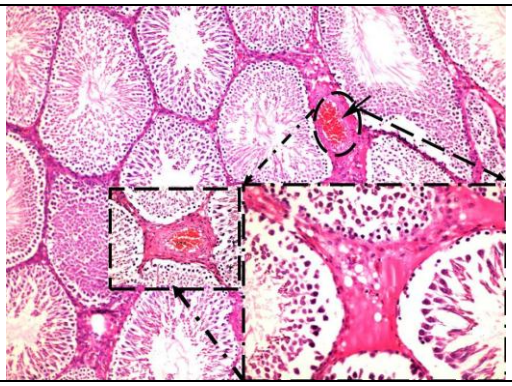


Fig (3). Testes in Lead group showed many seminiferous tubules with marked reduction in spermatogenic steps and sperm, Leydig cells in interstial space with marked dilated congested blood vessels and Wide area of seminiferous tubules with marked degenerative changes, detachment basement membrane and ruptures of basement membrane.. H & E X 100 & 400.

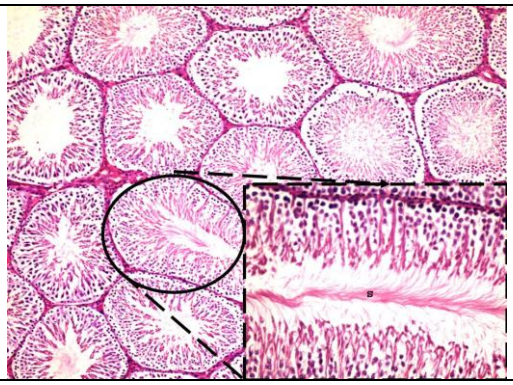


Fig (4). Testes in Lead + GSH group showed normal histological structure of somniferous tubules with approximately complete spermatogenesis series and some wide area of seminiferous tubules. H & E X 100 & 400

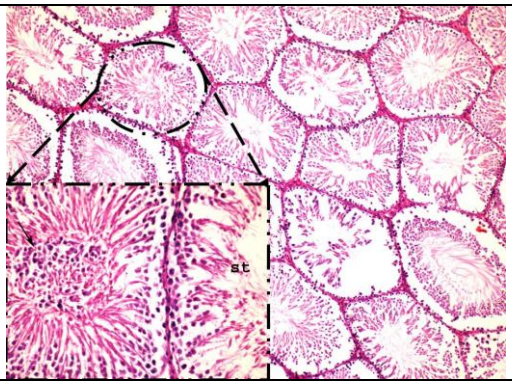


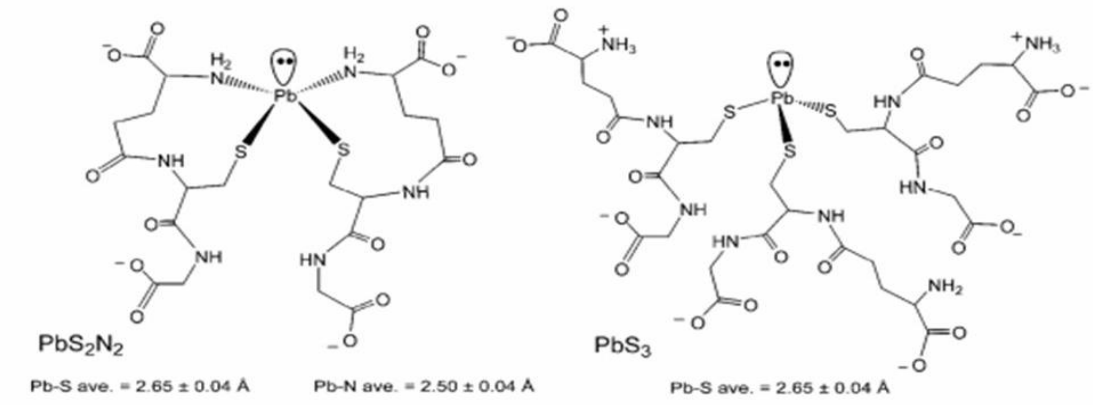
Fig (5). Testes in Lead + GSH group showed normal histological structure of somniferous tubules with approximately complete spermatogenesis series and some wide area of seminiferous tubules. H & E X 100 & 400

DISCUSSION

Contaminations with industrial chemicals and heavy metals are harmful effects on human health and reproductive performance. Exposure to lead acetate may introduce pathological changes in the testes, leading to its atrophy[22] disorganized epithelia, decreased sperm quality, altered sperm morphology, and low androgen levels.[23] This pathological alteration could be due to a decrease LH binding sites in Leydig cells that directly may decrease testosterone synthation and secretion.[24] Decrease of testosterone may be due to the accumulation of lead acetate in testes via penetrating blood-testis barrier.[25] This

accumulation may depressed testes activity for testosterone secretory, and changes in peripheral metabolism.[26] Lead acetate might induce mitochondrial dysfunction, increase free radical production or decrease endogenous antioxidant levels such as GSH, SOD and CAT, enhance the lipid peroxidation of the cell membrane leads to increase MDA and contribute to the oxidative damage of DNA or inhibit androgen biosynthesis in Leydig cells.[27]

Glutathione is considered an important antioxidant in animals and plants that is capable of preventing damage to important cellular components caused by free radicals and heavy metals.[28]



Structures of lead(II)-glutathione complexes [Pb(GSH)2 and Pb(GSH)3 at pH 8.5][29]

Enhancement of testicular tissue altered with lead Pb may be due to the strong complex formatted between glutathione and Pb. Amelioration of testosterone may be due to the cell protection that may decrease cell damage leading to sustained testosterone secretion. Intuitive glutathione group decrease reductive stress markers, purinergic metabolites by increase GSH, SOD, CAT, ATP, decrease MDA, NO, ADP and AMP. Glutathione is also able to activate the purinergic P2X7[30] receptor that may lead to increase ATP concentration in testicular cell.

Flaxseed oil contributes to antioxidant defense and scavenges ROS. The significant increase in testosterone concentration may be due to the adequate amount of unsaturated fatty acids such as linoleic and linolenic acids. Essential fatty acids (linoleic and linolenic acids) Increase testosterone levels that increase may be due to increase perilipin A protein which is a cofactor embody the steroid path synthesis.[31] These unsaturated fatty acids especially lenolenic could be involved in the synthesis of cholesterol which is considered the precursor materials for steroid synthesis.[32] The protection effect of flaxseed oil may be due to presence

of presence of unsaturated fatty acids and tocopherols that may increase endogenous (SOD and CAT) antioxidant and decrease reductive stress markers (MDA, NO, GSSG). The antioxidant properties of flaxseed oil and its function that decrease lipid peroxidation may be due to Lignans. Regarding of this concern Lignans served as an antioxidant role in the defenses against biotic and abiotic factors, and are under basic research for their potential anti-inflammatory or antioxidant activity in laboratory models of human diseases.[33]

Whey protein reached with branched chain amino acids (BCAA) especially Lucien. Increase of plasma Lucien due to treatment leading to increase 3- to 4-fold of EAAs in plasma concentrations.[34] Enhancement of EAAs utilization may increase endogenous antioxidant defense mechanism against lead acetate insults via increase SOD, CAT and GSH level. On the other hand β-lactoglobulin, α-lactalbumin and lactoferrin which are main active constituent of whey protein enhance anabolic hormonal profile such as GH, IGF-1 and testosterone. Present data showed enhancement of purinergic cell energy via the increase in ATP and decrease their metabolites. These finding is consistent with[35] who found that the whey protein activate AMPK for obese mice. AMPK which is a sensor links regulating energy intake and metabolism and mediate the effects of adipokines to regulate food intake, body weight and lipid, glucose metabolism.

CONCLUSION

The present work however, throws some light on the nature of incorporation of whey protein with flaxseed oil may play a modulator agent against the toxicity of the environmental pollution of lead acetate. The combination of whey proteins with flaxseed oil may act as a prophylactic effect for rat fertility that similar to reference antioxidant drug (Glutathione) against the toxicity by lead acetate. On the other hand proved to be most detoxification, owing to be the nature of whey proteins as antioxidant agent. Treatment with whey protein and oil enhance testes function and decrease histopathological finding and ameliorate this finding nearly to normal.

ACKNOWLEDGEMENT

The authors are thankful to Dr. Karem Noor, Pathology Department, Medical division, National Research Centre, Egypt, for the kind help in histopathology shoots and comments with the interpretations of the findings.

REFERENCES

1. Ahmed EAM, Mohamed AD. and Saleh AQ (2010). African Journal of Biotechnology, 9(42): 7216-7223.
2. El-Nagar DM, and Badr AA (2013). Pakistan J. Zool, 45(4): 1083-1089.
3. Ng TP, Goh HH, Ng YL, Ong HY, Ong CN, Chia KS, et al.. Br J Ind Med, 1991; 48: 485-491.
4. Rodamilans M, Osaba MJ, To-Figueras J, Rivera Fillat F, Marques JM, Perez P, et al. Hum Toxicol, 1988; 7: 125-128.
5. Farkas E, Buglyó P (2017). "Chapter 8. Lead(II) Complexes of Amino Acids, Peptides, and Other Related Ligands of Biological Interest". In Astrid S, Helmut S, Sigel RK. Lead:. Metal Ions in Life Sciences. 17. de Gruyter, 201–240.
6. Balendiran GK, Dabur R, Fraser D, 2004; 22(6): 343-52.
7. Visca A, Bishop CT, Hilton SC, Hudson VM. "Improvement in clinical markers in CF patients using a reduced glutathione regimen: an uncontrolled, observational study. J Cyst Fibros, 2008.
8. Cook HW. Fatty acid desaturation and chain elongation in eucaryotes. Biochemistry of lipids, lipoproteins and membranes, 1991; 141-69.
9. Krissansen GW (2007) Emerging health properties of whey proteins and their clinical implications. Journal of the American College of Nutrition, 26: 713S-723S.
10. Mendez, E., J. Sanhueza, H. Speisky, A. Valenzuela, 1996. Validation of the rancimat test for the assessment of the relative stability of fish oils. J Am. Oil Chem. Soc, 73: 1033-1037.
11. Kalucka, N.M, J. Korczak, MDratwia, E.Lampart- Szczapa, A.Siger and M.Buchowski (2005). Changes in antioxidant activity and free radical scavenging potential of rosemary extract and tocopherols in isolated rapessd oil triacyglycerols during accelerated tests. Food Chemistry, 93: 227-235.
12. Cocchetto, D.M. and Bjornsson, T.D. (1983). J Pharm Sci, 72: 465-92.
13. Karatepe M. (2004) Chromatographic Line, 12: 362-365
14. Jayatilleke E and Shaw (1993). Anal. Biochem, 2142: 452-457.
15. Papadoyannis LN Samanidou VF and Nitsos, Ch.C.(1999) J. Liq. Chrom. Rel. Technol., 2213: 2023 – 2041.
16. Marklund S and Marklund G. (1974). Eur.J. Biochem, 47(3): 469-474.
17. Aebi H. Methods Enzymol, 1984; 105: 121–6.

18. Teerlink T, Hennekes M, Bussemaker J, Groeneveld J. (1993). Analytical biochemis-try, 214(1): 278-83.
19. Banchroft, J.D.; Stevens, A. and Turner, D.R. (1996): 4th ed. Churchil Livingstone, New York, London, San Francisco, Tokyo.
20. Hsieh, CC. Hernández-Ledesma, B. Fernández-Tomé, S. Weinborn, V. Barile, D and María, J. (2015). BioMed Research International, 146840: 16.
21. Cullen WR, McBride BC, Reglinski J. (1984). J Inorg Biochem. A, 21: 45–60.
22. Acharya, U.R. And Mishra, P., 1995. Adv. Biosci, 14: 37–44.
23. Senapati, S.K., Dey, S., Dwivedi, S.K.Andswarup, D., 2001. J. Ethnopharmacol, 76: 229-232.
24. Kempinas, W.G., Farvaretto, A.L.V., Melo, V.R., Lamano-Carvalho, T.L., Petenusci, S.O., Oliveira-Filho, R.M. 1994. J. Appl. Toxicol, 14: 427–433.
25. Gorbel, F., Boujelbena, M., Makni-Ayadi, F., Guermazi, F., Croute, F., Feki, A. 2002. Demonstration of apoptotic activity. Crit Rev Biol, 325: 927-940.
26. Katsiya, G.V., Todua, T.N., Gorlushkin, V.M., Chirkov, A.M. 1989. Goncharov Biology Meditsiny, 107(2): 231-234.
27. Kasperczyk A, Kasperczyk S, Horak S, Ostałowska A, Grucka-Mamczar E, Romuk E, Olejek A, Birkner E (2008). Toxicol Appl Pharmacol, 228: 378–384.
28. Pompella A, Visvikis A, Paolicchi A, De Tata V, Casini AF (2003). Biochemical Pharmacology, 66(8): 1499–503.
29. Mah, V and Jalilehvand, F. (2012). Inorg Chem. 4; 51(11): 6285–6298.
30. Doná F., Conceicao I. M., Ulrich H., Ribeiro E. B., Freitas T. A., Nencioni A. L., et al. . (2016). Purinergic Signal, 12: 295–302. 10.1007/s11302-016-9504-9.
31. Chung, S, Brown, JM, Sandberg, MB, and McIntosh, M. J Lipid Res, 2005; 46: 885–895.
32. Servetnick, DA, Brasaemle, DL, Gruia-Gray, J, Kimmel, AR, Wolff, J, and Londos, C. J Biol Chem, 1995; 270: 16970–16973.
33. Korkina, L; Kostyuk, V; De Luca, C; Pastore, S (2011). Mini reviews in medicinal chemistry, 11(10): 823–35.
34. Kobayashi, H. Kato, H. Hirabayashi, Y. Murakami, H. and Suzuki, H. (2006). American Society for Nutrition, 22(6): 234S-236S.
35. Kahn BB, Alquier T, Carling D, Hardie DG (2005). Cell metabolism, 1: 15-25.