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Impact of Quercetin and Omega-3 Fatty-Acid on Lead-Induced Alterations in Reproductive Parameters in Male Wistar Rats

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Abstract:- Exposure to environmental toxins like Lead has been associated with male infertility. Whether or not potent antioxidants like omega 3 Fatty acids and/or quercetin could reduce the effect of lead on male sexual functions deserve scientific investigation. Thirty-five (35) male albino Wistar rats were assigned into 7 groups: Group 1 (normal control), Group 2 (sham-control1), Group 3 (sham-control 2), Group 4 (Lead group), Group 5 (Lead + Omega-3), Group 6 (Lead + Quercetin) and Group 7 (Lead + Omega-3 + Quercetin). Lead was given orally at 20mg/kg bwt, quercetin 20mg/kg bwt s.c, Omega-3 14.29mg/kg bwt orally. The animals all had free access to rat food and water for 56 days. After which they were sacrificed, and semen and blood samples were collected for assay. The results showed no significant difference between normal control group and sham controls. Sperm function parameters (sperm count, motile, viable and normal sperms) in the lead treated group was significantly reduced compared to the control. Omega 3 and/or quercetin administration reversed the reductions in sperm function parameters to near control levels. Hormone levels (LH and Testosterone) were significantly reduced in lead group compared to the normal control but were reversed after quercetin and omega 3 treatment. However, there was no significant difference in the FSH level among experimental groups. Conclusively, omega 3 and/or quercetin ameliorates the harmful effects of lead on reproductive parameters by improving sperm functions (total sperm count, viability, motility, and morphology). A combination of both quercetin and omega 3 provided better ameliorative effect than either omega 3 or quercetin.

Keywords:- *Infertility, Quercetin, Omega 3, Lead, Sex Hormones, Rats.*

I. INTRODUCTION

The International Committee Monitoring Assisted Reproductive Technologies (ICMART) and World Health Organisation define clinical infertility as the inability of pregnancy to occur after 12 months of regular unprotected sexual intercourse, where either one or both parties contribute to the challenge. When a sexually mature male is unable to impregnate a sexually mature female, it is described as male infertility. This accounts for This accounts for 1/3rd (20-30%) of infertility cases [1]. Male infertility is mostly associated with difficulty in ejaculation, small volumes of semen ejaculated, absence or low sperm levels, abnormally shaped (morphology) sperm and abnormal movements (motility), erectile dysfunction (impotency) [2].

Also, exposure to environmental elements such as heat, toxins, and industrial chemicals, heavy metals like lead, radiation and unhealthy lifestyles affect sperm production and function. Male infertility can be diagnosed via laboratory semen analysis, thereby testing for, sperm production levels sperm count, sperm functionality (morphology and motility) and sperm concentration, and assay of male sex hormones. Lead is a neurotoxin that easily accumulates in soft tissues and bones and can damage a lot of body systems. Lead toxicity affects almost every body function. [3]. Lead toxicity has a devastating effect on body system and is fast become a serious environmental disease. Lead is highly persistent in environment especially in developing countries where its use is still high [4].

Plant pigments (flavonoids) such as quercetin can be found in a lot of plants and food. Quercetin has antioxidant and anti-inflammatory effects that reduce swellings, kill cancer and control body parameters, making it fit as a potent disease resistant and improves overall health [5]. Quercetin is ubiquitous in nature, available in many fruits such as apple, vegetables, berries and tomatoes.

Aside other health benefits, studies have shown that quercetin improves male fertility (sperm motility) from leukocytospermic patients and protect oxidative damage to sperm [6].

Omega 3 fatty acids are a subclass of polyunsaturated fatty acids (PUFAs). They have a double bond and three atoms from their terminal methyl group. They are ubiquitous in nature and is an important constituents of animal lipid metabolism [7]. Omega 3 oils play very important roles in human physiology and diet. Although they are not synthesized in the body, they are obtained from diet. Docosahexaenoic acid (DHA) is a major constituent of omega-3 that is found in male reproductive organs and the brain. Animal studies has shown that in early reproductive events, omega 3 acids play important roles, can restore fertility and improve spermatogenesis in male rodents [8, 9].

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Some other studies showed misshaped sperm due to DHA absence resulting in infertility in Male Mice [10].

Lead exposure in both men and women is popular for many adverse reproductive effects [11, 12]. Some of these harmful effects for males are: infertility, changes in serum testosterone, reduction in libido, abnormal prostatic functions, and alteration in spermatogenesis [13, 14].

This research was therefore aimed at investigating the effect of a combination of quercetin and omega 3 to curb the effect of lead on reproductive parameters in male rats.

II. MATERIALS AND METHODS

➢ *Laboratory Animals*

Thirty-five (35) male albino Wister rats weighting between 180-220g were obtained from the animal house of the Department of Physiology, University of Calabar, Calabar, Nigeria for the study. All rats had access to free water and chow. The animals were acclimatized for one week, Ethical approval was obtained from the Animal Research Ethics Committee of Faculty of Basic Medical Sciences, University of Calabar, (approval number: 231PHY2523).

➢ *Experimental Design and Drug Administration*

The animals were weighed and distributed into seven groups of five animals. Lead was administered at a dose of 20mg/kg body weight.

Omega-3 was administered at a dose of 14.29 mg/kg (extrapolated from human dose of 1000 mg / 70 kg) was administered once daily by dissolving 1 capsule in 5mL of Olive oil and 0.01mL was given to 100g rat[15].

A dose of 20mg/kg body weight of quercetin was administered to the rats subcutaneously once daily. 0.1g of quercetin was dissolve in 5ml of 2% dimethly sulfoxide (DMSO) solvent, then given at 0.1ml/100g body weight, subcutaneously and once daily.

- Group 1 (control): Received normal rat chow + drinking water
- Group 2 (Sham control-1): DMSO (2%) 1mL/kg orally and once daily.
- Group 3 (Sham control-2): Olive oil (0.1 mL/kg orally and once daily)
- Group 4: (Lead group): Lead (20 mg/kg, o.p)
- Group 5: (Lead + Omega-3): Lead (20 mg/kg, o.p) + Omega-3 (14.29 g/kg o.p)
- Group 6: (Lead + Quercetin): Took Lead (20 mg/kg, o.p) + Quercetin (20 mg/kg s.c)
- Group 7: (Lead + Quercetin + Omega-3): Lead (20 mg/kg, o.p) + Omega-3 (14.29mg/kg o.p) + Quercetin (20mg/kg, subcutaneously)

All the animals had free access to normal rat feed and drinking water. The feeding regimens lasted for 56 days. The animals were euthanized. The feeding regimens lasted 56 days (8 weeks). The animals were then fasted overnight, weighed and anaesthetized with 5% chloroform. Blood samples were collected via cardiac puncture[16] into plain caped sample bottles, then left to stand for 2 hours to clot, the blood was then centrifuged and serum extracted from the supernatant. Semen samples were also collected from the epididymis into 9% NaCl solution in sample bottles for semen analysis.

➢ *Semen analysis*

- *Determination of Sperm Count*

The epididymal content was obtained with forcept, weighed and placed on a petri-dish containing physiological saline. The suspension was separated into fragments by fattening through 80 micronmeter stainless mesh. A tissue – free aliquot obtained was loaded into the Neubauer haemocytometer (deep 1/10 Labart. Germany). Different sperm counts were done using microscope[17].

- *Mean Count for each Rat was Calculated using the Formula: Sperm Count = (Total No. of Sperm cells in the Cytometer) / (Mean Value)*

- *Determination of Sperm Viability*

The improved one step eosin-nigrosin staining technique was utilized[18]. A fraction of each suspension of sperm sample was mixed with equal volume of eosin-nigrosin stain. Smears were made on slides and air-dried. The air dried smears were then prepared on a slide for each sample. The slides were coated randomly and examine under the microscope for viability. The percentage viability was calculated based on the number of viable (live) sperm cells divided by the number of sperm cells within 30 minutes multiply by 100.

- *Determination of Sperm Motility and pH*

Semen samples from different treated groups were collected and dropped on a glass slide and viewed under the (Olympus, Japan) microscope at x400 magnification. The percentage of sperm was analyzed for progressive motile sperm (PMS), non-progressive – motile sperm (NPMS), and distinguished – by the movement of the sperm.

The pH meter was used to determine the pH of the semen samples.

- *Determination of Sperm Morphology*

One drop of suspension of each semen sample was placed on a glass slide. The slide was air dried and stained with 1% eosin. The morphological abnormalities of the sperm were evaluated from a total of two hundred sperm per

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animal[19]. The results were recorded as percentage of the abnormal sperms.

➢ *Hormonal Assay*

- *Determination of Testosterone*

ELISA kit was employed for the determination of testosterone levels. It was carried out in Zone-3 diagnostic laboratory, Akpandem Street, Calabar, Nigeria. A working solution of the testosterone – HRP conjugate and wash buffer were prepared. The required number of microwell strips were displaced.100µl of the conjugate working solution was placed in each well. This was followed by addition of 50µl of each calibrator, control and specimen sample into corresponding labelled wells. Duplicates of the wells were made[20].

All wells were incubated on a plate shaker approximately 200rpm for 1hr at room temperature. Each well was washed 3 times with 300µl wash buffer per well and the plate was tap firmly against absorbent paper to ensure that it was dry. Thereafter, 150µl of 5,5-tetrameltylbenzidine (TMB) substrate was pipette into each well at time intervals and re-incubated on a plate shaker 10 – 15 minutes at room temperature (or until calibrator attain a dark) blue color for desired OD. At the expiration of the incubation, 5µl of stop solution was added to each well at the same time intervals. Finally, reading of the plates was on a microwell plate reader at 450nm within 20 minutes.

- *Determination of Luteinizing Hormone (LH) and Follicle Stimulating Hormone (FSH)*

ELISA kit was adopted for the determination of luteinizing hormone (LH) and Follicle stimulating hormone. LH- conjugate and wash buffer solutions were prepared. 25µl of each calibrator, control and specimen samples was pipetted into correspondingly labelled wells in duplicate. The plate shaker was incubated for 200rpm for 30 minutes at room temperature. The wells were then incubated on the plate shaker for 15 – 20 minutes at room temperature (or until calibrator attain dark blue color for desired OD). Thereafter, 50µl of stop solution was transferred into each well at the same time interval. Reading of the plate on a micro well plate reader was at 450nm within 20 minutes after dilution of the stop solution[20].

Similar procedures were followed for the determination of follicle stimulating hormone. However, FSH binds to anti β-FSH receptors and required a volume of 300ml of Mela and 2 diluent buffers in the buffer bottle to properly process the assay run[20].

➢ *Statistical Analysis*

The data obtained were presented as mean ±SEM, data were analyzed using one-way analysis of variance followed with Tukey post hoc test. This was done with the aid of a statistical package, IBM SPSS Version 25.0 for windows. $P<0.05$ was considered significant.

III. RESULTS

➢ *Sperm Count, Motile, Viable Sperms and Sperm Morphology in the Different Experimental Groups*

The total sperm count ($x10^6$/L) for the control, sham-1 and sham-2 were 192.20 ±21.00, 211.80 ±23.11, and 196.20 ±10.55 respectively. It was significantly ($p<0.05$) reduced in the lead group 91.20 ±8.25 compared with other groups (Lead + Omega 3, 170.60 ±16.68, Lead + quercetin, 150.60 ±18.56 and Lead +quercetin + Omega 3, 185.40 ±17.22), Table 1.

Percentage of motile sperm cells for the control was 88.00 ±2.55%, for the sham-1 it was 84.00 ±1.87%, for the sham-2, 87.00 ±2.55%, lead group, 71.00 ±1.00%, Lead + Omega 3, 78.00 ±1.22%, Lead + quercetin, 79.00 ±1.87% and Lead +quercetin + Omega 3 it was, 80.00 ±2.74%. No significant difference existed among the control groups. But it decreased significantly ($p<0.05$) in the lead group compared to the control and the treatment groups, Table 1.

The percentage of viable sperm cells was not significantly different among the different experimental groups. The control had a count of 90.60 ±, sham-1 (86.00 ±) and sham-2 (87.40 ±), the lead, Lead + Omega 3, Lead + quercetin and Lead +quercetin + Omega 3 had counts of 76.00 ±, 83.00 ±, 85.20 ± and 87.00± respectively, Table 1.

The percentage sperm cells with normal morphology for the control different control groups were not significantly different from each other, (control, 90.40 ±0.40%, sham-1, 90.80 ±0.37% and sham-2, 88.00 ±1.22%). It was significantly ($p<0.05$) lower in lead group (68.00 ±3.74%) compared with the control groups and the other treatment groups (Lead + Omega 3, 84.00 ±3.67%, Lead + quercetin, 88.00 ±3.39% and Lead +quercetin + Omega-3, 89.00 ±2.92%), Table 1.

➢ *Sex Hormones Concentration in the Different Experimental Groups*

There were no significant differences in concentrations of follicle stimulating hormone (FSH) among the different experimental groups. For the control FSH concentration was 8.26 ± 0.47, sham-1, 7.08 ± 0.27; sham-2, 7.50 ± 0.51; lead group 7.12 ± 0.46; Lead + Omega 3, 6.28 ± 0.46; Lead + quercetin it was 6.24 ± 0.14 and in the Lead +quercetin + Omega 3 it was 6.98 ± 0.55, Fig. 1.

The luteinizing hormone (LH) levels (mIU/mL) for the control groups were not significantly different from each other. Control had LH level of 53.00 ± 0.89, sham-1, 48.80 ± 2.01, and sham-2 49.20 ± 2.29. The lead group (16.00 ± 1.10) had significantly ($p<0.05$) lower LH concentration compared with the control groups. The increase in LH observed in Lead + Omega 3 (23.20 ± 0.49) and Lead + quercetin (24.60 ± 0.68) were not significantly different from the lead group, but Lead +quercetin + Omega 3 (33.00 ± 3.10) had significantly higher LH levels compared with lead group, Fig. 2.

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The Testosterone levels (ng/mL) for the control, sham-1 and sham-2 groups were 36.00 ± 0.55, 33.20 ± 1.39 and 36.40 ± 3.59 respectively, showing no significant differences among groups. It was significantly ($p<0.05$) lower in the lead group (11.20 ± 0.49) compared with the controls and other treatment groups. Values of testosterone obtained in Lead + Omega 3 was 28.20 ± 0.73, the Lead + quercetin had values of 26.80 ± 11.77 and in the Lead + quercetin + Omega 3 it was 30.80 ± 1.02, Fig. 3.

Table 1 Comparison of Sperm Count, Motile, Viable Sperms and Sperm Morphology

Group	Sperm count (x10^6/L)	Motile sperm (%)	Viable sperms (%)	Normal morphology (%)
Control	192.20	88.00	90.60	90.40
	±21.00	±2.55	±0.40	±0.40
Sham fed 1	211.80	84.00	86.00	90.80
	±23.11	±1.87	±4.58	±0.37
Sham fed 2	196.20	87.00	87.40	88.00
	±10.55	±2.55	±2.18	±1.22
Lead	91.20	71.00	76.00	68.00
	±8.25*ab	±1.00*ab	±2.45	±3.74*ab
Lead + Omega 3	170.60	78.00	83.00	84.00
	±16.68	±1.22*	±3.39	±3.67c
Lead + Quercetin	150.60	79.00	85.20	88.00
	±18.56	±1.87	±4.31	±3.39c
Lead + Omega-3 + Quercetin	185.40	80.00	87.00	89.00
	±17.22c	±2.74	±3.39	±2.92c

Values are Expressed as mean ±SEM, n = 5.
* = $p<0.05$ vs control
a = $p<0.05$ vs sham fed-1
b = $p<0.05$ vs sham fed-2
c = $p<0.05$ vs lead

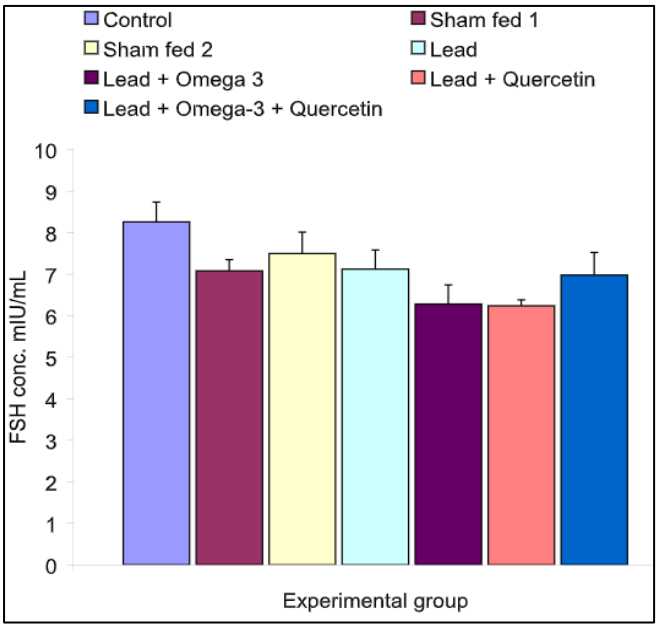

Fig 1 Follicle Stimulating Hormone Concentration in the Different Experimental Groups
Values are Expressd as mean +SEM, n = 5.
No Significant Differences among Groups

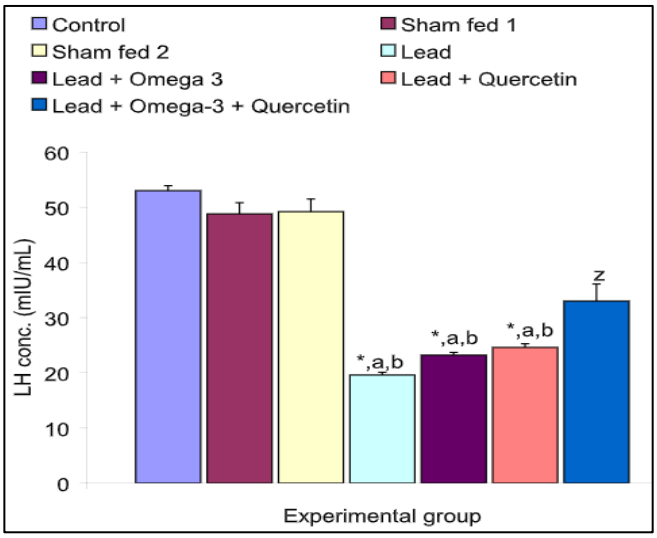

Fig 2 Luteinizing Hormone Concentration in the Different Experimental Groups
Values are Expressd as mean +SEM, n = 5.
*= $p<0.05$ vs control
a = $p<0.05$ vs sham fed-1
b = $p<0.05$ vs sham fed-2
z = $p<0.05$ vs all groups

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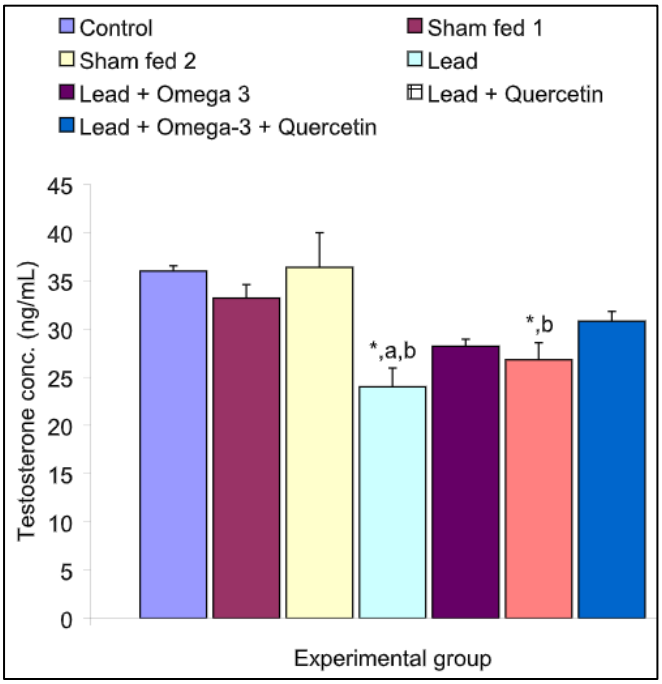

Fig 3 Testosterone Concentration in the Different Experimental Groups
Values are expressd as mean +SEM, n = 5
* = p<0.05 vs control
a = p<0.05 vs sham fed-1
b = p<0.05 vs sham fed-2

IV. DISCUSSION

When a sexually mature male is unable to impregnate a sexually mature fertile female, it is described as male infertility. This accounts 20-30% of infertility cases.[1] Male infertility is mostly associated with difficulty in ejaculation, small volumes of semen ejaculated, absence or low sperm levels, abnormally shaped (morphology) sperm and abnormal movements (motility), erectile dysfunction (impotency) [2]. Also, exposure to environmental elements such as heat, toxins, and industrial chemicals, heavy metals like lead, radiation and unhealthy lifestyles affect sperm production and function. Lead exposure is known to cause adverse health outcome in men and women [14]. Several adverse reproductive outcomes have been reported to occur in men exposed to lead. Some of which include; reduced libido, effects on spermatogenesis, chromosomal damage, infertility, abnormal prostatic function and changes in serum testosterone [14]

This study was aimed at investigating the effect of a combination of quercetin and omega 3 to curb the effect of lead on reproductive parameters in male rats.

Results obtained indicated that in the lead group, there was a significant decrease in sperm motility compared to the control groups. This could be as a result of the direct effect of lead on reproductive organs. There was an increase in the motility of sperm in the Quercetin and Omega 3 treated groups, suggesting that quercetin and omega 3 treatments can improve sperm motility.

Earlier reports showed that a supplementary treatment with omega 3 fatty acids on infertile men caused a significant improvement in sperm motility and plasma concentration of DHA [21].

Also studies indicate that membrane integrity of sperm was preserved to near normal following quercetin treatment [22].

Our study showed that sperm counts was decreased significantly in the lead group compared to control, suggesting that lead reduces sperm count levels. The administration of quercetin and omega 3 treatment significantly improved sperm count levels, thereby reversing the effects of lead. This corresponds to a study that showed DHA and EPA concentrations in omega 3 corresponded with those in spermatozoa. This proves the significant improvement of total sperm counts and sperm concentration in omega 3 treated group [23]. Sperm viability is the amount of live sperm cells in the semen sample which is converted to percentage. At least 58% of the sperm present in the semen should be viable [24]. Sperm viability in the lead groups was reduced compared to the control group though not significantly. This suggests that lead as a tendency to affect viability of sperm. However, the treatment showed no significant difference compared to the lead group. Morphology is one of the sperm function parameters, it assesses sperm cells that have physical and structural abnormalities with the head, mid piece or tail. Morphological abnormality in greater numbers in the sperm than 25% can lead to infertility [25]. In teratoospermia there is less than 4% morphologically normal spermatozoa. This study showed a significant decrease in sperm cells with normal morphology and an increase in abnormal sperm cells in lead group compared to the control groups. However, treatment with quercetin and omega 3 improved the number of normal sperms. This suggests that quercetin and omega 3 helps in sustaining the morphology of sperm cells.

A study on in vivo administration of quercetin had documented near-normal sperm functions and morphology, and that these effects however were most likely caused by the inability of this com pound to ameliorate oxidative stress and inflammation in sperm [22]. No significant alterations were observed in FSH levels following lead administration. Also no significant difference was observed in omega 3 and quercetin treated groups when compared to the lead group. Some studies indicate that dietary supplementation of omega 3 decreased serum FSH level in normal weight women but not in obese women [26]. This could suggest why there was no difference in the treatment group compared to the lead group.

LH hormones showed significant reduction in the lead group compared to the control group. Suggesting that lead affects hormone levels of LH. No significant difference was observed in the lead + quercetin, and lead + omega 3 treatment groups. However, the combined administration of Lead+ quercetin and Omega 3 showed improved increase in the LH levels. In males, LH aids in production of testosterone. Testosterone is converted to

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dihydrotestosterone (DHT) by 5-alpha reductase. DHT is a more potent form of testosterone and is synthesized by the prostate gland. DHT is also responsible for the growth and advancement of the prostate, scrotum, penis, male hair pattern, acne and baldness [27]. Factors that reduce levels of testosterone greatly affect spermatogenesis [28, 29]. The lead group showed significant reduction in testosterone levels compared with the control. The levels of testosterone were significantly increased following administration of quercetin and omega 3 treatments.

V. CONCLUSION

It can be concluded from this study that lead exposure leads to impairment of reproductive parameters (sperm count, viability and motility). It also caused reduction in sex hormone (LH and testosterone) concentrations. These deleterious effects of lead on reproductive parameters were ameliorated following omega-3 and quercetin administration in rats. Combination of both omega-3 and quercetin provided better ameliorative effect on total sperm count than single administration of omega-3 or quercetin.

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- *Conflict of Interest*

The author(s) declare(s) that there is no conflict of interest regarding the publication of this article.

REFERENCES

[1]. Sharlip ID, Jarow JP, Belker AM, Lipshultz LI, Sigman M, Thomas AJ. Best practice policies for male infertility. *Fertility and Sterility* 2002; 77:873–82.
[2]. World Health Organization (WHO). International Classification of Diseases, 11th Revision (ICD-11) Geneva: WHO 2018.
[3]. Osmel LL, José MS. Effects of Lead on Reproductive Health, Lead Chemistry, Pipat Chooto, *IntechOpen*, 2020. https://www.intechopen.com/chapters/71715
[4]. Wani AL, Ara A, Usmani JA. Lead toxicity: a review. *Interdisc Toxicol* 2015; 8(2):55–64.
[5]. Li Y, Yao J, Han C, Yang J, Chaudhry MT, Wang S, Liu H, & Yin Y. Quercetin, Inflammation and Immunity. *Nutr* 2016; 8(3), 167.
[6]. Diao R, Gan H, Tian F, Cai X, Zhen W, Song X, Duan YG. In vitro antioxidation effect of Quercetin on sperm function from the infertile patients with leukocytospermia. *Ame J Rep Immun* 2019; 82(3):e13155.
[7]. Scorletti E, Byrne CD. "Omega-3 fatty acids, hepatic lipid metabolism, and nonalcoholic fatty liver disease". *Ann Review Nutr* 2013; 33 (1): 231–348.
[8]. Nehra D, Le HD, Fallon EM, Carlson SJ, Woods D, White YA, Pan AH, Guo L, Rodig SJ, Tilly JL, Rueda BR, Puder M. Prolonging the female reproductive lifespan and improving egg quality with dietary omega-3 fatty acids. *Aging Cell* 2012; 11:1046–1054.
[9]. Gaskins AJ, Colaci DS, Mendiola J, Swan SH, Chavarro JE. Dietary patterns and semen quality in young men. *Human Rep* 2012; 27:2899–2907.
[10]. Roqueta-Rivera R. Docosahexaenoic acid supplementation fully restores fertility and spermatogenesis in male delta-6 desaturase-null mice. *J Lipid Res* 2010; 51(2):360.
[11]. American Society for Reproductive Medicine. Optimizing natural fertility. Retrieved June 11, 2022. https://www.reproductivefacts.org/globalassets/rf/news-and-publications/bookletsfact-sheets/english-fact-sheets-and-info-booklets/optimizing_natural_fertility_factsheet.pdf (PDF 253 KB), 2012.
[12]. Patrick OU, Abiodun ME. Male Infertility in Nigeria: A neglected reproductive health issue requiring attention. *J Basic Clin Reprod Sci* 2015; 4:45-53.
[13]. Agarwal A, Mulgund A, Hamada A. A unique view on male infertility around the globe. *Reprod Biol Endocrinol* 2015; 13:37.
[14]. Kumar S. Occupational and Environmental Exposure to Lead and Reproductive Health Impairment: An Overview. *Indian J Occup Envir Med* 2018; 22(3):128-137.
[15]. Allan-Ndoul GF, Barbier O, Vohl, MC. Effects of different concentrations of omega-3 fatty acids on stimulated THP-I macrophages. *Genes and Nutrition* 2017; 12:7
[16]. Ohwada K. Improvement of cardiac puncture in mice. *Experimental Animals* 1986; 35(3):353-355.
[17]. Ekalue U, Ikpeme E, Uno U, Umeh S. Protective role of aqueous, Guava leaf extract against caffeine induced spermatotoxicity in Albino rats. *J Med Plant* 2016; 10(1): 98-105.
[18]. Björndahl L, Söderlund I, Kvist U. Evaluation of the one-step eosin-nigrosin staining technique for human sperm vitality assessment, *Human Rep* 2003; 18:4:813-816.
[19]. Naga AM, Shata A, Mohamed AE, Hany AE. Potential impact of urocortin I on sperm count, motility and sex hormone profiles in normal adult rats. *Afr J Pharm Pharmacol* 2016; 10(32):665-669.
[20]. Ulubaev A, Lee DM, Purandare N, Pendleton N, Wu FCW. Activational effects of sex hormones on cognition in men. *Clin Endocrinol* 2009; *71*(5):607-623.
[21]. Banafshe H, Mahdieh N, Shima H, Mohsen T, Zatollah A, Seyed Ali K, Sadegh J. The effect of omega-3 fatty acids, EPA, and/or DHA on male infertility: a systematic review and meta-analysis. *J Diet Suppl* 2019; 16 (2):245-256.

ISSN No:-2456-2165 https://doi.org/10.38124/ijisrt/IJISRT24FEB084

[22]. Attaman JA, Toth TL, Furtado J, Campos H, Hauser, R. Dietary fat and semen quality among men attending a fertility clinic. *Human Reprod* 2012; 27:1466-74.
[23]. Suseela Y, Nelli G, Kamarulzaman K, Siti Z, Omar N, Bin, S. In vivo administration of quercetin ameliorates sperm oxidative stress, inflammation, preserves sperm morphology and functions in streptozotocinnicotinamide induced adult male diabetic rats. *Arch Med Sci* 2019; 15(1)240-249.
[24]. Safarinejad, MR. Effect of omega-3 polyunsaturated fatty acid supplementation on semen profile and enzymatic anti-oxidant capacity of seminal plasma in infertile men with idiopathic oligoasthenoteratospermia: a double-blind, placebo-controlled, randomised study. *Andrologia* 2011; 43(1):38-47.
[25]. Komal F, Khan MK, Imran M. Impact of different omega-3 fatty acid sources on lipid, hormonal, blood glucose, weight gain and histopathological damages profile in PCOS rat model. *J Transl Med* 2020; 18:349.
[26]. Murota K, Terao, J. Antioxidative flavonoid quercetin: implication of its intestinal absorption and metabolism. *Arch Biochem Biophy* 2003; 417:12–7.
[27]. Zain AA, Huayu L, Nichole EC, Justin C, Mary H, Andrew PB, Celeste R, Robert HE, Alex JP. Omega-3 Fatty Acid Supplementation Lowers Serum FSH in Normal Weight but Not Obese Women. *J Clin Endocrinol Met* 101(1):2016; 324-333.
[28]. Nassar GN, Leslie SW. Physiology, testosterone. *Statpearl Publishers*, 2020.
[29]. Ashby J, Tinwell H, Lefevre PA, Joiner R, Haseman J. The effect on sperm production in adult Sprague-Dawley rats exposed by gavage to bisphenol A between postnatal days 91–97. *J Toxicol Sci* 2003; 74:129-138.