RESEARCH ARTICLE

Amelioration of Spermiogram and Reproductive Hormonal Changes with *Nigella Sativa* and *Eurycoma Longifolia* Treatments in Rats Exposed to Lead Acetate

Mohammed A Assi[1,2]

[1]*Department of Community Health, College of Health and Medical Techniques, Al_Furat Al_Awsat Technical University, Iraq*
[2]*Department of Veterinary Preclinical Sciences, Faculty of Veterinary Medicine, Universiti Putra Malaysia, 43400, Serdang, Selangor, Malaysia*

Received: 04th July, 19; Revised: 08th August, 19, Accepted: 05th September, 19; Available Online: 25th September, 2019

ABSTRACT

Aim The current study was designed to estimate the influence of *Nigella sativa* (NS) and *Eurycoma longifolia* pre-treatment and Lead acetate administration on the reproductive hormonal and stereogram of rats.

Materials and Methods: Five groups of Sprague Dawley rats have been divided into six rats each. Distilled water was given to Group 1 (NC) and set as the negative control. Lead acetate 20 mg/kg/day orally for one month was administered to Group 2 (PC) and set as the positive control. Group 3 (T1) were administered 20 mg/kg LA and 300 mg/kg *Nigella sativa* both orally/day for one month. Group 4 (T2) were received 20 mg/kg LA and 500 mg/kg *Eurycoma longifolia* orally/day for one month. Group 5 (T3) were administered 300 mg/kg *Nigella sativa*, 20 mg/kg LA, and 500 mg/kg *Eurycoma longifolia* orally/day for one month.

Results: In this study, five groups of Sprague Dawley rats have been divided into 6 male rats each and grouped as follows; Group 1(Negative control); Group 2 (Positive control; 20mg/kg lead acetate); Group 3 (LA 20mg/kg + NS 300mg/kg); Group 4 (LA 20mg/kg + EL 500mg/kg); Group 5 (LA 20mg/kg+ NS 300mg/kg + EL 500mg/kg). All administrations were given daily for 30 days. The rats were euthanized, and serum and epididymal samples were collected for reproductive hormonal assays and spermiogram determination. The estrogen concentration was less ($p < 0.05$) in the EL treated group, whereas in the positive control (PC), the concentration of follicle stimulation hormones, as well as luteinizing hormones, were lower ($p < 0.05$). Testosterone concentration was found to be higher ($p < 0.05$) in the PC in comparison to other groups. The motility, concentration, and viability of the sperm were all low in the PC and high ($p < 0.05$) in the treatment groups. The sperm abnormality was higher in the PC in compared with other groups.

Conclusion: In conclusion, this study showed the preventive effect of *Nigella sativa* and *Eurycoma longifolia* administration against alterations in spirogram and hormones caused by LA.

Keywords: *Eurycoma longifolia*, Infertility, Lead acetate, *Nigella sativa*

International Journal of Pharmaceutical Quality Assurance (2019); DOI: 10.25258/ijpqa.10.3.20

How to cite this article: Assi, M.A. (2019). Amelioration of Spermiogram and Reproductive Hormonal Changes with *Nigella Sativa* and *Eurycoma Longifolia* Treatments in Rats Exposed to Lead Acetate. International Journal of Pharmaceutical Quality Assurance 10(3): 50-54.

Source of support: Nil

Conflict of interest: The author wishes to declare that there is no competing interest what so ever about this manuscript.

INTRODUCTION

Numerous medical situations could be affected by a lot of herbs, which have shown to hold possible medicinal effects, and therefore, the roles of NS and EL have appeared as therapeutic values. For hundreds of years, people all over the world used numerous healing herbs for alleviating the symptoms and signs of several illnesses.[1]

Nigella sativa (NS) is a plant that is flowering yearly and growing nearly in all the regions around the globe, generally found in Southern Europe, the Middle East, and Northern Africa, and could also be found most commonly in South and Southwest Asia and,[2] Besides *N. sativa* is recognized as nigella, nutmeg flower, fennel flower, Roman coriander, black cumin, black seed, black caraway, "habbat al-barakah" (in the Middle East), "kalonji" (in India), "Kalo jeera" (in Bangladesh), and "Hak Jung Chou" (in China). *N. sativa* is a plant from the family of Ranunculaceae.[3] *N. sativa* seed and its oil have many active components that could be isolated and used such as alpha-hederin, nigellidine, nigellicine, nigellimine-N-oxide (NO), carvacrol, thymol, dithymoquinone, thymohydroquinone, and thymoquinone. However, *in* vitro and in vivo studies have been carried out to investigate the pharmacological properties of *N.*

**Author for Correspondence: razaq_assi@yahoo.com, kuh.muh@atu.edu.iq*

sativa and its ingredients on laboratory animals and humans. Those studies revealed that there is a wide range of activities in *N. sativa* and its ingredients, such as anticancer, antioxidant, antiparasitic, antimicrobial, antiasthmatic, antihypertensive, hypoglycemic, anti-inflammatory, immune-stimulatory, and pharmacological effects.[4] As a traditional medication, crude extracts of *N. sativa* were used to treat numerous conditions like cancer, microbial infections, skin disorders, diarrhea, digestive problems, liver and kidney diseases, cardiovascular disease, hypertension, asthma, diabetes, as well as using it as analgesics, liver tonics, bronchodilators, and appetite stimulants, etc.[5]

Eurycoma longifolia (EL) is traditionally used as a remedy for improving the fertility sexual ability, and male libido. The ethnic population recognizes it as 'Tongkat Ali.' Currently, numerous carbonated drinks, coffee, and tea, pre-mixed with the root extract, are commercially obtainable for the libido and general health perfection.[6] It now knows to improve the rat spermatogenesis by affecting the hypothalamic-pituitary-gonadal axis. Generally, the extract of *the root of E. longifolia* were used as traditional medicine for syphilis and glandular swelling, stress, osteoporosis, leukemia, increased strength, increased energy, fever, exercise recovery, constipation, aches, anxiety, diabetes, cancer, malaria, aging, sexual dysfunction, as well as health supplement, appetite stimulant, antibiotic, and aphrodisiac.[7]

Recently, the interest was increased in the influence of environmental and occupational contacts to contaminated toxins to the declining concentration of the sperms and the infertility of men.[8] There are epidemiological indications that exposure to industrial metal aerosols may be detrimental to the male reproduction system, and lead is one of the main elements that have been verified as negative to fertility.[9] According to the exposure time and dose, lead exposure affects sperm count, reductions in seminiferous tubule diameter (STD), cellular degeneration, testicular atrophy, and sterility and infertility, and therefore, the role of the lead in subfertility factor of men is of certain current interest, particularly in professional workers.[8] Genetic, environmental, and nutritional factors have a great influence on the time to reach puberty. Therefore, it is difficult to predict the age at which a particular individual will achieve his reproductive maturity, i.e., the sperm production corresponding to that of an adult. Besides, biomedical information about the age of first conscious ejaculation, the presence or absence of spermatozoa in seminal fluid, the biological characteristics of spermatozoa during this maturational process, and the time required to reach adult spermatogenesis.[10]

MATERIALS AND METHODS

Preparation of *Nigella sativa*, Lead acetate, and *Eurycoma longifolia* solutions

In order to get a water-soluble powder of the black seeds (*Nigella sativa*), the seeds have been obtained, washed, and grounded for 10 minutes via an electric grinder (Model HGB2WTS3, U.S.A., National Blender 8011S). Then, *Nigella sativa* powder has been suspended with a concentration of 30 g/L and equipped for this experiment. Using a gavage tube, a concentration of 20 mg/kg body weight of Lead acetate (Oxford Lab. Co., India) has been directed to the rats dissolved in distilled water, whereas a concentration of 500 mg/kg of *Eurycoma longifolia* has been given orally to the rats via a gavage tube after dissolved in distilled water.

Ethical Statement

The Institutional Animal Care and Use Committee (IACUC) has permitted the experimental protocol of animals applied in this research with reference number: UPM/IACUC/AUP-R047/2015, according to the standard rules on using and carefulness of laboratory animals.

Animal grouping and Treatment

Five groups of Sprague Dawley rats have been divided into six rats each. The bedding was varied weekly to maintain hygienic conditions. Before the experiment started, the animals have been kept for 15 days for acclimatization. Distilled water was given to Group 1 (NC) and set as the negative control. Lead acetate 20 mg/kg/day orally for one month was administered to Group 2 (PC) and set as the positive control.[8] Group 3 (T1) were administered 20 mg/kg LA and 300 mg/kg *Nigella sativa* both orally/day for one month.[11] Group 4 (T2) were received 20 mg/kg LA and 500 mg/kg *Eurycoma longifolia* orally/day for one month.[12] Group 5 (T3) were administered 300 mg/kg *Nigella sativa*, 20 mg/kg LA, and 500 mg/kg *Eurycoma longifolia* orally/day for one month.

Sperm counts (Sperm abnormality, General Motility, Individual motility, Viability, and Concentration)

For the right caudal epididymis to release the spermatozoa, a surgical micro-scissor has been used to cut the epididymal tubules into nearly 200 pieces and positioned in 2 mL of PBS. Then, for further testing, we directly incubate epididymal semen suspension (ESS) at 37ºC. To get a dilution ratio of 1:20, 10 µL of ESS has been mixed with 190 µL of formal saline. As stated previously by,[13] the total sperm concentration (TSC) has been calculated by a Neubauer hemocytometer. Under a phase-contrast microscope at a 100 × enlargement, a 5 µL of the suspension has been placed on a glass slide and inspected in order to estimate the mass motility. The spermatozoa percentage of motility has classified mass motility. Furthermore, 10 µL of ESS on a slip-covered glass slide has been inspected under a phase-contrast microscope at 400 × magnification to calculate general motility. For determination of the morphology and capability of the sperm, Eosin-negrosin stain has been used for staining sperm.

Statistical analysis

Using one-way analysis of variance (ANOVA) with Tukey multiple comparison *post hoc* tests, Data achieved from the spermiogram were briefed as mean ± S.E and investigated with Graph Pad Prism 6.0.

RESULTS AND DISCUSSION

The previous studies stated that the exposure of man to lead could lessen the quality of man semen, where they indicate that exposure to lead decrease the motility and counts of the sperm and alter morphology.[14] This study reported the potential positive effects of EL and NS and the improvement of the reproductive system in rats exposed to lead acetate toxicity, and however, there are important associations between lead and reproductive system in relation to motility, sperm density, viability counts and morphology.[15] In this study, the concentration of the sperms was less in PC ($p < 0.05$) in comparison with NS and EL groups where was a high ($p < 0.05$), the viability and motility were less in the PC and more ($p < 0.05$) in the treatment groups, this is in agreement with the study reported by Telisman, S., et al.[16] The sperm concentration, viability and motility were all higher in the mixed group ($p < 0.05$) as compared with other groups, whereas no difference at the significance between group 3 treated with NS and group 4 treated with EL as shown in Figure 1 and 2, and this is in agreement with Assi, M. A., et al.[17] The percentage of malformed sperm shapes was significantly increased at the PC group, but this percentage became lower by giving an increased concentration of both NS and EL groups, this in agreement with[18] as shown in Figure 3. The characteristics of both individual and general motility percentages were significantly decreased at the PC group in comparison with other groups, while there was almost no difference between NS and EL treated groups, and agreed to A.G. Miah, et al.[19] as shown in Figures 4 and 5. *N. sativa* seed extract effects on reproductive organs of male albino rats have been reported and it has been revealed the germinal layer thickness of somniferous tubules increased significantly, whereas the epithelial layer thickness that lined the tubules also decreased significantly.[20] A study by M.S. Akhtar, et al.[21] was carried out on mammalian fertility to demonstrate the impact of certain plants and herbs extract, and however, that study attributed the improvement to follicle-stimulating hormone (FSH) and luteinizing hormone (LH) induction level.

In this study, the levels of hormones related to the reproductive process were estimated. The study revealed that there is noticed increase ($p < 0.05$) in the concentrations of FSH at the group 3 and group 4 while this became higher ($p < 0.05$) at the Group 5 (mixed group) in comparison with

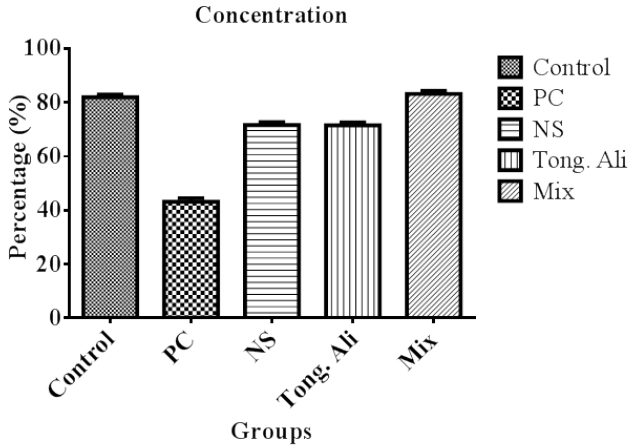

Figure 1: Graph showing sperm concentration in rats administered LA, NS, and *Eurycoma long* treatments.

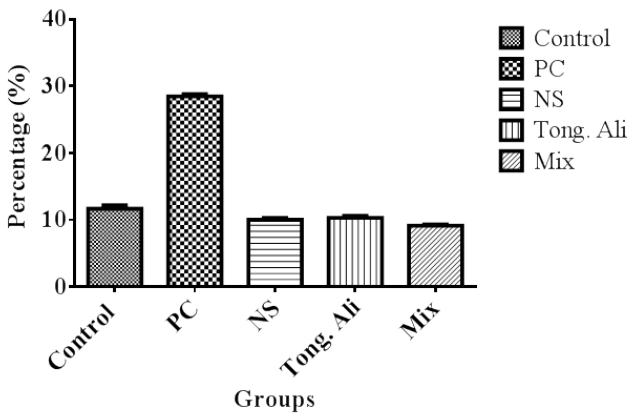

Figure 3: Showing Graph Percentage of abnormality (%) in rats administered LA, NS, and *Eurycoma long* treatments.

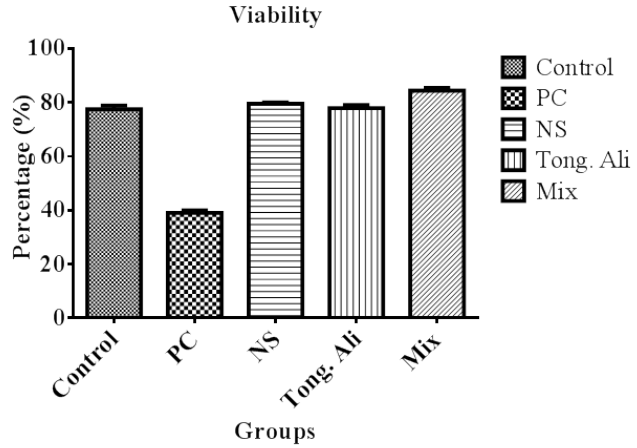

Figure 2: Graph showing sperm viability in rats administered LA, NS, and *Eurycoma long* treatments.

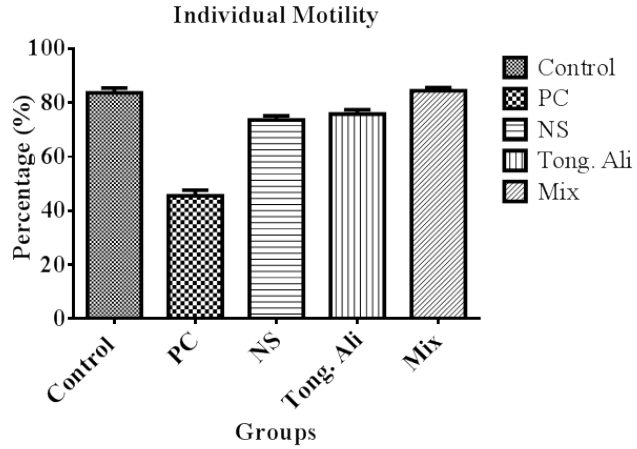

Figure 4: Graph showing Individual motility (%) in rats administered LA, NS, and *Eurycoma long* treatments.

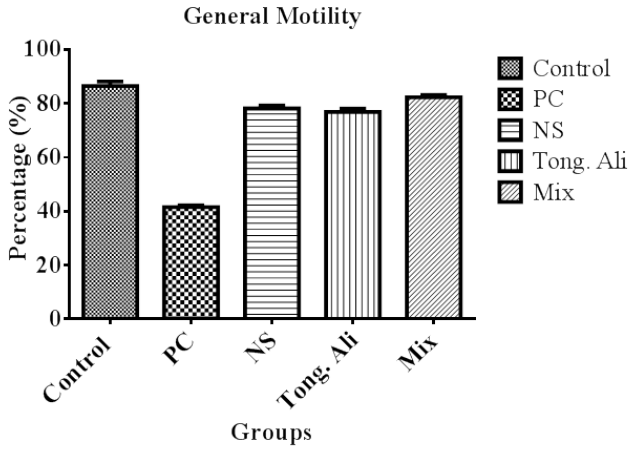

Figure 5: Graph showing General motility (%) in rats administered LA, NS, and *Eurycoma long* treatments.

Group 1 (PC) where it lower as shown in Figure 6. The LA effects on the male hormones was dependent on the treatment dose and exposure time, whereas in our study the testosterone concentration was achieved higher levels at the PC group ($p<0.05$) in comparison with the other four groups, but there is little difference in the concentration between NS group and EL group as shown in Figure 7 which is in agreement with L. Allouche, et al.[22] On the other hand, another study done by M. Dorostghoal, et al.[8] was dissimilar to our study reported that testosterone concentration was decreased after three to four months of lead administration; this is due to the dose and duration period. Also, the testosterone level was found to be elevated significantly with EL treatment, and this was in agreement with F. Al-Joufi, et al.[23] In normal rats, the levels of LH, FSH, and testosterone was reported to increase after the administration or treatment with NS after one to two months.[24] The LH at the treated groups achieved high levels ($p<0.05$) in contrast to the control group and PC, but these levels were higher in the mixed group as shown in figure 8, and agreed to M. A. Assi, et al.[15]

The results in Figure 9 showed that there is a significant decrease ($p<0.05$) in the concentration of estrogen (EST) in the EL treated group in comparison with the control group and PC, but there is a high increase ($p<0.05$) in NS treated group. Yet, there is no significant difference between the control group and group 5 (mixed group).

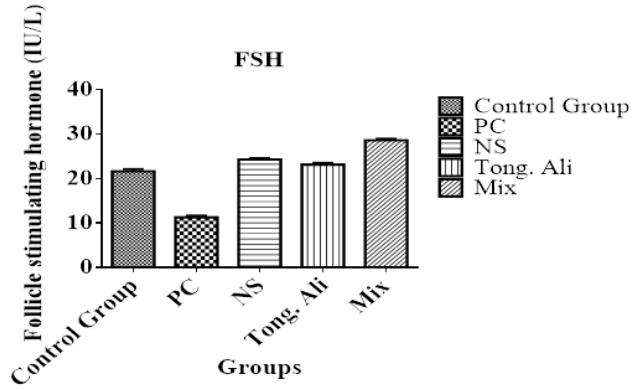

Figure 6: Graph showing the hormonal concentration of the follicle-stimulating hormone in rats administered LA, NS, and *Eurycoma long* treatments.

CONCLUSION

Lead acetate has shown deleterious effects in the spermiogram of rats, while pre-treatment with *Nigella sativa* and *Eurycoma longifolia* played an important role in reducing these harmful effects.

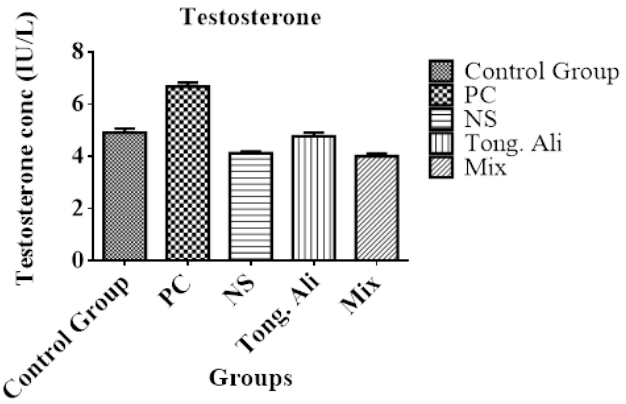

Figure 7: Graph showing a hormonal concentration of testosterone in rats administered LA, NS, and *Eurycoma long* treatments.

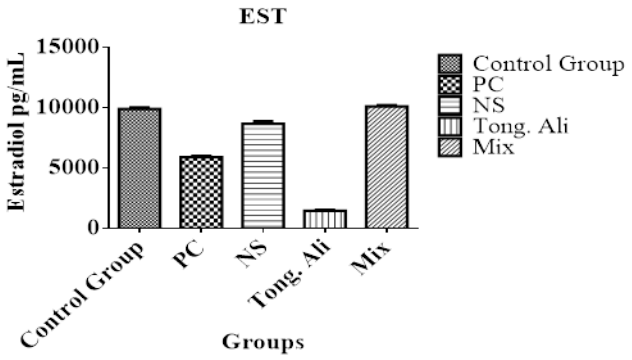

Figure 9: Graph showing hormonal concentration of estradiol in rats administered LA, NS, and *Eurycoma long* treatments.

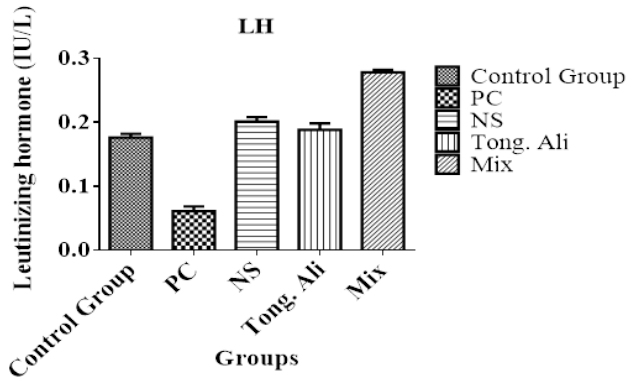

Figure 8: Graph showing hormonal concentration of luteinizing hormone in rats administered LA, NS, and *Eurycoma long* treatments.

AUTHOR CONTRIBUTION

A considerable contribution to this manuscript has been made by the author in this manuscript. The author, Mohammed Abdulrazzaq Assi, contributed to both design and conception. Mohammed Abdulrazzaq Assi has drafted the manuscript; also, he has well edited the paper, revised, and contributed to the intellectual content. The author has approved the final draft for submission and the list of authors.

ACKNOWLEDGMENT

We want to acknowledge the Ministry of Higher Education (MOHE), Malaysia, through its Fundamental Research Grant Scheme for the financial support of this research.

REFERENCES

1. Kooti, W., Hasanzadeh-Noohi, Z., Sharafi-Ahvazi, N., Asadi-Samani, M., & Ashtary-Larky, D. (2016). Phytochemistry, pharmacology, and therapeutic uses of black seed (Nigella sativa). *Chin J Nat Medicines*, 14(10), 732-745.
2. Banerjee, S., Azmi, A. S., Padhye, S., Singh, M. W., Baruah, J. B., Philip, P. A., & Mohammad, R. M. (2010). Structure-activity studies on therapeutic potential of Thymoquinone analogs in pancreatic cancer. *Pharm Res*, 27(6), 1146-1158.
3. Salem, M. L. (2005). Immunomodulatory and therapeutic properties of the Nigella sativa L. seed. *Int Immunopharmacol*, 5(13-14), 1749-1770.
4. Aljabre, S. H., Alakloby, O. M., & Randhawa, M. A. (2015). Dermatological effects of Nigella sativa. *J Dermatol Dermatol Sur*, 19(2), 92-98.
5. Khan, A., Chen, H. C., Tania, M., & Zhang, D. Z. (2011). Anticancer activities of Nigella sativa (black cumin). *Afr J Tradit Complement Altern Med*, 8(5S).
6. Low, B. S., Choi, S. B., Wahab, H. A., Das, P. K., & Chan, K. L. (2013). Eurycomanone, the major quassinoid in Eurycoma longifolia root extract increases spermatogenesis by inhibiting the activity of phosphodiesterase and aromatase in steroidogenesis. *J Ethnopharmacol*, 149(1), 201-207.
7. Rehman, S., Choe, K., & Yoo, H. (2016). Review on a traditional herbal medicine, Eurycoma longifolia Jack (Tongkat Ali): its traditional uses, chemistry, evidence-based pharmacology and toxicology. *Mol,* 21(3), 331.
8. Dorostghoal, M., Dezfoolian, A., & Sorooshnia, F. (2011). Effects of maternal lead acetate exposure during lactation on postnatal development of testis in offspring wistar rats. *Iranian Journal of Basic Medical Sciences*, 14(2), 122-131.
9. Benoff, S., Jacob, A., & Hurley, I. R. (2000). Male infertility and environmental exposure to lead and cadmium. *Hum Reprod Update*, 6(2), 107-121.
10. Vásquez, F., Soler, C., Camps, P., Valverde, A., & García-Molina, A. (2016). Spermiogram and sperm head morphometry assessed by multivariate cluster analysis results during adolescence (12-18 years) and the effect of varicocele. *Asian J Androl*, 18(6), 824.
11. Saheb, S. H., Desai, S. D., Das, K. K., & Haseena, S. (2016). Antioxidant effect of Nigella sativa seed powder and thymoquinone in normal and sterptozotocine induced diabetic albino rats. *Int J Intg Med Sci*, 3, 242-247.
12. Zanoli, P., Zavatti, M., Montanari, C., & Baraldi, M. (2009). Influence of Eurycoma longifolia on the copulatory activity of sexually sluggish and impotent male rats. *J Ethnopharmacol*, 126(2), 308-313.
13. Yokoi, K., Uthus, E. O., & Nielsen, F. H. (2003). Nickel deficiency diminishes sperm quantity and movement in rats. *Biol Trace Elem Res*, 93(1-3), 141-153.
14. Saleh, A. H. (2018). The Potential Effect of Grape Seeds Extract against Lead toxicity That Induces Infertility to Male Rats. *Tikrit Journal of Pure Science*, 23(1), 70-74.
15. Assi, M. A., Hezmee, M. N. M., Haron, A. W., Sabri, M. Y. M., & Rajion, M. A. (2016). The detrimental effects of lead on human and animal health. *Vet World*, 9(6), 660.
16. Telisman, S., Cvitković, P., Jurasović, J., Pizent, A., Gavella, M., & Rocić, B. (2000). Semen quality and reproductive endocrine function in relation to biomarkers of lead, cadmium, zinc, and copper in men. *Environ Health Perspect*, 108(1), 45-53.
17. Assi, M. A., Hezmee, M. N. M., Abba, Y., Rajion, M. A., Wahid, H., & Yusof, M. S. M. (2017). Assessment of therapeutic effects of Nigella sativa against chronic lead acetate-induced reproductive dysfunction in male Sprague-Dawley rats. *Com Clin Pathol*, 26(1), 87-97.
18. Mosbah, R., Djerrou, Z., & Mantovani, A. (2018). Protective effect of Nigella sativa oil against acetamiprid induced reproductive toxicity in male rats. *Drug Chem Toxicol*, 41(2), 206-212.
19. Miah, A. G., Bathgate, R., Hamano, K. I., & Salma, U. (2018). Effects of pre-freeze Nigella sativa oil supplementation on cryosurvival of ovine spermatozoa. *Reprod Domes Anim*, 53(6), 1424-1433.
20. Mohammad, M. A., Mohamad, M. M., & Dradka, H. (2009). Effects of black seeds (Nigella sativa) on spermatogenesis and fertility of male albino rats. *Research Journal of Medicine and Medical Sciences*, 4(2), 386-390.
21. Akhtar, M. S., Nasir, Z., & Abid, A. R. (2003). Effect of feeding powdered Nigella sativa L. seeds on poultry egg production and their suitability for human consumption. *Vet Arhiv*, 73(3), 181-190.
22. Allouche, L., Hamadouche, M., & Touabti, A. (2009). Chronic effects of low lead levels on sperm quality, gonadotropins and testosterone in albino rats. *Exp Toxicol Pathol*, 61(5), 503-510.
23. Al-Joufi, F., Saxena, A. K., Al-Ani, I. M., Talib, N. A., Mokhtar, R. H., & Ku-Zaifah, N. (2017). Anti-atherosclerotic Effects of Eurycoma Longifolia (Tongkat Ali) in Rats Fed on High-fat Diet. *International Medical Journal Malaysia*, 16(1).
24. Mahdavi, R., Heshmati, J., & Namazi, N. (2015). Effects of black seeds (Nigella sativa) on male infertility: A systematic review. *J. Herb. Med., 5*(3), 133-139.