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Restoration of spermatozoa: Antioxidant effects of virgin coconut oil and corn oil in Phenytoin induced toxicity of seminiferous tubules in rats.

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ABSTRACT… Objectives: Evaluate the antioxidant effects of Virgin Coconut Oil and Corn Oil in Phenytoin induced toxicity of seminiferous tubules in rats. **Study Design:** Experimental study. **Setting:** Al-Tibri Medical College and Hospital, **Period:** November 2019 to May 2020. **Material & Methods:** 48 male albino rats (weighing between 150-250gms) were selected based on a randomized sampling technique and divided into four groups, 12 rats per group and euthanized on the 4th, 5th, and 6th week of the study. Group A control, B was given Phenytoin, C received phenytoin +virgin coconut oil, and D received Phenytoin + corn oil once daily for six weeks. The sample of sperm was taken from epididymal fluid and counted by using the Neubauer chamber. The data was analyzed through SPSS version 21.0. Mean values were compared through the application of One way ANOVA followed by post hoc Tukey's test. P-value was considered significant <0.05. **Results:** In Group B, there was a significant decrease in spermatozoa count due to Phenytoin induced toxicity. At the same time, there was a considerable restoration in the number of spermatozoa in group C compared to D. **Conclusion:** In Group B, there was a significant decrease in spermatozoa count due to Phenytoin induced toxicity of seminiferous tubules, and those animals received the virgin coconut oil along with phenytoin help in the restoration of numbers of spermatozoa as compared to group D. Virgin coconut oil showed significant antioxidant role in comparison with corn oil.

Key words: Corn Oil, Phenytoin, Seminiferous Tubules, Sperm Count, Virgin Coconut Oil.

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INTRODUCTION

Phenytoin is an anti-epileptic agent used in the treatment of generalized tonic-clonic seizures and status epilepticus.[1,2] The drug first introduced back in 1908 in Germany at the University of Kiel is a commonly used anti-convulsant agent belonging to the family of hydantoin organic compounds, comprising both aromatic and non-aromatic sedative properties. Phenytoin suppresses the electrical activity of the neuron preventing the spread of electrical discharge in seizures. It blocks the post-tetanic enhancement that is the potentiating factor of a post-synaptic action potential occurring due to a repetition of action potential.[3] Regardless of Phenytoin's potential benefits in treating epilepsy, it is known for causing different adverse effects in the body. One of these is related to male impotence. Drugs such as Phenytoin and other anti-convulsant medications have caused drug-dependent complications such as decreased testicular volume, abnormality in sperm morphology, reduction in motility, and a reduced sperm count.[4] All these effects will collectively lead to hypo sexuality among male patients, impacting their lives. Supplementation may be used to counteract this impotence in male patients. One of these includes Antioxidant agents that can counteract the oxidative stress created in the male testes helping to neutralize the Reactive Oxygen Species (ROS).[5] Several scientific studies have proven the beneficial effects of antioxidant agents in protection against the deleterious effects of ROS, leading to male infertility. Virgin Coconut Oil and Corn Oil are different types of oils, containing antioxidant properties that help

boost the immune system, reduce weight, and prevent heart diseases.[6,7] If Virgin Coconut Oil and Corn oil contain antioxidant effects, they may be able to improve upon the male fertility among those individuals taking Phenytoin. Therefore, a study was conducted to assess the restoration of male fertility in Phenytoin induced toxicity of seminiferous tubules by evaluating the antioxidant effects of Virgin Coconut Oil and Corn Oil.

MATERIAL & METHODS

After taking approval from the ethical committee, an experimental study was conducted for a period of 6 months in the Anatomy Department of Al-Tibri Medical College and Hospital, Isra University Karachi Campus, from November 2019 to May 2020. For this study, male albino Rats were taken from the animal house of Al-Tibri Medical College and Hospital. Total 48 numbers of rats with the weight between 150-250gms were included in the study through randomized sampling. After the sampling and selection of the 48 male albino rats based on the inclusion as mentioned above and exclusion criteria, they were divided into 4 groups of 12 animals per group; the grouping was based on the following treatment plan.

Group A: The Control Group received an intraperitoneal injection of 1 unit of normal saline with a regular daily diet.

Group B: Received Phenytoin 10mg/kg/body wt. Intra-peritoneal injection once daily.

Group C: Received Virgin Coconut Oil (6.7ml) along with Phenytoin 10mg/kg/body wt. Intra-peritoneal injection once daily.

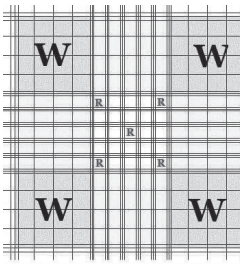
Group D: Received Corn Oil (2.5ml) along with Phenytoin 10mg/kg/body wt. Intra-peritoneal injection once daily.

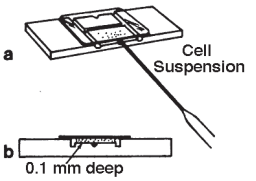
All the animals were put in a large cage under the control of water, diet, and light duration (12 hours light, 12 hours dark). After giving anesthesia with ethanol, animals from each group were euthanized on the 4th, 5th, and 6th week of the study. The epididymis was cut and put into the normal saline solution and left on the petri dish for a few minutes until a change in coloration from transparent to greyish was visible. To evaluate the spermatozoa count, a single drop of diluted spermatozoa was placed onto a Neubauer counting chamber, after which the number of spermatozoa was counted on a large square as it is used in assessing the WBC Count. The formula for calculating the spermatozoa count, when five small squares within the large center square are counted is as follows:

Number of counted Spermatozoa x dilution factor/ volume x 1000= sperm/ml

If dilution is 1:20, and the usual 5 small squares are counted, then the formula may be simplified as follows:

Sperm/ml= spermatozoa counted x 1,000,000





Data analysis was carried out on SPSS version 20.0, with all values being expressed as mean ± S.D. the four groups were compared through the One Way ANOVA followed by Post hoc Tukey's Test. The statistical significance was taken at $P < 0.05$.

RESULTS

Table-I shows the comparison of the number of spermatozoa among the different groups. Group B shows a significant reduction in the number of spermatozoa due to toxicity of Phenytoin and Phenytoin induced alteration in male fertility on prolonged use. Group C and D were treated with potent antioxidants, while the virgin coconut oil was able to restore male fertility in comparison with corn oil. Figure-1 shows Mean value of numbers of spermatozoa among different therapeutic groups.

Weeks	Mean Spermatozoa Count in Group B	Groups	Mean Spermatozoa Count	Comparison of Groups	P-Value
4th week	B 49.6 ± 1.34	A	87.4 ± 2.96	B vs. A	<0.001
		C	76.0 ± 1.140	B vs. C	<0.001
		D	53.6 ± 1.48	B vs. D	0.294
5th week	B 30.2 ± 1.224	A	87.4 ± 2.28	B vs. A	<0.001
		C	84.6 ± 4.52	B vs. C	<0.001
		D	31.8 ± 1.140	B vs. D	0.847
6th week	B 18.8 ± 1.14	A	89.2 ± 1.516	B vs. A	<0.001
		C	86.8 ± 3.93	B vs. C	<0.001
		D	19.8 ± 1.140	B vs. D	0.748

Table-I. Shows the comparison of number of spermatozoa among the different groups One way ANOVA (post hoc Tukey's) p <0.05

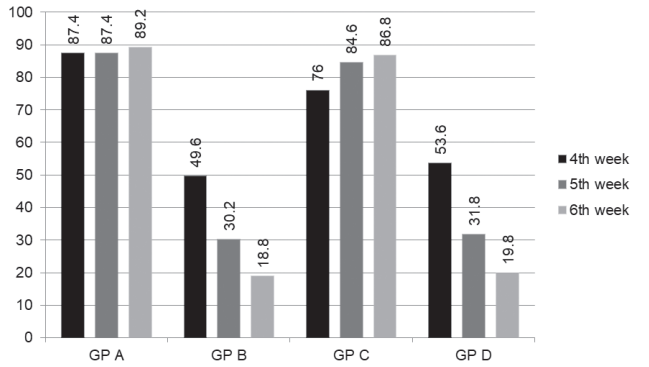


Figure1: Shows Mean sperm Count among different therapeutic groups

DISCUSSION

The number of couples that seek consultation for infertility problems has steadily risen over the years, affecting 10-15% of all the sexually active population.[8,9] While semen analysis has multiple measured parameters, one of the most significant is the abnormality in sperm count.[10,11] Epilepsy has been known to have an association with Male Factor Infertility (MFI), with studies showing epileptic males to have a significantly lower fertility rate and to be at a higher risk of developing hyposexuality than the general population.[12] Phenytoin also causes a similar effect as it decreases the sperm count. Our study showed a reduction in the spermatozoa in the Phenytoin-based group (Group B) and the group taking both Phenytoin and Corn Oil (Group D). However, we saw a significant rise in the spermatozoa population with those rats receiving virgin coconut oil and Phenytoin (Group C). A similar study was conducted by G.D Nayeri Kaman et al.: 2002 to assess the phenytoin toxicity on the testis, which showed contradictory results to our study by demonstrating no reduction in the sperm count.[13] Whereas another study conducted to investigate the effects of chronic use of Phenytoin on male reproductive functions in Sprague-Dawley rats also demonstrated a significant (P-Value <0.05) reduction infertility of male rats, these results are similar to the results of our study[14] The group given with Corn Oil showed no substantial rise in the sperm count, rather there was a decrease in the number of spermatozoa count. In accordance with our study, another investigation with Moringa Oleifera leaves extracts in rats along with cryptorchid and non-cryptorchid was given for 15 days of treatment compared with control. Similar results were seen concerning our study, which showed that the group treated with Corn Oil significantly decreased germinal cells compared to the control group and Coconut Oil group along with phenytoin medication for different durations of treatment.[15] Moreover, Naji et al.: 2013 also demonstrated a significant number of sperm count in tocopherol extracts compared to corn oil. These findings are following the present study.[16] The group being given Virgin Coconut Oil (Group C) showed significant improvement, and a gradual restoration in the number of spermatozoa count even after being injected with Phenytoin. This study is similar to another study in which animals treated with ethanol showed a reduction in the sperm count. In contrast, the administration of Virgin Coconut Oil improved the antioxidant

statuses, which eventually lead to a significant increase in sperm count.[17] Another study showed that virgin coconut oil being given to animals also injected with Highly Antiretroviral therapy showed significant restoration of the number of sperms. Similar effects were also seen in our study in the group that administered Phenytoin and Virgin Coconut Oil.[18] Virgin Coconut Oil is vastly right in polyunsaturated fatty acids. The ratio of polyunsaturated fatty acids is crucial in causing an inhibitory action that will influence lipid peroxidation. Our study demonstrated the beneficial antioxidant effects of virgin coconut oil in restoring male fertility. This same antioxidant effect, also seen in another study in which Virgin Coconut Oil showed a significant antioxidant effect compared to Copra oil and Groundnut Oil, the same things were also demonstrated in our study.[19] Overall, we say a better antioxidant effect of Virgin Coconut Oil that helped restore function of the male testes and helped in reversing the adverse effects and oxidative stress developed by Phenytoin therapy.

CONCLUSION

The study has concluded that Phenytoin's toxic effects can be reversed upon the usage of antioxidant agents. Virgin Coconut Oil helped restore the spermatozoa count in male rats due to their antioxidant properties compared with others. The study results revealed that if virgin coconut oil uses along with Phenytoin, thus it will help to restore male fertility, which can be altered by a given drug. More studies are needed to assess further and evaluate the effects of Virgin Coconut Oil and Corn Oil's effect on testicular functions and morphology.

REFERENCES

1. Gupta A, Yek C, Hendler RS. **Phenytoin toxicity.** Jama. 2017 Jun 20; 317(23):2445-6.
2. Browne TR, Holmes GL. **Handbook of epilepsy.** Jones & Bartlett Learning; 2008.
3. Katzung BG, Susan B, Trevor AJ. **Basic & clinical pharmacy.** 13th Edition Chapter 24.2016; 403-27.
4. Brezina PR, Yunus FN, Zhao Y. **Effects of pharmaceutical medications on male fertility.** Journal of reproduction & infertility. 2012 Jan; 13(1):3.
5. Agarwal A, Sekhon LH. **The role of antioxidant therapy in the treatment of male infertility.** Human fertility. 2010 Dec 1; 13(4):217-25.
6. Dayrit CS. **Coconut oil: Atherogenic or not.** Philippine Journal of Cardiology. 2003; 31(3):97-104.
7. Orhun GE. **Maize for life.** International Journal of Food Science and Nutrition Engineering. 2013; 3(2):13-6.
8. Rabin DS, Qadeer U, Steir VE. **A cost and outcome model of fertility treatment in a managed care environment.** Fertility and sterility. 1996 Dec 1; 66(6):896-903.
9. Singh K, Jaiswal D. **Human male infertility: A complex multifactorial phenotype.** Reproductive sciences. 2011 May 1; 18(5):418-25.
10. Natali A, Turek PJ. **An assessment of new sperm tests for male infertility.** Urology. 2011 May 1; 77(5):1027-34.
11. Baker HW. **Male infertility.** Endocrinology and metabolism clinics of North America. 1994 Dec 1; 23(4):783-93.
12. Webber MP, Hauser WA, Ottman R, Annegesr JF. **Fertility in persons with epilepsy: 1935–1974.** Epilepsia. 1986 Dec; 27(6):746-52.
13. Nayeri Kaman GD, Motiollah F. **Phenytoin and the reproductive system.** Medical Journal of The Islamic Republic of Iran (MJIRI). 2002 May 10; 16(1):35-40.
14. Olutunde PF, Emmanuel OS, Moyosore SA, Olusola AA, Olutoyin OO, Ebenezer AA, Abiodun O, Olakunle JO. **Chronic use of phenytoin reversibly suppresses fertility in male Sprague-Dawley rats.** Scientific Research and Essays. 2010 May 31; 5(9):999-1004.
15. Afolabi AO, Aderoju HA, Alagbonsi IA. **Effects of methanolic extract of Moringa Oleifera leave on semen and biochemical parameters in cryptorchid rats.** African Journal of Traditional, Complementary and Alternative Medicines. 2013 Aug 14; 10(5):230-5.
16. Naji NS, Abood FN. **Effect of tocopherol extraction of Lepidium sativum seeds in sperm parameters of white male rabbits.** Journal of Biology, Agriculture and Healthcare. 2013; 3:43-8.
17. Dosumu OO, Akinola OB, Akang EN. **Alcohol-induced testicular oxidative stress and cholesterol homeostasis in rats–The therapeutic potential of virgin coconut oil.** Middle East Fertility Society Journal. 2012 Jun 1; 17(2):122-8.

18. Ogedengbe OO, Jegede AI, Onanuga IO, Offor U, Naidu EC, Peter AI, Azu OO. **Coconut oil extract mitigates testicular injury following adjuvant treatment with antiretroviral drugs.** Toxicological research. 2016; 32(4):317.

19. Nevin KG, Rajamohan T. **Virgin coconut oil supplemented diet increases the antioxidant status in rats.** Food chemistry. 2006; 99(2):260-6.

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2	Hina Khan	Conceptualization and Manuscript writing.	
3	Shahid Hussain Soomro	Critical review.	
4	Asad Raza Jiskani	Data analysis	
5	Raja Faisal	Study conduction.	
6	Farheen Hameed	Manuscript writing.	