



ORIGINAL ARTICLE

## Resurgence of sperm motility: An effect of antioxidant oils in phenytoin induced toxicity of rat testis.

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**ABSTRACT... Objective:** To evaluate the restoration of sperm motility with the antioxidant oils (Virgin coconut oil and Corn oil) therapy in Phenytoin induced testicular toxicity of rats. **Study Design:** Experimental Study. **Setting:** Al-Tibri Medical College and Hospital. **Period:** November 2019 to March 2020. **Material & Methods:** Forty-eight male albino rats weighing between 150-250gms were taken from the animal house of Al-Tibri medical college and placed in 4 groups, each with 12 rats. Group A was the control group receiving saline, Group B received Phenytoin only, Group C received Phenytoin and Virgin coconut oil, and Group D received Phenytoin and Corn oil. The rats were euthanized on the 4<sup>th</sup>, 5<sup>th</sup>, and 6<sup>th</sup> week of the study. Their epididymis was cut, and the epididymal fluid was placed under the microscope to determine sperm motility. Data was analyzed using SPSS Version 20.0 with one-way ANOVA followed with Post-Hoc Tukey's test to compare statistical significance. **Results:** The significant difference was seen in mean % sperm motility when comparing Group B vs. A ( $\leq 0.001$ ) and Group B vs. C ( $\leq 0.001$ ) during the 4<sup>th</sup>, 5<sup>th</sup>, and 6<sup>th</sup>. **Conclusion:** Virgin Coconut Oil and Corn Oil both restored sperm motility. A higher percentage was observed in Virgin Coconut Oil received rats, which proved effective in resurging sperm motility.

**Key words:** Antioxidant, Phenytoin, Virgin Coconut oil.

### INTRODUCTION

Phenytoin introduced first in Germany way back in 1908 at the University of Kiel, is a very potent and commonly employed anti-epileptic agent used for the treatment of generalized tonic-clonic seizures and status epilepticus.<sup>1-2</sup> Phenytoin belongs to the family of hydantoin organic compounds. Epilepsy occurs due to the uncontrolled excitation of neurons in the nervous system. Phenytoin is employed to limit this uncontrolled activity by suppressing the neuron's electrical activity, preventing the spread of electrical discharge in seizures. The repetitive action potential generated is blocked by Phenytoin.<sup>3</sup> Phenytoin is widely used and prescribed all around the world. However, just like many other drugs, it too has multiple side effects. Phenytoin is widely known to cause drug-induced gingival enlargement/hyperplasia

with an incidence rate of 3% to 93%.<sup>4</sup> Other than gingival hyperplasia, it can also stimulate aplastic anemia, leukemia, agranulocytosis, and other neurological deficits<sup>5</sup> Phenytoin, other than causing all of the earlier mentioned side effects, it can ultimately result in male impotence. Phenytoin is excreted in the human semen and can possibly lead to complications of reduced testicular volume, morphological changes in the sperm and its number, as well as reducing its motility. These issues can result in infertility and impotence, causing harm to the social life of male patients.<sup>6</sup>

Male impotence can be counteracted by supplementation, one of which is the use of antioxidant agents. Reactive Oxygen Species (ROS), such as free radicals, accumulate in

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the body and generate oxidative stress. This oxidative stress is what lays the framework for the origination of many diseases and conditions, including arthritis, neurodegenerative diseases, autoimmune diseases, inflammation, cancer, and last but not the least male impotence.<sup>7-8</sup> Antioxidant agents are proven to reduce the harmful effects of ROS species, thereby neutralizing the threat of oxidative stress in the body.<sup>9-10</sup> Virgin Coconut Oil (VCO) and Corn Oil (CO) are among some oils that possess antioxidant properties. Studies have shown the beneficial effects of VCO and CO on the body.<sup>11-14</sup> If VCO and CO are antioxidants, they can thus also stimulate a positive effect on the reproductive system of men and reduce male infertility due to phenytoin use. This study was conducted to evaluate the effect of antioxidant oils on sperm motility in Phenytoin induced toxicity affecting rat's testis.

## MATERIAL & METHODS

The study was conducted at the anatomy department of Al-Tibri Medical College and Hospital, Isra University Karachi Campus, once approval was granted by the Institutional review board (IRB) (IERC/ATMC/18/008). The study was conducted at Al-Tibri medical college and Hospital from November 2019 to March 2020. Male Albino rats were acquired from the animal house of the institute. 48 Albino rats were taken that weighed between 150-250gms. The 48 rats were divided into 4 groups, with each group consisting of 12 rats. The groups were divided and the following was carried out in each group:

### Group A

Control Group received an intraperitoneal injection of 1-unit normal saline solution along with a normal daily diet.

### Group B

Received dose of Phenytoin 10mg/kg body wt intraperitoneally once every day.

### Group C

Received Virgin Coconut Oil (6.7ml) along with Phenytoin 10mg/kg body wt., intra-peritoneally once every day.

### Group D

Received Corn Oil (2.5ml) along with Phenytoin 10mg/kg body wt., intraperitoneally once every day.

The rats were kept in cages under a controlled diet and light duration. The animals were euthanized under the influence of anesthesia on the 4<sup>th</sup>, 5<sup>th</sup>, and 6<sup>th</sup> weeks of the study. Their epididymis was cut and put in a solution of normal saline and left on the petri dish to see a change in the coloration from transparent to grey. For determining sperm motility, one drop of epididymal fluid was placed under a microscopic slide.

Data was analyzed using the statistical package of social science (SPSS Version 20.). All values were written in mean  $\pm$  S.D. The four groups were compared via One-way ANOVA followed with Post Hoc Tukey's Test. The statistical significance was taken at  $P \leq 0.05$ .

## RESULTS

Comparing Sperm motility among different Groups.

### Week 4

Sperm Motility in mean % in Group A were  $69.8 \pm 4.21$ , in Group B were  $19.8 \pm 1.14$ , in Group C  $68.8 \pm 3.67$ , and in Group D  $22.2 \pm 3.78$ . Level of significance between groups B vs. A  $\leq 0.001$ , B vs. C  $\leq 0.001$ , and B vs. D  $\leq 0.126$ . (Table-I, Figure-1).

### Week 5

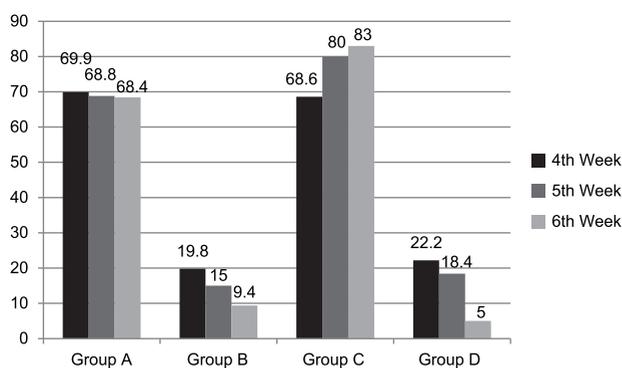
Sperm Motility in mean % in Group A were  $68.8 \pm 2.30$ , in Group B were  $15.0 \pm 2.16$ , in Group C  $80.0 \pm 3.04$ , and in Group D  $18.4 \pm 3.04$ . Level of significance between groups B vs. A  $\leq 0.001$ , B vs. C  $\leq 0.001$ , and B vs. D  $\leq 0.224$ . (Table-I, Figure-1).

### Week 6

Sperm Motility in mean % in Group A were  $68.4 \pm 1.92$ , in Group B were  $9.4 \pm 1.30$ , in Group C  $83.0 \pm 1.67$ , and in Group D  $13.6 \pm 1.14$ . Level of significance between groups B vs. A  $\leq 0.001$ , B vs. C  $\leq 0.001$ , and B vs. D  $\leq 0.431$ . (Table-I, Figure-1).

Weeks	Mean Sperm Motility	Groups	Mean Sperm Motility	Comparison of Groups	P-Value
4 <sup>th</sup> Week	B 19.8 ± 1.14	A	69.8±4.21	B vs A	≤0.001
		C	68.8±3.67	B vs C	≤0.001
		D	22.2±3.78	B vs D	0.126
5 <sup>th</sup> Week	B 15.0±2.16	A	68.8±2.30	B vs A	≤0.001
		C	80.0±3.04	B vs C	≤0.001
		D	18.4±3.04	B vs D	0.224
6 <sup>th</sup> Week	B 9.4±1.30	A	68.4±1.92	B vs A	≤0.001
		C	83.0±1.67	B vs C	≤0.001
		D	13.6±1.14	B vs D	0.431

**Table-I. Comparison of sperm motility among different groups.  
P-Value ≤0.05**



**Figure-1. Shows the Mean Percentage of Sperm Motility among different groups. P-Value ≤0.05.**

## DISCUSSION

Anti-epileptic drugs have been shown to negatively affect the sexual characteristics of males by reducing serum testosterone, sperm count, sperm motility, and sperm viability.<sup>15</sup> Sterility is a very controversial topic in our society. Many fail to discuss it and seek treatment for it due to the social implications that it might have on them and their family. Infertility or impotence is predominantly a male factor.<sup>16</sup> Epilepsy is well known for causing infertility, with studies showing a high risk of impotence and development of hyposexuality among individuals.<sup>17</sup> Phenytoin is an important pharmacological agent that is well studied for its benefits as well as its drawbacks. Our study concluded that Phenytoin leads to a reduction in sperm motility. Another study by Olutunde et al. (2010) also demonstrated similar results to our study by showing that a decreased motility of sperm upon the increased dose of Phenytoin. However, this effect was reversible and

was eradicated upon withdrawal of the drug.<sup>18</sup> Other agents, vigabatrin and carbamazepine, that treat similar types of epilepsy as Phenytoin also cause impotence, as shown by another study conducted on Wistar rats.<sup>19</sup> This can be a cause of concern as that limits the amount of anti-epileptic agents that can be given to sexually active men, as many of them have an association with infertility.

Our study aimed at determining if virgin coconut oil and corn oil can have an antioxidant effect while Phenytoin is being administered. Both showed favorable results as both led to a revival of sperm motility, however, virgin coconut oil proved to be more effective than corn oil. Ogendengbe et al. (2018) also showed the positive effects of virgin coconut oil when tested as an adjuvant with highly active antiretroviral therapy (HAART) and alcohol consumption. The study provided similar results to ours by showing the virgin coconut oil improved sperm motility.<sup>20</sup> Virgin Coconut oil is an effective antioxidant agent that can be used to restore sperm motility reduced by phenytoin induction into the body and by other agents. Virgin coconut oil is abundant in polyunsaturated fatty acids that strongly inhibit the action of lipid peroxidation. This shows the superior antioxidant nature of virgin coconut oil, as shown by another study that gave evidence of VCO's significant antioxidant role compared to copra oil and groundnut oil.<sup>21</sup> Virgin Coconut Oil should be used as it is readily available, inexpensive, and has no adverse effects. In the future, other oils

can be used to study their effect on sperm motility via antioxidant effects.

## CONCLUSION

Virgin Coconut Oil and Corn Oil both helped in the restoration of sperm motility. However, Virgin Coconut Oil showed more substantial results in restoring sperm motility due to its resilient antioxidant nature.

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4	Sayyada Humaira Masood	Manuscript writing.	
5	Jamil Ahmed Siddiqui	Critical review.	
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