Original Article

Preventive Role of Antioxidants in Phenytoin Induced Toxicity of Rat Testes: A Microscopic Analysis

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ABSTRACT

Objective: To analyze the preventive role of Virgin Coconut Oil (VCO) and Corn Oil (CO) in the testes of rats upon administration of Phenytoin.

Study Design: Analytic study

Place and Duration of Study: This study was conducted at the Al-Tibri Medical College, Karachi from November 2020 to March 2021.

Materials and Methods: 32 male albino rats were acquired by random sampling technique and divided into 4 interventional groups. Group 1 was given Normal Saline Only, Group 2 was given Phenytoin only, Group 3 was given VCO + Phenytoin, and Group 4 was given VO + Phenytoin. The diet, water intake, and light exposure were well regulated and the subjects were then euthanized on the 4th, 5th, and 6th week of the study to assess the germinal layer thickness on the microscope. The testes were removed from the subjects, and tissue samples were acquired and placed on the microscope accordingly and the germinal layer thickness and morphology was assessed at 400x magnification.

Results: Our results showed a significant decrease in the germinal layer thickness in the phenytoin group 2, owing to the generation of oxidative stresses. In the VCO group, a significant improvement was seen compared with the phenytoin group as the germinal layer thickness was restored in this group. Unfortunately, CO wasn't able to restore the germinal layer thickness and the microscopic findings of the CO group were similar to the phenytoin group. VCO due to its antioxidant potential because of the presence of polyunsaturated fatty acids helps in restoring the germinal layer thickness in the presence of phenytoin.

Conclusion: VCO is a potent antioxidant and can mitigate the toxicity of phenytoin on testes

Key Words: Virgin Coconut Oil, Germinal Layer, Phenytoin, Coconut Oil

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INTRODUCTION

Epilepsy is a very devastating neurological disorder that significantly impacts the quality of life of the individual facing it as well as the family members that are associated with the patient[1].

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Apart from the constant seizures that can spontaneously arise in the patients, they also have to suffer the adverse effects of many anti-epileptic drugs as well[2]. One of the most promising, and well-known drugs used in the field of medicine for Epilepsy is Phenytoin, which is an established drug for treating acute repetitive seizures as well as Status Epilepticus[3]. Although it is the most widely used anti-epileptic drug available on the market, it needs to be monitored due to it possessing a narrow therapeutic range[4]. Side effects of Phenytoin include gingival hyperplasia, dermatological reactions such as toxic-epidermal necrolysis and Steven-Johnson Syndrome, Cardiovascular effects, and Respiratory problems[5-7]. Furthermore, studies have also indicated that phenytoin is said to have a negative effect on the male reproductive system[8]. Phenytoin can cause a significant decrease in the sperm count, active sperm motility, viable sperm number, and increase the number of abnormal sperms[9]. The factor that has been identified to cause toxicity in the male reproductive system is the development of oxidative stress. It occurs due to the state of imbalance between free radical production and

antioxidant, thereby damaging the cells responsible for sperm formation and ultimately having an effect on the male reproductive system[10]. If the antioxidant level in the body is restore to counteract the free radical generation and the subsequent oxidative stress, the toxic potential of the phenytoin drug on the male testes can be successfully neutralized. Many antioxidants such as β-carotene, Vitamin C, and E all provide major protective against the free radicals that generate oxidative stresses in the body by reducing reactive oxygen species[11]. Virgin Coconut Oil (VCO) and Corn Oil (CO) are some more examples of antioxidant agents that have seen promising results in reducing oxidative stress[12-14]. As the use of phenytoin is massive among reproducing male, we decided to conduct a study to determine the role of antioxidant oils in phenytoin induced toxicity on testes in rats.

MATERIALS AND METHODS

This experimental study was conducted after approval from the institutional review board at Al-Tibri Medical College and Hospital, Karachi from November 2020 to March 2021. For this study we randomly selected 32 male albino rats that weight 15-250 grams. The animals were randomly divided into 4 treatment groups in which we carried out the following interventions

Group 1:
The control group of our study was to receive an intra-peritoneal injection of 1unit Normal Saline with a normal diet

Group 2:
Received only 10/mg/kg/body weight of Phenytoin through an intra-peritoneal injection once daily.

Group 3:
Received both 10mg/kg/body weight of Phenytoin plus 6.7ml of Virgin Coconut Oil once daily.

Group 4:
Received both 10mg/kg/body weight of Phenytoin plus 2.5ml Corn Oil once daily.

The diet and water intake was well regulated along with the duration of light. The subjects were monitored thoroughly with samples being acquired on the 4th, 5th, and 6th week of the study by euthanizing the rats under anesthesia and then incising the subjects and removing both the testes for microscopic analysis. The tissues were carefully removed from the tests and preserved in 10% formalin. The tissues were then processed for slide preparation and staining using H&E staining technique. The germinal layer thickness was assessed under the microscope to see how its thickness and appearance will be affected in all of the mentioned groups. The magnification was fixed at 400x.

RESULTS

Figure 1: Photomicrographs of the seminiferous tubules using H&E staining and representing the germinal layer thickness on the 4th week among Different Groups

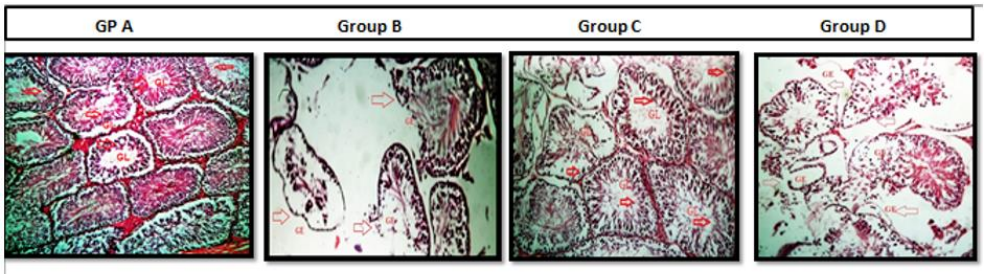

Figure 1: Photomicrographs of the seminiferous tubules using H&E staining and representing the germinal layer thickness on the 4th week among Different Groups. Magnification 400x, GL: Germinal Layer. In group B significant reduction in numbers of seminiferous tubules with marked thinning of Germinal epithelium. In Group C the virgin coconut oil significantly restore the germinal tissue of the testes. In Corn oil treated group the non-significant reduction of germinal epithelium, as compare to group B.

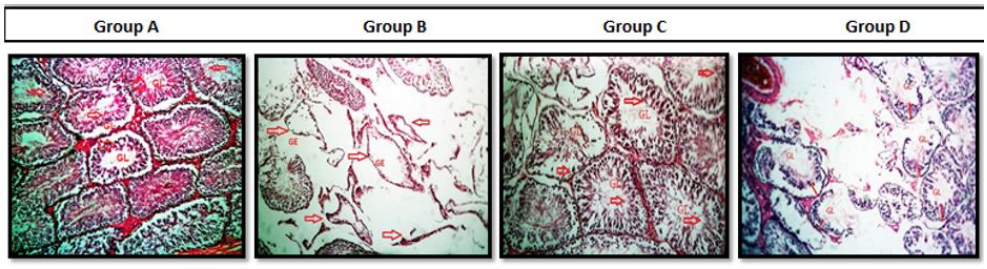

Figure 2: Photomicrographs of the seminiferous tubules using H&E staining and representing the germinal layer thickness on the 5th week among Different Groups. Magnification 400x, GL: Germinal Layer. In group B significant reduction in numbers of seminiferous tubules with marked thinning of Germinal epithelium. In Group C the virgin coconut oil significantly restore the germinal tissue of the testes. In Corn oil treated group the significant reduction of germinal epithelium, as compare to group B minimum restoration of germinal layer.

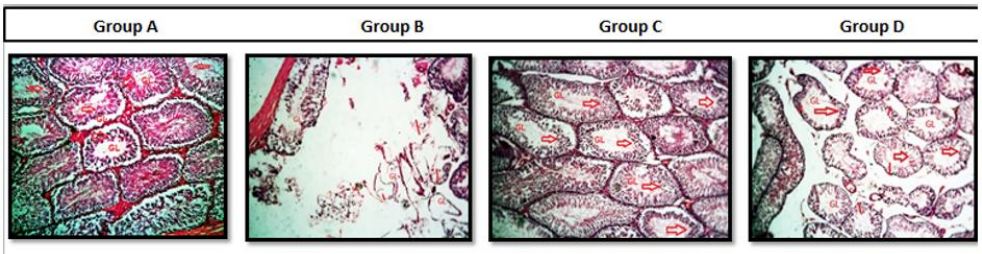

Figure 3: Photomicrographs of the seminiferous tubules using H&E staining and representing the germinal layer thickness on the 6th week among Different Groups. Magnification 400x, GL: Germinal Layer. In group B significant reduction in numbers of seminiferous tubules with marked loss of Germinal epithelium. In Group C the virgin coconut oil significantly restore the germinal tissue of the testes. In Corn oil treated group the significant reduction of germinal epithelium, as compare to group B minimum restoration of germinal layer

Figure 2: Photomicrographs of the seminiferous tubules using H&E staining and representing the germinal layer thickness on the 5th week among Different Groups.

Figure 3: Photomicrographs of the seminiferous tubules using H&E staining and representing the germinal layer thickness on the 6th week among Different Groups.

DISCUSSION

Apart from Phenytoin, other anti-epileptic drugs such as sodium valproate, topiramate, carbamazepine, gabapentin, and levetiracetam all lead to some sort of testicular toxicity[15-18]. Therefore, it is critical to find agents that can limit the amount of toxicity these drugs can do. We studied the histological features of all our interventional groups. The microscopic findings revealed that phenytoin causes a reduction in the germinal layer thickness thus proving to create disastrous consequences on the reproductive organs. However, upon the administration of the interventional agents which were VCO and CO, we did see favorable results with the VCO+ Phenytoin group in preventing a reducing in the germinal layer thickness. CO however failed to show significantly sound results as the histological findings of germinal layer thickness were fairly similar to the group in which only phenytoin was administered. In another study Ogendengbe et al (2016) induced testicular toxicity using anti-retroviral therapy and showed that significant decrease in the seminiferous tubular architecture, as well as a decline in the epithelial height, a finding which mirrors our study. Furthermore, in accordance with our study it was also found in the same study that intervention using VCO along with phenytoin also did lead to a decrease in the seminiferous tubular diameter, but other morphometric and histological parameters were in resemblance to the control group or VCO group[19]. VCO is highly rich in polyunsaturated fatty acids that have a potent inhibitory effect on lipid peroxidation. This is what may have led to a much better histological architecture in the group administered with VCO. VCO showed its antioxidant characteristics in another similar study to ours in which comparison was done with Groundnut oil and Copra oil[20]. There have been other antioxidant agents that have further cemented the potential to treat toxicity caused by phenytoin in tests. Olatunde et al (2015) compared the antioxidant role of Kolaviron and Vitamin E in phenytoin induced hepatic and testicular dysfunction in Wistar rats, his results showed a significant reduction in the seminal epithelium thickness along with a decrease in the diameter of seminiferous tubules, a finding similar to our study. Furthermore, the antioxidant status of the liver and testes were restored to normal levels in rats that were treated with Kolaviron and Vitamin E, a finding that matches our results with the administration of VCO [21]. Dosumu et al (2010) also showed that VCO also has a therapeutic potential in limiting the oxidative stress in the testes by improving the antioxidant stress by reducing the malondialdehyde (MDA) and allowing the lipid profile status to attain near normal levels once again[22].

CONCLUSION

We can safely say that the antioxidant potential of Virgin coconut oil is vital in maintaining and restoring the germinal thickness of testes induced with phenytoin.

Author's Contribution:

Concept & Design of Study:	Khalique-ur-Rehman
Drafting:	Khalid Shehzad, Syed Muhammad Masood Ali
Data Analysis:	Sonia Khan, Sarah Sughra, Raja Faisal
Revisiting Critically:	Khalique-ur-Rehman, Khalid Shehzad
Final Approval of version:	Khalique-ur-Rehman

Conflict of Interest: The study has no conflict of interest to declare by any author.

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