Original Article

Protective Role of Antioxidant Oils in Phenytoin Induced Toxicity of Seminiferous Tubules in Rats

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ABSTRACT

Objective: To evaluate the protective role of virgin coconut oil and corn oil in phenytoin induced toxicity of rat on the basis of histomorphology of seminiferous tubules.

Study Design: Experimental study

Place and Duration of Study: This study was conducted at the department of Anatomy of Al-Tibri Medical College and Hospital, for a period of six months from October 2018 to November 2019.

Materials and Methods: 48 numbers of male albino rats were randomly selected with weight between 150-250gms. Four different groups were made on the basis of therapeutic agents. Group A control, Group B phenytoin induced intra-peritoneal, Group C virgin coconut oil plus phenytoin and Group D corn oil plus phenytoin. The sample was taken by given anesthesia and both testes were removed through dissection at 4th, 5th and 6th week. The sample was preserved for tissue processing and staining. Tubular dimension were measured through micrometry at 400x, mean of five different tubules from five different field areas were taken and one way ANOVA followed by post hoc tukey's test was applied to evaluate the significant difference among different groups. P value considered to be significant <0.05

Results: Mean value of tubular dimension was significantly reduced in phenytoin induced toxic group, while in group A and C shows significant restoration of tubular dimension as compared to group D on three different week.

Conclusion: Virgin coconut oils showed significant restoration of seminiferous tubules dimension when used along with phenytoin for 6 weeks in comparison of corn oil. Virgin coconut oil showed significant ant oxidative effects and alter the toxic effects of drugs if administered simultaneously

Key Words: Virgin coconut oil, seminiferous tubules, Phenytoin

Citation of article: Khalique-ur-Rehman, Khan H, Hameed U, Korai S, Iqbal S, Faisal R. Protective Role of Antioxidant Oils in Phenytoin Induced Toxicity of Seminiferous Tubules in Rats. Med Forum 2021;32(4):79-82.

INTRODUCTION

It is an anticonvulsant, non-sedative drug which is used in the treatment of epilepsy. Male patients after the use of this drug complained of impotence. Chemically it is regarded as NO3 group name Antiepileptic. Its generic name is 5, 5 diphenyl-substituted hydantoin Trade Name; Epigran, Dilantin, Dantonio[1]. Phenytoin is excreted in human semen in small quantities, and this may affect the testosterone levels. Reduce the plasma concentration of free testosterone that has been detected in male epileptic patients receiving phenytoin. For those patients who are receiving phenytoin, the serum level of testosterone is reduced[2]. It has been observed that phenytoin has a mutagenic effect on human sperm cells. It is observed that hyposexulity and the low fertility rate are greater in epileptic patients than in the general population. There are reports that they reduce testicular weight, spermatic count, and gives abnormal morphology of spermatozoa. They reduce spermatozoa's motility to interfere with the normal HPG (hypophyseal gonadal) pathway[3]. Its biological name is Cocoas nucifera.

The coconut oil is abstracted by mechanical means from the mature kernel of the coconut with or without heat and without chemical refining called Virgin coconut oil. Virgin coconut oil is derived from COPRA. Myristic acid and Lauric acid are related to the average serum cholesterol concentration in humans. The lauric acid level in coconut is controlled by environment and genetics[4]. Lauric acid is a fatty acid derived from coconut for developing monolaurin. Monolaurin is an antimicrobial agent for killing bacteria, yeasts, and viruses. Coconut oil contains flavonox and poly phenol

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Received: October, 2020
Accepted: December, 2020
Printed: April, 2021

that have potent antioxidant effects particularly in male fertility[5]. The corn oil is composed of Triacylglycerol 99% and Poly Unsaturated fatty acids (PUFA). Sperm cells contain a high proportion of (PUFA), and normal spermatozoa possess a high % of PUFA [6]. These PUFA are formed from linoleic acid (LA), and they give fluidity to the sperm plasma membrane, which helps in the fusion events of fertilization. More recently, antioxidants (AOX) and Phytosterols (PST), commonly found in substantial amounts in special oils, such as Pecan nut (PNO) Corn (CO) and grape seed (GSO) oils[7,8]. These oils are associated with a lower risk of inflammation, dyslipidemia, and low risk of oxidative stress required for the maintenance of endothelial integrity [9,10].

MATERIALS AND METHODS

The experimental study was carried out at Al-Tibri Medical College and Hospital, six months from October 2018 to November 2019. After taking ethical consideration from the ethical review committee of the concerned institute and the animals, they were taken from the animal house of Al-Tibri Medical College and Hospital. A total of 48 male Wister albino rats were taken through a randomized sampling technique with a weight of 150-250gms and equally divided into four different groups and kept them in separate cages for six weeks. Group A (control group) given standard diet and1 unit normal saline intraperitoneal daily once a day, Group B (Experimental group) given inj. Phenytoin 10mg/kg/body wt intra-peritoneal once daily. Group C (Virgin coconut oil) 6.7ml orally and the same dose of phenytoin once daily intra-peritoneal. Group D received 2.5 ml once daily, along with a similar application of inj. Phenytoin. The entire treatment plan was given for six weeks, and the sample was taken on the 4th, 5th, and 6th weeks. Throughout the period, 12hours of day and light cycle were maintained.

Sampling: Before starting the study, the weight of the animals was divided into four groups. At 4th, 5th and 6th week of the study, weight of animals was taken, and the animals were anesthetized with ethanol containing jar. After given anesthesia, the animals were sacrificed, and through dissection, the testis was removed and stored in formalin, the tissue of the testis were sent for preservation, embedding, and staining process for the histomorphological examination of the tissue. The tissue was stained with H&E.

Histomorphology: The tubular dimension was taken from different field areas of the slide (choose five field area). The tubular diameter was measured through micrometer, and then the mean of five different field areas at 400x were taken for the comparison among different groups. The reading was recorded in um.

Tubular Dimension= length x Breath/2

Photomicrograph was taken from DSLR camera for the comparison among the different groups.

Statistical Analysis: The Mean diameter of seminiferous tubules was recorded, and to compare the mean difference among the groups, the one-way ANOVA followed by post hoc tukey's was applied. The level of significance was considered p=<0.05.

RESULTS

Table No.1 shows Mean values of tubular dimension among different therapeutic groups in comparison with group B (experimental group). P value <0.05 was considered significant.

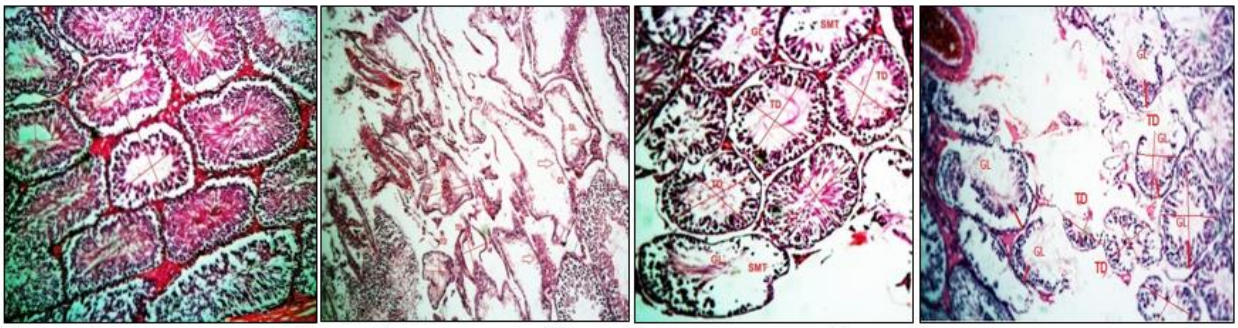
Photomicrograph: 1.1 shows the histomorphology of seminiferous tubules among different groups on 4th week of sampling. Significant reduction of tubular dimension was seen in Group B (phenytoin induced group), while significant restoration of tubules were found in Group A (control) and Group C (Virgin coconut oil) as compared with Group D (corn oil).

Photomicrograph: 1.2 shows the histomorphology of seminiferous tubules among different groups on 5th week of sampling. Significant reduction of tubular dimension was seen in Group B (phenytoin induced group), while significant restoration of tubules were found in Group A (control) and Group C (Virgin coconut oil) as compared with Group D (corn oil).

Photomicrograph: 1.3 shows the histomorphology of seminiferous tubules among different groups on 6th week of sampling. Significant reduction of tubular dimension was seen in Group B (phenytoin induced group), while significant restoration of tubules were found in Group A (control) and Group C (Virgin coconut oil) as compared with Group D (corn oil).

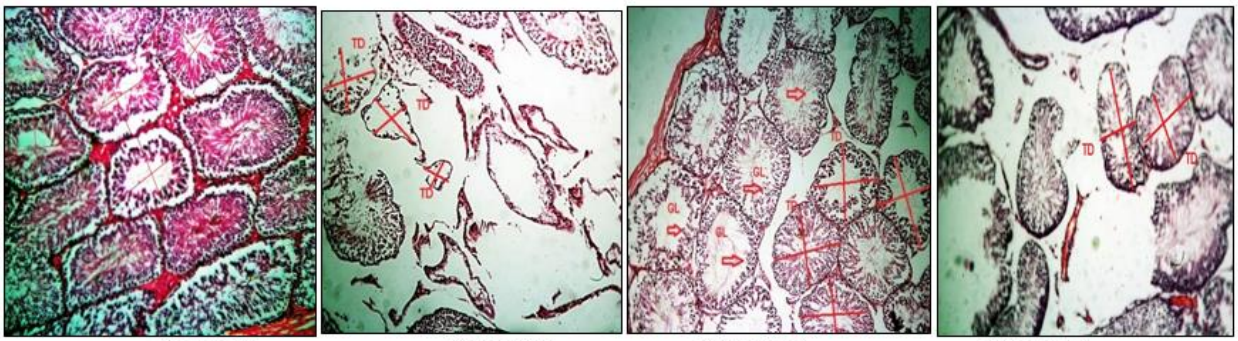
Table No1: Comparison of tubular dimension of seminiferous tubules among the different groups (um)

Weeks	Mean tubular dimension of seminiferous tubules	Groups	Mean tubular dimension of seminiferous tubules	Comparison of groups	P-Value
4th week	B 18.6 ± 3.21	A	44.6 ± 3.93	B vs A	<0.001
		C	33.6 ± 2.93	B vs C	<0.001
		D	19.6 ± 2.37	B vs D	0.481
5th week	B 12.4 ± 3.12	A	45.2 ± 2.72	B vs A	<0.001
		C	38.8 ± 2.71	B vs C	<0.001
		D	11.8 ± 1.79	B vs D	0.597
6th week	B 9.4 ± 1.19	A	44.4 ± 2.75	B vs A	<0.001
		C	43.2 ± 2.59	B vs C	<0.001
		D	9.8 ± 2.36	B vs D	0.229



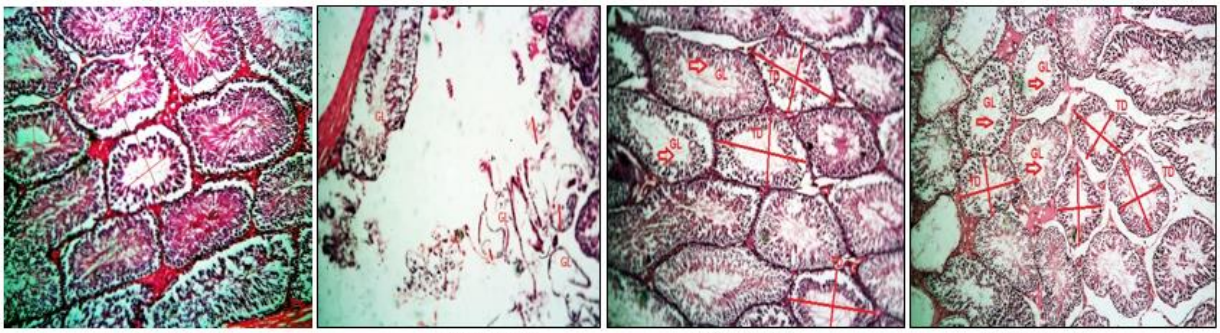
Group A Group B Group C Group D

Photomicrograph 1.1 represents the tissue of seminiferous tubules with H&E stain and showing the tubular dimension at 4th week among different groups (400x). (GL) germinal layer, (TD) tubular dimension



Group A Group B Group C Group D

Photomicrograph 1.2 represents the tissue of seminiferous tubules with H&E stain and showing the tubular dimension at 5th week among different groups (400x). (GL) germinal layer, (TD) tubular dimension



Group A Group B Group C Group D

Photomicrograph 1.3 represents the tissue of seminiferous tubules with H&E stain and showing the tubular dimension at 6th week among different groups (400x). (GL) germinal layer, (TD) tubular dimension

DISCUSSION

Following the results of Oluwatosin et al:2016, testicular toxicity induced with the application of antiretroviral therapy, which leads to drastic effects on male fertility the same as in our study produced with phenytoin. In a similar study, the virgin coconut oil was used to observe the antioxidant effects on male fertility and sperm morphology. There was a significant reduction of sperm motility $p<0.01$ in animals after being treated with HAART. On the other hand, in the group of virgin coconut oil HAART, the significant restoration of numbers sperm and maintain the sperm motility almost near to the normal value. Similar findings were found in our study in a group of phenytoin and virgin coconut oil. The readings were taken at an interval of 4, 5, and 6 weeks [11,12]. The ratio of polyunsaturated fatty acids had potent inhibitory action that influences on lipid peroxidation, and Virgin coconut oil is highly rich in polyunsaturated fatty acids, so similar in our study the virgin coconut oil showed their antioxidant effects in male fertility as related in this study the virgin coconut oil evidence more significant antioxidant role in comparison with copra

oil and groundnut oil. At the same time, the same results were found in our study [7,13]. In the same study, the virgin coconut oil and HARRT showed a significant reduction in the diameter of seminiferous tubules; moreover, there was no effect on the germinal layer. By the results of our study, the virgin coconut oil along with phenytoin had a potent role in the restoration of tubular diameter and thickness of germinal epithelium in the animal taken long term phenytoin intra-peritoneal once daily. The effects were measure with an interval of 4, 5, and 6 weeks.

Additionally, virgin coconut oil mixed with groundnut oil or olive oil was observed to be more effective in inhibiting LDL oxidation, and stimulate hepatic antioxidant enzyme activity. The antioxidant activity of virgin coconut oil was linked to high polyphenol compounds in the oil. Polyphenols were reported to be a stronger antioxidant than vitamin C and E in vitro on a molecular basis [9, 14].

CONCLUSION

Virgin coconut oils showed significant restoration of seminiferous tubules dimension when used along with phenytoin for 6 weeks in comparison of corn oil. Virgin coconut oil showed significant ant oxidative effects and alter the toxic effects of drugs if administered simultaneously**.**

Author's Contribution:

Concept & Design of Study:	Khalique-ur-Rehman
Drafting:	Hina Khan
Data Analysis:	Uzma Hameed Shahid Korai, and
Revisiting Critically:	Sadia Iqbal, Raja Faisal
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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