



TESTICULAR MORPHOLOGICAL EVALUATIONS IN SPRAGUE-DAWLEY RATS UNDER AN ANTITUSSIVE-DEXTROMETHORPHAN

Adebajo Adesina Oluwaseye*^{1,2}, Gbotolorun Stella Chinwe¹, Oremosu Ademola Ayodele¹

¹Department of Anatomy, Faculty of Basic Medical Sciences, College of Medicine of the University of Lagos, Lagos, Nigeria.

²Anatomy Programme, College of health Sciences, Bowen university Iwo, Osun state

Corresponding Author: Dr. Adebajo Adesina Oluwaseye. **E-mail:** adebajoseye@gmail.com

ORCID ID: <https://orcid.org/0000-0001-5682-8032>

ABSTRACT

Background: Dextromethorphan is the dextrorotatory enantiomer of the methyl ether of levorphanol, an opioid analgesic. It is also a stereoisomer of levomethorphan, an opioid analgesic and has been reported to affect male fertility by altering the cytoarchitecture of the seminiferous tubules and affect hormones that are responsible for fecundity in males. The aim of this study is to determine the possible morphological alterations in males following exposure to dextromethorphan, using Sprague-Dawley rats as experimental models. **Methods:** A total of twenty rats (150 ± 30 g) divided into four groups (N=5; A-D) were used. Group A, control received distilled water (DW); group B received 20 mg/kg of the drug, group C received 40 mg/kg and group D received 80 mg/kg of DM for a duration of 16 weeks. At the end of treatment period, the animals were selected and sacrificed, the following histomorphometric parameters were analyzed: diameter and height of the seminiferous tubule and epididymis, volume of testes and the number of spermatogonia, spermatocytes and spermatids within the seminiferous tubules. **Results:** Significant reduction in all the Histomorphometric parameters were recorded when treatment groups were compared to control but when recovery-alone group was compared to treatment groups, slight increases were recorded. **Conclusion:** DM has deleterious effect on the morphometric parameters analyzed from the epididymis and testes.

Keywords: Dextromethorphan, Histomorphometry, Epididymis, Testes.

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INTRODUCTION

Dextromethorphan (DM) has been reported to be an active composition of antitussives and are said to be found in over 150 over the counter (OTC) medications. Dextromethorphan is the dextrorotatory enantiomer of the methyl ether of levorphanol, an opioid analgesic. It is also a stereoisomer of levomethorphan, an opioid analgesic. Dextromethorphan is a synthetic opiate that is synthesized from a benzyloisoquinoline with a planar structure through a phenomenon called the Grewe's cyclization to give the morphinan with a three-dimensional structure. The isoquinoline is 1,2,3,4,5,6,7,8-octahydro-1-(4-methoxybenzyl) isoquinoline is converted

into the N-formyl derivative, cyclised to the N-formyl normorphinan, and the formyl group reduced to an N-methyl group, to give 3-methoxy-17-methylmorphinan, or racemethorphan [1]. It acts by suppressing cough by reducing the activity of the cough center in the brain by acting directly on the cough center or numbing the cough receptors. It has been documented to suppress pain in cancer patients [2-4], manage Parkinson's disease [5], reduce the severity of pseudobulbar affect [6,7] and treat cerebral ischemia [8,9]. Dextromethorphan as an OTC has been reported to have deleterious effect on male reproduction by negatively affecting the

reproductive hormones, seminal milieu parameters and cytoarchitecture of the seminiferous tubules that are pointers of male-factor fertility, hence, the aim of this study is to determine the possible

morphological alterations in males following exposure to dextromethorphan, using Sprague-Dawley rats as experimental models.

MATERIALS AND METHODS

Drugs and Animals used

The pure substance of Dextromethorphan was obtained from Fairy Pharmaceuticals, Ltd., Zhuhai City, Guangdong Province, China republic.

A total of twenty healthy male Sprague-Dawley rats weighing between 150 ± 30 g were used for this study. They were housed in standard well-ventilated wire mesh plastic cages in the Animal House of the Department of Anatomy, College of Medicine of the University of Lagos under standard room temperature ranging between 26°C-28°C and relative humidity of 50-55%. The animals were exposed to twelve hours light and twelve hours dark cycle and were left to acclimatize for a period of two weeks before the commencement of the experiment. The route of administration of the drug was per oral, with the use of a feeding tube. The animals were identified by different ear tags. All experimental procedures and techniques were approved by the Health Ethics committee of the college of medicine, University of Lagos, Nigeria (CM/HREC/09/16/054) with strict compliance with the guiding principles for research involving animals [10].

Experimental Design

The animals were divided into four groups designated as A-D of five rats each. Group A served as control, and they received 1 ml of distilled water. Group B received 20 mg/kg of DM, group C received 40 mg/kg of DM and group D received 80 mg/kg of DM for duration of 16 weeks. At the end of treatment period, the animals were euthanized. The testes and caudal epididymis were harvested, and the following histomorphometric parameters were

analyzed: diameter and height of the seminiferous tubule and epididymis, volume of testes and the number of spermatogonia, spermatocytes and spermatids within the seminiferous tubules.

Evaluation Of Histomorphometric Analysis

The slides were examined under the Light microscope (10X) and the following measurements were taken; Seminiferous tubular diameter, Height of Seminiferous tubule, Epididymal tubular diameter and epididymal epithelial height were measured in epididymis. For each parameter, ten measurements were made per section using a calibrated eye-piece micrometer (Graticules Ltd. Toubridge Kent). The means of the measurements of parameter in each section were recorded for each animal. The volume (V) of testes was calculated by using vernier caliper. Testes were dissected after sacrificing animals and they were kept in Petri dish and their dimension obtained. The length (L), breadth (B) and thickness (T) of testes were calculated with help of vernier caliper and recorded. These values were put into a formula as mentioned by Setchell and Waites, and the volume of testes was obtained. The Setchell and Waites, formulae for calculating the volume testes was $V = \frac{4}{3} \times \pi \times \frac{L}{2} \times \frac{W}{2} \times \frac{T}{2}$. Here pi (π) is a constant figure, and its value is 3.141. Histomorphometric (Stereological) Evaluation of the Testes Histomorphometric analysis using 'ImageJ®' (an open-source image processing software, designed for scientific multidimensional images): the science that studies the reaction between antigen and antibodies in serum. Sections of 5 μ m were stained with H and E was used for

stereological studies. Histomorphometric data was collected with the aid of a Leica (DM 750) digital microscope (Leica Microsystems, Switzerland) and connected to a computer.

Statistical Analysis

The data obtained from all the groups were compiled and statistically analyzed using ONE WAY-ANOVA using Graph pad software version 9.5. The results of the data were expressed as mean \pm SEM (standard error of mean) where $p < 0.05$ was taken as significant.

RESULTS

Effect On Diameter Of Seminiferous Tubule

In the study, significant decrease in a dose-dependent manner was recorded when Treatment group was compared to control, significant decrease was seen when medium and high doses were compared to low dose, also when high dose was compared to medium dose.

Table 1. Diameter of Seminiferous Tubule

Duration	16weeks			
Group	CL	LD	MD	HD
Treatment	280.60 \pm 0.75	240.20 \pm 0.58a	244.40 \pm 1.21a	228.20 \pm 0.66abc

Values are expressed as mean \pm Standard Error of Mean (SEM). ^a $p < 0.05$ significant compared to control (treatment); ^b $p < 0.05$ significant compared with low dose (treatment); ^c $p < 0.05$ significant compared with medium dose (treatment)

Effect On Height Of Seminiferous Tubule

In the study, significant decrease in a dose-dependent manner was recorded when Treatment group was compared to control, significant decrease was seen when medium and high doses were compared to low dose, also when high dose was compared to medium dose.

Table 2. Height of Seminiferous Tubule

Duration	16weeks			
Group	CL	LD	MD	HD
Treatment	89.80 \pm 0.37	76.40 \pm 0.24a	66.60 \pm 0.24ab	58.20 \pm 0.86abc

Values are expressed as mean \pm Standard Error of Mean (SEM). ^a $p < 0.05$ significant compared to control (treatment); ^b $p < 0.05$ significant compared with low dose (treatment); ^c $p < 0.05$ significant compared with medium dose (treatment)

Effect On Volume Of Testicular Component Tubule

In the study, significant decrease in a dose-dependent manner was recorded when Treatment group was compared to control, significant decrease was seen when medium and high doses were compared to low dose, also when high dose was compared to medium dose.

Table 3. Volume of Testicular Component

Duration	16weeks			
Group	CL	LD	MD	HD
Treatment	0.54 \pm 0.01	0.35 \pm 0.01a	0.26 \pm 0.01ab	0.15 \pm 0.01abc

Values are expressed as mean \pm Standard Error of Mean (SEM). ^a $p < 0.05$ significant compared to control (treatment); ^b $p < 0.05$ significant compared with low dose (treatment); ^c $p < 0.05$ significant compared with medium dose (treatment).

Effect On Spermatids Of Seminiferous Tubule

In the study, significant decrease in a dose-dependent manner was recorded when Treatment group was compared to control, significant decrease was seen when medium and high doses were compared to low dose, also when high dose was compared to medium dose.

Table 4. Spermatids of seminiferous tubule

Duration	16weeks			
Group	CL	LD	MD	HD
Treatment	107.80 ± 1.07	82.40 ± 0.68a	70.60 ± 0.51ab	59.40 ± 0.51abc

Values are expressed as mean ± Standard Error of Mean (SEM). ^ap<0.05 significant compared to control (treatment); ^bp<0.05 significant compared with low dose (treatment); ^cp<0.05 significant compared with medium dose (treatment)

Effect On Spermatoocytes Of Seminiferous Tubule

In the study, significant decrease in a dose-dependent manner was recorded when Treatment group was compared to control, significant decrease was seen when medium and high doses were compared to low dose, also when high dose was compared to medium dose.

Table 5. Spermatoocytes of seminiferous tubule

Duration	16weeks			
Group	CL	LD	MD	HD
Treatment	122.80 ± 1.02	91.40 ± 0.51a	81.40 ± 0.51ab	72.20 ± 1.02abc

The diameter of seminiferous tubule is used as a relevant parameter for the evaluation of spermatogenic activity in experimental and toxicological assays. There is a positive relationship exists between the tubular diameter and the spermatogenic activity of the testis [11]. In this study, seminiferous tubule diameter was found to be significantly reduced in males treated with dextromethorphan. Reduced tubule diameter could be caused by the shrinkage of cellular cytoplasm [12,13] or defective spermatogenesis as evidenced by reduction in the number of various spermatogenic cells (14). The animals treated with Dextromethorphan had decreased area of seminiferous tubule. This reduction could be

Values are expressed as mean ± Standard Error of Mean (SEM). ^ap<0.05 significant compared to control (treatment); ^bp<0.05 significant compared with low dose (treatment); ^cp<0.05 significant compared with medium dose (treatment).

Effect On Spermatogonia Of Seminiferous Tubule

In the study, significant decrease in a dose-dependent manner was recorded when Treatment group was compared to control, significant decrease was seen when medium and high doses were compared to low dose, also when high dose was compared to medium dose.

Table 6. Spermatogonia of seminiferous tubule

Duration	16weeks			
Group	CL	LD	MD	HD
Treatment	48.20 ± 0.49	30.80 ± 0.37a	22.60 ± 0.51ab	12.40 ± 0.51abc

Values are expressed as mean ± Standard Error of Mean (SEM). ^ap<0.05 significant compared to control (treatment); ^bp<0.05 significant compared with low dose (treatment); ^cp<0.05 significant compared with medium dose (treatment)

DISCUSSION

explained by the fact that there was reduction in spermatogenic cells, and the absence of spermatozoa present in the lumen as seen from the respective micrographs (15). The volume of testis has been reported to be an important parameter of fertility in males. This study reported the decrease in testicular volume when treatment group was compared to control. This reduction might be due to a decrease in the number of germ cells because the volume is largely dependent on the differentiated spermatogenic cells [16, 17]. Also, studies have shown that decrease in testicular volume can be caused by inhibited differentiation of the seminiferous tubules and decreased in diameter of seminiferous

tubules [18,19]. Counting of germ cells in seminiferous tubule has been reported to be an appropriate method in detecting testicular toxicity. The different germ cell population of spermatogonia, spermatocyte and spermatids each display their own sensitivity to different toxicants [20]. The seminiferous epithelia height is under the influence of hormonal regulation of the male reproductive system; the spermatogenic epithelium is induced by follicle stimulating hormone (FSH). The result showed that there was a decrease in height of seminiferous tubules of treatment group and this can be said to be caused by various degrees of degeneration of spermatogonia with the presence of numerous apoptotic cells. This study showed decrease in spermatids, spermatocytes and spermatogonia within the seminiferous tubules of treatment group when compared to control. This reduction is so because of free radicals in DM that are toxic and inevitably affect the number of spermatogenic cells and parameters of seminiferous tubules. This study also reported the decreased number of spermatogonia, spermatocytes and spermatids within the seminiferous tubules

of treatment group when compared to control. This is due to the adverse effect of dextromethorphan on the number of spermatogenic cycle [21-23]. Also, DM seems to interfere with cellular division resulting into the reduced number of spermatogenic cells [24]. This reduction is so because of free radicals in DM that are toxic and inevitably affect the number of spermatogenic cells and parameters of seminiferous tubules.

CONCLUSION

The gradual deterioration of male reproductive quality because of toxicity has become a global phenomenon of concerns. The results of this study have demonstrated that dextromethorphan could affect male reproductive functions as seen from this study with the decrease in histomorphometric evaluations.

CONFLICTS OF INTEREST

The authors affirmed that there are not any conflicts of interest.

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