Access this Article online
Quick Response Code:

Website:
jecajournal.com
Doi:
doi.org/10.4314/jeca.v21i2.9

Original Article

Testicular histology and seminal assessments following codeine-containing cough syrup administration in male Wistar rats

[1,2,3]Ibeabuchi, K.C.; [1]Gbotolorun, S.C.

Abstract

BACKGROUND AND AIM: Studies have shown that given its sedating and euphoric effects and the development of tolerance within a relatively short time-frame on repeated use, codeine and codeine-containing products carry identified abuse potential both in pill and syrup forms. Its use, misuse and dependence have become an emerging global public health concern. Accordingly, this study investigated the effect of codeine containing cough syrup on the testes of adult Wistar rats.

METHODOLOGY: Twenty rats (110-200g) were divided into four groups **A-D** of five rats each. Group **A** (control) received only feed and water. Group **B** (low dose group) received 10.95mg/kg body weight, group **C** (medium dose group) received 21.90mg/kg body weight while group **D** (high dose group) received 43.80mg/kg body weight of the syrup daily via an oral cannula for eight weeks. At the end of the experiment, the testes were harvested, weighed and processed for seminal and histological assessments.

RESULTS: Only the low dose group had significantly ($P<0.05$) lower values of seminal analysis when compared to the control. No deleterious effects were observed in the histological profiles of all the testes.

CONCLUSION: Taken together, these results provide preliminary evidence from seminal analysis and histology that codeine-containing cough syrup had no adverse effect on the testes.

Keywords:
Codeine, Cough syrup, Spermatogenesis, Testes

Submitted: 20th August, 2024
Revised: 5th November, 2024
Accepted: 15th November, 2024
Published: 31st December, 2024

[1]Department of Anatomy, Faculty of Basic Medical Sciences, College of Medicine of the University of Lagos, Idi-ara, Lagos, Nigeria; [2]Anatomy unit, Department of Nursing, Claretian University of Nigeria, Maryland, Nekede, Owerri, Nigeria; [3]Anatomy unit, College of Nursing Sciences, Our Lady of Lourdes Hospital complex, Ihiala, Anambra, Nigeria.

Address for Correspondence:
Ibeabuchi, K.C.
Department of Anatomy, Faculty of Basic Medical Sciences, College of Medicine of the University of Lagos, Idi-ara, Lagos, Nigeria.
kingsleychinemerem@gmail.com

INTRODUCTION

Codeine (3-methylmorphine), a naturally occurring alkaloid is a methylated morphine derivative that occurs naturally in the poppy seed. It was first isolated from the opium poppy (*papaviersomniferum*) a plant in the *papaveraceae* family in the 1830s (Eddy *et al.*, 1968). It is a mild opiate with increasing global consumption and represents the most prescribed opioid to children, adults and women of reproductive age (Broussard *et al*., 2011).

It is used to treat pain (Havig *et al.* 2016; Hudak 2016; Kimergard *et al*. 2016), as a cough medicine (antitussive), and for diarrhoea (Prommer 2011), and appears on the world health organization's list of essential medicines; the most effective and safe medicines needed in a health system (WHO, 2015).

Given its sedating and euphoric effect and development of tolerance within a relatively short time-frame on repeated use, products containing codeine carry identified abuse potential as reported in drug administration trials (Babalonis *et al*., 2013) and the case reporting of patient dependence (Sproule *et al*., 1999; Frei *et al.*, 2010; Nielsen *et al.,* 2010). Studies have also shown that codeine is abused unaltered in both pill and syrup forms (Compton and Volkow, 2005).

Reports have indicated the use, misuse of and dependence on codeine-containing medicines as an emerging global public health concern, given widespread availability of the drug for the symptomatic relief of mild to moderate pain or cough (Lessenger *et al*., 2008; United Nations on Drugs and Crime, 2011; Van Hout *et al*., 2014).

While codeine is abused unaltered in both pill and syrup forms (Compton and Volkow, 2005), the syrup in most cases is used in a mixture of soft drinks, alcohol or candy (Peters et al., 2003) referred to as "purple drink". The mixture or concoction is also known as "syrup," "sizzurp," "barre" and "lean" (nicknamed for the posture that its users assume when intoxicated), (Agnich *et* al., 2013).

Couple infertility, which is defined by the failure to achieve pregnancy after at least 12 months of regular, unprotected intercourse, (Sansone *et al.,* 2018) is a reoccurring societal problem facing the

How to cite this article: Ibeabuchi, K.C.; Gbotolorun, S.C. Testicular histology and seminal assessments following codeine-containing cough syrup administration in male Wistar rats. J Exp Clin Anat 2024; 21(2): 202-209.
https://dx.doi.org/10.4314/jeca.v21i2.9

nations having approximately half of its cases caused by factors related to the male partner (Miyamoto *et al.,*2011). However, exposure to certain drugs and the recreational use of illicit drugs has been reported to be a major contributor to male factor infertility with marijuana, opioid narcotics, methamphetamines, cocaine, and anabolic-androgenic steroids (AAS) topping the list (Amini *et al,* 2014; Fronczak et al., 2012).

Studies have reported the effects of codeine-containing cough syrups on motor and locomotive behaviours and memory (Tijani *et al.,* 2012), on the lungs (Okorie *et al.,* 2021), and on the liver, kidney, blood and brain (Adele *et al.,* 2022). However, there exists scarcity of documented data on the effect of the drug on the testes and seminal values. The present study therefore evaluated the effect of the administration of codeine-containing cough syrup on the testicular histology and seminal values in male Wistar rats.

MATERIALS AND METHODS

Drug

Codeine containing cough syrups (Emzolyn(R) with codeine) were purchased with permission from the head office of Medplus Pharmacy on the Lagos Island. It contained 14mg of Diphenhydramine hydrochloride, 10.9mg of Codeine phosphate, 57mg of Sodium Citrate, and 1.1mg of Menthol as active constituents (salts) in each 5ml spoonful.

Experimental animals

A total of twenty male Wistar rats (110 – 200g) were used for this experiment. They were purchased from an animal/livestock farm in Oshogbo in Osun state, Western Nigeria. The animals were kept in well-ventilated wire meshed-plastic cages under standard room and atmospheric conditions of temperature in the Animal House of the Department of Anatomy, College of Medicine of the University of Lagos with a 12-hour light and 12-hour dark cycle. Rat feed and clean tap water were provided *ad libitum*. Two weeks of acclimatization to laboratory environmental conditions was provided before the commencement of the experiment. Body weights measurements were taken on a weekly basis to monitor changes in the weight throughout the experimental period.

Generally, the study was conducted in accordance with the recommendation from the declarations of Helsinki on guiding principles in care and use of experimental animals (American Physiological Society, 2002) and by the local Animal and Research Ethics Committee of the University of Lagos college of Medicine (CMUL/HREC/02/19/495).

Experimental design

The animals were marked with black hair dye at various body parts for easy and proper identification. They were divided into four groups of five animals each consisting of control and treatment group. The control group (**A**) received feed and water only while the treatment groups (**B-D**) received varying doses of the drug. Group **B** (low dose group) received 10.95 mg/kg, group **C** (medium dose group) received 21.90 mg/kg while group **D** (high dose group) received 43.80mg/kg of the syrup. Doses used were extrapolated based on the maximum therapeutic dose of 40 ml/day for adults, LD50 for codeine in rats (427 mg/kg), and also followed that of Tijani *et al. (*2012) and Adele *et al.* (2022). The drug was administered daily via an oral cannula for a period of eight (8) weeks. The duration of eight (8) weeks was chosen since spermatogenesis takes an average of 51.6 to 56 days (Heller and Clermont, 1963) in rats and 64 days in man (Clermont and Troat, 1972).

Animal sacrifice

At the end of the treatment period, the animals were sacrificed by cervical dislocation. A transverse abdomino-pelvic incision was performed and the testes were accessed, dissected and harvested. The testes were weighed using an electronic weighing scale (Scout Pro SPU 2001, Ohaus Corporation, Pine Brook, NJ USA) before fixing in Bouin's fluid for histological studies.

Sperm Count

Total sperm number was determined with the improved Neuber's counting chamber (haemo-cytometer). Immediately after dissection, the epididymis was cut into smaller pieces to allow the semen to flow out which was diluted in semen diluting medium (50 g of sodium bicarbonate (NaHCO3) and 10 ml of 35% (v/v) formalin in 1000 ml of purified water) in the ratio of 1:20 weight by volume (WHO, 2010). Approximately 10µl of the diluted sperm suspension was transferred to each counting chamber of the haemo-cytometer and was allowed to settle for 5 minutes. This chamber was then placed under a binocular light microscope using an adjustable light source. The counting chamber grids were then focused and the number of spermatozoa counted in five 16-celled squares. The sperm concentration was the calculated number multiplied by 5 and expressed as [X] x 10^6/ml, where [X] is the number of spermatozoa in a 16-celled square (WHO, 2010; Ekaluo *et al.,* 2005).

Sperm Motility

The semen was placed in 0.5 ml of 0.9% normal saline. The solution was allowed to incubate at room temperature for 2 minutes, after which a drop of it was placed on a glass slide using a pipette, and covered with cover slip. The slide was placed on a binocular microscope and observed at 40x magnification. The sample was allowed to stop drifting and sperm cells were randomly examined in fields or areas at least 5mm from the edge of the glass slide (WHO, 2010). Each area viewed was quickly counted and scored to prevent the over estimation of motile cells as a result of other cells swimming into the area during scoring. The motility was based on stationary (immotile), slow progression, rapid progressive and vibrating movement and were expressed in percentage (WHO, 2010; Ekaluo *et al.,* 2013).

Sperm Morphology

About 10µl of the semen aliquot was smeared on a clean glass slide, air-dried, fixed for 15 minutes in 95% ethanol and was

stained with eosin-nigrosin stain for 30 minutes for proper staining to take place (WHO, 2010). 200 sperm cells per animal were morphologically examined at 100x magnification and the abnormalities seen were coiled tail, swollen head, middle part of the head, pin head, only head, bent neck. The values were expressed as a percentage of morphologically normal sperm cells (WHO, 2010; Ekaluo *et al.,* 2009).

Tissue processing

The harvested tissues were processed for microscopic examination using a standard protocol (Aziz & Zeman-Pocrnich, 2021) and 5 µm thick paraffin sections were made (Reichert Ultra Microtome). Slides were stained with routine Haematoxylin and Eosin according to routine procedure for light microscopy (Aziz & Zeman-Pocrnich, 2021) and photomicrographs were made using Leica microscope (ATC 2000 Binocular Microscope, Leica microsystems, Danaher Corporation).

Statistical Analysis

The statistical analyses were done with the Graphpad Prism (version 7.03) software. The data obtained from the seminal analyses were compiled and statistically analyzed using analysis of variance (ANOVA). The results of the data were expressed as mean ±SEM (standard error of mean) while $p<0.05$ was taken as statistically significant.

RESULTS

Physical observations

No death was recorded following the drug administration all through the period of the study (eight weeks). The animals maintained their normal feeding pattern. However, some signs of lethargy (decreased activity and reduced response to handling) were observed in some of the animals in the treated groups from the fifth week of drug administration to the end of the study.

Effect of treatment on Sperm Count, Motility and Morphology

The mean sperm count and motility for the control group were 77.38±3.21 and 80.00±10.63, respectively **(Table 1)**. When compared to the control, only the low dose group also recorded a significant decrease in sperm count at ($P<0.05$).

Effect of treatment on Histology of the testes

The photomicrographs of testicular cross-sections of all the groups appear normal showing normal and closely packed seminiferous tubules **(ST)** containing normal maturing germinal cell layers with the lumen and the interstitial space **(IS)** and cells appearing normal also. The seminiferous epithelium **(SE)** appear normal and well stratified. The Sertoli cells **(SC)** are present and appear normal. The cuboidal spermatogonia cells are present and show active division in all the groups. A zone of mitotic spermatocytes is seen giving rise to a large number of spermatids **(SD)** with their cellular processes extending into the lumen **(L)** of the seminiferous tubules. The testicular interstitial spaces **(IS)** also appear normal and contain the Leydig cells.

Table 1: Effect of treatment on seminal parameters - sperm count, motility and morphology

Experimental Groups	Sperm Count ($X10^6$/Ml)	Sperm Motility (%)	Abnormal (%)
Control	77.38±3.21	80.00±10.63	9.00±1.22
Low	61.96±2.26*	80.80±2.00	24.80±6.22
Medium	74.24±1.91	94.60±24.92	11.80±1.36
High	82.38±2.04	97.00±2.00	15.00±0.02

Values are expressed as Mean±Standard Error of Mean (SEM); n= 5; *P<0.05 significant difference when compared to control.

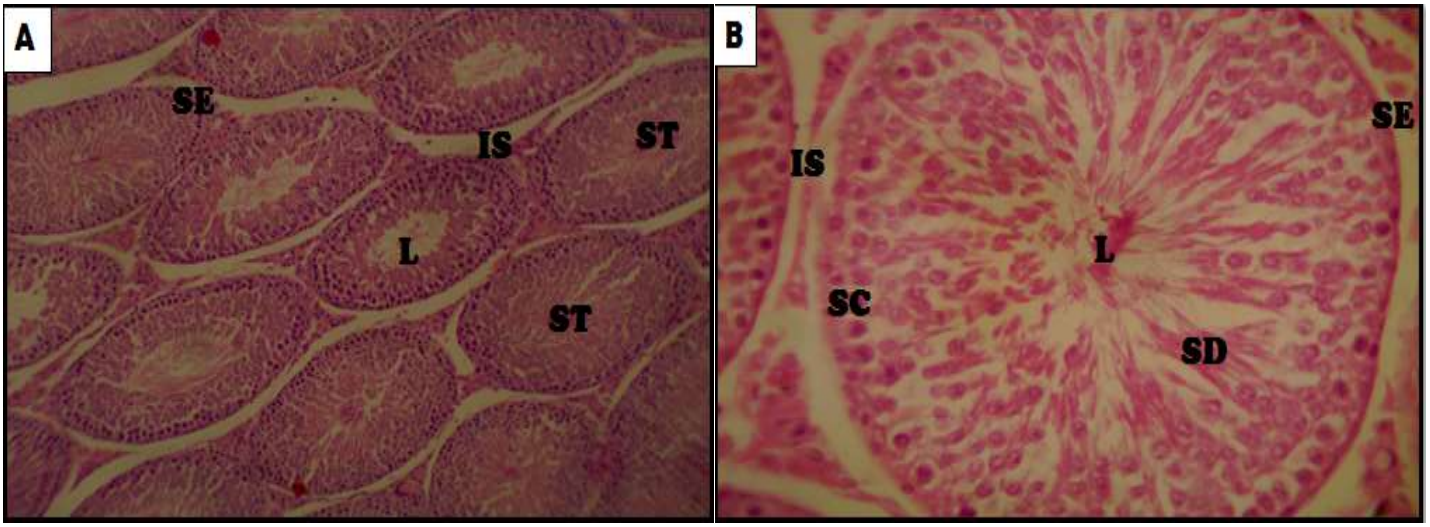

PLATE 1: Normal photomicrograph of testicular sections of the control group showing seminiferous epithelium **(SE)**, Lumen **(L)** of seminiferous tubule, Interstitial Space **(IS)**, Sertoli Cells **(SC)**, Spermatids **(SD)** and Seminiferous Tubules **(ST). H&E X100(A), X200(B)**

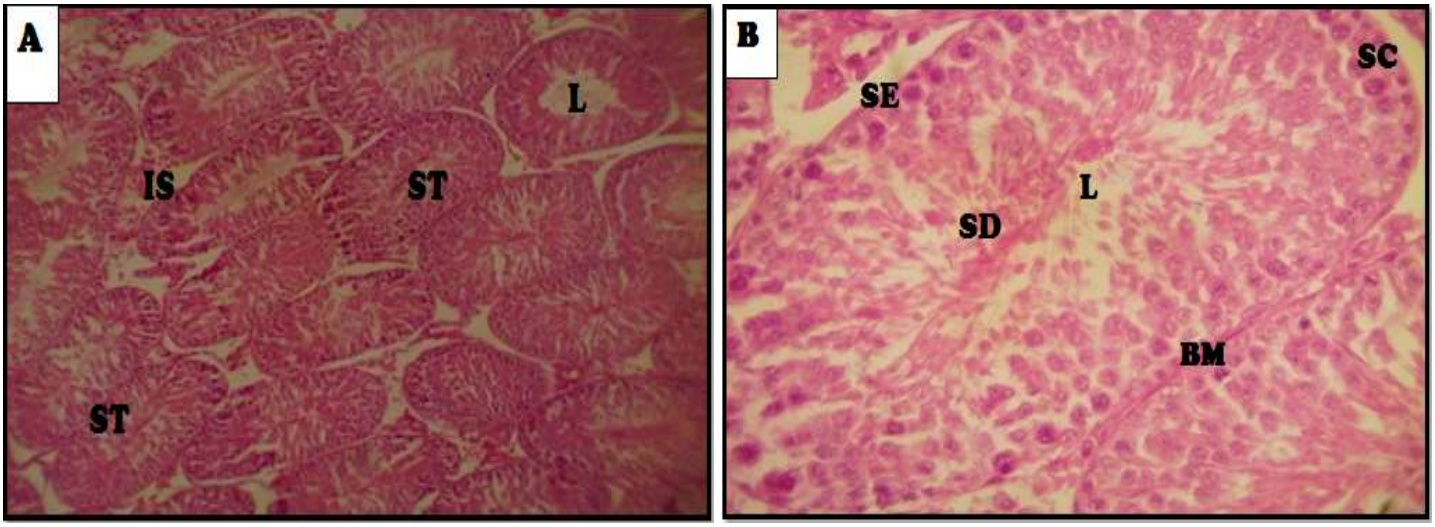

PLATE 2: Normal photomicrograph of testicular sections of the Low dose (10.95mg/kg) group showing seminiferous epithelium (SE), Lumen (L) of seminiferous tubule, Interstitial Space (IS), Sertoli Cells (SC), Spermatids (SD), Seminiferous Tubules (ST) and Basement membrane (BM). H&E X100 (A), X200 (B)

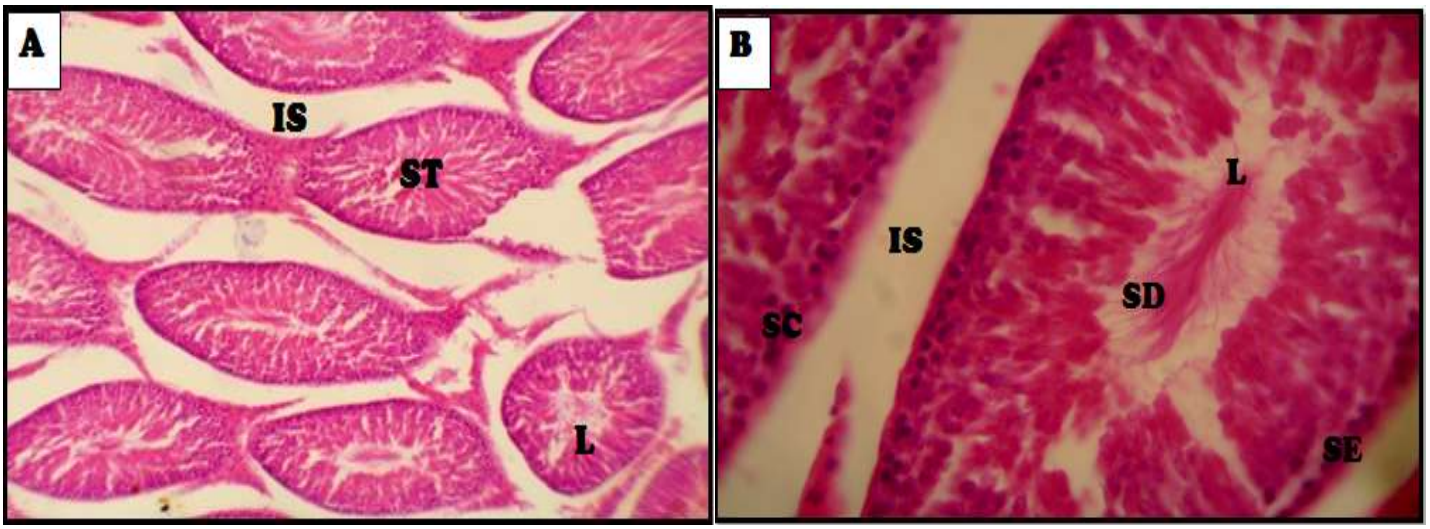

PLATE 3: Normal photomicrograph of testicular sections of the Medium dose (21.90mg/kg) group showing seminiferous epithelium (SE), Lumen (L) of seminiferous tubule, Interstitial Space (IS), Sertoli Cells (SC), Spermatids (SD), and Seminiferous Tubules (ST). H&E X100 (A), X200 (B)

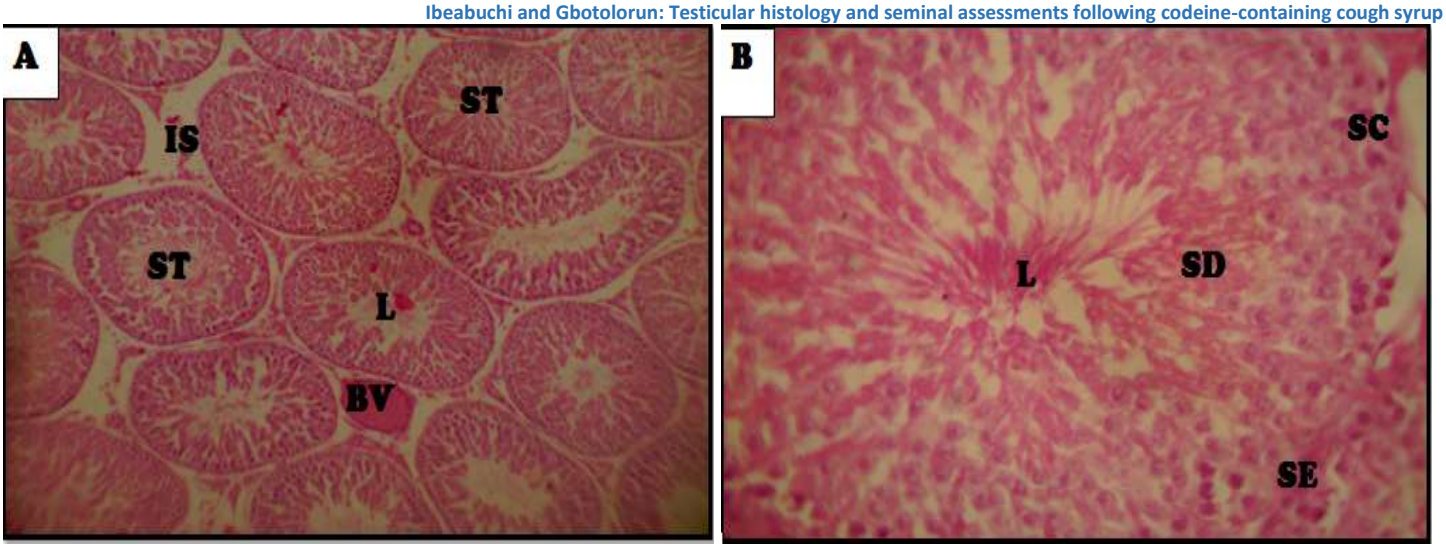

PLATE 4: Normal photomicrograph of testicular sections of the High dose (43.80mg/kg) group showing seminiferous epithelium (SE), Lumen (L) of seminiferous tubule, Interstitial Space (IS), Sertoli Cells (SC), Spermatids (SD), Seminiferous Tubules (ST) and blood vessel (BV). H&E X100 (A), X200 (B)

DISCUSSION

This study investigated the effect of Codeine-containing cough syrup administered for eight weeks on the testes of adult male Wistar rats. The animals remained alive till the end of the study. However, signs of lethargy were observed across the treated groups. This may be as a result of the suppressive action of codeine on the central nervous system and respiratory system (Straube *et al.,* 2014).

The seminal analysis showed a non-significant increment in the sperm count, motility and morphological abnormality in the high dose group when compared to the control. However, there was a non-significant decrease in sperm count, a non-significant increase in sperm motility and a non-significant increase in sperm morphological abnormality in the medium dose group. Meanwhile, the low dose group recorded a significant decrease in sperm count, a non-significant increase in sperm motility and a significant increase in sperm morphological abnormality. Thus, the result suggests that codeine-containing cough syrup may possess the capacity of increasing sperm count and motility at higher doses. This is evidenced in the fact that the low dose group had the lowest sperm count and the highest morphologically abnormal sperm cells.

This result agrees with Sharpe 2012 and Mínguez-Alarcón *et al,* 2018 who reported that increased sperm production and motility were markers of normal testicular function.

Sodium citrate the highest active ingredient in the codeine-containing cough syrup used for this study is an anticoagulant, alkalinizing agent, an osmotic relaxative and an agent for the treatment of urine and metabolic acidosis (Oöpik *et al.,* 2003). It has over the years been used as a cryopreservative and extension medium for sperm cells (Lopez *et al.,* 1999). Lopez *et al.,* (1999) reported that sodium citrate as a cryopreservative medium was found to increase sperm viability, progressive sperm motility, and sperm plasma membrane integrity. With these taken into account, one can link the *in vivo* beneficial effect of codeine-containing cough syrup on the sperm parameters to sodium citrate.

Codeine phosphate an opioid and methylated Morphine derivative constitutes one of the components of the cough syrup used for this study. Studies have consistently shown the deleterious effects of opioids on the male reproductive system (Fronczak *et al.,* 2012; Safarinejad *et al.,* 2013). However, the result of this present comes out in contrast to their findings. This could be because the codeine in the syrup was a part of a mixture with the other constituents having higher concentrations than it and thus may have countered or cushioned whatever negative impact it could have had on the testes, thus showing the absence of testicular damage or dysfunction and confirming increased spermatogenesis.

The histological profile of all the groups showed normal testicular histology with normal and closely packed seminiferous tubules (ST), containing normal maturing germinal cell layers with the lumen (L), interstitial space (IS) and cells appearing normal also. The seminiferous epithelium (SE) appears normal and well stratified. The Sertoli cells (SC) are present and appear normal. The cuboidal spermatogonia cells are present and show active division in all the groups. A zone of mitotic spermatocytes is seen giving rise to a large number of spermatids (SD) with their cellular processes extending into the lumen of the seminiferous tubules. The testicular interstitial spaces also appear normal and contain the Leydig.

The testicular histology of this present study is in concord with the results obtained for the seminal analyses. All these results when taken together show that the testes of the treated animals were normal and devoid of damage. This agrees with Vidal and Whitney

2014 who reported that the presence of morphologic manifestations like necrosis, inflammation, vascular and tubular constriction and dilation, haemorrhage, depletion of germ cells and luminal contents, epithelial atrophy and scanty cytoplasm in testicular histological sections are markers of testicular and ependymal toxicity and dysfunction. And since none of the afore mentioned indicators of testicular toxicity and damage was seen in any of the histological sections across the groups in this present study, one can safely conclude that the testes of all the treated animals were devoid of toxicity or damage. This further confirms the fact that codeine-containing cough syrup had no damaging effect on the testes and the male reproductive system.

Conclusion: Put together, the findings from this study show that codeine-containing cough syrup in contrast to its adverse effects on the central nervous, respiratory and gastrointestinal systems had no harmful effects on the male reproductive system and did not alter the histology of the testes of Wistar rats. It is therefore safe to conclude that it can improve spermatogenesis and other seminal parameters.

REFERENCES

Agnich, L. E., Stogner, J. M., Miller, B. L., and Marcum, C. D. (2013). Purple drank prevalence and characteristics of misusers of codeine cough syrup mixtures. Addictive Behaviors, 38(9), 2445–2449.

Adele BO, Alonge AJ, Adewoye EO (2022) Codeine induced hematological, hepatic alterations, lung and brain damage in mice. Adv Toxicol Toxic Effects 6(1): 001-007.

Allan CM, Haywood M, Swaraj S, Spaliviero J, Koch A, Jimenz M, Poutanen M, Levallet J, Huhtaniemi I, Illingworth PH, Handelsman D J (2001). A novel transgenic model to characterize the specific effects of follicle- stimulating hormone on gonadal physiology in the absence of luteinizing hormone actions. Endocrinology, 142:2213-2220

Amini, M., Shirinbayan, P., Behnam, B., Roghani, M., Farhoudian, A., Joghataei, M. T., &Koruji, M. (2014). Correlation between expression of CatSperfamily and sperm profiles in the adult mouse testis following Iranian Kerack abuse. Andrology, 2(3), 386–393.

Atig, F., Raffa, M., Ali, H.B., Abdelhamid, K., Saad, A., Ajina, M., (2012). Altered antioxidant status and increased lipid per-oxidation in seminal plasma of tunisian infertile men. Int. J. Biol. Sci. 8, 139–149.

Aziz, S.J., Zeman-Pocrnich, C.E. (2021). Tissue Processing. In: Del Valle, L. (eds) Immunohistochemistry and Immunocytochemistry. Methods in Molecular Biology, vol 2422. Humana, New York, NY. https://doi.org/10.1007/978-1-0716-1948-3_4

Babalonis, S., Lofwall, M. R., Nuzzo, P. A., Siegel, A. J., &Walsh, S. L. (2013). Abuse liability and reinforcing efficacyof oral tramadol in humans. Drug and Alcohol Dependence, 129, 116–124.

Bahmani M, Mirhoseini M, Shirzad H, Sedighi M, Shahinfard N, Rafieian-Kopaei A (2015). Review on promising natural agents effective on hyperlipidemia. J Evid Based Complementary Altern Med., 20(3):228–238.

Broussard CS, Rasmussen SA, Reefhuis J, et al.; National Birth DefectsPrevention Study (2011). Maternal treatment with opioid analgesicsand risk for birth defects. Am J Obstet Gynecol 204:314.e1–314.11

Clermont, Y. and Troat, M. (1972). Duration of the cycle of the seminiferous tubule epithelium in mouse and Hamster determined by means of H-3 thymine and auto-radiography. **20:**205

Compton, W., & Volkow, N. (2005). Major increases in opioid analgesic abuse in theUnited States. Drug and Alcohol Dependence, 81(2), 103–108.

Cunha-Silva M, Brito V N, Macedo D B, Bessa D S, Ramos C O, Lima L G, Barroso, Arnhold I J P, Segaloff D L, Mendonca B B, Latronico A C (2018). Spontaneous fertility in a male patient with testotoxicosis despite suppression of FSH levels. Human Reproduction 2018; 33(5):914-918

Eddy, N. B., Friebel, H., Hahn, K. J., & Halbach, H. (1968). Codeine and its alternates for pain and cough relief: 1. Codeine, exclusive of its antitussive action. *Bulletin of the World Health Organization*, *38*(5), 673.

Ekaluo, U.B., A.E. Udokpoh, U.U. Udofia and R.O. Ajang, (2005). Comparative toxicity of five commonly used analgesics on rat sperm count and sperm morphology. Glob. J. Pure Applied Sci., 11: 81-84.

Ekaluo, U.B., E.V. Ikpeme and E.A. Udokpoh, (2009). Sperm head abnormality and mutagenic effects of aspirin, paracetamol and caffeine containing analgesics in rats. Int. J. Toxicol., 7: 1-9.

Ekaluo, U.B., E.V. Ikpeme, Y.B. Ibiang and F.O. Omordia, (2013). Effect of soursop (Annonamuricata L.) fruit extract on sperm toxicity induced by caffeine in albino rats. J. Med. Sci., 13: 67-71.

Frei, M. Y., Nielsen, S., Dobbin, M. D. H, & Tobin, C. L. (2010). Serious morbidity associated with misuse of overthe-counter codeine-ibuprofen analgesics: A series of 27cases. Medical Journal of Australia, 193, 294–296.

Fronczak CM, Kim ED, Barqawi AB (2012). The insults of illicit drug use on male fertility. J Androl., 33(4):515–528.

Havig SM, Wiik E, Karinen R, Brochmann GW, Vevelstad M. (2016). Codeinespikedbeer in a date rape case? Int J Legal Med., 130:1513–1518.

Heller, C. G. and Clermont, Y. (1963). Spermatogenesis in man: an estimate of its duration. *Science.* 140**:** 184-186.

Hudak, M. L. (2016). Codeine pharmacogenetics as a proof of concept for pediatric precision medicine. *Pediatrics*, *138*(1): e20161359.

Kimergard A, Deluca P, Hindersson P, Breindahl T. (2016). How resistant totampering are codeine containing analgesics on the market? Assessing thepotential for opioid extraction. Pain Ther. 5: 187–201.

Koksal, I.T., Usta, M., Orhan, I., Abbasoglu, S., Kadioglu, A., (2003). Potential role of reactive oxygen species on testicular pathology associated with infertility. Asian J. Androl. 5: 95–99.

Kumar T. R., Wang Y., Lu N., and Matzuk M. M.(1997). Follicle stimulating hormone is required for ovarian follicle maturation but not male fertility. *Nat Genet.,* 15**:** 201–204.

Lei Z. M, Mishra S, Ponnuru P, Li X, Yang ZW, Rao Ch V (2004). Testicular phenotype in luteinizing hormone receptor knockout animals and the effect of testosterone replacement therapy. Biol Reprod, 71 (5): 1605-1613

Lessenger, J. E., Feinberg, S. D. (2008). Abuse of prescription and over-the-counter medications. Journal of American Board of Family Medicine, 21(1): 45–54.

Lopez A, Söderquist L, Rodriguez-Martinez H (1999). Sperm viability in ram semen diluted and stored in three different extenders. Acta Vet Scand. 1999; 40 (1): 1-9

Mínguez-Alarcón, L., Gaskins, A. J., Chiu, Y. H., Messerlian, C., Williams, P. L., Ford, J. B., Souter, I., Hauser, R., & Chavarro, J. E. (2018). Type of underwear worn and markers of testicular function among men attending a fertility center. *Human Reproduction*, *33*(9), 1749-1756.

Miyamoto, T., Tsujimura, A., Miyagawa, Y., Koh, E., Namiki, M., & Sengoku, K. (2012). Male infertility and its causes in human. *Advances in urology*, *2012*(1), 384520.

Allouh, M. Z., Daradka, H. M., & Ghaida, J. H. A. (2015). Influence of Cyperus esculentus tubers (Tiger Nut) on male rat copulatory behavior. *BMC complementary and alternative medicine*, *15*, 1-7.

Mylonas, C., Kouretas, D., (1999). Lipid peroxidation and tissue damage. *In Vivo* 13, 295–309

Nasri H, Rafieian-Kopaei M (2014). Protective effects of herbal antioxidants on diabetic kidney disease. J Res Med Sci., 19(1):82–83

Nielsen, S., Cameron, J., & Pahoki, S. (2013). Opportunities and challenges: over-the-counter codeine supply from the codeine consumer's perspective. *The International journal of pharmacy practice*, *21*(3), 161–168.

O'Donnell L, Meachem S. J, Stanton, P. G, McLachlan R. I (2006). Endocrine regulation of spermatogenesis. In: Neill JD, editor; Plant TM, Pfaff DW, Challis JRG, de Kretser DM, Richards JS, Wassarman PM, section editors. Knobil and Neill's Physiology of Reproduc- tion. Sydney, Australia: Elsevier; page 1017-1070.

Okorie, N., Obeagu, E. I., Nnamani, A. D., Ude, U. A., Agada, U. N., obi, I. A., & Ibiam, G. A. (2021). Histopathological Effect of Emzolyn Codein Cough Syrup on Lungs and Its Oxidative Stress Biomarkers. Journal of Pharmaceutical Research International, 33(49B), 228–240.

Oöpik V, Saaremets I, Medijainen L, Karelson K, Janson T, Timpmann S (2003). Effects of sodium citrate ingestion before exercise on the endurance performance in well trained college runners. Br J Sports Med 2003; 37:485-489.

Pakarainen T, Zhang FP, Makela S, Poutanen M, Huh- taniemi I (2005). Testosterone replacement therapy induces spermatogenesis and partially restores fertility in luteiniz-ing hormone receptor knockout mice. Endocrinology 2005; 146(2):596-606

Peters, R. J., Kelder, S. H., Markham, C. M., Yacoubian, G. S., Peters, L. A., & Ellis, A. (2003). Beliefs and social norms about codeine and promethazine hydrochloride cough syrup (CPHCS) onset and perceived addiction among Houstonian adolescents: An addiction trend in the city of lean. Journal of Drug Education, 33(4), 415–425.

Prommer E. 2011. Role of codeine in palliative care. J Opioid Manag.7:401–440

Ramaswamy S, Marshall GR, Pohl CR, Friedman RL, Plant TM (2003). Inhibitory and stimulatory regulation of testicular inhibin B secretion by luteinizing hormone and follicl stimulating hormone, respectively, in the rhesus monkey (Macaca mulatta). Endocrinology 2003; 144(4):1175-1185

Ramaswamy, S., & Weinbauer, G. F. (2014). Endocrine control of spermatogenesis: Role of FSH and LH/testosterone. *Spermatogenesis*, *4*(2), e996025.

Romeo C, Antonuccio P, Esposito M, Marini H, Impellizzeri P, Turiaco N (2004). Hydrophilic vitamin E-like antioxidant reduces testicular ischemia perfusion injury. Urol Res. 4;32:367–71.

Rukkumani R, Aruna K, Varma PS, Rajasekaran KN, Menon VP (2004). Comparative effects of curcumin and an analog of curcumin on alcohol and PUFA induced oxidative stress. J Pharm Pharm Sci. 2004 Aug 20;7(2):274-83

Safarinejad MR, Asgari SA, Farshi A, Ghaedi G, Kolahi AA, Iravani S, Khoshdel AR (2013). The effects of opiate consumption on serum reproductive hormone levels, sperm parameters, seminal plasma antioxidant capacity and sperm DNA integrity. Reprod Toxicol. 2013;36:18–23.

Sansone, A., Di Dato, C., de Angelis, C., Menafra, D., Pozza, C., Pivonello, R., Isidori, A. & Gianfrilli, D. (2018). Smoke, alcohol and drug addiction and male fertility. *Reproductive biology and endocrinology*, *16*, 1-11.

Sharpe R. M (1994). Regulation of spermatogenesis. In: Kno- bil E, Neill JD, editors. The Physiology of Reproduction. New York: Raven Press, Ltd; 1994. p. 1363-434.

Sharpe, R. M. (2012). Sperm counts and fertility in men: a rocky road ahead. EMBO Reports, 13(5), 398–403

Sproule BA, Busto UE, Somer G, Romach MK, Sellers EM (1999). Characteristics of dependent and nondependent regular users of codeine. Journal of Clinical Psychopharmacology. 1999 Aug;19(4):367-372.

Siegel ET, Kim HG, Nishimoto HK, Layman LC (2013). The molecular basis of impaired follicle-stimulating hormone action: evidence from human mutations and mouse models. Reprod Sci; 20(3):211-33

Straube, C., Derry, S., Jackson, K. C., Wiffen, P. J., Bell, R. F., Strassels, S., & Straube, S. (2014). Codeine, alone and with paracetamol (acetaminophen), for cancer pain. *The Cochrane database of systematic reviews*, *2014*(9), CD006601.

Tijani AY ,Salawu OA, John–Africa LB , Sadiq Abubakar and Chindo BA (2012).Behavioural effects of Benylin-Codein in mice. Nature and Science, 10 (4):83-88

Turner, T. T., Lysiak, J. J. (2008). Oxidative Stress: A Common Factor in Testicular Dysfunction. Journal of Andrology, 29(5), 488–498.

United Nations on Drugs and Crime. (2011). The non-medical use of prescription drugs: Policy direction issues. Vienna ,Austria: United Nations Office On Drugs and Crime. https://www.unodc.org/documents/drug-prevention-and-treatment/nonmedical-use-prescription-drugs.pdf

Valko M., Leibfritz D., Moncol J., Cronin M. T. D., Mazur M., and Telser J. (2007). "Free radicals and antioxidants in normal physiological functions and human disease". *International Journal of Biochemistry & Cell Biology,* 39 (1): 44–84.

Van Hout, M. C., Bergin, M., Foley, M., Rich, E., Rapca, A. I., Harris, R., and Norman, I. (2014). A scoping review of codeine use, misuse and dependence, final report. CODEMISUSED Project European Commission 7th Framework Programme, Brussels, Belgium.

Vidal, J. D., Whitney, K. M. (2014). Morphologic manifestations of testicular and epididymal toxicity. Spermatogenesis, 4(2), e979099.

W.H.O. (2010). WHO laboratory manual for the examination and processing of human semen, 5th ed. World Health Organization. https://iris.who.int/handle/10665/44261

W.H.O (2015). Model List of Essential Medicines, 19th List". World Health Organization. April 2015. Archived(PDF) from the original on 13 December 2016. Retrieved 8 December 2022.

Wright, C. Milne, S. Leeson H. (2014). Sperm DNA damage caused by oxidative stress: modifiable clinical, lifestyle and nutritional factors in male infertility. Reproductive BioMedicine Online, 28(6), 684–703

Zhang FP, Poutanen M, Wilbertz J, Huhtaniemi I (2001). Normal prenatal but arrested postnatal sexual devel-opment of luteinizing hormone receptor knockout (LuRKO) mice. Mol Endocrinol, 15(1):172-83