MERIT RESEARCH JOURNALS
www.meritresearchjournals.org

Merit Research Journal of Medicine and Medical Sciences (ISSN: 2354-323X) Vol. 9(1) pp. 031-048, January, 2021
Available online http://www.meritresearchjournals.org/mms/index.htm

DOI: 10.5281/zenodo.4445796

Original Research Article

Histomorphological Study of Oral Administration of Codeine on the Histology of the Testis of Adult Albino Wistar Rats

Saleh M. S[1], Ayuba, Y[2*]

[1]Department of Anatomy, Faculty of Basic Medical Sciences, Bayero University Kano, Kano, Kano State, Nigeria.

[2]Department of Human Anatomy, Faculty of Basic Medical Sciences University of Maiduguri, Maiduguri, Borno State, Nigeria

***Corresponding Author's Email: yohanaayuba125@yahoo.com**

Abstract

This study was conducted to the Histomorphological Study of subacute Oral Administration of Codeine on Testis of Adult Albino Wistar Rats. The objectives of the study include; to investigate the effects of codeine on the histology of the seminiferous tubules, interstitial cells of Lydig of the testis in adult albino Wistar rats at light microscopic level, to assess sperm count and motility following codeine administration in adult albino rats, to evaluate the effects of codeine on body and organ (testis) weight ratio in adult albino rats and to find out the effect of codeine on sperm morphology and sperm motility. Data obtained were subjected and calculated for one-way analysis of variance (ANOVA), followed by student t-test. The results were expressed as mean ± standard error of mean. Analyses of data were carried out using computer statistical software package SPSS version 20. All statement of differences was based on significance at $p<0.05$. Twenty male albino rats were used. The animals were divided into 4 groups of 5 rats each, Groups 1, II, III and IV. Group I serve as the control group and were given normal feed and water ad libitum for 28 days. Group II rats were administered codeine 30mg/kg by orogastric intubation daily for 28 days in addition to normal feed and water ad libitum. Group III rats were administered codeine 60mg/kg. Group IV rats were administered codeine 90mg/kg. Ethical clearance was obtained from the animal ethical committee, Bayero University Kano for the study.Results from this study showed that There was a significant decrease in mean of initial - final body weights of the animals. Sperm count decreases significantly as doses increases, there was a significant decreased in normal sperm cell morphology as doses increases. sperm cell motility showed significant decreases as doses increases. Result of histopathology of the testis showed; mild focal seminiferous tubular hypertrophy, tubules becoming star shaped, mild focal oedema which is also seen in blood vessels, scanty interstitial connective tissue, increased inter tubular space, mild focal degeneration and disruption of seminiferous tubules, disruption and crumbling of the cell of the spermatogenic series.

Keywords: Codeine, Histomorphological Study, Subacute Oral Administration, Testis

INTRODUCTION

History of drug use for therapeutic purpose is as old as medical practice itself. Drugs used for therapeutic purposes also in some cases become toxic to the patient. The reason is attributed to the fact that drug is a substance that brings about change in biological function through its chemical actions (Pappano, 2009).

Codeine has overtaken tramadol since 2015 as the most abuse drug in Nigeria. Thousands of young people in Nigeria are addicted to codeine cough syrup, a medicine that has become a sweet drug. Three million bottles of codeine syrup are drunken every day in just two states Kano and Jigawa in northern Nigeria alone according to recent Nigerian government re-port (www.vanguardngr.com, 2017; Olanrewanju, 2018;

www.bbc.com, 2018).

Codeine (3- methylmorphine) is also the most common opiate consumed worldwide, it is widely used for its analgesic, antitussive and anti-diarrhoeal components (Tremlett *et al*., 2010; Derry *et al.,* 2013). The name codeine was derived from the greek word *kodeia* for 'poppy head' and it is found in the poppy plant ("*papaversomniferumvar album*") naturally (Derry *et al*., 2013).

Codeine is extracted from opium and is a derivative of phenanthrene, which is also produced synthetically by the methylation of morphine.

Codeine exists as base and as salt, but mainly used as codeine sulphate, which is present in hydrated form. It is a white or near white crystal solid that is soluble in water. Codeine is formulated in different number of ways which are used in various health conditions and are administered in different routes of administration (Martindale, 2014). The use and formulation of codeine varies from one country to another and their laws that regulate the production, use and sales of codeine in different nations of the world (Derry *et al*., 2013). This regulation ranges from total prohibition like in case of Nigeria to minimal regulation from pharmacies and codeine producing factories (Martindale, 2014).

Derry *et al*., (2013), reported that codeine prepared in 30mg and above should be classified as 'prescription only medicine'. The maximum recommended dose is 240mg daily and increasing the dose to 60mg four times a day does not increase the efficacy of codeine (Campbell, 2006).

Codeine exists in different form in the pharmaceutical industries. The main forms of codeine are, tablets (60%), capsule, effervescent tablets, syrups, suppository and solutions (EMA, 2013).

Codeine is used in adults for treatment of cancer pain originally and for management of mild and moderate pains in adults and children (Campbell, 2006; Kelly and Madadi, 2012). Recently codeine recommendation for treatment of pain has reduced due to its limited effectiveness, variation in metabolism and availability of more predictable opioids. It is today commonly used as cough medicine because codeine suppresses cough at sub-analgestic doses (NICE, 2010). Codeine in pediatrics was prescribed due to the lower incidence of opioid-related side effects in situations where airway management and neurological assessment are critical (Semple *et al*., 1999). Codeine due to its ease of administration as an oral syrup, capsule, suppository or tablet for post-operative has been used for mild and moderate pain management in children (Tremlett *et al*., 2010).

MATERIALS AND METHODS

This research was conducted in the Department of Anatomy, Beyero University Kano, to investigate the Histomorphological Study of subacute Oral Administration of Codeine on Testis of Adult Albino Wistar Rats.

Materials

Dihydrocodeine tablet (Actavis UK) 30mg, distilled water, Animal cages and Drinkers, Animal feed (vital feed, Grand cerealJos), Syringe and intubation tubes, Electronic weighing balance (BOSCH India), Manual weighing balance (MATLER Germany), Specimen bottles, and EDTA sterile test tubes, Beakers, Pipette, Glass slides, Cover-slips, Hemocytometer improved Neubauer (Deep 1/10 mm, LABART, Germany), , Microscope (Olympus, Germany), Rotary Microtome (Matler, Germany), Reagents of different kinds (Bouins fluid, DPX mountant, glycerol, xylene, paraffin wax, haematoxylin, eosin stains, Formalin, absolute alcohol and Hand gloves,

Animals

Matured adult male albino Wistar rats weighing between 120 to 250 grams was obtained from the laboratory animal holdings, Department of Anatomy, Bayero University Kano, Nigeria. The study was conducted on 20 adult albino Wistar rats. The animals were kept in plastic cages covered with wire mesh in the animal house of the department of Anatomy andmaintained on standard pellet diet and water *ad libitum*. The rats were housed 5 per cage and allowed to acclimatize to existing climatic condition in the animal house for the period of 14 days before the commencement of administration of codeine solution. Animals were kept in well ventilated cages and housing with the average humidity, with a temperature range of between 27 - 30 ± 2^0C. The lighting consists of natural day light: darkness rhythm.

Experimental Design (Protocol)

After acclimatization the 20 rats were divided into 4 groups (I, II, III and IV). Each group comprising of 5 rats each, which were weighed and grouped according to their body weight.

Group I serve as the control group. The rats were given normal feed and water *ad libitum*for 28 days.

Group II rats were administered codeine 30mg/kg by orogastric intubation daily for 28 days in addition to normal feed and water *ad libitum*.

Group III rats were administered codeine 60mg/kg by orogastric intubation daily for 28 days in addition to normal feed and water *ad libitum*.

Group IV rats were administeredcodeine 90mg/kg orally

daily for 28 days in addition to normal feed and water *ad libitum.*

The chosen dose level for this study fell within the range of doses applied by Peter *et al.* (2019).

Ethical clearance was obtained from the animal ethical committee, Bayero University Kano.

Preparation of Codeine Solution

A stock solution of codeine was prepared by dissolving one tablet of 30mg of dihydrocodeine in 5ml of distilled water. From the stock solution doses were calculated and administered to each animal per body weight.

Body Weight of Animals

Body weight of rats were weighusing the digital balance. The body weights of all the rats were taken beforeadministration codeine solution and at weekly intervals during the treatment period and again just before the animals were sacrificed on the 29th day. Body weight changes were observed and recorded.

Body and Testes Ratio

The rats were sacrificed on the 29th day a day after the last dose was administered. The animals were sacrificed by deeply anaesthising the animals with 120mg/kg ketamine injection per body weight. The testes were removed from the scrotum along with the epididymis by a median incision on the scrotal sack and pushed out of the scrotal sack by the help of the fingers. The testes were weighed using the sensitive electronic weighing balance. Body and gonad ratio were calculation by dividing total weight of both testes by body weight of the specific rats under investigation.

Estimation of Sperm Motility and Testicular Sperm Concentration

To estimate sperm motility, one of the testes that was removed was crushed in 10ml of normal saline solution (Filler, 1993). The crushed mixture was pipette and used to feed the haemocytometer with five quadrants. The solution in the hemocytometer was placed under a light microscope (Seed *et al*., 1999; Ohtani *et al*., 2003; Pant and Srivastava, 2003). The sperm motility was observed and recorded. For testicular sperm count a diluting fluid is constituted as follow; i.Sodium bicarbonate 5gii. Formalin neutral 1ml iii. Distilled water 100ml (Sood, 2006). Drawn semen till 0.5mil marks, dilute with the diluting fluid till 11 mark mix properly (Sood, 2006). Charge the chamber, let stand for 2 minutes (sperm cells settle down). The number of sperm head was counted by viewing through the eye piece of the microscope at x40 magnification. The numbers of sperm head were recorded per each quadrant. The average number of sperm head was obtained, by adding the number of sperm head per each quadrant and dividing it by the number of quadrant which in this study is five (Seed *et al*., 1999; Ohtani *et al*., 2003; Pant and Srivastava, 2003). The average number of sperm head will be divided by the dilution factor which is 10ml and multiplied by a standard factor of 10^6 to obtain the total sperm volume (Seed *et al*., 1999; Ohtani *et al*., 2003; Pant and Srivastava, 2003). One of the testes was fixed in bouins fluid for histological and histochemical analysis.

Sperm Morphology Analysis

Epididymis was dissected in 10ml of normal saline (0.9% Nacl) incubated at 37^{0}c (Filler, 1993). Smear was prepared from the suspension, stain with 1% crystal violet solution and examine for sperm morphology abnormalities (Filler, 1993).

Tissue Processing for Paraffin Section

To determine the effect of codeine on the histology of the testis. The testes were fixed in bouins fluid for 48 hours and cut into smaller sizes. The tissues were dehydrated in graded series of ethanol (30, 50, 75, 95 and 100%) in ascending order, cleared in xylene, and infiltrated in molten paraffin. The specimens were embedded in fresh wax and allowed to solidify to form blocks. Paraffin sections between 3-5µm thick were cut using rotary microtome. The sections were placed on a glass slide and stained with haematoxylin and eosin mounted on the glass slide and was covered with a cover slip. The sections were dried overnight at oven temperature between 35 to 40^{0}C (Drury and Wallingo, 1980). Tissues were viewed under the light microscope at objective lens x10, x20 and x40. Photomicrographs of the tissue sections was taken using photomicroscope to obtain a photomicrograph of x100, x200 and x400.

Statistical Analysis

The data obtained from this research, weights of rats, organ weight, testicular sperm concentration, sperm motility and morphology were subjected and calculated for one-way analysis of variance (ANOVA) (Eva and Oskar, 2013) followed by student t-test. The results were expressed as mean ± standard error of mean. Analyses of data were carried out using computer statistical software package SPSS version 20. All statement of

Table 1. Effects of Codeine on Body and Testicular Weight

Groups	Weight of Rats Day 1 (g)	Day 29 (g)	Weight of Rats Both Testes	Weight of Bwt. (g)	Final - Initial Increase(g)		Body to Testis Ratio
I	239.26 ± 2.19		283.58 ± 4.45	2.90 ± 0.13	44.32	0.00	0.01
II	216.40 ± 3.18		245.94 ± 6.21	2.60 ± 0.07	29.54 *	14.78	0.01
III	201.40 ± 8.0		227.66 ± 4.81	2.50 ± 0.03	26.26*	18.06	0.01
IV	183.54 ± 4.01		199.00 ± 8.13	2.20 ± 0.28	15.46*	28.86	0.01

Values are Mean ± SEM. *= p<0.05. (Comparison relative to control). N= 5 where N is the number of rats per group.

Table 2. Effect of Codeine on Testicular Sperm Count

Groups N=5	Mean weight of paired testes Day 29 (g)	Testicular Sperm Count million/mil
I	2.90 ± 0.13	$8.24 \times 10^6 \pm 353000$
II	2.60 ± 0.07	$7.72 \times 10^6 \pm 318400$
III	2.50 ± 0.03	$3.60 \times 10^6 \pm 109500$*
IV	2.20 ± 0.41	$0.60 \times 10^6 \pm 204900$*

Values are Mean ± SEM. *= p<0.05. (Comparison relative to control). N= number of rats per group.

differences was based on significance at p<0.05 (Eva and Oskar, 2013).

RESULTS

Effects of Codeine on Body and Testis Weight and Testis to Body Weight Ratio

The changes in the mean body weights of animals treated with codeine and their control are presented in Table 1. There was a decrease in mean initial minus final body weight of rats administered 30mg/kg of codeine, which decreased by 14.78g. In the group administered 60mg/kg the mean body weight decreased by 18.06g. While in the group administered 90mg/kg codeine the mean body weight decreased by 28.86g. The differences in increased in weight was significant (p<0.05) in all the treated groups when compared to the control which increased by 44.32g.

Testis to body weight ratio was not significant in all the treated groups when compared to the control group at p<0.05.

Effect of Codeine on Testicular Sperm Count

The present study shows that as doses increases sperm count decreases (Table 2). The sperm count was 8.24×10^6 for the control group animals, 7.72×10^6 for the rats treated with 30mg/kg, 3.60×10^6 for the rats administered 60mg/kg and 0.6×10^6 for those administered 90mg/kg. The difference in sperm count of 3.60×10^6 and 0.6×10^6 were statistically significant between the rats administered 60mg/kg and 90mg/kg codeine solution respectively, compared to the control, but was not significant in rats administered 30mg/kg.

Effect of Codeine on Sperm Cell Morphology

The results of sperm cell morphology are presented on Table 3. Sperm cell morphology showed that there was a decrease in normal sperm cell morphology which was dose dependent i.e as doses increases normal morphology decreased. This decreased was statistically significant at p<0.05 in all the treated groups when compared to the control.

Table 3. Effect of Codeine on Sperm Cell Morphology

Group N=5	Normal Morphology (%)	Bent Neck (%)	Headless Tail (%)	Detached Head (%)	Pairing Phenomenon (%)
I	84.80 ± 2.94	14.00 ± 2.45	2.20 ± 0.20	11.20 ± 0.73	2.40 ± 0.24
II	68.00 ± 6.43 *	22.80 ± 6.22	7.20 ± 0.49	19.00 ± 2.45	3.20 ± 0.20
III	57.80 ± 2.56 *	5.40 ± 0.24	19.2 ± 1.72*	17.40 ± 1.29	2.40 ± 0.24
IV	51.40 ± 9.10 *	48.2 ± 1.36*	23.4 ± 2.91*	12.60 ± 0.40	7.60 ± 0.24

Values are Mean ± SEM. *= p<0.05. (Comparison relative to control). N= 5 where N is the number of rats per group

Table 4. Effect of Codeine on Sperm Cell Motility

Group N=5	Excellent (%)	Weak (%)	Dead (%)
I	17.20 ± 1.72	13.20 ± 1.96	68.20 ± 0.73
II	15.60 ± 0.40	23.60 ± 0.40*	89.20 ± 7.03*
III	7.00 ± 0.32*	20.00 ± 0.32	87.40 ± 5.88 *
IV	6.00 ± 0.32*	26.80 ± 3.18*	74.20 ± 0.80

Values are Mean ± SEM. *= p<0.05. (Comparison relative to control). N= 5 where N is the number of rats per group.

Result for bend neck showed an increased in the number of sperm cell with bend neck in the groups administered 30mg/kg and 90mg/kg but was significant at p<0.05 in the group administered 90mg/kg codeine.

For headless tail there was an increased in the number of sperm cells with headless tail which was dose dependent. These increase was significant in the groups administered 60mg/kg and 90mg/kg.

There was an increased in the number of sperm cells with detached head that was more in the low doses i.e 30mg/kg but this increase was not significant when compared to the control.

Effects of Codeine on Sperm Cell Motility

The results for sperm cell motility are presented in table 4. Percentage of excellent motile sperm cell decreases as doses increases from 30mg/kg to 90mg/kg these increases was statistically significant at p<0.05 in treated groups administered 60mg/kg and 90mg/kg codeine.

Weakness in sperm motility increased in the treated group but was significant in the group administered 30mg/kg and 90mg/kg.

The number of dead sperm cells increased in the treatment group but was significant in the group administered 30mg/kg and 60mg/kg codeine. The group administered 90mg/kg showed and increased but was not statistically significant.

Effect of codeine on the histology of the testis

The results of this study showed that photomicrograph of Group one (control group) rat testis showed normal seminiferous tubules, blood vessels, interstitial connective tissue seminiferous tubules with turf of spermatids in the lumen and cells of the spermatogenic series (Figure 1 and 2). Photomicrograph of group II rat's testis treated with 30mg/kg codeine showed some seminiferous tubules with turf of spermatids in the lumen, some seminiferous tubules undergoing hypertrophy becoming star shaped, blood vessel and scanty interstitial connective tissue (Figure 3 and 4).

Photomicrograph of rat testis treated with 60mg/kg codeine showing focal seminiferous tubular hypertrophy (seminiferous tubules appears as star shaped), focal mild degeneration of some of the seminiferous tubules and

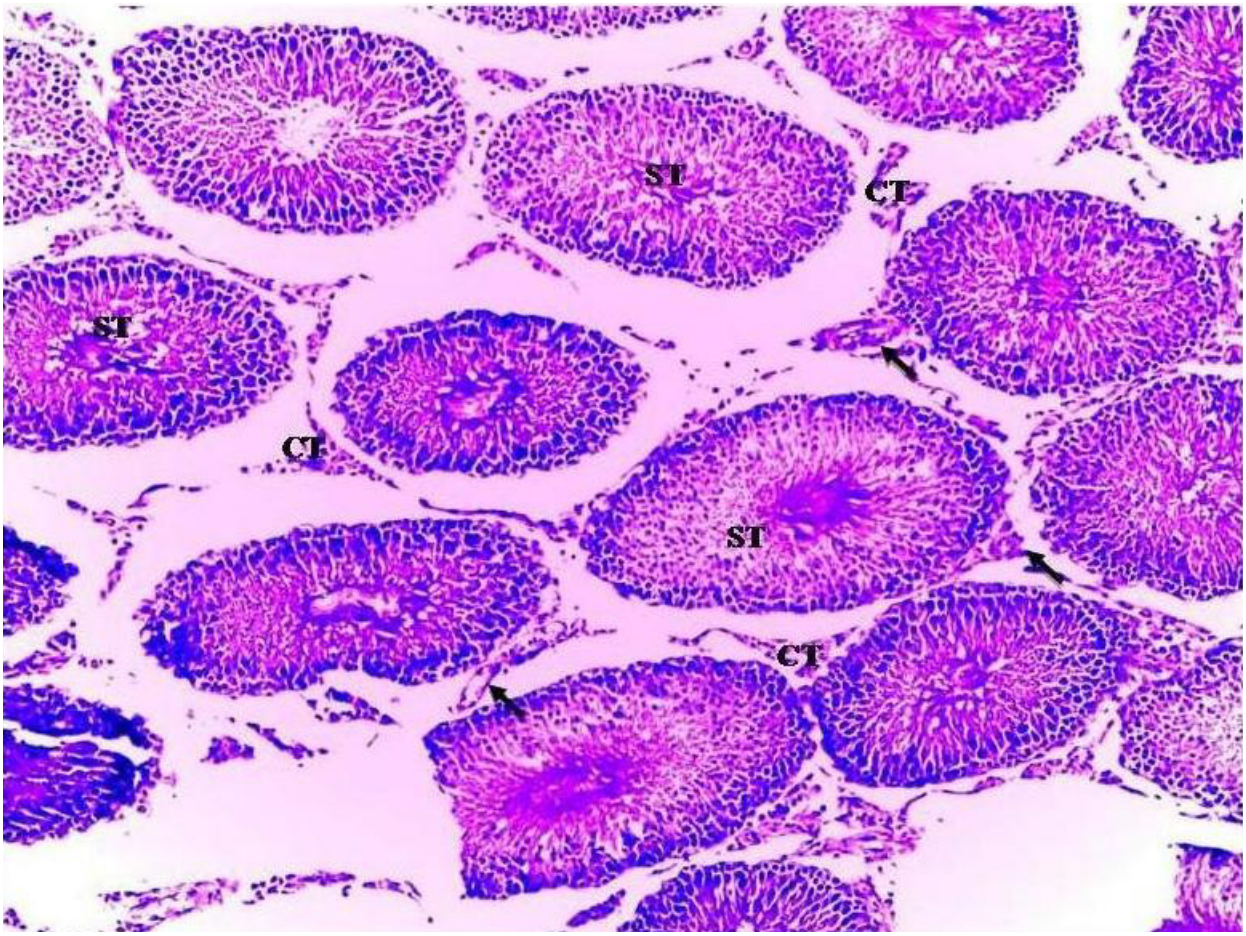

Figure 1. Photomicrograph of control rat testis showing normal seminiferous tubules (ST), blood vessels (arrows) and interstitial connective tissue (CT) H&E x100.

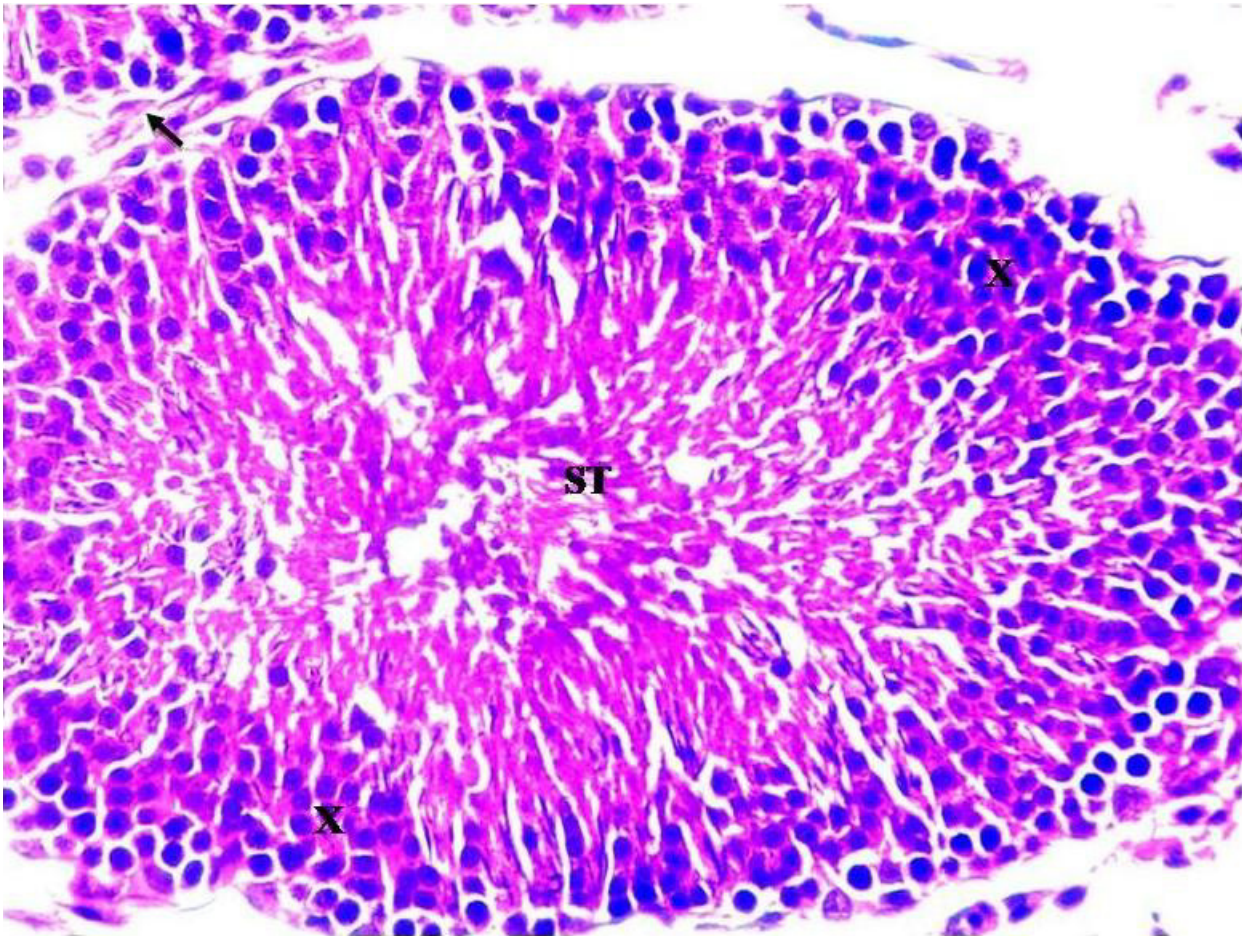

Figure 2. Photomicrograph of control rat testis showing normal seminiferous tubules with turf of spermatids in the lumen (ST), cells of the spermatogenic series (X) and interstitial connective tissue (arrow) H&E x400.

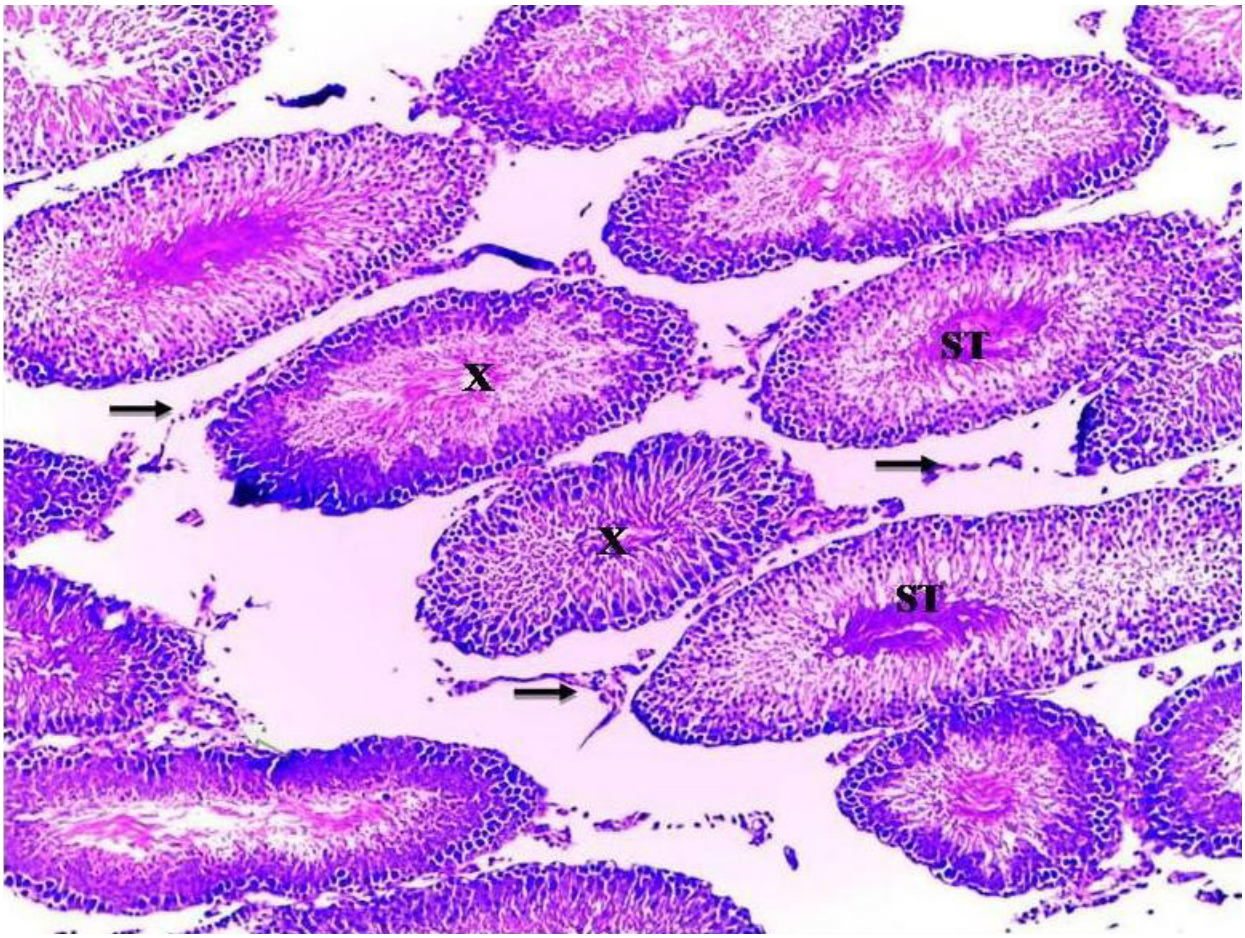

Figure 3. Photomicrograph of group II rat testis treated with 30mg/kg codeine showing some seminiferous tubules with turf of spermatids in the lumen (ST), some seminiferous tubules undergoing hypertrophy by becoming star shaped (X), blood vessel (white arrow) and interstitial connective tissue (black arrow) H&E x100.

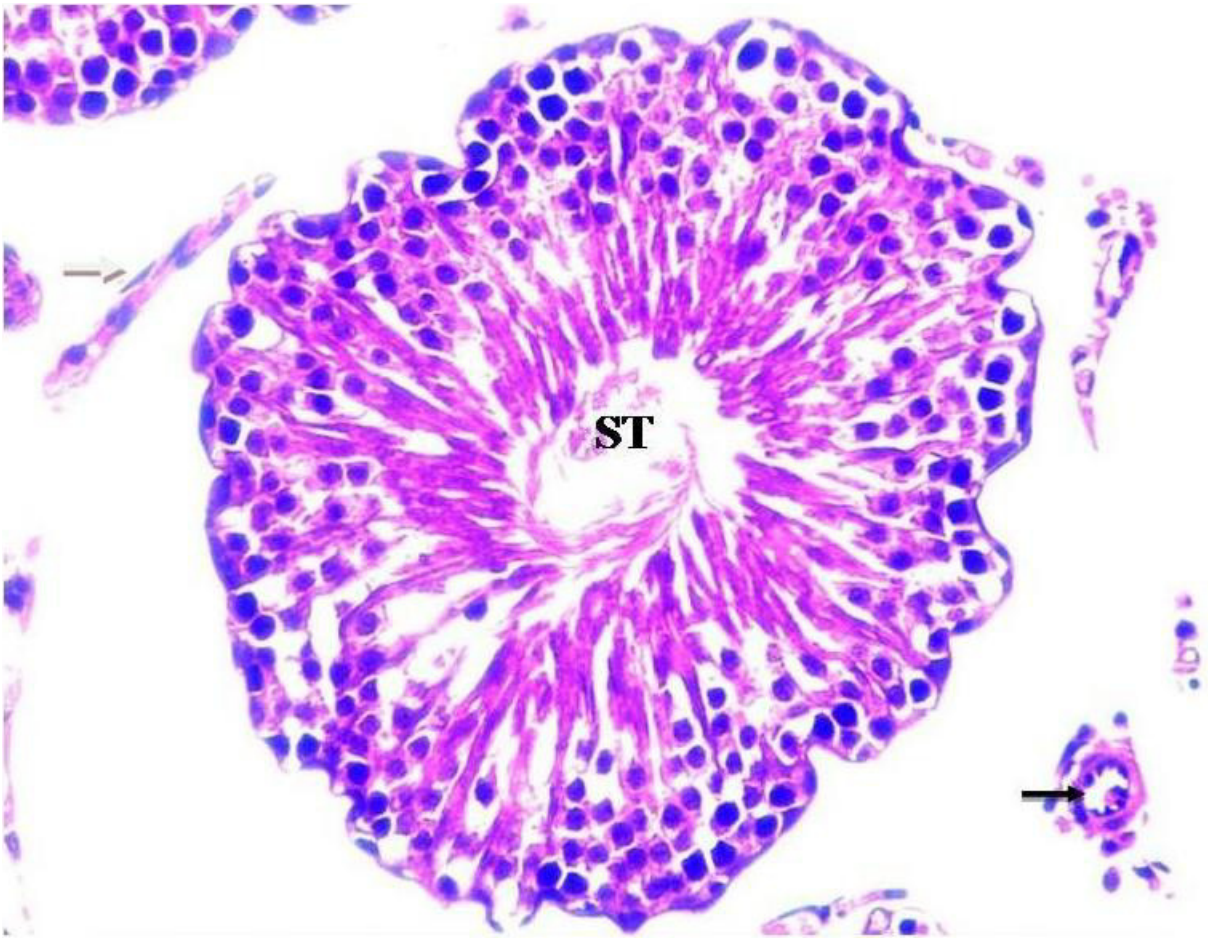

Figure 4. Photomicrograph of rat testis treated with 30mg/kg codeine showing seminiferous tubules (ST), focal seminiferous tubular hypertrophy becoming star shaped (X), blood vessel (black arrow) and scanty interstitial connective tissue (white arrow) H&E x400.

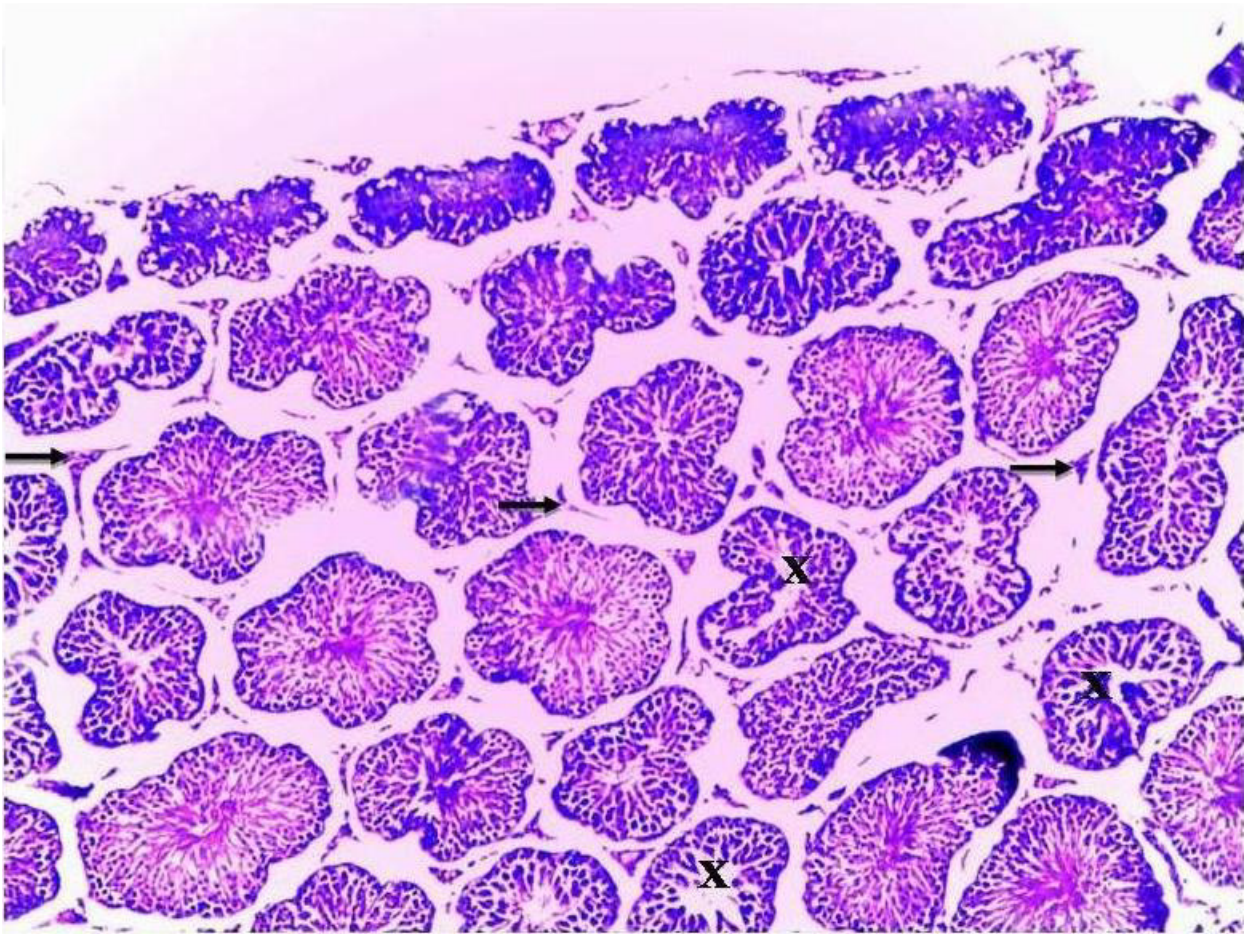

Figure 5. Photomicrograph of rat testis treated with 60mg/kg codeine showing focal seminiferous tubular hypertrophy (seminiferous tubules appears as star shaped), focal mild degeneration of some of the seminiferous tubules (X) and increased interstitial intertubular space of connective tissue (arrows) H&E x100.

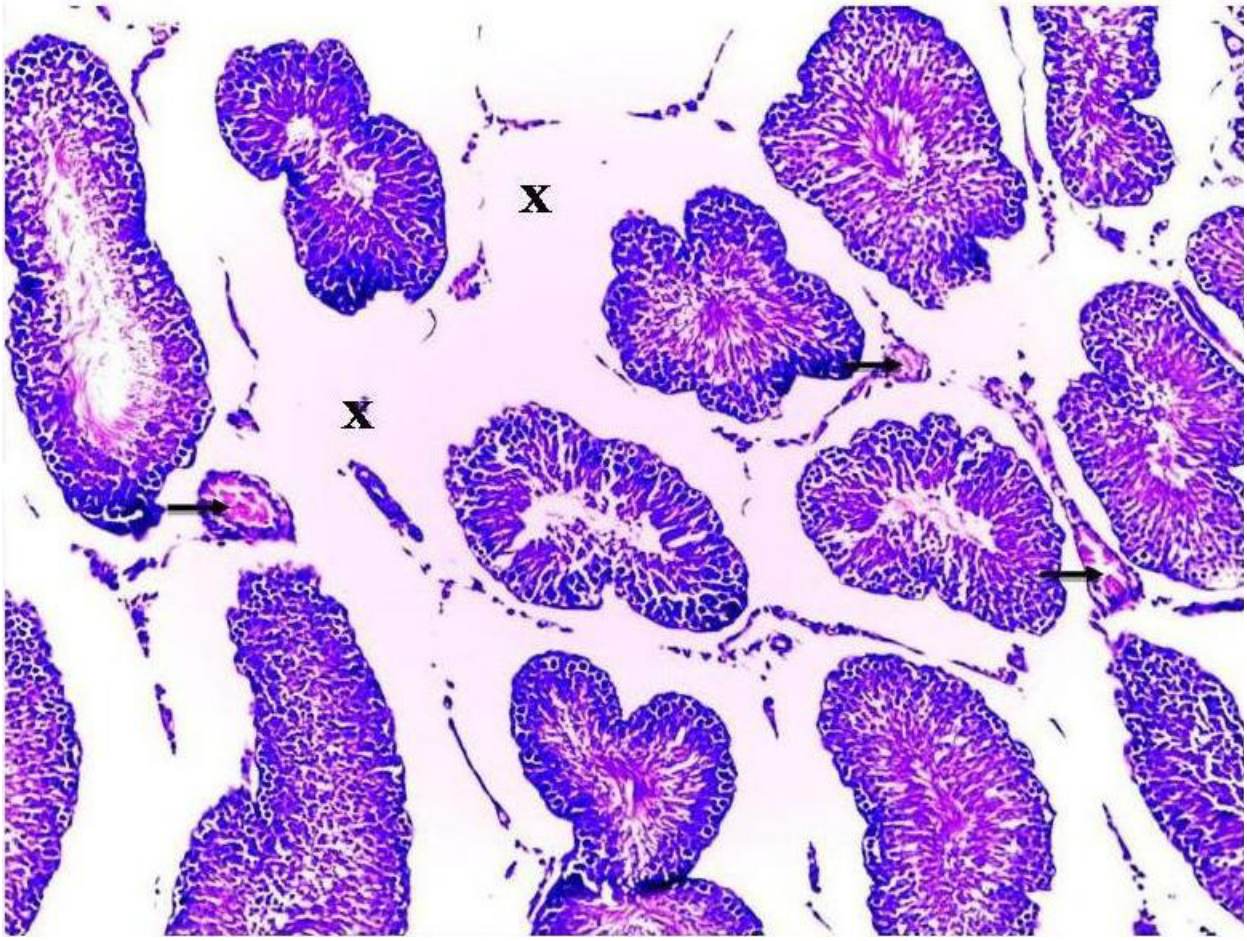

Figure 6. Photomicrograph of rat testis treated with 60mg/kg codeine showing focal seminiferous tubular hypertrophy (seminiferous tubules appears as star shaped) and degeneration (ST), mild oedema of blood vessel (arrows), increased inter tubular spaces (X) and interstitial connective tissue H&E x200.

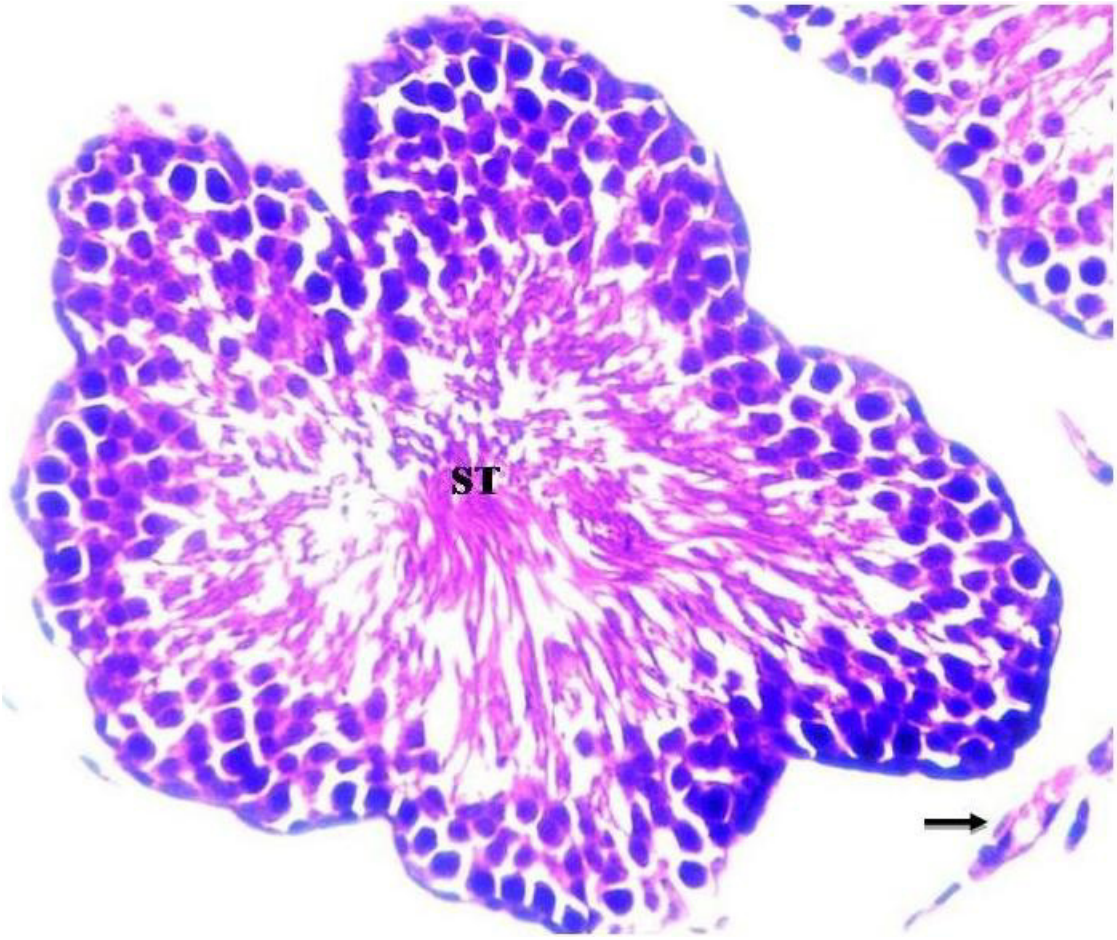

Figure 7. Photomicrograph of rat testis treated with 60mg/kg codeine showing focal seminiferous tubular hypertrophy (seminiferous tubules appears as star shaped) with turf of spermatid in the lumen (ST), and interstitial connective tissue (arrow) H&E x400.

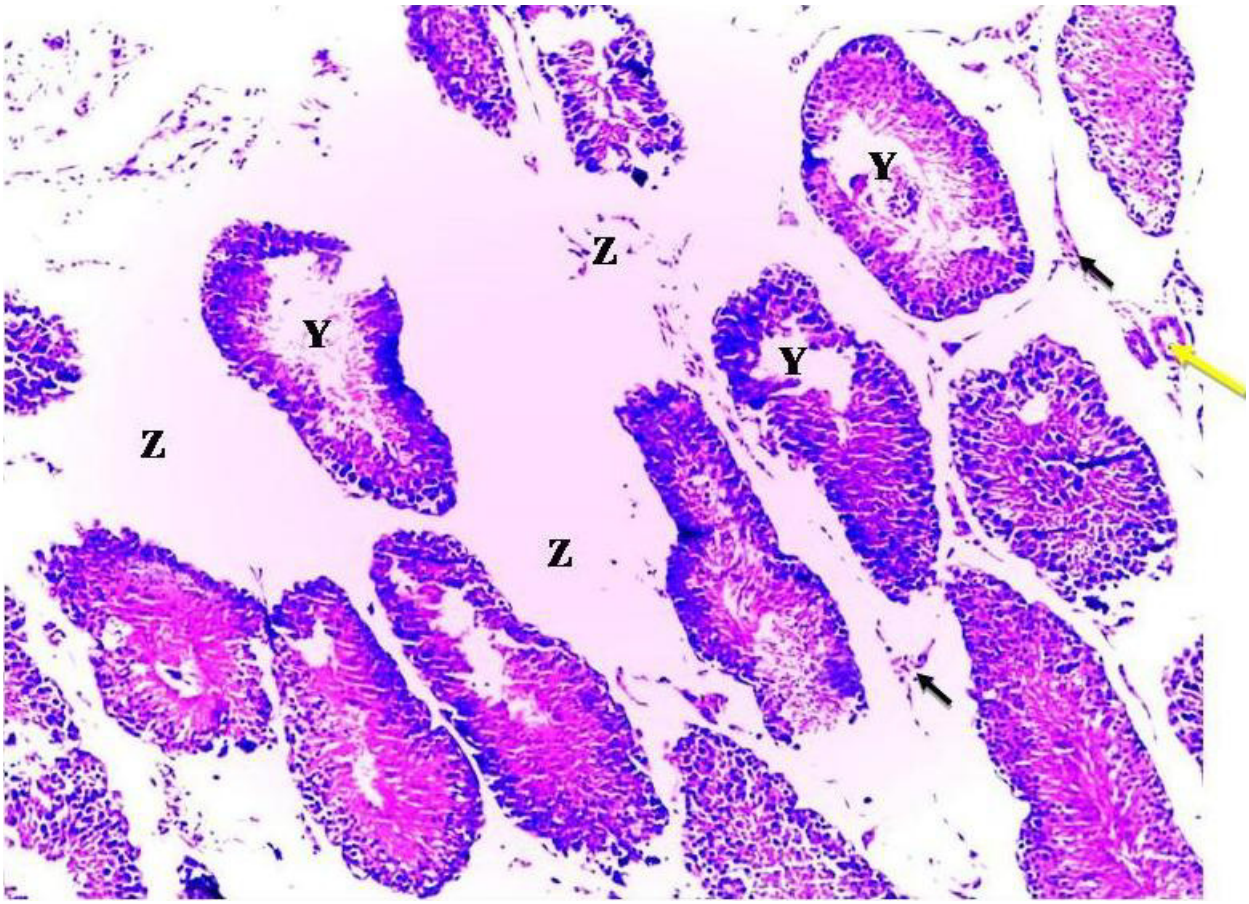

Figure 8. Photomicrograph of rat testis treated with 90mg/kg codeine showing focal moderate seminiferous tubular degeneration (Y), increased inter tubular spaces (Z), mild focal oedema and interstitial connective tissue (arrow) and blood vessel (yellow arrow) H&E x100.

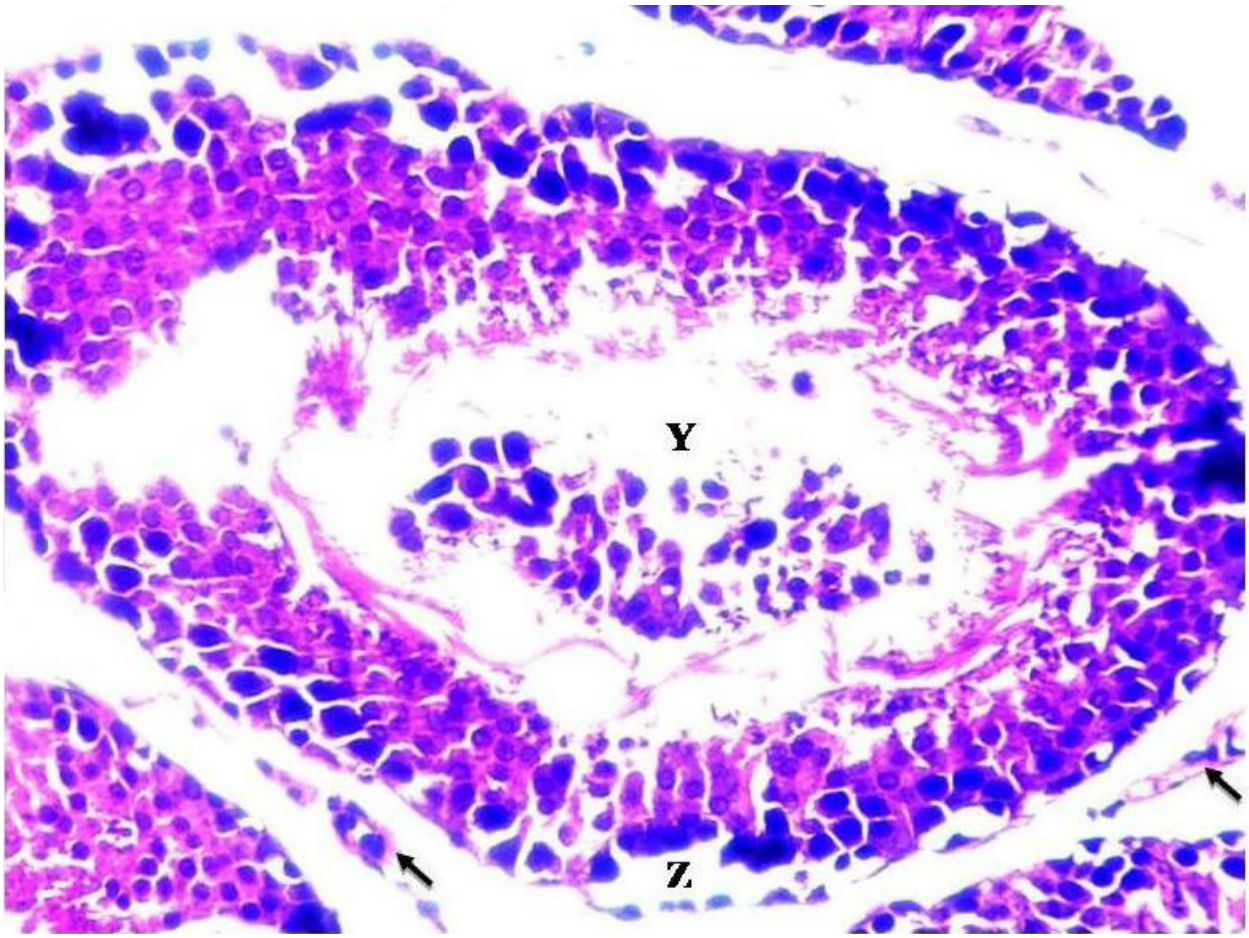

Figure 9. Photomicrograph of rat testis treated with 90mg/kg codeine showing seminiferous tubular eruption and crumbling of the cell of the spermatogenic series (Y), disruption of the basement membrane (Z), and interstitial connective tissue (arrows) H&E x400.

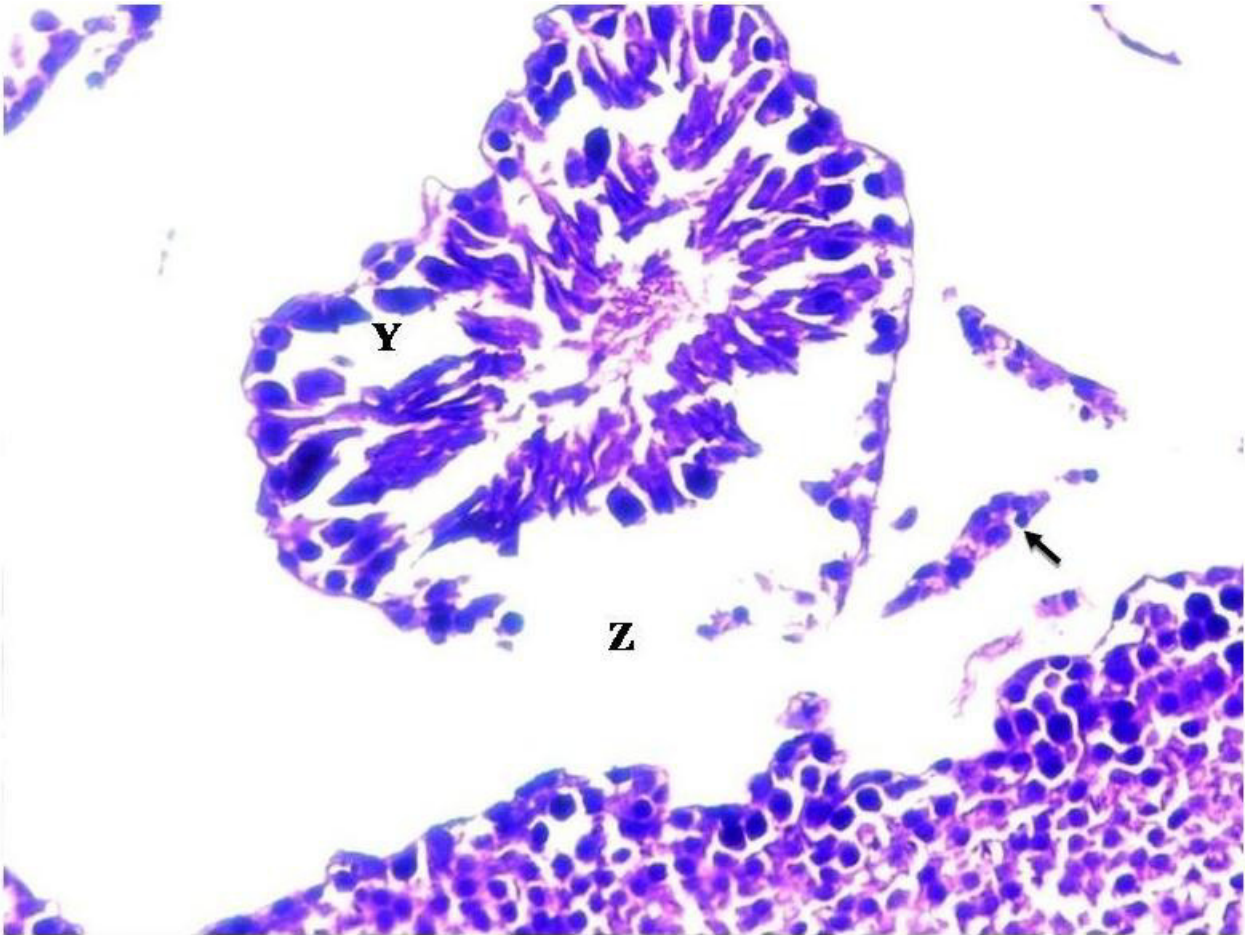

Figure 10. Photomicrograph of rat testis treated with 90mg/kg codeine showing seminiferous tubular eruption and crumbling of the cell of the spermatogenic series (Y), disruption of the basement membrane (Z), and interstitial connective tissue (arrows) H&E x400.

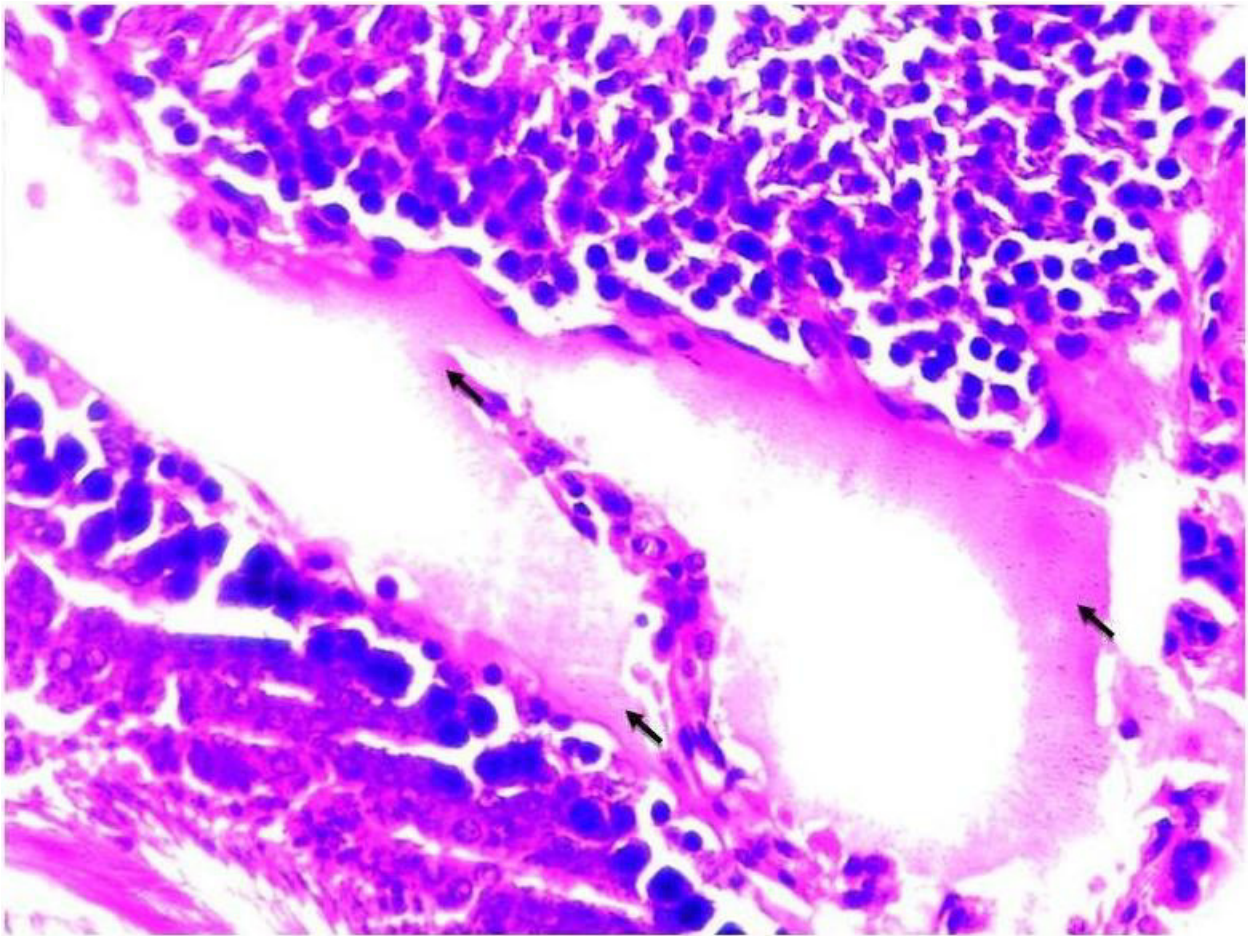

Figure 11. Photomicrograph of rat testis treated with 90mg/kg codeine showing mild interstitial connective tissue oedema (arrows) H&E x400.

increased interstitial inter tubular space of connective tissue and mild oedema of blood vessel (Figure 5 and 6). Photomicrograph of rat testis treated with 90mg/kg codeine showed focal moderate seminiferous tubular degeneration, increased inter tubular spaces, mild focal oedema, scanty interstitial connective tissue, blood vessels, seminiferous tubular eruption, crumbling of the cell of the spermatogenic series, mild interstitial connective tissue oedema and disruption of the seminiferous tubular basement membrane (figure 1 - 11).

DISCUSSION

Effect of codeine on body to testis weights and ratio

The result from this study showed that the increased in mean body weights decreased as doses increases. This decreased observed were significant at $p<0.05$ in the treated group when compared with the control group. Decreased in body weights were observed in both male and female rats at every exposure in a dose dependent manner (Dunnick and Elwell, 1989). Codeine as reported by Marcus, (2010) caused decreased in weight of the testis in male rats when 12500ppm was administered. In mice it was reported that codeine caused decreased in body weights at a dose of 6250ppm in male (Dunnick and Elwell, 1989). The changes in testicular weight, body to testis weights ratio did not decreased significantly when compared to the control. Marcus, (2010) reported a decreased in weight of the testis in male rats when 12500ppm codeine was administered. National Toxicology Program (NTP) in 1996, reported decreased in testicular weight when codeine sulfate cough syrup was used by drug abusers (NTP, 1996). This decreased in body weights may be due to loss of appetite observed. It may also be due to the toxic effect of codeine. The weights of the testes that did not decreased significantly may be due to the range of doses administered in this study.

Effect of codeine on sperm count

It has become a fact from this study that codeine caused decreased in sperm count at doses of 60 and 90mg/kg

oral administration which was significant at $p<0.05$. These findings were in line with what was obtained by Marcus (2010) and Greaves, (2007b) who reported decreased in sperm count at doses of 12500ppm and 40mg/kg three times daily for eight weeks respectively. Codeine mostly affects the brain. Follicle stimulating hormones and luteinizing hormones which are produced by the anterior pituitary gland of the brain regulates the production of testosterone which also regulates sperm production. This could have affected sperm production in the testis by reducing it. The result also showed effects in sperm morphology and motility which could also be a factor for the low sperm count which was dose dependent.

Effect of codeine on sperm morphology

This study showed that there was decreased in the number of normal sperm cells morphology as doses increases. The number of sperm cells with bent neck increased in the treatment groups but was significant in the high dose group. The number of sperm cell with headless tail increased progressively as doses increases, which was significant at $p<0.05$. The sperm cells with detached head increased in the treatment group but the increased was statistically not significant. For pairing phenomenon there was a slight increased mean value in the treatment group when compared to the control group which was not significant. Abnormalities observed may be due to the toxic effect of codeine or its interference on the central nervous system especially the posterior pituitary which produces FSH and LH. This result was parallel to the study conducted by Marcus (2010), who reported that toxicity was not observed in sperm morphology when codeine was administered orally. While Heba, in 2015 worked on tramadol a close associate of codeine and reported exfoliation of the damaged spermatocytes and spermatids which was observed within the tubular lumina of many seminiferous tubules.

Effect of codeine on sperm motility

Excellent sperm cell motility decreased as doses increased, weakness of sperm cell increases as doses increased in the treatment group and the number of dead sperm cells increased in dose dependent manner. This effect on sperm cell motility was probably to the toxic effect of codeine on the sperm cell or shortage of the regulatory hormones of FSH and LH. These findings were supported by the study of Ahmed and Kurka (2014) who administered 40mg/kg tramadol 3 times daily for eight weeks who reported decreased in sperm cell motility and Cariello *et al*., 1986 in their study reported that opioids abuse caused decreased in sperm motility.

Effect of codeine on the histology of the testis

The results of this study showed that the rats testis treated with 30mg/kg codeine, showed some seminiferous tubules undergoing hypertrophy, by becoming star shaped and scanty interstitial connective tissue. Rats treated with 60mg/kg codeine showed focal seminiferous tubular hypertrophy (seminiferous tubules appears as star shaped), focal mild degeneration of the seminiferous tubules and increased inter tubular spaces and mild oedema of blood vessel. Rat's testis treated with 90mg/kg codeine showed, focal moderate seminiferous tubular degeneration, increased inter tubular spaces, mild focal oedema, scanty interstitial connective tissue, seminiferous tubular eruption, crumbling of the cell of the spermatogenic series, mild interstitial connective tissue oedema and disruption of the seminiferous tubular basement membrane. This study was in accordance to the study of Greaves, (2007b), who reported that codeine causes testicular atrophy and degeneration of the seminiferous tubules in the testis of albino rats. National Toxicology Program (NTP) in 1996 reported, degeneration of testicular tissues. This study was also in line with the study carried out by Marwa *et al*., (2015), were they administered tramadol 40mg/kg for one month. The result showed irregular contour of widely spaced seminiferous tubules, the interstitial tissue was disorganized and there was diffused vacuolitions of the seminiferous tubules which was also observed (Marwa *et al*., 2015). The study also observed an apparent decrease in germ cells number, exfoliation of germ cells in the lumina and separation of the germinal epithelium from the underlying basement membrane (basal lamina). In some tubules spermatogonia appeared with hyperchromatic karyolysis and apoptosis. Most of the cells detected were sertoli cells and some of them revealed mitotic division (Marwa *et al*., 2015). Histological examination of testicular tissues when tramadol was used in the study of Youssef and Zidan, (2016), demonstrated atrophy of the seminiferous tubules with interstitial calcification. Focal testicular degeneration with single and multiple layer of v acuolated spermatocytes was observed when gradual increased dose from 7.2mg/kg-144mg/kg tramadol was administered for sixty days (Youssef and Zidan, 2016).

CONCLUSION

This study concludes that codeine affects the testis by distorting the normal cytoarchitecture of the organ causing degeneration of the organ. Codeine also caused oedema, increased inter tubular spaces of seminiferous tubules, hypertrophy of the testes, degeneration of the interstitial tissues. Codeine caused decreased in sperm count, normal morphology and motility. Codeine caused decreased in body weight. Codeine use could be link to infertility. Codeine at these doses are not safe.

REFERENCES

Ahmed MA, Kurkar A (2014). Effect of opoiod tramadol treatment on testicular functions in adult male rats; the role of nitric oxide and oxidative stress. *Clin. Exp. Pharmacol. Physiol*. 41: (4): 317.

Campbell W (2006). Appropriate drug treatment of mild-to-moderate pain. *Prescriber,* 17(18), 28-38.

Cariello L, Zanetti L, Spgnunolo A, Nelson L (1986). Effects of opioids and antagonist on the rate of sea urchin sperm progressive motility. *Boil Bull*. 171:208-216.

Derry S, Karlin SM, Moore RA (2013). Single dose oral ibuprofen plus codeine for acute postoperative pain in adult. *The Cochrane Database of Systematic Reviews,* Issue 3.Art. No.: CD010107.

Drury RAB, Wallingo EA (1980). Histological techniques 24(49): 279-280.

Dunnick JK, Elwell MR (1989). Toxicity studies of amphetamine sulfate, ampicillin trihydrate, codeine, 8-methoxypsoralen, alpha-methyldopa, penicillin VK and propantheline bromide in rats and mice. *Toxicology* 56:123-136.

EMA, European Medicines Agency (2013). *Assessment report for codeine-containing medicinal products indicated in the management of pain in children.* Procedure under Article 31 of Directive 2001/83/EC resulting from pharmacovigilance data Procedure number: EMEA/H/A-31/1342. London: European Medicines Agency.

Eva O, Oskar O (2013). Methodology and application of one-way ANOVA. *Ame. J. Mech. Eng*. vol. 1, No. 7, 256- 261.

Filler R (1993). Methods for evaluation of Rat Epididymal Sperm Morphology. In: Methods in toxicology. Male reproductive toxicology, Chapin, R. E. and Heindel, J. J. (Eds). Academic Press, New York, pp: 334-343

Greaves P (2007b). Interpretation and relevance in drug safety, elsevier science.

Heba (2015). Effect of tramadol on the reproductive function of Wistar albino rats. Pelagia Research Library. *Euro. J. Bio*., 5(1); 56-64.

Kelly LE, Madadi P (2012). Is There a Role for Therapeutic Drug Monitoring With Codeine? *Therapeutic Drug Monitoring,* 34(3), 249–256.

Marcus SD (2010). Drug information. Pharmacological review(s). pharmacology/toxicology and DNA review and evaluation, department of health and human services public health service food and drug administration; center for drug evaluation and research p. 7

Martindale (2014). The complete drug reference. 38th ed. London; pharmaceutical press 4109.

Marwa M, El Sawy and Hany, W. Abdel Malak (2015). Effect of tramadol abuse on testicular tissue of adult albino rat: A Light and Electron Microscopic Study. *The Egypt. J. Histol.* 31: 1528- 2015.

NICE, National Institute for Health and Clinical Excellence (2010). Analgesia - mild-to-moderate pain. *Retrieved from http://cks.nice.org.uk/analgesia-mild-to-moderate-pain.*

NTP, National Toxicology Program (1996). Toxicology and carcinogenesis studies of codeine (CAS No. 76-57-3) in F344/N rats and B63CF1 mice (Feed Studies). U S Department of Health and HumanServices, Public Health Service, National Institutes of Health, Research Triangle Park,NC. Technical ReportSeries No 455 NIH publication No 96-*3360.*

Ohtani K, Yamazaki S, Kubota H, Miyagawa M, Saegusa J (2003). Comparative investigation of several sperm analysis methods for evaluation of spermatotoxicity of industrial chemical: 2-bromopropane as an example. Industrial health 42, 219-255.

Olanrewanju E (2018). Nigerian's codeine epidemic has seen millions of youth became hooked to the drug. www.bbc.com.

Pant N, Srivastava SP (2003). Testicular and spermatoxic effect of quinaphos in rats. *J. Appl*. *Toxicol*. 23: 271-274.

Pappano AJ (2009). "Cholinoceptor-blocking drugs". In: Katzung BG, Masters SB, Trevor AJ, editors. Basic and Clinical Pharmacology. 11th edition. New York, NY: McGraw-Hill Companies Inc.

Peter UA, Nosa TO, Erabor T, Henry E, Eze C, Okoyeocha O (2019). Codeine and its Histopathological Effect on Brain of Albino Rats. An Experimental Study. *Acta Scientific Nutritional Health* 3.2: 125-133.

Seed J, Chapin RE, Clegg ED, Dostal LA, Hurtt ME (1996). Methods for assessing sperm motility, morphology and counts in rats, rabbits and dog: a consensus report. *Reprod.Toxicol*.10: 237-244.

Semple D, Russel S, Doyle E, Aldridge LM (1999). Comparison of morphine sulphate and codeine phosphate in children undergoing adenotonsillectomy. *Paediatric Anaesthesia, 9*(2), 135-138.

Sood R (2006). Semen analysis. Textbook of Medical Laoratory Technology, Maulana Azad Medical Collegenew Delhi. Japee Brother Medical Publishers (P) Ltd. (13): 408-410

Tremlett M, Anderson BJ, Wolf A (2010). Pro-con debate: is codeine a drug that still has a useful role in pediatric practice? *Pediatric* 146 *Anaesthesia, 20*, 183–194.

www.bbc.com (2018). Sweet sweet codeine-inside nigeria's deadly syrup trade. Africa's new investigations unit, Africa Eye.

www.vanguardngr.com/2017/10/drug-abuse-3m-bottles-codeine-consume-daily-north-sanate/

Youssef SH, Zidan AHM (2016). Histopathological and biochemical effects of acute and chronic tramadol drug toxicities on the liver kidney and testicular funtion in adult male albino rats. *J. Med Toxicol Clin Forencic Med* 1:2.