Research Article

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Journal of Anatomical Science and Research

2021
Vol. 4 No. 1:1

Ovarian Toxicity Following Subacute Oral Administration of Codeine in Albino Wistar Rats

Muhammed S. Saleh[1] and Yohana Ayuba[2*]

[1]Department of Anatomy, Bayero University, Kano, Nigeria; [2]Department of Human Anatomy, University of Maiduguri, Maiduguri, Nigeria

Corresponding author:
Yohana Ayuba, Department of Human Anatomy, University of Maiduguri, Maiduguri, Nigeria, Tel: 07033013321; E-mail: yohanaayuba125@yahoo.com

Citation: Ayuba Y, Saleh MS (2021) Ovarian Toxicity Following Subacute Oral Administration of Codeine in Albino Wistar Rats. J Anat Sci Res Vol. 4 No.1: 1.

Abstract

This study was on Ovarian Toxicity Following Subacute Oral Administration of Codeine in Albino Wistar Rats. The objectives of the study include to investigate the effects of codeine on the histology of the ovarian follicles in adult albino Wistar rats at light microscopic level, to determine the effects of codeine on estrogen, progesterone, FSH and LH blood levels, in female adult albino Wistar rats to ascertain the effects of codeine on estrous cycle in female albino rats and to evaluate the effects of codeine on body and ovaries weight ratio in adult albino Wistar rats. 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 female 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 they were given normal feed and water ad libitum for 28 days. Group II rats were administered codeine 30 mg/kg by orogastric intubation daily for 28 days in addition to normal feed and water ad libitum. Group III rats were administered codeine 60 mg/kg. Group IV rats were administered codeine 90 mg/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 slight decrease in mean of initial-final body weights of the animals which was not significant. Body to ovarian weight ratio increased which was significant at doses of 60 and 90 mg/kg. Estrous cycle was extended between 5-6 days on an average. Estrogen, Progesterone, Follicle stimulating hormone and Luteinizing hormone blood levels did not show much variation when compared to control. The histopathology of the ovary showed mild and moderate focal degeneration of ovarian follicles undergoing process of ovulation, degeneration of the granulosa cells, loss of oocytes completely in most of the follicles, oocyte undergoing process of degeneration, degeneration of the connective tissue stroma focal oedema and also in some blood vessels.

Keywords: Codeine; Oral administration; Ovarian toxicity; Wistar rats

Received: January 13, 2021; **Accepted:** January 26, 2021; **Published:** February 2, 2021

Introduction

Drugs have been used long ago as therapeutic agents and has shown to impaired both male and female fertility through interference with the hormonal and none hormonal mechanisms. They have been found to interrupt by suppressing the hypothalamic-pituitary-gonadal axis. Hormones such as LH and FSH were they reduced the synthesis of oestrogen and progesterone [1-3].

Some of these drugs damage the gonad tissue directly. Codeine (3-methylmorphine) is a weak opiate is one of the most accessible and commonly used opiate in the world, this is due to its analgesic, antitussive and anti-diarrhoeal properties. Its common side effects are dependence such as sedation, euphoria and constipation among others. Misuse and abuse of codeine is one of the leading emerging health challenges in various nations of the world. This is because it is easily accessible as over the counter (OCT) drug in various countries of the world. Thousands

of young people both male and females in Nigeria have become addicted to codeine especially the codeine cough syrup [4-10].

This was reported in a documentary of the British Broadcasting Corporation (BBC) in May, 2018, titled "Sweet Sweet Codeine". This documentary by the BBC led to the total ban on importation, production and sale of codeine and its related products as been over the counter drug, it is still used as prescription drug only nevertheless this use lack legal backing. Opioids abuse has led to global public health concerns due its overall consequences on youth and the society at large. It is therefore imperative to evaluate ovarian toxicity following subacute oral administration of codeine in Wistar albino rats [9-12].

Material and Methods

Materials

Dihydrocodeine tablet (Actavis UK) 30 mg, distilled water, Animal cages and Drinkers, Animal feed (vital feed, Grand cereal Jos), 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 female albino Wistar rats weighing between 110 to 150 grams was obtained from the laboratory animal holdings, Department of Anatomy, Bayero University Kano, Nigeria. The study was conducted on 20 albino Wistar rats. The animals were kept in plastic cages covered with wire mesh in the animal house of the department of Anatomy and maintained 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 ± 20°C. 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 comprises 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 30 mg/kg by orogastric intubation daily for 28 days in addition to normal feed and water ad libitum.

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

Group IV, rats were administered codeine 90 mg/kg orally daily for 28 days in addition to normal feed and water ad libitum.

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 30 mg of dihydrocodeine in 5 ml 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 was weigh using the digital balance. The body weights of all the rats were taken before administration 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 ovary 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 120 mg/kg ketamine injection per body weight. Ovaries from all the female animals were obtained by median incision on the abdomino-pelvic region. The ovaries were weighed using the electronic weighing and digital balance. Body and ovarian weight ratio were calculation by dividing total weight of both ovaries by body weight of the specific rats under investigation.

Hormonal assay

Blood samples were collected by cardiac puncture method in a plain sterilized EDTA bottle. The samples were centrifuged at 1000 rpm × g for 5 mins and the serum was used to determine the levels of estrogen, progesterone, FSH and LH using enzyme link immunosorbant Assay (ELISA), according to manufacturer's instruction in the Department of Chemical Pathology, Ahmadu Bello University Zaria using animal testing kits [13].

Oestrous cycle study

All the treatment groups were given codeine by orogastric intubation once daily for 28 days to cover five regular oestrous cycles. Vaginal secretions were collected with a plastic pipette fill with 10 µl of normal saline (Nacl 0.09%) by inserting the tip of the pipette into the rat's vagina, but not deeply. Vaginal fluid was placed on glass slides. A different glass slide was used for each rat. One drop was collected with a clean tip from each rat. The drop was smeared on a glass slide allow to dry and fixed with methanol. The unstained material was stained with Gimsa stain for 1 minute and excess stain washed off and allowed to dry on natural air. Dried stained specimen was observed under the light microscope, to assure a good contrast, using the x40 objective lens, the characterization of the cell type was easier than using the x10 objective lens. The cycles were monitored for 14 days after commencement of treatment. Vaginal smear was observed every morning. Three types of cells were recognized, round and nucleated ones are epithelial cells, irregular ones without nucleus

are the cornified cells and little round among them are leukocytes [14-16].

Histopathological analyses of the ovary

All animals (both control and treated) were sacrificed on the 29th day. Ovaries from all the animals were obtained, weighed and fixed (in 10% formalin) for histopathological analyses for paraffin section as was previously used [15].

Statistical Analysis

Data obtained from this research, weights of rats, ovarian weight, hormonal levels and testicular sperm concentration were subjected and calculated for one-way analysis of variance (ANOVA) followed by student t-test [17].

Results

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 $p<0.05$. The changes in the mean body weights of animals treated with codeine and their control are presented in **Table 1.**

Groups	Weight of rats day 1 (g)	Weight of rats day 29 (g)	Weight of both ovaries	Final - initial Bwt (g)	Body to ovary ratio
I	111.00 ± 3.44	138.80 ± 6.01	0.06 ± 0.01	27.8	0.0004
II	150.80 ± 8.63	177.80 ± 7.74	0.08 ± 0.01	27	0.0004
III	124.80 ± 3.51	145.00 ± 7.85	0.08 ± 0.01	20.2	0.0006*
IV	104.80 ± 1.83	128.40 ± 3.41	0.07 ± 0.01	23.6	0.0005*
Values are Mean ± SEM. *= p<0.05. (Comparison relative to control). N= 5 where N is the number of rats per group.					

Table 1: Effects of codeine on body and ovarian weights.

Effects of codeine on body and ovarian weight and ovary to body weight ratio

There was an increase of 27.80 g in mean body weight of rats of the control group. In the group administered 30 mg/kg of codeine there was an increase of 27.00 g. In the group administered 60 mg/kg the mean body weight increased by 20.20 g while in the group administered 90 mg/kg codeine the mean body weight increased by 23.60 g. The increased in weight reduced in the treated groups when compared to the control group.

Effect of Codeine on some selected reproductive hormones in female rats

The result was not significant ($p<0.05$) in the group treated groups. Ovarian to body weight ratio was significant in the groups that received 60 mg/kg and 90 mg/kg codeine when compared to the control group at $p<0.05$. Estrogen, progesterone, follicle stimulating hormone and luteinizing hormone blood levels in the female treated groups did not show much variation when compared to control as seen in **Table 2.**

Group N=5	Estrogen (nmol/l)	Progesterone (nmol/l)	FSH (miu/l)	LH (iu/l)
I	120.40 ± 0.34	6.29 ± 0.19	80.51 ± 5.39	6.42 ± 0.06
II	117.90± 0.41	6.47 ± 0.23	82.38 ± 0.66	5.56 ± 0.23
III	118.50 ± 0.29	6.08 ± 0.15	70.11 ± 1.47	5.85 ± 0.30
IV	120.10± 0.42	6.08 ± 0.17	73.08 ± 6.71	6.13 ± 0.37
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 some selected reproductive hormones in female albino rats.

Effect of codeine on the histology of the ovary

Photomicrograph of rat ovary of group I, control group showed normal ovarian follicles, corpus luteum, connective tissue stroma, ovarian follicles, blood vessels, ovarian oocytes, granullosa cells, follicular antrum, theca interna and theca externa **(Figures 1-3).**

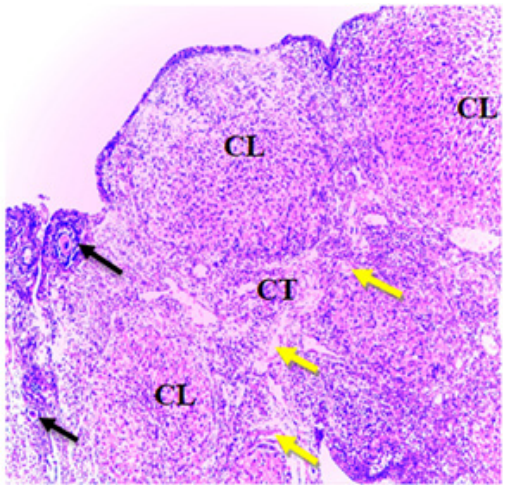

Figure 1: Photomicrograph of rat ovary of control group showing normal ovarian follicles (arrows) corpus luteum (CL), connective tissue stroma (FTS), blood vessels (yellow arrow) H&E x100.

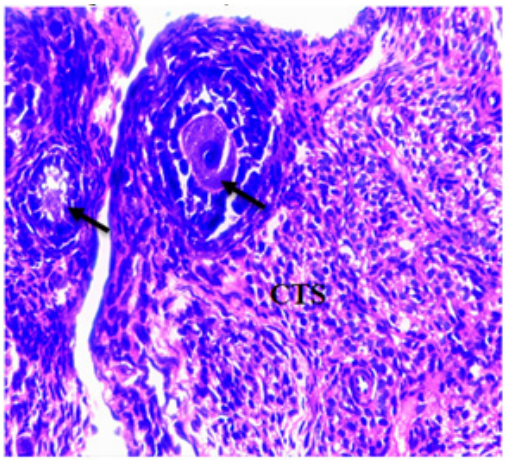

Figure 2: Photomicrograph of rat ovary of control group showing normal ovarian follicles (arrows) connective tissue stroma (CTS) H&E x400.

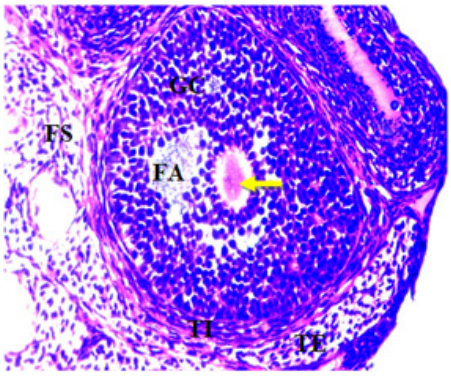

Figure 3: Photomicrograph of rat ovary of control group showing ovarian oocytes (yellow arrow) granullosa cells (GC), follicular antrum (FA), theca interna (TI), theca externa (TE) follicular stroma (FS) H&E x400.

Photomicrograph of rat ovary of group II rats, treated with 30 mg/kg codeine, showed mild focal degeneration of ovarian follicles undergoing process of ovulation, connective tissue stroma blood vessels, granulosa cells, ovarian follicles with oocytes at various stages of development, corpus luteum, medulla, cortex blood vessels, focal numerous ovulation and mild oedema, zona pellucida, follicular antrum, theca interna and granullosa cells **(Figures 4-6).**

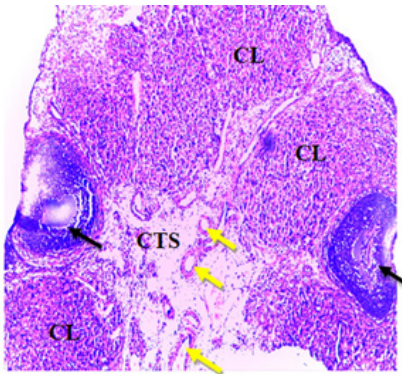

Figure 4: Photomicrograph of rat ovary of group II treated with 30 mg/kg codeine showing ovarian follicles (black arrows) undergoing process of ovulation connective tissue stroma (CTS) blood vessels (yellow arrows) H&E x100.

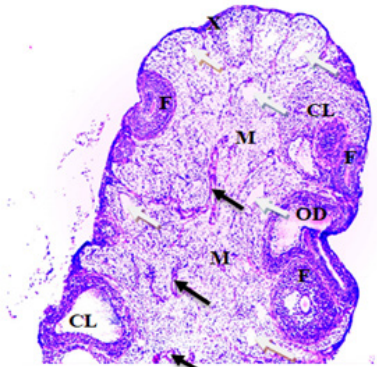

Figure 5: PPhotomicrograph of rat ovary treated with 30 mg/kg codeine showing numerous ovarian follicles with oocytes at various stages of development (F) corpus luteum (CL), medulla (M), cortex (X) blood vessels (black arrows), focal numerous ovulation (white arrows) and mild oedema (OD) H&E x100.

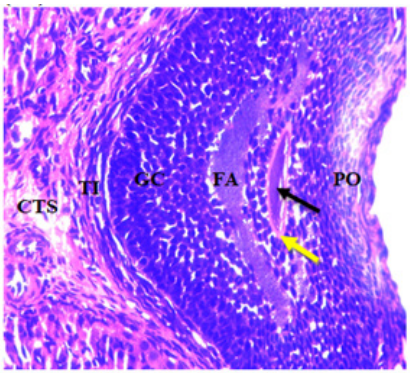

Figure 6: Photomicrograph of rat ovary of group II treated with 30mg/kg codeine showing ovarian follicle nucleus (black arrows), zona pellucida (yellow arrow), follicular antrum (FA), theca interna granullosa cells (GC), ovarian follicle undergoing process of ovulation (PO) and connective tissue stroma (CTS) H&E x400.

Photomicrograph of rat ovary of group III treated with 60 mg/kg codeine showed mild focal degeneration of ovarian follicles, numerous ovulated ovarian follicles, focal degeneration of the corpus luteum, blood vessels and mild focal oedema **(Figures 7 and 8).**

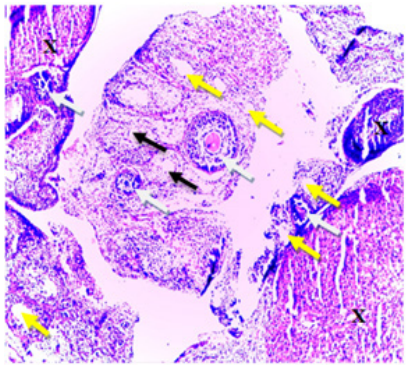

Figure 7: Photomicrograph of rat ovary of group II treated with 60 mg/kg codeine showing mild focal degeneration of ovarian follicles (white arrows), numerous ovulated ovarian follicles (yellow arrows), focal degeneration of the corpus luteum (X), blood vessels (black arrows) and mild focal oedema H&E x100.

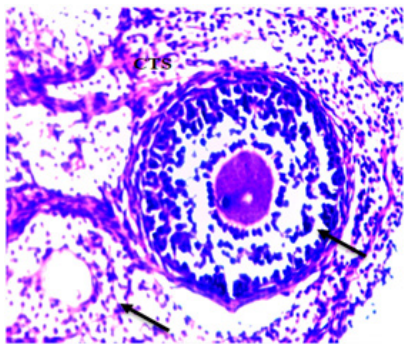

Figure 8: Photomicrograph of rat ovary of group II treated with 60 mg/kg codeine showing focal degeneration of the ovarian follicles (black arrows) and connective tissue stroma (CTS) H&E x400.

Photomicrograph of rat ovary of group IV treated with 90 mg/

kg codeine showed focal moderate degeneration of the ovarian follicles, degeneration of the granulosa cells, oocytes has been loss completely in most of the follicles, oocyte undergoing process of degeneration, degeneration of the connective tissue stroma and blood vessels **(Figures 9 and 10).**

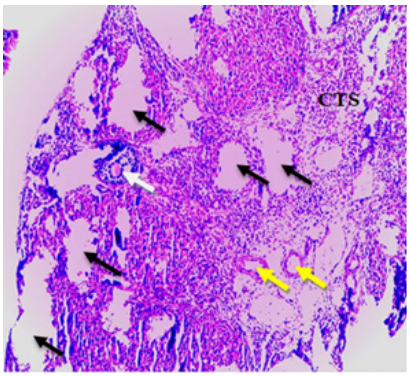

Figure 9: Photomicrograph of rat ovary of group II treated with 90 mg/kg codeine showing focal moderate degeneration of the ovarian follicles, granulosa cells, oocytes has been loss completely in most of the follicles (black arrows), oocyte undergoing process of degeneration (white arrow), degeneration of the connective tissue stroma (CTS) blood vessels (yellow arrows) H&E x100.

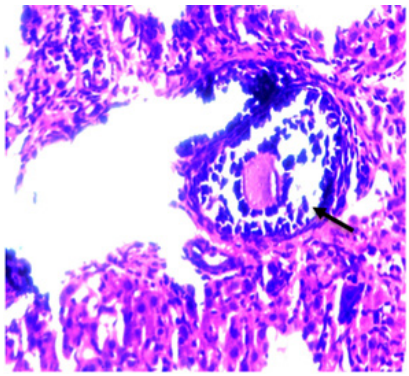

Figure 10: PPhotomicrograph of rat ovary of group II treated with 90 mg/kg codeine showing ovarian follicle and oocyte undergoing degeneration (black arrow) H&E x400.

Discussion

Effect of codeine on body and ovarian weight and their ratio

From this study the result showed the differences observed between the mean, final and initial body weight of the animals decreased as doses increased which was significant at $p<0.05$ in the groups treated with 60 mg/kg and 90 mg/kg codeine. Increased in ovarian weights was observed in the treated groups but the increased was not significant. The body weight to ovarian weight ratio increased which was significant in the group administered 60 mg/kg and 90 mg/kg of codeine at $p<0.05$. Dose dependent reduction of body weights of both male and female rats were reported by Dunnick and Ewell, Melad and Mohammed, reported a decreased in ovarian weights. In female rats, doses of 1562, 3125 and 6250 ppm decreased their body weight. In the same study NTP found parallel result to this study was an increased in body weight in female rats treated with 3125 ppm codeine. The effect of codeine has not been well documented hence exist paucity of data on the effect of codeine on ovarian weight but opioids such as tramadol has been reported by Heba, to cause reduction in ovarian weight which was parallel to the findings of this study which showed an increased ovarian weights in the treatment groups [18-21].

Effect of codeine on some selected blood reproductive hormones in female rats

Codeine did not affect Estrogen, progesterone, Follicle Stimulating Hormones and Luteinizing Hormones blood level as the results did not show much variation when compared to the control group. Youssef and Ziddan, showed that there was a significant reduction in blood reproductive hormones levels when tramadol was administered. The lack of variation observed in this study that was not significant may be due to the dose administered. May be at higher doses the effects may be seen [22].

Effect of codeine on the estrous circle

The length of estrous circle in the female rats monitored for 16 days to cover two complete circles did not show variation in the treatment group when compared to the control group. Since codeine did not affect the blood level reproductive hormones, it may be the reason for the normal estrous circles observed in this study. Melad and Mohammed reported that estrous circle was significantly prolonged when 80 mg/kg tramadol was administered for 8 weeks [19].

Effect of codeine on the histology of the ovary

The effects of codeine on the ovaries according to this study include; focal mild and moderate degeneration of ovarian follicles, degeneration of the connective tissue stroma, degeneration of the granulosa cells, degeneration of the corpus luteum, focal numerous ovulated ovarian follicles, mild focal oedema, oocytes have been loss completely in most of the follicles, oocyte undergoing process of degeneration, degeneration of the connective tissue stroma and blood vessels. This study agrees with the study of Heba, whose result showed that there was marked deterioration of the growing ovarian follicles of the ovary. Many of the mature follicles appeared atretic in the form of cystic-like structure. Atretic follicles possessed granulose cells with the apparent increase of pyknosis and deformed antral cavity. The stroma of the ovary showed marked increased hypercellularity of fibroblast activity. Depletion was found to be associated with impaired ovarian function as measured by the number of oocytes ovulated and duration of the subsequent estrous cycle [21].

Conclusion

This study concludes that codeine affects the ovaries by distorting its normal cyto-architecture. Codeine also caused oedema, premature ovulation of the ovarian follicles, degeneration of oocytes and connective tissue stroma. Codeine caused slight decreased in body weight which was not significant and a significant increase in body to ovary weight ratio. Estrogen, progesterone FSH and LH, blood levels were not affected. Estrous

circle was also affected as it was prolonged. Codeine at these doses is not safe and could lead to infertility.

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