

Arid Zone Journal of Basic and Applied Research

Faculty of Science, Borno State University
Maiduguri, Nigeria

Journal homepage: <https://www.azjournalbar.com>



Research Article

Effect of Oral Administration of 3-Methylmorphine (Codeine) Induced Renal Toxicity in Adult Wistar Albino

¹Y. Ayuba and ²M. S. Saleh²

¹Department of Human Anatomy, Faculty of Basic Medical Sciences University of Maiduguri, Borno State Nigeria

²Department of Anatomy, Faculty of Basic Medical Sciences, Bayero University Kano, Kano, Kano State Nigeria

*Corresponding Author: yohanaayuba15@gmail.com

ARTICLE INFO:

Keyword:

Albino Rats,
Codeine,
Renal toxicity,
Oral Administration.

ABSTRACT

Codeine (3-Methylmorphine) is the most common opiate consumed worldwide, it is widely used for its analgesic, antitussive and anti-diarrheal activities. The study aims to determine the effect of oral administration of codeine induced renal toxicity in adult Wistar Albino Rats. Twenty albino rats weighing between 150-200g were used. Animals were divided into 4 groups (A, B, C and D). Group A served as the control group. Group B received 30mg/kg, group C received 60mg/kg and group D received 90mg/kg codeine orally for 28 days.

Data obtained were calculated for one-way analysis of variance (ANOVA), followed by a Post Hoc test. Results were expressed as mean \pm standard error of the mean. Statement of differences was based on significance level at $p < 0.05$. The results showed a slight decrease in the mean values of Na^+ , Cl^- , and HCO_3^- levels which are not significant, while K^+ level showed an increase in group B and C followed by a decrease in group D which were not statistically significant ($p < 0.05$). Creatinine level did not show any variation when compared with the control group but there was an increase in urea level which was also not statistically significant at $p < 0.05$. Photomicrographs of rat's kidneys in the treatment group showed; degeneration of the glomerulus, hydrophobic changes in the renal tubular lumen, fatty tissue, multi-focal interstitial haemorrhages, infiltration of inflammatory lymphoid cells, mild mononuclear cells infiltration in the renal cortex and mass aggregation of lymphoid cells in the cellular medullar when compared to control

Corresponding Author: Yohana Ayuba, yohanaayuba15@gmail.com

Department of Human Anatomy, University of Maiduguri.

INTRODUCTION

Codeine is one of the most commonly consumed opiate drugs globally. Its wide range of consumption was based on its analgesic, antitussive, anti-cough and anti-diarrhoeal properties (Tremlett *et al.*, 2010; Derry *et al.*, 2013). Codeine is a white or near-white crystalline solid substance that is soluble in water (EMA, 2013). It is available in base and salt form and several hydrated forms (Martindale, 2014). Codeine is administered through oral, intramuscular and intravenous routes (EMA, 2013). Codeine is purchased on prescription by physicians and even without prescription as the counter drug in pharmacies (Moore *et al.*, 1998). Codeine consumption and its appropriate usage is a matter of public concern but little is known about codeine, misuse and dependence. Codeine is prescribed and dispensed legally for patients suffering symptoms such as pain, cough and diarrhoea (Peterson, 2005).

Both males and female's abuse codeine in Nigeria, but the number of females has overtaken that of the males (BBC, 2018). Other abusers include lesbians, gays, bisexuals, those that have used new psychoactive drugs (Agnich *et al.*, 2013). Thugs, criminals, and those that do hard labour also abuse codeine. The side effects of codeine include; fatigue, loss of coordination, sedation, dissociation, altered level of consciousness (Williams, 2005). The maximum daily recommended dose of codeine in humans is 240mg/kg daily which is 60mg four times daily.

Codeine abuse is a problem in Nigeria. Due to codeine being available as a prescription-only drug and for research purposes the drug is still abused. Drug abuse has been associated with violence, terrorism, arm robbery, kidnapping, political violence, and herder's /farmers clashes. Drug abuse has led to an increase in mental illness and even death in Nigeria. Nigeria as a country has lost resources in the fight, control, and treatment of illnesses due to codeine abuse. Kidney disease and its complication is on the rise especially in Maiduguri, Borno state one of them with high indices in Nigeria. Therefore, the objective of this

study is to determine the effects of codeine on kidney function and the histology of the kidney at the light microscopic level.

MATERIALS AND METHODS

Study Area

This study was conducted in the Department of Anatomy, Bayero University Kano, to investigate Renal Toxicity Following Codeine Oral Administration in Adult Albino Wistar Rats.

Materials

Dihydrocodeine tablets (Actavis UK) 30mg, 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, Microscope (Olympus, Germany), Rotary Microtome (Matler, Germany), Reagents of different kinds (Formalin, DPX mountant, glycerol, xylene, paraffin wax, haematoxylin, eosin stains, absolute alcohol) and Hand gloves.

Animals

The matured adult male and female albino Wistar rats weighing between 150 to 200 grams were obtained from the laboratory animal holdings, Department of Anatomy, Bayero University Kano, Nigeria. Twenty (20) adult albino Wistar rats were used for this study. Animals were kept in plastic cages covered with wire mesh in the animal house of the Department of Anatomy and maintained on a standard pellet diet and water *ad libitum*. The rats were housed 5 per cage. Animals were kept in well-ventilated cages and housing with average humidity, with a temperature range between 27 - 30 ± 20 °C. The lighting consists of natural daylight: darkness rhythm.

Experimental Design (Protocol)

The twenty 20 rats were divided into four (4) groups (A, B, C and D). Each group comprised five (5) rats, which were weighed and grouped according to their body weight. Group A served as the control group was not administered codeine. Group B, C and D were administered 30mg/kg, 60mg/kg and 90mg/kg respectively with codeine by orogastric intubation daily for 28 days.

Ethical consideration

Ethical clearance was obtained from the animal ethical committee, Bayero University Kano before the commencement of the study.

Determination of liver function

At the end of the experiment (day 29th), the animals were sacrificed after anaesthesia was administered by injecting 120g/kg ketamine intradermal. Animals were allowed to fall under the effect of anaesthesia. Blood samples were collected by cardiac puncture with syringe and needle into sterilized EDTA specimen bottle (plain). Blood samples were centrifuged at 1000 rpm x g for 5mins.

Preparation of tissue for Kidney histopathology

The kidneys were removed from the posterior abdominal wall by a median incision on the thoracoabdominal wall. Kidneys were fixed in formalin 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 3-5 μ m thick were cut using a rotary microtome. The sections were placed on a glass slide and stained with hematoxylin and eosin mounted on the glass slide and was covered with a coverslip. The sections were dried overnight at oven temperature between 35 to 400C (Drury and Walling, 1980). Tissues were viewed under the light microscope at objective lenses x10, x20 and x40. Photomicrographs of the tissue sections were taken using arms cope to obtain a photomicrograph of x100, x200 and x400.

Statistical Analysis

The data obtained from this study were calculated for one-way analysis of variance (ANOVA) (Eva and Oskar, 2013), followed by a Student t-test. The results were expressed as mean \pm standard error of the mean. Analyses of data were carried out using the computer statistical software package SPSS version 20. All statement of differences was based on a significant level at $p < 0.05$.

RESULTS

Effect of codeine on liver function test

The results for the kidney function test are presented in table 1. There was a slight decrease in the mean values of Na^+ which was not statistically significant. K^+ showed an increase followed by a decrease which was also not statistically significant. CL^- slightly decreased but was not significant. HCO_3^- decreased and increased but was also not significant. Creatinine level did not show any variation when compared with control. There was an increase in urea level but was not statistically significant.

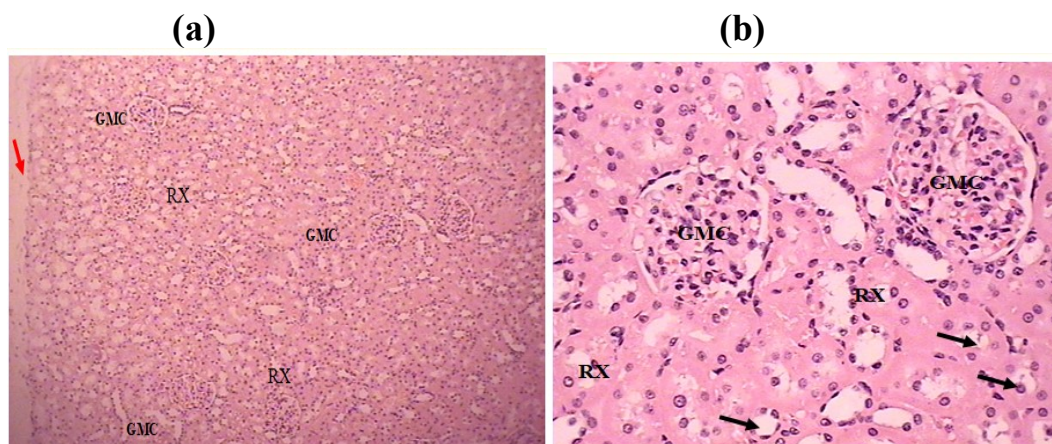
Table 1: Effects of Codeine on kidney function test

Group	Na ⁺	K ⁺	CL ⁻	HCO ₃ ⁻	Creatinine	Urea
N=5	(mEq/l)	(mEq/l)	(mEq/l)	(mmol/l)	(mg/dl)	(mg/dl)
A	62.26 ± 3.52	4.06 ± 1.85	78.56 ± 5.51	36.32 ± 8.48	1.68 ± 0.36	43.85 ± 3.67
B	51.12 ± 2.16	5.51 ± 0.88	77.42 ± 7.48	9.25 ± 4.50	1.37 ± 0.17	48.31 ± 11.01
C	59.84 ± 9.81	6.17 ± 1.90	76.08 ± 3.34	11.80 ± 5.56	1.08 ± 0.17	56.55 ± 5.99
D	59.19 ± 6.67	3.77 ± 0.50	71.34 ± 9.25	35.72 ± 17.32	1.30 ± 0.21	46.84 ± 14.72

Note: Values are mean ± SEM, N=5 where N is the number of rats per group, *<0.05 Level of significance relative to control.

Effect of codeine on the histology of the kidney

The result from this present study showed that photomicrographs of group A (control) rats kidneys showed normal glomeruli, renal tubules, capsule and normal loop of Henle (Figure 1a, b and c). Photomicrographs of rat's kidneys treated with 30mg/kg codeine showed degeneration of the glomerulus, hydrophobic changes in the renal tubular lumen, fatty tissue, multi-focal interstitial hemorrhages and infiltration of inflammatory lymphoid cells (Figure 2a, b and c). Photomicrographs of rat's kidneys administered 60mg/kg codeine showed multi-focal hydrophobic changes within renal tubules, mild mononuclear cells infiltration in the renal cortex (Figure 3a and b). Photomicrographs of rat's kidneys treated with 90mg/kg codeine demonstrated degeneration of some of the glomerulus, hydrophobic changes in the renal tubular lumen, hemorrhage, mass aggregation of lymphoid cells in the cellular medullar (Figure 4a, b, c and d



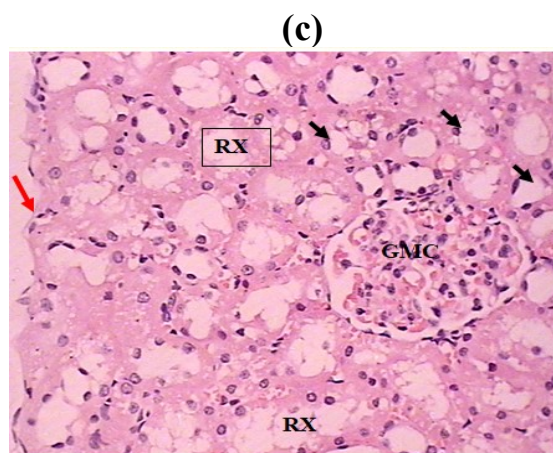


Figure 1: Photomicrograph of rat kidney of control showing normal features (a) glomeruli (GMC), renal tubules (RX) and capsule (red arrow) H&E x100 (b) glomeruli (GMC), renal tubules (RX) Loop of Henley (arrows) H&E x400 (c) glomeruli (GMC), renal tubules (RX) Loop of Henley (arrows) and capsule (red arrow) H&E x400.

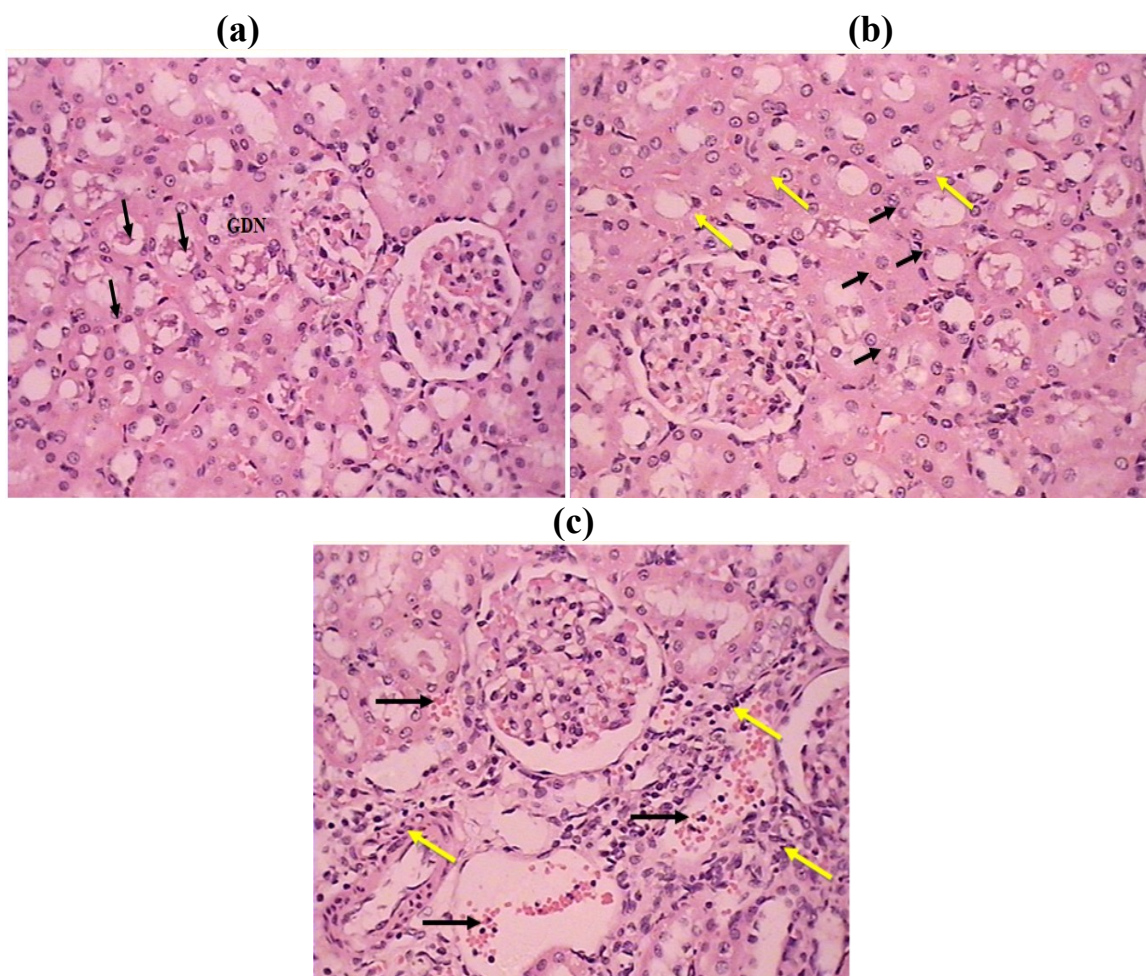


Figure 2: Photomicrograph of rat kidney treated with 30mg/kg of codeine (a) showing glomerular degeneration (GDN) and hydrophobic change (arrows) H&E x400 (b) mild hydrophobic change in the renal tubular lumen (yellow arrows) and fatty tissues (blue arrow) H&E x400. (c) Multi-focal interstitial hemorrhages (arrows) infiltration of inflammatory cells (yellow arrows) H&E x400.

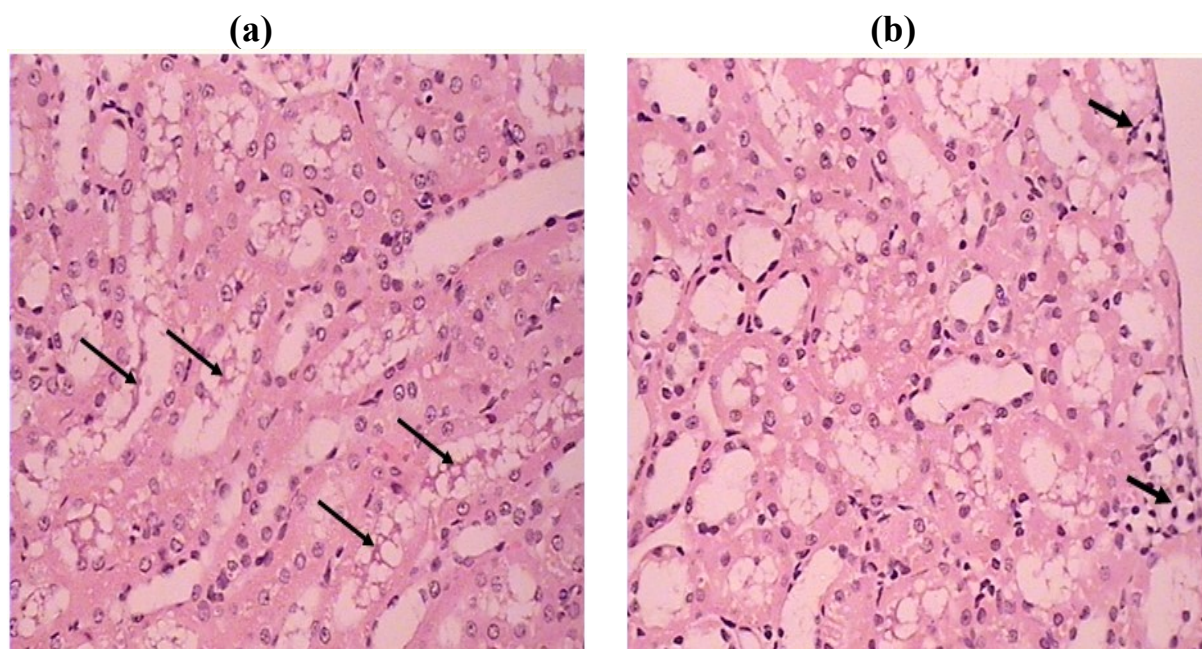
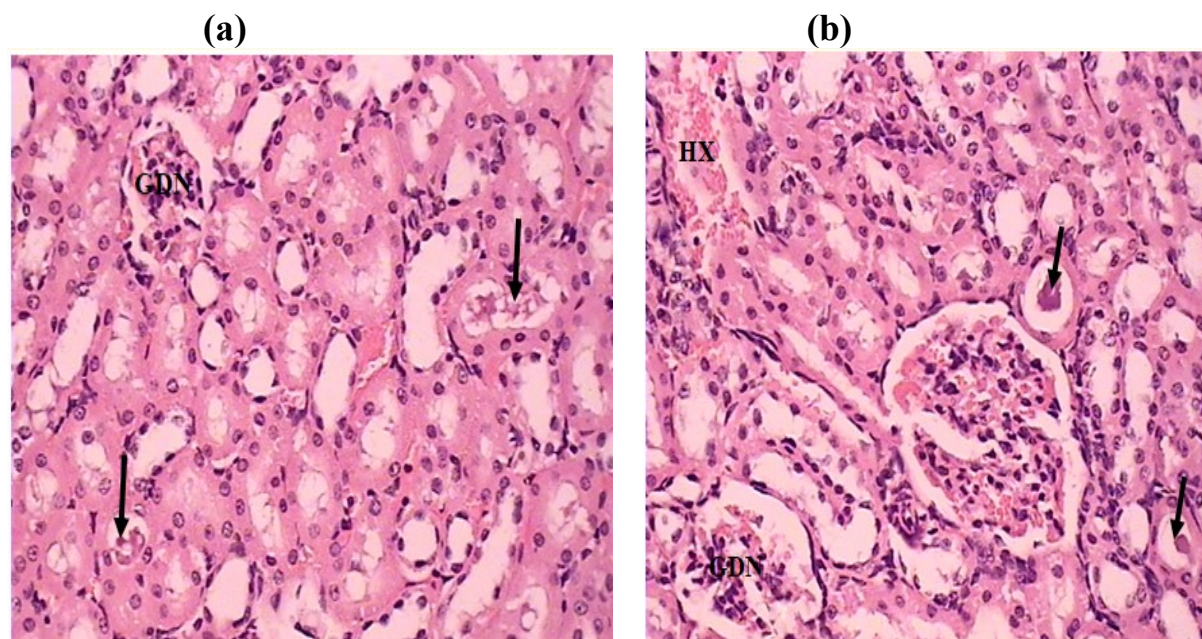


Figure 3: Photomicrograph of rat kidney treated with 60mg/kg of codeine showing (a) multi-focal hydrophobic changes within the renal tubular lumen (arrows) H&E x400. (b) mild mononuclear cells infiltration in the cortex (arrow) H&E x400.



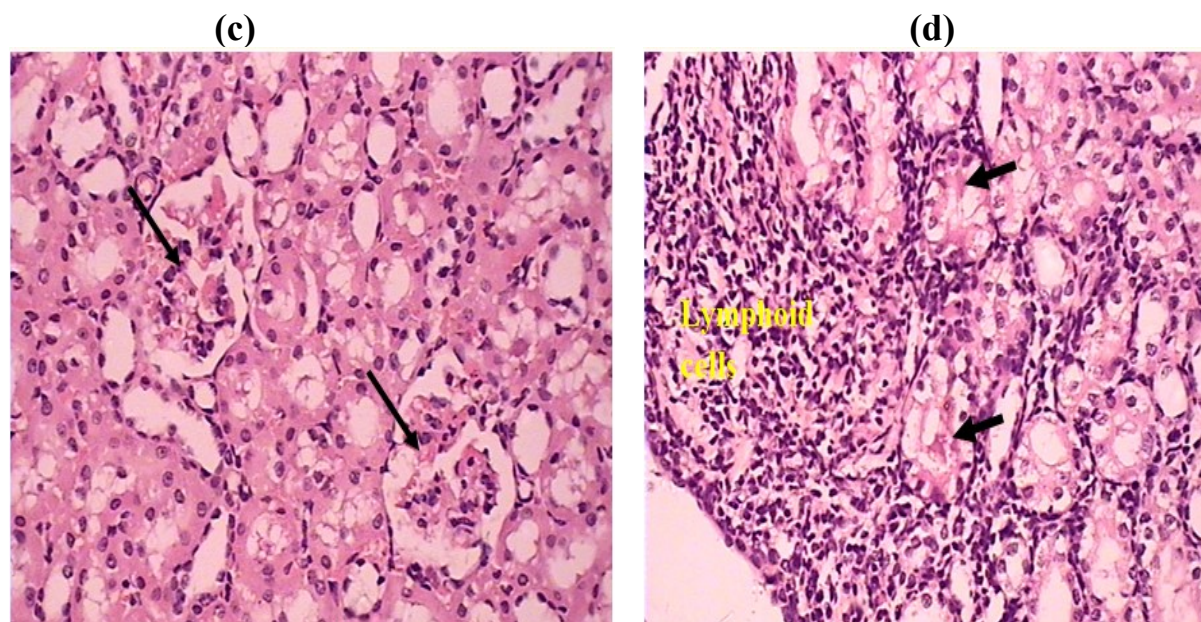


Figure 4: Photomicrograph of rat kidney treated with 90mg/kg of codeine showing (a) glomerular degeneration (GDN) hydrophobic change in the tubular lumen (arrows) H&E x400. (b) glomerular degeneration (GDN), hemorrhage (HX) and hydrophobic change in the tubular lumen (arrows) H&E x400. (c) glomerular degeneration (arrows) H&E x400. (d) mass aggregation of lymphoid cells in the medulla and hydrophobic change in the tubular lumen (arrows) H&E x400

DISCUSSION

This study showed a slight decrease in the mean values of Na^+ , K^+ levels. This could be due to ultra-rapid metabolizers who showed high levels of morphine after the intake of codeine due to CYP2D6 duplication, as reported by Kirchheiner *et al.* (2007). It also showed an increase followed by a decrease, CL^- slightly decreased and HCO_3^- decreased and followed by an increased which were not statistically significant ($p < 0.05$). Creatinine level did not show any variation when compared with the control group but there was an increase in urea level which was also not statistically significant at $p < 0.05$. This study was parallel to earlier studies by Kayode *et al.* (2021) who observed significant alteration in kidney function parameters such as creatinine, urea and uric acid ($p < 0.05$). Codeine administered in a dose of 1.9 mg/kg per body weight four times daily did not cause significant changes in urea levels (Mar'i *et al.*, 2020). This study fell in line with this study where the alterations observed in the kidney function parameters were not statistically

significant ($p < 0.05$). On the other hand, Alusoji *et al.* (2019) in their research reported that high administration of codeine at doses of 25-50mg/kg for 28 days caused a significant increase in serum levels of urea in male's albino Wistar rats. This result was parallel to what was obtained in this present study where no significant change was observed. This variation in result observed could be due to feedback mechanism, the age and sex of animals used or doses and duration of drug administration.

The study demonstrated that photomicrographs of control rat's kidneys showed normal glomeruli, renal tubules, capsule and normal loop of Henle. While Photomicrographs of rat's kidneys treated with doses 30, 60 and 90mg/kg codeine showed degeneration of the glomerulus, hydrophobic changes in the renal tubular lumen, fatty tissue, multi-focal interstitial hemorrhages, infiltration of inflammatory lymphoid cells, mild mononuclear cells infiltration in the renal cortex and mass aggregation of lymphoid cells in the cellular

medullar. This study was in line with the study of Alusoji *et al.*, (2019), where they reported that codeine increases the risk of nephrotoxicity. While Hameed *et al.* (2015) in an earlier study demonstrated vacuolation of mesangial cells of glomeruli, necrosis of proximal convoluted tubules and dilatation of renal cortical tubules which were not observed in this study this could be because codeine was administered with paracetamol and the use of Sprague Dawley rats as

against codeine alone and albino Wistar rats used in the present study. The findings of this study on the other hand agree with the findings of Kayode *et al.* (2021) as they also observed that high doses of codeine cough syrup may predispose renal injury. This finding was supported by degeneration of some of the glomeruli, haemorrhage and infiltration of lymphocytes as observed in this present study.

CONCLUSION

This study concludes that codeine did not cause a significant change in kidney function test parameters (Na^+ , K^+ , Cl^- , HCO_3^- , Creatinine and Urea). Codeine was seen to cause degeneration of the glomerulus, hydrophobic changes in the renal tubular lumen, fatty tissue, multi-focal interstitial

hemorrhages, infiltration of inflammatory lymphoid cells, mild mononuclear cells infiltration in the renal cortex and mass aggregation of lymphoid cells in the cellular medullar when compared to control.

REFERENCES

- Agnich, L. E., Stogner, J. M., Miller, B. L., & Marcum, C. D. (2013). Purple drank prevalence and characteristics of misusers of codeine cough syrup mixtures. *Addictive behaviors*, 38(9), 2445-2449.
- British Broadcasting Corporation (2018). Nigeria's deadly codeine cough syrup epidemic. BBC Africa's new investigations unit, Africa Eye. www.bbc.com/news/world-africa-43982302. 2018. Accessed 21 Jan 2019.
- Derry, S., Karlin, S. M. and Moore, R. A. (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, R. A. B. and Walling E.A. (1980). Histological techniques 24(49): 279-280.
- European Medicines Agency (EMA) (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. and Oskar, O. (2013). Methodology and application of one-way ANOVA. *American Journal of Mechanical Engineering*, vol. 1, No. 7, 256- 261.
- Hameed, A. K., Hussein, A. J., & Majeed, S. K. (2015). Histopathological and Biochemical Study to Effect of Codeine-Paracetamol in Sprague Dawley Rats. *Basrah Journal of Veterinary Research.*, 14(1).

- Kayode, A. A., Kayode, O. T., & Oridota, O. J. (2021). Alterations in the biochemical indices in Wistar rats exposed to an overdose of codeine and dextromethorphan. *Journal of Taibah University Medical Sciences*, 16(2), 198-208.
- Kirchheiner, J., Schmidt, H., Tzvetkov, M., Keulen, J. T. H. A., Lötsch, J., Roots, I., & Brockmüller, J. (2007). Pharmacokinetics of codeine and its metabolite morphine in ultra-rapid metabolizers due to CYP2D6 duplication. *The pharmacogenomics journal*, 7(4), 257-265.
- Mar'i, A., Nugroho, T. E., Kresnadi, E., & Budiono, P. (2020). The Effect of Analgesics Combination of Paracetamol and Codeine Administration to Serum Urea in Male Wistar Rats. *Diponegoro Medical Journal (Jurnal Kedokteran Diponegoro)*, 9(4), 385-390.
- Martindale (2014). The complete drug reference. 38th ed. London; pharmaceutical press 4109.
- Moore, A., Collins, S., Carroll, D., McQuay, H., & Edwards, J. (1998). Single dose paracetamol (acetaminophen), with and without codeine, for postoperative pain. *Cochrane database of systematic reviews*, (4).
- Peterson, G. M. (2005). Selecting nonprescription analgesics. *American journal of therapeutics*, 12(1), 67-79.
- Tremlett, M., Anderson, B. J., & Wolf, A. (2010). Pro-con debate: is codeine a drug that still has a useful role in pediatric practice?. *Pediatric Anesthesia*, 20(2), 183-194.
- Williams, D. G., Patel, A., & Howard, R. F. (2002). Pharmacogenetics of codeine metabolism in an urban population of children and its implications for analgesic reliability. *British Journal of Anaesthesia*, 89(6), 839-845.