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Oral administration of 3- methylmorphine (dihydrocodeine) has less likelyhood of been a carcinogine in testicular germ cells and the interstial cells of leydig in adult albino wistar rats

Yohana Ayuba[1]*, Mohammed Sagir Saleh[2], Mohammed Alhaji Buba[3], Ibrahim Ishyaku[3], and Sunday Joseph Manye[1]

[1]Department of Human Anatomy, Faculty of Basic Medical Sciences University of Maiduguri, Maiduguri, Borno State Nigeria.
[2]Department of Anatomy, Faculty of Basic Medical Sciences, Bayero University Kano, Kano, Kano State Nigeria.
[3]Department of Human Anatomy Faculty of Basic Medical Sciences, Yusuf Maitama University Kano, Kano State, Nigeria.
Corresponding author email: yohanaayuba15@gmail.com

ABTRACT

This study was on Sub-acute Oral Administration of Dihydrocodeine in Placental like Alkaline Phosphatase (PLAP) Immunohistochemistry in the Testis of Albino Wistar Rats. The objectives are to; investigate the level of PLAP in testicular germ cells in the testis, using immunohistochemistry and analysing staining intensity using Image J statistical software. Control (Group 1) rats received distilled water per body weight while group 2-4 received 30, 60 and 90mg/kg dihydrocodeine orally respectively for 28 days. Animals were anesthetized using ketamine injection and abdominopelvic incision was made and the testes were harvested and processed using histochemistry. Photomicrographs were obtained using light microscope and arm scope. Testicular tissue staining intensity were analysed by ANOVA, followed by Post Hoc test using Image J statistical software. The results showed that only few germ cells in the treatment groups were seen to stain faintly with PLAP. This means that only those few cells that pick up the stain have the tendency of becoming cancerous. The interstitial connective tissue and the Leydig cells that produces the hormone testosterone were also found to stain with high intensity. This study concludes that Immunohistochemistry study using PLAP demonstrated dihydrocodeine has less likely hood of been a carcinogen.

Keywords: Albino Rats, Dihydrocodeine. Placental like alkaline phosphatase (PLAP). Testis

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INTRODUCTION

The history of drug use for therapeutic purposes is as old as medical practice itself. Drug used for therapeutic purpose also in some cases can become toxic to the patient and this is because it causes changes in biological function through their chemical actions (Pappono, 2009). Many drugs have been used for the management of pain. Drug abuse has been describe as the use of alcohol, tobacco or illicit and/or prescription of drugs in a problematic way (Mersy, 2003). David *et al*., (2023), refer to drug abuse as a pathological pattern or excessive use, intake of a drug even if it may be causing physical damage, jeopardizing safety (such as driving a car while intoxicated) or impairing social relationships and occupational functioning.

Drug abuse in Nigeria is a Matter of grave concern to stakeholders in the educational, medical, sociological, religious, counselling and parental aspect of human endeavour (Adewuya, 2005).

Non-medical use of drugs like codeine, tramadol and others were most likely to be married (monogamous) or single (never married) and living rent free either with friends or families. On an average those living in urban areas spend more than those in rural areas on drug abuse (UNDOC, 2018). Non-medical use of cough syrup containing codeine in Nigeria is the third most common form of substance abuse and in 2017 about 2.4 million people had used cough syrup containing codeine for non-medical purpose (UNDOC, 2018).

In Nigeria, the most common types of abused drugs according to Haladu (2003) are categorised into; stimulant (from caffeine substances), hallucinogens (marijuana, LSD etc,) narcotics (heroin, codeine, opium etc,) sedatives (valium, alcohol, promotazine, chloroform etc), miscellaneous (glues, spot removers, tube repairs, perfumes chemicals etc.) and traquilizers (librium, valium etc) (Haladu, 2003).

Codeine (3-methylemorphine) is also the most common opiate consumed worldwide, it is widely used for its analgesic, antitussive and antidiarrheal activities (Tremlett *et al*., 2010; Derry *et al*., 2013). One of the drugs derived from opioid is Dihydrocodeine (DHC) developed in 1900s and its pharmacokinetics is similar to that of codeine (Rowel *et al*., 1983). DHC is a semi-synthetic analogue of codeine which is produced from hydrogenation of the double tie in the main chain of the codeine molecules. Dihydrocodeine contain a single bond instead of the double bond between carbon 7 and 8 (Wojciech and Jaroslaw, 2016), when compared to codeine. WHO has tagged codeine as a 'weak' opioid on the second step analgesic ladder (Wojciech and Jaroslaw, 2016), and it is used as analgesic, antitussive, antidiarrheal, anti-cough and in opioid addiction. When DHC is administered orally it is found to have similar analgesic potency to codeine but twice as potent as tramadol (Leppert and Majkowicz 2010). Common side effects seen in patient and abusers of dihydrocodeine are, dry mouth, sleepiness, stomach pain, nausea and vomiting (Williams *at al*., 2002), others include; fatigue, loss of coordination, sedation, dissociation, altered level of consciousness (Williams *at al*., 2002). Decreased in libido, difficulty in sexual activity related to its effects on the release of gonadotropins has been observed in most opioids (Wojciech and Jaroslaw, 2016). Confusion, hallucinations, convulsion, headaches and vertigo is also observed in elderly patients.

The British Broadcasting Commission (BBC) carried out an undercover investigation under the African Eye and discovered that 3 million bottles of codeine cough syrup are consumed in just two states in northern Nigeria (BBC, 2018). The findings of the study led the Nigeria Government to ban the production, sale and use of codeine and codeine containing products through its legislatures (Senate) in 2018. Despite the ban, codeine is still abused mostly by young people in their reproductive age. Both male and females abused codeine but the number of females may have overtaken that of the males (BBC, 2018). Other abusers of codeine include, lesbians, gays, bisexuals, those that have used new psychoactive drugs (Thorvaldsen, 2012). Thugs, criminals such as terrorist and those that do hard labour also abuse codeine. Dihydrocodeine and other products containing codeine are still available as prescription only drugs and for research purposes in Nigeria. Despite its ban in 2018, the drug is still abused.

Mba (2008) identified various effects of different drugs abused on the body chemistry as; Alcohol related problems which include; physical problems like liver cirrhosis, pancreatic, peptic ulcer, tuberculosis, hypertension and neurological disorders. Mental retardation for infants in the womb etc. Tobacco; stimulation of heart and narrowing of blood vessels, hypertension etc. Stimulants; causes irritability exaggerated self-confidence, damage to nose lining sleeplessness, psychiatric complications etc. Inhalant; anaemia, kidney damage, stomach bleeding etc. narcotics; poor perception, constipation, cough, suppression, vomiting, drowsiness, unconsciousness and death (Mba, 2008). Codeine abuse has been documented in the US (Blackley and Schilling, 2008), India (Mattoo *et al*., 1997) and Japan (Miyatake *et al.,* 2002). In southern states of the US and in Nigeria, codeine syrup is mixed with alcohol and soft drinks. When mixed with sprite it is called *'purple drank' 'Syrup,' 'barre', 'Purple tonic', 'Sizzurp', 'Texas tea' etc* (Peters *et al*., 2010). This study aimed to determine the effect of codeine on the testis using PLAP immunohistochemical methods in Albino Wistar Rats.

MATERIALS AND METHODS

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), Specimen bottles, and Cover-slips, Microscope (Olympus, Germany), Rotary Microtome (Matler, Germany), Reagents of different kinds (Neutral buffered formalin, DPX mountant, xylene, paraffin wax, Hand gloves, Placental-like alkaline phosphatase (PLAP) mouse monoclonal IgG antibody (Dako, DK-2600 Glostrup, Denmark), Super frost plus slide, hydrogen peroxide, methanol, equine normal serum diluted with bovine serum albumin (BSA)/tris buffered saline (TBS), 0.5M EDTA buffered at pH 8.0, microwave oven, citrate buffer,

peroxidase enzyme, streptavidin ABC kits (DAKO), 3-amino-9-ethylcarbozole (AEC) 3,3 diaminobenzidine substrate, hematoxylin.

Animals

Adult male albino Wistar rats weighing between 150 to 200 grams were obtained from the animal house of the Department of Anatomy, Bayero University Kano, Nigeria. Twenty (20) albino Wistar rats were used for this study according animal ethical guideline (Anderson, 2002). Animals were kept in the animal in well-ventilated plastic cages covered with wire mesh and maintained on standard pellet feed and water *ad libitum.* Five rats were housed per cage with average humidity and temperature ranging between 27 - 30 ± 2^0C. Lighting consists of natural day light: darkness rhythm.

Experimental design (Protocol)

The twenty adult male albino rats were divided into 4 groups (1, 2, 3 and 4) comprising of 5 rats each. The rats were weighed and grouped randomly. Group 1 serve as the control group and were not administered codeine for the period the experiment lasted. Group 2, 3 and 4 rats were administered 30mg/kg, 60mg/kg and 90mg/kg orally daily for 28.

Immunohistochemistry of testicular germ cells using placental like alkaline Phosphatase (PLAP)

Placental like alkaline phosphatase (PLAP) staining was performed with mouse monoclonal IgG antibody and sectioned between 3-4µm from 10% neutral buffered formalin (NBF). Paraffin wax embedded tissues were labeled by the avidin-biotin-peroxidase complex (ABC) procedure with the Vectastan immunoperoxidase kits (Thorvaldsen *et al*., 2012). PLAP antigen retrieval was done by immersing the tissues in 0.5M EDTA buffered at pH 8.0 and heating the tissue and buffer to 92^0C for 5 minutes in a microwave oven at 750 watts (Thorvaldsen *et al*., 2012).

Inhibition of endogenous peroxidase was done with hydrogen peroxide (H_2O_2) in methanol. Blocking of nonspecific binding was done with equine normal serum diluted with bovine serum albumin (BSA)/tris buffered saline (TBS). Primary antibody, Avidin-biotin complex (ABC) was diluted in 1:25 in TBS and sections were incubated at 4^0C overnight. Sections were then incubated for 30 minutes with biotinylated PLAP (secondary antibody) and subsequently incubated with ABC-complex according to the kits instructions (Thorvaldsen *et al*., 2012). Specific signal was developed by incubation in 3-amino-9-ethylcarbozole (AEC) and the sections were counterstained with hematoxylin.

Statistical analysis

Estrogen receptor staining intensity were analyzed using Image J statistical software (Chaowei, 2018). One-Way Analysis of Variance (ANOVA) was used to determine the differences in the measured parameters across the study groups. A Bonferroni post-hoc tests was carried out to identify the comparative group responsible for the significant difference identified in the ANOVA test.

Ethical consideration

Ethical clearance was obtained from the animal ethical committee, Bayero University Kano. Animal handling was based on the guideline of the Institute of Animal Care and use committee (IACUC) guidelines (Anderson, 2002). and the Bayero University, ethics committee.

RESULTS

Effect of codeine on placental alkaline phosphatase (PLAP) histochemistry of the germ cell of the testis

The result of this study showed that only few germ cells in the treatment groups (Figures 1 and 2), were seen to stain faintly with Placental like alkaline phosphatase (PLAP). This means that only those few cells that pick up the stain (Figure 2) have the tendency of becoming cancerous. The more the stain the germ cell picked the more its affinity of becoming cancerous. The interstitial connective tissues and the Leydig cells that produce the hormone testosterone were also found to stain with high intensity. The intensity of coloration in the interstitial connective tissues increased in group 2 followed by a decrease in group 3 and 4 which were not statistically (Figure 3).

Effect of Codeine on the Staining Intensity of Placental Alkaline Phosphatase (PLAP) in the Testis of Adult Albino Wistar rats

The intensity of coloration in the interstitial connective tissues increased in the treatment groups and was not dose dependent. The increased observed were not statistically significant (Figure 3).

DISCUSSION

Previous findings such as reports from (Greaves, 2007), showed that codeine causes testicular atrophy and degeneration of the seminiferous tubules in the testis of albino rats. Marwa *et al*. (2015) administered tramadol 40mg/kg for one month and reported widely spaced seminiferous tubules with irregular contour, disorganized interstitial tissue and diffuse vacuolations of the

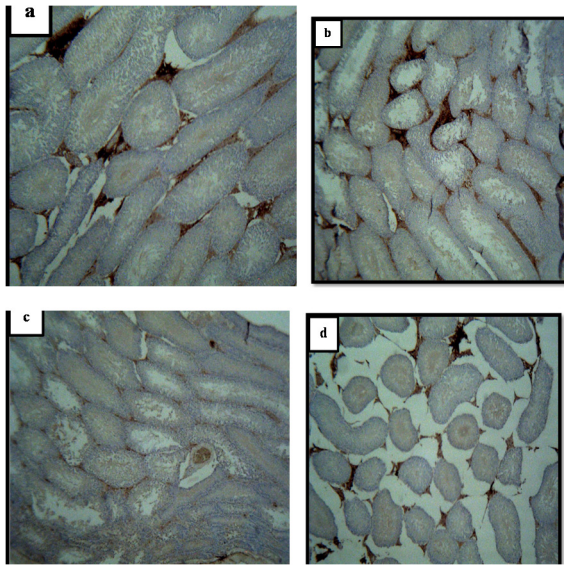

Figure 1: Effect of Codeine on the Histology of Rat Testis Photomicrograph (Group 1, 2, 3 and 4): Placental Alkaline Phosphatase (PLAP) X40. **a.** Group 1 showing Focal Low Staining in Testicular Germ Cells and Moderate Staining of the Interstitial Connective Tissue. **b.** Group 2 Showing Focal Low Staining of Germ Cells and Moderate Staining of the Interstitial Connective Tissue. **c.** Group 3 Showing Focal Low Staining of Germ Cells and Low Staining of the Interstitial Connective Tissue. **d.** Group 4 Showing Focal Low Staining in Germ Cells and Low to Moderate Staining of the Interstitial Connective Tissue.

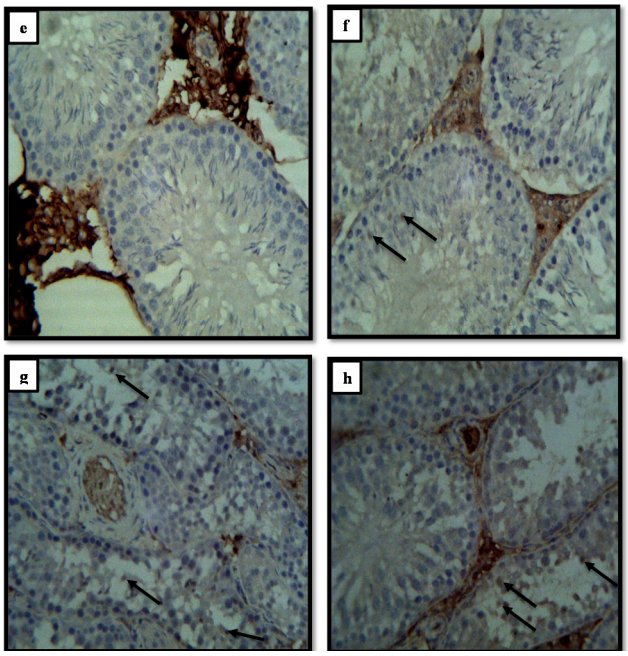

Figure 2: Effect of Codeine on the Histology of Rat Testis Photomicrograph (Group 1, 2, 3 and 4): Placental Alkaline Phosphatase (PLAP) X100. **e.** Control Group showing Focal High Staining in Testicular Germ Cells and Moderate Staining of the Interstitial Connective Tissue. **f.** Group 2 Showing Focal Low Staining of Germ Cells and low Staining the Interstitial Connective Tissue. **g.** Group 3 Showing Focal Low Staining of Germ Cells and Low Staining of the Interstitial Connective Tissue. **h.** Group 4 Showing Focal Low Staining in Germ Cells and Low to Moderate Staining of the Interstitial Connective Tissue.

seminiferous tubule. They also observed decreased in germ cells number, exfoliation of germ cells in the lumina and separation of the germinal epithelium from the underlying basement membrane (basal lamina) (Marwa *et al*., 2015). Placental like alkaline phosphatase (PLAP) antibody stained only few germ cells faintly (having the

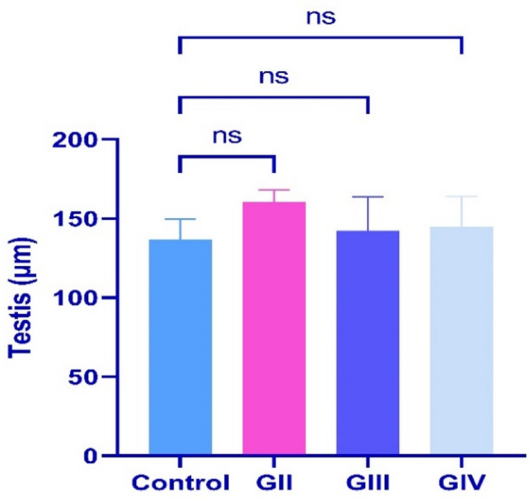

Figure 3: Effect of Codeine on the Staining Intensity of Placental Alkaline Phosphatase (PLAP) in the Testis of Albino Wistar rats. Data are presented as Mean ± SEM; G=Group. ns= Not Significant at $p \leq 0.05$ Across the Groups compared to control.

affinity of becoming cancerous) in the treatment groups. It also stained the interstitial connective tissues which contain the Leydig cells which produces testosterone hormone. The intensity of coloration of the germ cells slightly increased in the treatment groups which were not significant at $p<0.05$, when analyzed using Image J statistical software.

Three types of germ cell were suggested in earlier studies and described as gonocytes, intermediate germ cells and spermatogonia (Terri *et al*., 2004). Gonocytes are the most abundant germ cell after the 10th week of gestation and are found around seminiferous tubules cords and separated from the basal lamina by sertoli cells. These cells become intermediate cells and it has been hypothesized that when they reach the basal lamina they lose their pluripotency and start differentiating into spermatogonia which constitute the common germ cell population. Gonocytes becomes cancerous as a result of an altered differentiation which is linked to Testicular dysgenesis syndrome (TDS) which arises from abnormal fetal development of the male gonads due to genetic and environmental factors. Germ cell neoplasia in situ is due to gonocytes which fail to differentiate into spermatogonium during development and stained positive with PLAP (Skakkebaek Karapetis *et al*., 1987). In earlier study Immunohistochemistry was positive for PLAP (placental alkaline phosphatase) but was negative for epithelial membrane antigen (EMA) (Karapetis *et al*., 2001). This immunohistochemistry profile obtained in Karapetis's study represented a sarcoid reaction to micro-metastatic testicular cancer relapse. An immunohistochemistry analysis on sarcoidosis using PLAP found a similar profile and Sarcoidosis (Karapetis *et al*., 2001).

Intensity analysis using Image J statistical software showed increased in intensity when compared with the control group but this increase observed was not statistically significant at $p<0.05$. This increase in intensity coloration in testicular germ cell observed may be due to the direct effects of codeine on the testis or the regulation of the gonadotropins hormones of the brain on the testis. The increased in coloration in the interstitial connective tissue observed may support the insinuation that codeine enhance sexuality due some researcher reported sexual difficulty. Codeine might cause increase in sexual activity in low doses and shorter duration of usage but high doses with longer duration might give low sexual activity. This study concludes that Histochemistry evaluation using PLAP was able to demonstrate that codeine has less likely hood of being a carcinogen.

REFERENCES

Adewuya, A. (2005). Validation of alcohol use disorders identification test (AUDIT) as a screening tool for alcohol-related problems among Nigerian university students. Alcohol & Alcoholism; 40(6); 575-577.

Alexander, J. T. and Hill, R. G. (1987). Choices in management of postoperative pain. Postoperative pain. London: Blackwell scientific publications; 130-131.

Anderson, U. S. (2002). What is animal husbandry? The Ethics of Animal Labor; 1-22.

BBC, 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. Accessed 21 Jan 2019.

Chaowei, S. (2018). Analyze Immunohistochemistry images in Image J

LSU Health Sciences Center-Shreveport; 1-7.
David, O. I., Moses, T. I., James, E. E. and David O. I. (2023). Dynamics of drug use and experience of stress among students of tertiary institutions in Nigeria. *Africa Journal of Drug and Alcohol Studies;* 16(2); 117-126.
Greaves, P. (2007). Interpretation and relevance in drug safety; elsevier science.
Haladu, A. A. (2003). Outreach strategies for curbing drug abuse among out-of school youth in Nigeria. A challenge for community based organization (CBOS) in A. Garba (ed). Youth and drug abuse in Nigeria: strategies for counselling, management and control. Kano: Matasa Press.
Karapetis, C. S., Strickland, A. H., Yip, D., Vanderwalt, J. D. and Harper, P. G. (2001). PET and PLAP in suspected testicular cancer relapse: Beware sarcoidosis. Kluwer Academic Publishers. Printed in the Netherlands. *Annals of Oncology*; 12: 1485-1488.
Leppert, W. and Majkowicz, M. (2010). The impact of tramadol and dihydrocodeine treatment on quality of life of patients with cancer pain. *Int. J. Clin. Pract*; 64:1681-7.
Leppert, W. and Majkowicz, M. (2010). dihydrocodeine as an analgesic for treatment of moderate to severe chronic pain. *Curr Drug Metab*. 2010; 11: 494-505.
Marwa, M., El Sawy, A. and Hany, W. (2015). Effect of tramadol abuse on testicular tissue of adult albino rat: a Light and Electron Microscopic Study. *The Egyptian Journal of Histology*; 31: 1528-2015.
Mba, A. I. (2008). Counselling techniques for the rehabilitation of drug addicts in Nigeria, Ogun State. The counsellor; 18; 10-18.
Mersy, D. J. (2003). Recognition of alcohol and substance abuse. Am Fam Physician; 67(7); 1529 - 32.
Pappano, A. J. (2009). Cholinoreceptor-blockin drugs. In katzung, B. G., Masters, S. B, Trevor, J, Editors. Basic and Clinical Pharmacology. 11th edition. New York, NY: McGraw-Hill Companies Inc.
Rowel, F. J., Seymour, R. A and Rowlins, M. D. (1983). Pharmacokinetics of intravenous and oral dihydrcodeine and its metabolites. *European Journal of Clinical Pharmacology*; 25: 419-424.
Skakkebaek, N. E., Berthesen, J. G., Giwercman, A. and Muller, J. (1987). Carcinoma-in-situ of the testis: possible origin from gonocytes and precursors of all types of germ cell tumor except spermatoctoma. *Int. Androl.*; 10: 19-28.
Terri, L., Gaskell, A. E., Lynn, L. L., Robinson, R. A., Anderson, and Philippa, T. K. (2004). Saunders Immunohistochemical Profiling of Germ Cells within the Human Fetal Testis: Identification of Three Subpopulations. Biology of Reproduction; 71.
Thorvaldsen, T. E., Nødtvedt, A., Grotmo, T. and Gunnes, G. (2012). Morphological and immunohistochemical characterisation of seminomas in Norwegian dogs. *Acta Veterinaria Scandinavica*; 54:52.
Tremlltt, M., Anderson, B. J. and Wolf, A. (2010). Pro-con debate: is codeine a drug that still has a useful role in pediatric practice? Pediatric 145 Anaesthesia; 20, 183-194.
UNDOC, United Nation Office on Drugs and Crime (2018). Drug use in Nigeria. World drug report. Vienna; 1-57.
Williams, D. G., Patel, A. and Howard, R. F. (2002) Pharmacogenetics of codeine metabolism in an urban population of children and its implications for analgesic *reliability British Journal of Anaesthesi*; 89(6): 839-45.
Wojciech, L. and Jaroslaw, W. (2016). Dihydrocodeine: Safety Concerns Expert Review of Clinical Pharmacology; 9(1): 9-12.