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Ameliorative Potential of *Elaeis Guineensis* on the Histomorphology and Biochemical Status of Liver and Kidney in Wistar Rats Administered with Selected Narcotics

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Abstract
The unguided use of drugs is not new in many Nigerian communities. Oil palm has been in used for eons before now and still being used for vast purposes. This study aimed to ascertain level of histomorphological and biochemical alteration of narcotics and possible remedies to combat its effects using Elaeis guineensis Wistar rats. Fifty (50) adult male albino rats were study. They were divided into five (5) equal groups (labeled A to E) of ten (10) rats in each group. Group A (Control) received only normal feed (growers' mash) and distilled water, B received 0.2 mg/kg bw of tramadol and 0.2mg/kg of codeine, feed and distilled water daily, C received 0.2mg/kg of tramadol and 0.2mg/kg of rohypnol, D received 0.2mg/kg of codeine and 0.2 mg/kg bw of rohypnol, E received 0.2mg/kg of tramadol, 0.2mg/kg of rohypnol and 0.2mg/kg of codeine and Elaeis guineensis extract respectively for 42 days after which the animals were sacrificed, dissected, blood samples and tissues taken and processed. Significant difference ($p< 0.05$) observed in creatinine, alkaline phosphatase value in group C and E, also urea in group B and E, conjugated bilirubin also in group C. Deleterious effect was observed in the liver and kidney tissue in group B, C and D. Amelioration was observed in group E. E. guinensis had ameliorative potential in the effect of the tested narcotics out did not have reversal effect on the tissue architectures prior to the usage of the drugs

Keywords: *Elaeis guineensis,* Wistar rats, Codeine, Rophynol, Tramadol

INTRODUCTION
Elaeis Guineensis is a species of palm that is frequently referred to as oil palm nut, African oil palm, or macaw-fat (Chong and Ng, 1991). Most palm oil is derived from *Elaeis guineensis*
It is indigenous to West and Southwest Africa, more specifically the region between Angola and the Gambia. The species name *guineensis* refers to the name of the region, Guinea, rather than the current nation by that name (Brian *et al*., 2011).

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Recently, there has been ongoing discussion about the effects of palm oil consumption on the heart, particularly in the emergence of coronary artery disease, primarily in mainstream literature. The majority of the debate is driven by advocacy and consumer protection groups with competing agendas and interests (McNamara, 2010). The four major dietary saturated fatty acids—palmitic, stearic, lauric, and myristic—have different effects on serum cholesterol, though. Lauric and myristic acids, which are saturated fatty acids with 12 and 14 carbon atoms, increase all cholesterol fractions more than palmitic acid, while stearic acid increases all cholesterol fractions more than palmitic acid (Kromhout, 1995).

Due to its low potential for abuse, tramadol is a frequently prescribed therapeutic substitute for other opioid analgesics (Amber *et al.*, 2021). But codeine, an opiate and morphine prodrug, is used to treat pain, coughing, and diarrhea. It can be naturally found in the sap of Papaver somniferum, the opium poppy (Amber *et al*., 2021). In medicine, flunitrazepam (Rohypnol) is a highly lipophilic benzodiazepine derivative that is primarily used as a sedative and hypnotic (Owolabi *et al.,* 2017). It can be discovered naturally in the sap of Papaver somniferum, the opium poppy (Amber *et al*., 2021). Tramadol, codeine, and rophynol are excreted and metabolized by the liver and kidney, respectively, and as a result, they may be toxic to the liver and kidney (Wu, 2001). Historical evidence suggests that consuming psychoactive substances has been a part of human life for a very long time (Scott *et al.,* 2016). The desire to experience other states of awareness seems to be ingrained in human nature. Unfortunately, the costs to one's health and community from abusing these psychoactive substances are alarmingly high (Hartney, 2020). As a centrally acting painkiller with properties similar to morphine and codeine, tramadol, rophenol, and codeine were developed in Germany in the 1970s and introduced in the 1990s (Hartney, 2020). Despite the fact that it is illegal without a prescription in some areas, tramadol is fairly simple to get in Nigeria, either through phony prescriptions from pharmacies or on the black market.

Nigerians use drugs in a similar way to how many other societies do. Like the rest of the world, consumers of narcotics in Nigeria use do when the situation demands it. In Nigeria, narcotics are abused simply because they are used without prescriptions from qualified and registered medical personnel who must allow their use according to the laws of the land. Several studies on Nigerians' disease behavior corroborate this conclusion (Abdulkarim *et al*., 2005). Marijuana, amphetamines, mandrax, proplus, barbiturates, and codeine, according to Abdulkarim *et al*., 2005), are the most often consumed drugs in Nigeria, with detrimental consequences for youths, immediate society, and Nigeria as a whole. However, there are little or no studies on the oral combination of tramadol, codeine and rophynol coupled with the ameliorative effect of *E. guineensis* in association with histopathological, nephrotoxicity and hepatotoxicity, hence, this study aimed at ameliorative potential of *elaeis guineensis* (Akwu Ojukwu) on the histomorphology and biochemical of liver and kidney of rats previously induced with codeine, tramadol and rophynol.

MATERIALS AND METHODS

Conditions of experimental animal and housing

Five (5) week old adult Wistar rats weighing between 150 and 200g were purchased from the Ohilux Services Nigeria animal farm in Ekpoma, Edo State, and transported to the Ohilux Research Center's experimental laboratory where they were given two (2) weeks to acclimatize, in other to avoid contamination, they were housed in cages made of wire mesh with a tripod separating the animal from its feces. The rats were fed growers' mesh during

this period of acclimatization, and water was available at all times. The standard guide for the care and use of laboratory animals was followed in the upkeep and use of the animals.

Substance of Study
Tramadol, codeine and rohypnol were purchased in a pharmacy outlet and stored at a temperature below 30 °C in a cool place pending usage.

Ethical Consideration
The study followed the policies outlined in the guide for the care, handling, and use of laboratory animals (National Research Council, 2012). The ethical and promotion committee of the University of Nigeria Teaching Hospital, Etuku Ozuolla, Enugu, and granted approval for the study with approval number 056/02/2021.

Acute Toxicity Study
The Lorke's method was used to conduct the acute toxicity study (Lorke, 1983). According to this formula, the median lethal dose (LD_{50}) was determined: (LD_{50}) = (Highest nonlethal dose) x (Lowest lethal dose). The dosage used in this experiment was determined by the (LD_{50}) or lethal dose, as well as the potential dosage an animal might unintentionally be exposed to.

Research Design
Fifty (50) adult male albino rats were used for this study. They were divided into five (5) equal groups (labeled A to E) of ten (10) rats in each group. Group A served as the control and the rats were given distilled water. Group B was administered 0.2mg/ kg of tamadol and 0.2mg/kg of codeine, Group C was administered 0.2mg/kg of tramadol and 0.2mg/kg rohypnol. Group D was administered 0.2mg/kg each of codeine and rohypnol. Group E was administered 0.2mg/kg each of tramadol, codeine, rohypnol and 100mg/kg *E. guineensis* extract. The substance administration was given daily for 42 days (6 weeks) and the weights of both the test animal and control monitored every 2 weeks. After the administration, the rats were put under light chloroform anesthesia and the kidney and liver, harvested for immunohistochemistry processing.

Collection and analysis
The weight was measured before and after acclimatization and similar weight measurements were done at the end of each week and the average weight recorded accordingly. The kidney, liver, and testes of each rat were obtained at the ends of 2, 4, and 6 weeks under chloroform anaesthesia and fixed in 10% formalin for histological processing. The growth performance and feed utilization of the rats were determined at the end of the experiment as described by Dada and Ikuerowo (2009). At the end of each week, similar weight measurements were taken, and the average weight was recorded. The growth performance and feed utilization of the rats were determined at the end of the experiment as described by Dada and Ikuerowo (2009).

Haematoxylin and Eosin staining Protocol

Biochemical Examination
Alanine Aminotransferas Alkaline Phosphatase (ALP), Aspartate Aminotransferase (AST) was determined by spectrophotometric method reported by Dada and Ikuerowo (2009).
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Creatinine estimation
Creatinine was estimation using Modified Jaffe's method.

Urea Estimation

Urea was estimated Calorimetrically using Urease-Berthelor's method reported by Dada and Ikuerowo (2009).

Data Analysis

Analysis of Variance (ANOVA) was used to analyze the results of the weight, and the differences were considered significant at $p < 0.05$ level of confidence. All data are presented as standard deviation of the mean (SEM).The results were presented in Tables and comparisons were made statistically.

RESULTS

Table 1: Showing control against test of Kidney indicators

Parameters	Group A (control)	Group B	P-value	Group C	p-value	Group D	p-value	Group E	p-value
Cr (µmol/L)	1.1±0.1	0.9±0.1	0.84	0.60±0.1	0.03*	1.1±0.1	1.0	0.8±0.09	0.04*
Urea (mmol/L)	19.0±9.0	51.5±3.6*	0.00	6.67±9.7	0.0 1	42.00±3.2	0.001	42.3±2.67	0.001*
Na (mmol/L)	146.5±1.5	145.3 ±2.1	0.89	144.0±0.6	0.74	150.70±4.4	0.08	146.7±6.0	0.9
K (mmol/L)	7.0±1.0	6.5±0.3	0.49	24.40±17.8	0.001	8.20±0.7	0.04	8.5±1.04	0.49

Table 2: Showing control against test of liver indicators

Parameters	Group A	Group B	p-value	Group C	p-value	Group D	p-value	Group E	p-value
ALT (U/L)	56.5±6.5	44.7±2.9	0.06	50.5±10.5	0.61	45.00±5.0	0.06	44.3±5.8	0.06
AST (U/L)	166.5±26.5	152.7±13.6	0.09	161.5±37.5	0.96	47.50±0.5	0.10	156.0±9.5	0.96
ALP (U/L)	236.5±25.5	218.3±20.0	0.34	154.5±46.5	0.001*	194.50±9.5	0.01*	197.0±14.00	0.02*
CB (µmol/L)	3.8±2.5	2.2±0.4	0.34	7.7±3.8	0.03*	3.05±1.4	0.34	2.10±0.65	0.34
TB (µmol/L)	11.6±6.5	11.8±3.3	0.90	14.6±6.5	0.08	9.40±3.4	0.30	9.17±2.82	0.09

*** Means statistically significant (p<0.05) with control and across the group Means statistically significant (p<0.01) with control and across the group # Means statistically significant (p<0.05) with group C**

Key: Cr: creatinine,Na : serum sodium, K: serum potassium, CL: serum chloride, HCO3 : serum biocarbonate, TP: total protein, GLO: globulin, AL: albumin, TC: total cholesterol, TG: ttiglyceride, HDL: high density cholesterol , LDL: low density cholesterol, ALT: Alanine transaminase, ALP: Alkaline phosphates, AST: Aspatate transaminase CB: conjugated bilirubin, TB: total bilirubin

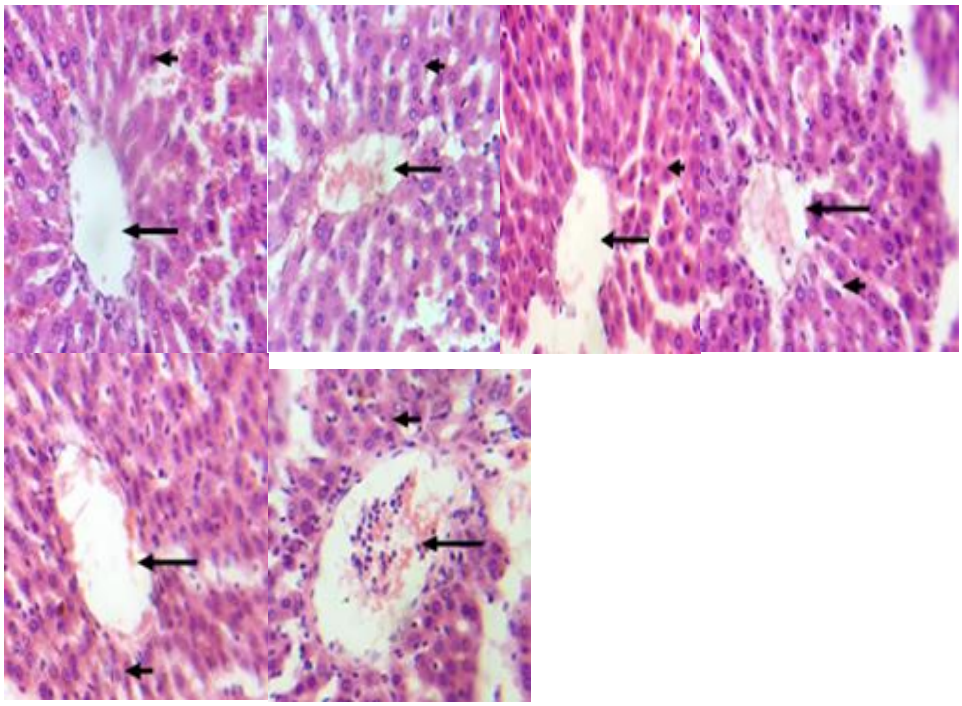

Figure 1: Photomicrograph liver sections of an experimental animal showing the large (long arrow).appearance of centriole and the hepatocytes also revealing a slightly pyknotic nucleus (short arrow) to represent Group A (Control).photomicrograph liver sections of an experimental animal's (long arrow) showing the hepatocytes also revealing a pyknotic nucleus with dilated sinusoids and mild steatosis (short arrow) to represent group B, Photomicrograph liver sections of an experimental animal's showing a clear centriole surrounded by mild mononuclear cells (long arrow) also revealing the hepatocytes with a slightly pyknotic nucleus and mild steatosis (short arrow) representing group C, Photomicrograph liver sections of the liver of an experimental animal showing centriole clear (long arrow), the hepatocytes also revealing dilated sinusoids and a pyknotic nucleus and mild steatosis (short arrow) representing group D, photomicrograph liver section, showing normal hepatocytes also reveal nucleus with dilated sinusoids (short arrow)representing group E. X400 magnification

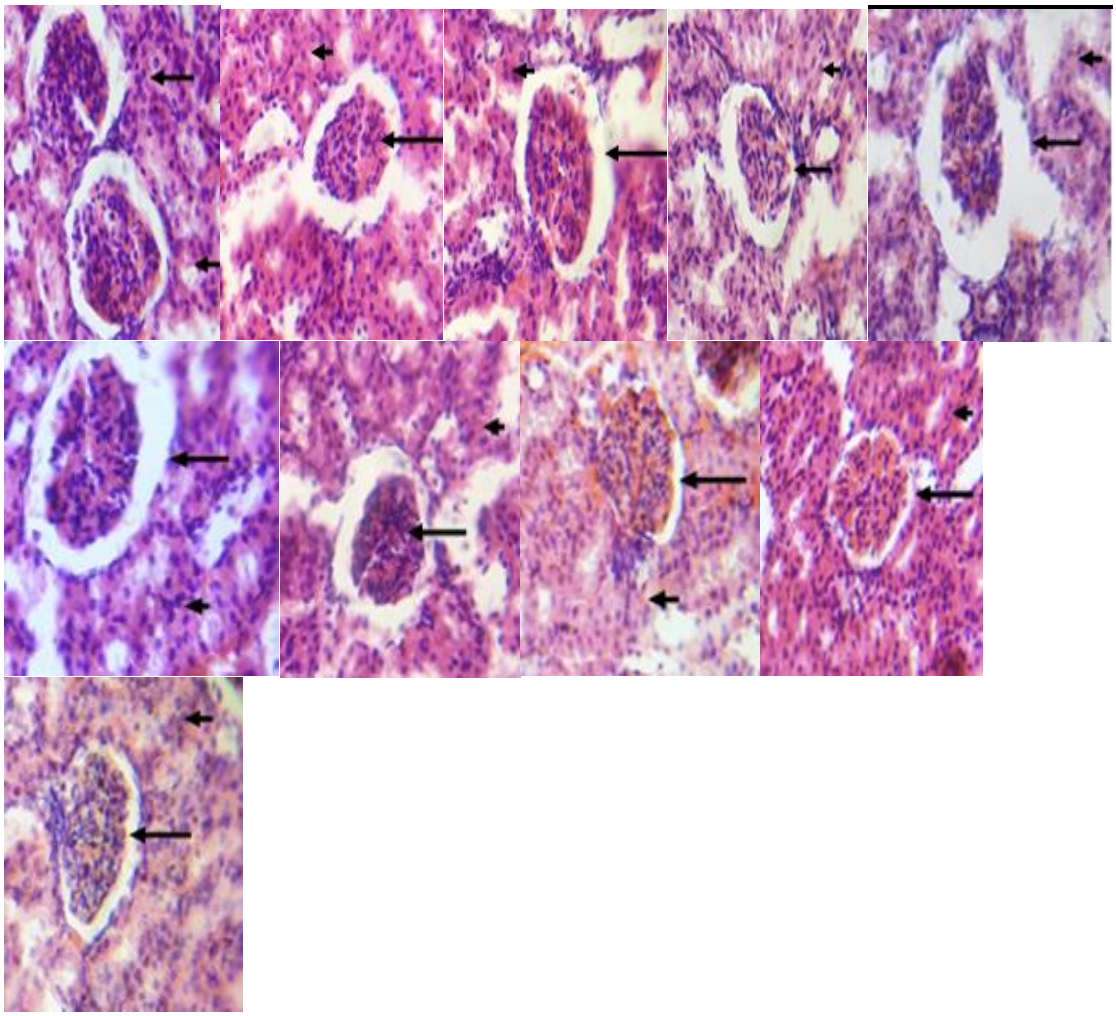

Figure 2: Photomicrograph kidney section depicting renal corpuscle (long arrow), interstitial, and tubules in slices of an experimental animal (short arrow) representing group A (control), photomicrographs kidney section of an experimental rat (short arrow), showing obvious atrophied renal corpuscle (long arrow), interstitial and tubules with evident distortion (short arrow) represnting group B, photomicrograph kidney section of an experimental animal showing visible enlarged renal corpuscle (long arrow) interstitial and tubules with visible distortion and diffused mononuclear infiltrates representing group C, photomicrograph kidney section tissue distorted renal corpuscle (long arrow), interstitial and tubules representing group D, photomicrograph kidney section showing normal renal tubules and bowman capsules

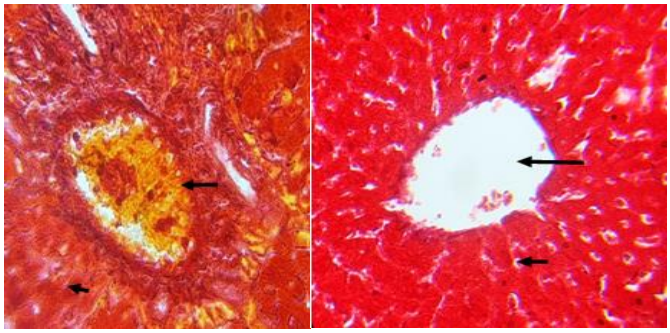

Figure 3: Photomicrograph liver sections of an experimental animal showing visible centriole rich in keratin colored red (long arrow), representing group A (control), Group B, liver section (0.2 mg/kg tramadol and 0.2mg/kg Codeine for 6 weeks):section revealing centriole with mild blue collagen deposition around the walls (long arrow) with spread of cytoplasm and keratin colored red also revealing hepatocytes with normal nucleus appearing blue/black (short arrow). Masson Trichrome Staining (X400 Magnification)

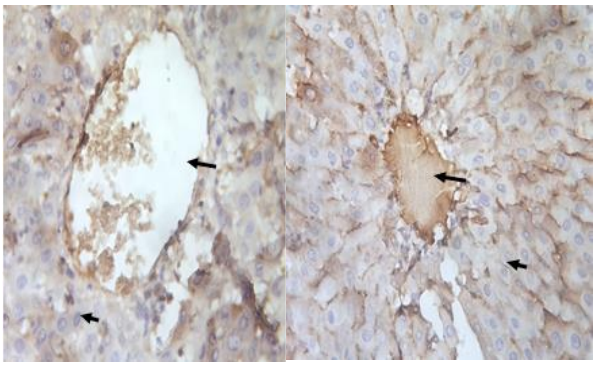

Figure 4: Photomicrograph liver sections of an experimental animal showing positive visible centriole wall with (long arrow) representing group A (control), also revealing hepatocytes with non-specific cytoplasmic staining (short arrow) representing group A (control), photomicrograph liver sections of an experimental animal showing prominent positive centriole of CD34 (long arrow) with spread of cytoplasm also revealing hepatocytes with the nucleus appearing blue/black (short arrow). Liver Cd34 Immunohistochemistry Staining (X400 Magnification.

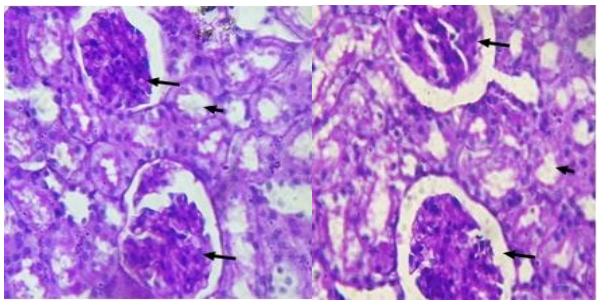

Figure 5- Photomicrograph kidney sections of an experimental animal showing renal corpuscles with mild magenta staining indicative of mild deposition of the glomeruli glycogen (long arrow) also revealing prominent tubules and interstitial (magenta colour) with the nucleus staining blue/black (short arrow) representing group A (control), photomicrograph kidney sections of an experimental animal showing normal renal corpuscles and glomeruli casts (long arrow) also revealing prominent tubules and interstitial (magenta colour) representing group B.. Pas Staining (X400 Magnification.

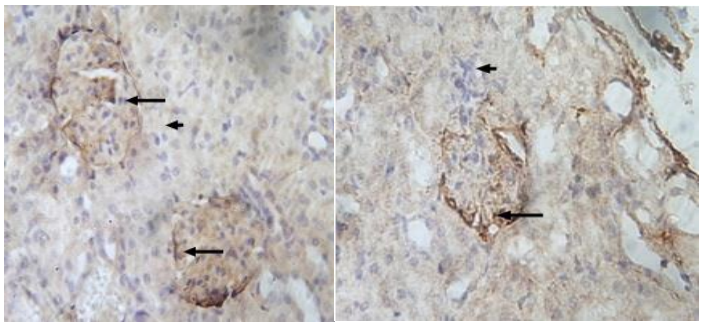

Figure 6: Photomicrograph kidney section of an experimental animal showing renal corpuscles with mild KIM-1 staining (long arrow) also revealing prominent tubules and interstitial with a negative KIM-1 immunoexpression (short arrow)representing group A(control), photomicrograph kidney sections of an experimental animal showing renal corpuscles mild KIM-1 staining (long arrow) and scanty KIM-1 immunoexpression in tubules and interstitial (short arrow). Immunohistochemistry Staining (X400 Magnification

DISCUSSION

This study compared the weight of test and control subjects and that none of the test groups was statistically different from the control group ($p > 0.05$). Although it was not statistically significant, there was an apparent increase from the base level in control rats to the apex level in group C followed by a decline to nearly the base level in group E

Photomicrographs of the liver histology from the control groups in this study showed that the centriole was prominent and easily visible, and the hepatocytes also exhibited a slightly

pyknotic nucleus with dilated sinusoids (short arrow). The group B centriole was mildly surrounded by mononuclear cells and ii was clear and visible. Additionally, the hepatocytes exhibited a mildly steatosed nucleus that was slightly pyknotic. Group C liver histology depicted a visible centriole The hepatocytes also revealed a pyknotic nucleus with dilated sinusoids and steatosis. Group D showed visible centriole. The hepatocytes also revealed pyknotic nucleus with dilated sinusoids and mild steatosis. However, for group E showed normal liver architectures such as the hepatocyte and sinusoids. These findings are in concord with Youssef and Azza (2016), where the effects of acute and chronic tramadol drug toxicity caused adult male albino rats to exhibit complete cell membrane degeneration, necrosis, and cytolysis in their liver tissues upon histopathological examination. The fact that the liver was in charge of tramadol's metabolism and excretion may help to explain these findings. Tramadol was administered in the current study, and it was discovered that the dose was hepatotoxic. This backs up the research done by Schungel *et al*. (2009) who observed pre-necrotic and necrotic changes in the liver. These authors further reported an increase in the number of inflammatory cell infiltrating the hepatocytic cells of the sinusoids as well as congestion in the central vein. Although little is known about the mechanisms underlying codeine toxicity, toxic effects of codeine use had been reported by Schungel et al. (2009).

The increase in the activities of ALT, aspartate aminotransferase (AST), ALP and lactate dehydrogenase (LDH) has been reported in previous studies following exposure to opioids, including morphine and tramadol (Ali *et al*., 2020; Samaka *et al*., 2012; Salahshoor *et al*., 2016; Eric *et al*., 2020) The reports of these authors indicate a slight variation to the findings in this study. The liver is an organ that detoxified toxic elements and chemical drugs in the body, The increase in the activities of AST and ALP in plasma in this study is indicative of liver alteration Salahshoor *et al*., 2016). The increased secretion of these liver enzymes may be accompanied by acute cell necrosis, therefore, the increased plasma level of these enzymes in rats treated with codeine could be due to necrosis or damage to liver cell membrane which could have possibly leaked the enzymes into the blood circulation (Salahshoor *et al*., 2016).

An oral combination of tramadol and rhypnol can result in hepatotoxicity because both drugs have been shown to cause hepatic injury in previous studies. According to Eric *et al*. (2020) central vein dilatation and mononuclear cellular infiltration are two of the postoperative effects of morphine and tramadol on the histopathology of the liver in rabbits with hepatocyte degeneration. In a different study done by Eric *et al*., 2000), it was reported that the toxic effects of rohypnol and its metabolites on the liver could be attributed to the histopathological results that were obtained. Oxidative stress causes lipid, protein, and DNA disruption, necrosis, and apoptosis in hepatocytes, amplifies the inflammatory response, and stimulates Kupffer cells and circulating inflammatory cells to produce pro-fibrogenic mediators, which leads to the beginning of fibrosis (Hafez *et al*., 2015).

The tissue histology for group A in this study indicated normal renal histo-morphology with discernible renal corpuscle interstitial and tubules. The renal corpuscle, interstitial, and tubules of the group animals kidneys were visibly atrophied and distorted. Group C experimental animals recorded distorted tubules and renal corpuscles as well as interstitial and renal histology. Group D experimental animals equally recorded kidney histology that includes enlarged renal corpuscle, interstitial and tubules with visible distortion, and dispersed mononuclear infiltrates. Again, experimental animals in group D kidney histology that included atrophied renal corpuscle and enlarged renal corpuscles.

The results of the current study supported those of Atici *et al*. (2005) who found that tramadol caused significant histopathological changes in kidney tissues changes like tissue degeneration, glomerular chamber enlargement and swelling of the lining epithelium, mononuclear cell infiltration, damaged proximal convoluted tubule brush borders, necrotic lesions, and pyknotic nuclei of the urinary tubules. Atici *et al*., 2005), have reported similar findings.

Application of tramadol and codeine was also observed in earlier investigations to result in tubular lesions in the kidney, becoming more noticeable in the latter days following treatment. The direct toxic effect on cell function was one potential mechanism for the tubular lesions (Alden and Frith, 1992). The measurement of renal function and the determination of glomerular filtration rate are both done using the level of plasma creatinine (Perrone, 1990). In this study, codeine administration significantly raised the plasma creatinine levels of the experimental and this can be interpreted as evidence of renal damage because (Pannabecker, 2008). While *E. guinensis* was able to ameliorate the aforementioned effects, it was unable to completely reverse the damage. This could be due partly to the phytonutrient contents of *E. guinensis*.

CONCLUSION

Owing to the results obtained in this study, it can be concluded that while codeine caused a decrease in body weight, chronic tramadol and rohypnol toxic effects persisted even after drug use was stopped. This implies that such compounds must only be administered when prescribed and under the supervision of qualified medical staff.

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