Codeine-mediated haematoxicity, hepatotoxicity and nephrotoxicity in male albino rats

ABSTRACT

Objectives:This study aimed to investigate the effects of codeine administration on some haematological and biochemical indices in rats. *Materials and methods*:Therapeutic dose (5 mg/kg/day), high dose (25mg/kg/day) and extreme dose (50mg/kg/day) of codeine were administered orally to rats for 28 days. Twenty-four hours after the last codeine administration, blood, liver and kidney were removed from the animals after an overnight fast and analysed for their haematological and biochemical parameters. *Results*:Results obtained revealed that codeine administration significantly reduced the levels of white blood cells (WBC), red blood cell (RBC) and platelet count (PLT) and increased the levels of mean corpuscular haemoglobin (MCH) and mean corpuscular volume (MCV)while it resulted in non-significant changes in other haematological parameters examinedwhen compared with control rats. Codeine intake significantly increased plasma levels of alkaline phosphatase (ALP), aspartate aminotransferase (AST),creatinine and urea while its reduced total protein levels. Hepatic and renal thiobarbituric acid reactive substances (TBARS) levels were significantly increased by codeine administration while levels of endogenous antioxidant enzymes superoxide dismutase (SOD), catalase (CAT), and reduced glutathione (GSH) were reduced. *Conclusions:*This study confirmed the risk of increased oxidative stress, haematoxicity, hepatotoxicity and nephrotoxicity due to codeine administration. Although codeine is reported to be effective in pain management, its toxicity should be kept in mind.

Keywords: codeine, haematological,oxidative stress, hepatotoxicity, nephrotoxicity.

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1. Introduction

Codeine is an opiate analgesic that is commonly used in aformulation with paracetamol (acetaminophen), although combinations with other analgesics like acetylsalicylic acid, ibuprofen, caffeine, barbiturates and sedative antihistamines may also exist [1]. The drug is often used to suppress a cough either alone or by combining it with other drugs[2].The major pharmacological effects of codeine such as analgesia, drowsiness, mood changes, respiratory depression, nausea, and decreased gastrointestinal motilityare produced on the central nervous system(CNS)and gastrointestinal tract[3].

The metabolism of codeine occurs mostly in the liver, and to a lesser extent in the intestine and CNS [1]. Althoughcodeine metabolism resulted in several metabolites,it is morphine, a product of codeine O-demethylation by enzyme cytochrome P450 2D6 (CYP2D6), that is responsible for its analgesic effect [4,5]. Codeine dosage is highly regulated, its overdose could cause depressive effects on the central nervous system ordeath from respirationarrest. The adult minimum lethal oral dose for codeine is estimated to be 0.5–1.0 g, i.e 17–34 pills containing 30 mg codeine [6]. The serum codeine concentrations exceeding 0.3 mg/L have been reported to cause toxicity,while concentrations above 1.6 mg/L are considered to be lethal [2].

The abuse and misuse of prescription opioids such as codeine havereached an alarming rate in the last ten years. In the UnitedStates, it was reported that about 1.2 million visit emergency department (ED) as a result of non-medical use of prescription medications in 2011 alone[7]. Theproduction and importation of cough syrup that include codeine as an ingredient was ban by the NigerianGovernment in 2018 due to concerns regarding its use by youths to get intoxicated [8]. Many addicts of codeine in many countries are into the habit of using the drug every day without doctor prescriptions which is of great concerns. Therefore,

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this study assesses the toxic effects of different doses, including overdoses of codeine, on haematological parameters, biochemical changes and oxidative damage in the liver and kidney of rats exposed to normal, high and extreme doses of codeine orally for 28 days.

2. Materials and Methods

2.1. *Materials*

Centrifuge machine, human automated haematology system analyzer (ERMA PCE 210, ERMA, Japan), weighing balance, dissecting sets, cuvette, spectrophotometer, pH meter, refrigerator, homogenizer, razor blade, 1 ml syringes, 2 ml syringes, and 5 ml syringes, surgical gloves, cotton wool, measuring cylinder, test tubes, beaker, spatula, plastic cages, EDTA bottles, plain sample bottles.

2.2.*Reagents*

Thiobarbituric acid (TBA), nicotinamide adenine dinucleotide reduced (NADH) and Codeine were obtained from Sigma–Aldrich Chemical Co. Ltd. (England). Nitrobluetetrazolium (NBT), 5,5′-Dithiobis (2-nitrobenzoic acid) (DTNB) are the product of Fluka (Buchs, Switzerland). All other chemicals used were analytical grade.

2.3.*Animals*

Twenty (20) male Wistar rats with an average weight of 170-200g were used for the experiments. They were housed in the Ladoke Akintola University of Technology, (LAUTECH) animal house. They were allowed fourteen (14) days to acclimatize before the commencement of drug administration. The animals were maintained on a standard pellet diet throughout the acclimatization and administration period. The animal experimental procedures were conducted in accordance with the National Institutes of Health guide for the

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care and use of laboratory animals (NIH Publications No. 8023) revised in 2002 and approved by the institutional research committee.

2.4. *Experimental Design*

Twenty(20) male wistar strain albino rats were divided into four groups of five rats each according to their weight. Group I labelled control received saline solution for 28 daysthrough the oral route. Group II labelled normal codeine received a normal dose of codeine at 5mg/kg/day body weight of rat. Group III labelled high codeine received a high dose of codeine at 25mg/kg/day. Group IV labelled extreme codeine received an extreme dose of codeine at 50mg/kg/day. Codeine was constituted in saline solution and administered through the oral route. During the experiment, the animals were allowed free access to food and distilled water. After 28 days of codeine treatment and after an overnight fast, animals were sacrificed by cardiac puncture under light ether anaesthesia into ethylene diamine tetra-acetic acid (EDTA) sample bottles for haematological analysis and heparinised sample bottles for biochemical analysis. Liver and kidney were removed from the animals for biochemical analyses. Blood samples in heparinized bottles were centrifuged to separate plasma and red blood cells. All samples were stored at -20°C until analysed.

2.5.*Haematological Study*

Freshly collected blood samples in EDTA bottles were analysed for haematological assay using an automatic haematological assay analyser (ERMA PCE 210, ERMA, Japan). Different tested haematological parameters were as follows: White Blood Cell (WBC), Red Blood Cells (RBC), Haemoglobin (HGB), Haematocrit (HCT), Red cells (RDW%), Red cells Distribution Width (RDW), Mean Corpuscular Haemoglobin (MCH), Mean Corpuscular

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Haemoglobin Concentration (MCHC), Platelet (PLT), Mean Platelet Volume (MPV), Mean Corpuscular Volume (MCV),Platelet crit (PCT), Platelet distribution width (PDW).

2.6.*Determination of Blood Biochemical Parameters*

Plasma concentrations of alkaline phosphatase (ALP), aspartate aminotransferase (AST), alanine aminotransferase (ALT), urea, creatinine and total protein were determined using enzymatic kits (CYPRESS® Diagnostics, Langdorp, Belgium) according to the manufacturer's instructions.

2.7.*Preparation of Liver and Kidney Homogenates*

Prior to biochemical analyses, the liver and kidney samples were cut into small pieces and homogenized in Phosphate buffer saline (PBS) with a homogenizer to give a 10 % (w/v) liver and kidney homogenate. The homogenates were then centrifuged at 12,000 rpm for 15 min. The supernatant obtained was used for the assay of superoxide dismutase, catalase, reduced glutathione, thiobarbituric acid reactive substances (TBARS) content, and protein estimation.

2.8.*Determination of Hepatic and Renal Antioxidant Enzyme Activities and MDA Levels*

Hepatic and renal superoxide dismutase (SOD) activities were assayed in the tissue homogenates by the method of Kakkar, *et al.* [9] at 560 nm. One unit of enzyme activity was defined as that amount of enzyme which caused 50% inhibition of nitrobluetetrazolium reduction/mg protein. Catalase (CAT) activity was determined at room temperature by using the method of Aebi[10] and the absorbance of the sample was measured at 240 nm in a UV spectrophotometer. The concentration of reduced glutathione (GSH) in liver and kidney homogenates was measured, as described by Jollow et al. [11]. The extent of lipid peroxidation was estimated as the concentration of thiobarbituric acid-reactive product

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malondialdehyde (MDA), using the method of Draper and Hadley, [12]. All of the enzyme activities were expressed as per mg of protein and the tissue protein was estimated according to the method of Lowry *et al.,* [13], using bovine serum albumin (BSA) as a standard.

2.9.*Statistical Analysis*

Results are expressed as mean ±S.E.M. The levels of homogeneity among the groups were assessed using One-way Analysis of Variance (ANOVA) followed by Turkey's test. All analyses were done using Graph Pad Prism Software Version 5.00 and p values < 0.05 were considered statistically significant.

3. Results

3.1.*HaematologicalParameters*

The effects of codeine administration on haematological parameter were depicted in Table 1. No significant changes in the parameters ofHGB, HCT, RDW-CV, MPV, and PDWwere found when compared with control animals. However, administration of codeine significantly lower ($p < 0.05$) white blood cell (WBC) count, red blood cell (RBC) count, and platelet (PLT), while the value of mean corpuscular volume (MCV) andMean Corpuscular Haemoglobin (MCH)were increased when compared with control animals.

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Table 1: Effect of codeine administration on haematological parameters of rats.

Parameters	Control	Normal codeine	High codeine	Extreme codeine
WBC ($X10^9$/L)	8.30±0.35	4.18±0.22 **	4.94±0.74 **	4.56±0.34 **
HGB (g/dl)	10.58±0.39	9.88±0.08	10.66±0.64	10.67±0.13
RBC ($x10^{12}$/L)	6.92±0.31	5.59±0.05 **	5.51±0.33 **	5.47±0.11 **
HCT (%)	31.64±0.97	29.00±0.32	32.00±1.58	32.00±0.32
MCV (fl)	55.50±0.87	56.48±0.54	67.70±1.15 **	67.70±1.15 **
MCH (pg)	15.28±0.31	17.60±0.19	19.30±0.28 **	19.42±0.22 **
MCHC (g/L)	275.64±1.93	313.00±2.21	286.60±2.6	290.80±2.46
RDW-CV (%)	17.22±0.31	16.30±0.25	17.84±0.38	17.32±0.16
RDW-SD (fl)	31.82±0.15	31.60±0.43	39.18±1.01	37.42±0.47
PLT ($X10^9$/L)	583.00±1.26	592.86±18.11	544.67±14.10	405.50±6.30 **
MPV (fl)	7.00±0.06	6.80±0.11	6.88±0.10	7.16±0.10
PDW	16.08±0.32	16.14±0.19	15.96±0.08	15.98±0.13
PTC (%)	0.39±0.00	0.40±0.02	0.33±0.01	0.33±0.04

Each value represents the mean of five rats. ** = significantlydifferent from control ($p < 0.05$).

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3.2.*Effect of Codeine Administration on Blood Biochemical Parameters*

Administration of codeine at normal, high and extreme doses significantly increased the activity of ALP by 75.39%, 149.36% and 122.65% respectively and AST activity by 31.82%, 83.41% and 145.47% respectively when compared with the normal rats. The plasma concentration of creatinine and Urea were also significantlyincreased by all the three doses of codeine while total proteins level wasdecreased by administration of normal, high and extreme doses of codeine by 30.35%, 48.02% and 38.84% respectively when compared with the normal rats (Table 2).

Table 2: Effect of codeine administration on blood biochemical parameters

PARAMETERS	Control	Normal codeine	High codeine	Extreme codeine
ALP (IU/L)	100.74 ± 7.86	176.69 ± 11.57 **	251.21 ± 12.52 **	224.30 ± 15.76 **
AST (IU/L)	44.25 ± 1.15	58.33 ± 2.42 **	81.16 ± 4.45 **	108.62 ± 3.56 **
Creatinine (mg/dL)	0.83 ± 0.08	1.61 ± 0.25 **	2.44 ± 0.21 **	2.19 ± 0.32 **
Urea (mg/dL)	23.79 ± 1.64	46.46 ± 3.94 **	41.60 ±3.51 **	33.39 ± 2.91 **
Total protein (g/dL)	8.60 ± 0.73	5.99 ± 0.36 **	4.47 ± 0.50 **	5.26 ± 0.47 **

Each value represents the mean of five rats. ** = significantlydifferent from control ($p < 0.05$).

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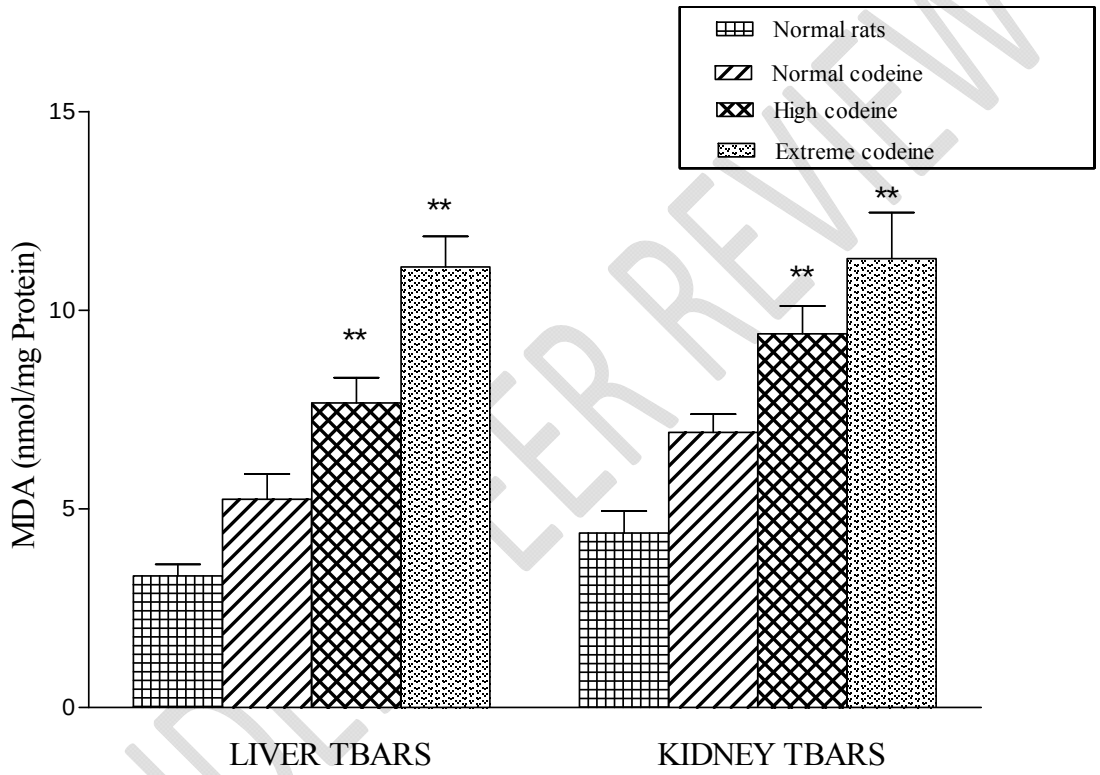
194 3.3.*Effect of Codeine Administration on TBARSLevels*

195 Hepatic TBARS levels of rats treated with normal, high and extreme doses of codeine were
196 dose-dependently significantly increased by 58.26%, 131.42% and 234.74% respectively
197 when compared with the normal rats. Similarly, administration of codeine at normal, high and
198 extreme doses significantly increases renal TBARS levels by 57.59%, 114.14% and 157.23%
199 respectively when compared with the control rats (Figure 1).



200
201 **Figure 1.** Effect of codeine administration on hepatic and renal TBARS levelsof rats. Values
202 are mean ± SEM (n=5).** = significantlydifferent from control ($p < 0.05$).
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3.4.*Effect of CodeineAdministration on SOD Activity*

Administration of codeine at normal, high and extreme doses significantly reduced hepaticSOD levels by 39.06%, 61.74% and 57.29% respectively and reduced renal SOD levels by 37.70%, 50.27% and 62.04% respectively when compared with normal rats (Figure 2).

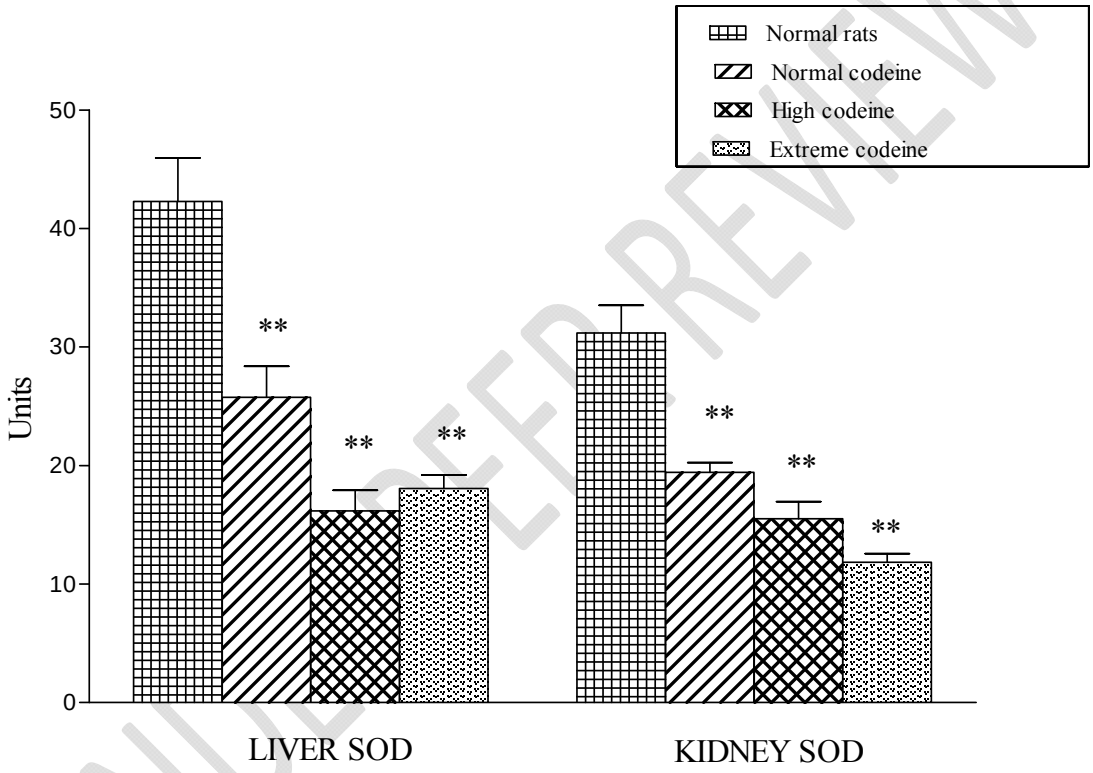

Figure 2. Effect of codeine administration on hepatic and renal SOD activity of rats. Values are mean ± SEM (n=5). ** = significantlydifferent from control ($p < 0.05$).

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3.5.*Effect of CodeineAdministration on Catalase Activity*

Administration of codeine at normal, high and extreme doses significantly reduced hepatic catalase levels by 28.93%, 40.89% and 53.33% respectively and reduced renal catalase levels by 45.92%, 61.73% and 40.30% respectively when compared with normal rats (Figure 3).

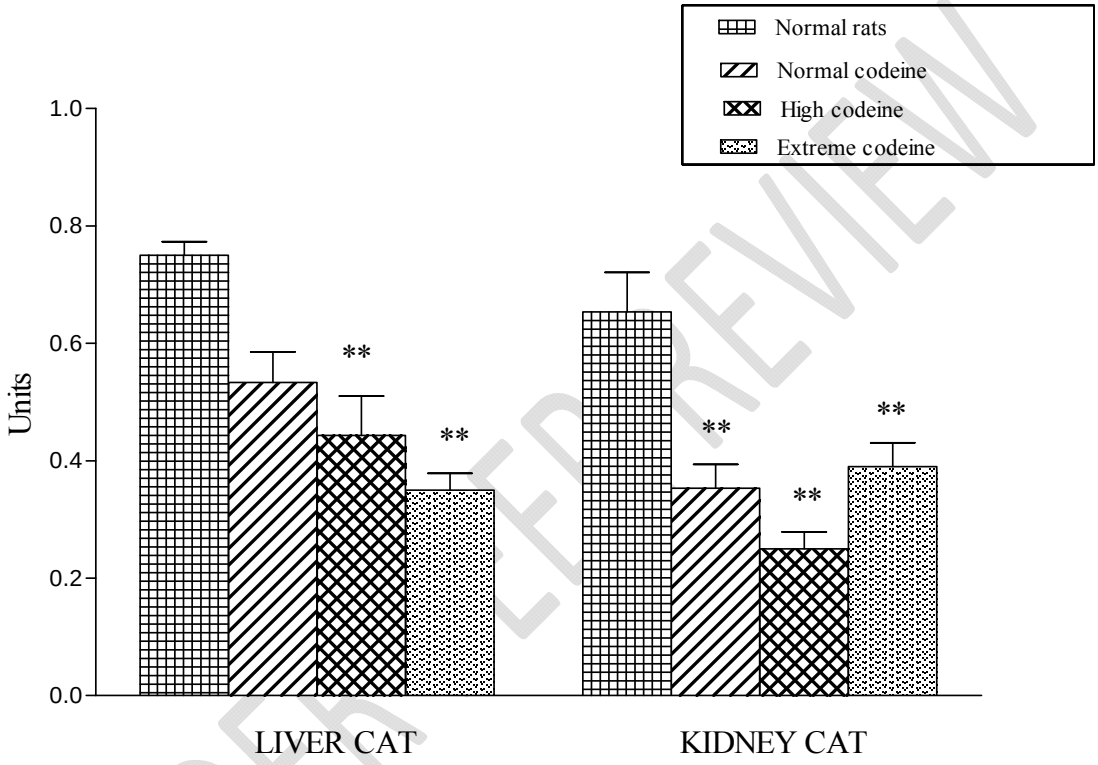

Figure 3. Effect of codeine administration on hepatic and renal catalase activity of rats.Values are mean ± SEM (n=5). ** = significantlydifferent from control ($p < 0.05$).

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239 3.6.*Effect of CodeineAdministration on GSH Activity*

240 Hepatic GSH levels of rats treated with normal, high and extreme doses of codeine were
241 significantly reduced by 37.04%, 65.21% and 63.58% respectively when compared with the
242 normal rats. Similarly, administration of codeine at normal, high and extreme doses
243 significantly reduced renal GSH levels by 33.06%, 49.73% and 66.71% respectively when
244 compared with the control rats (Figure 4).

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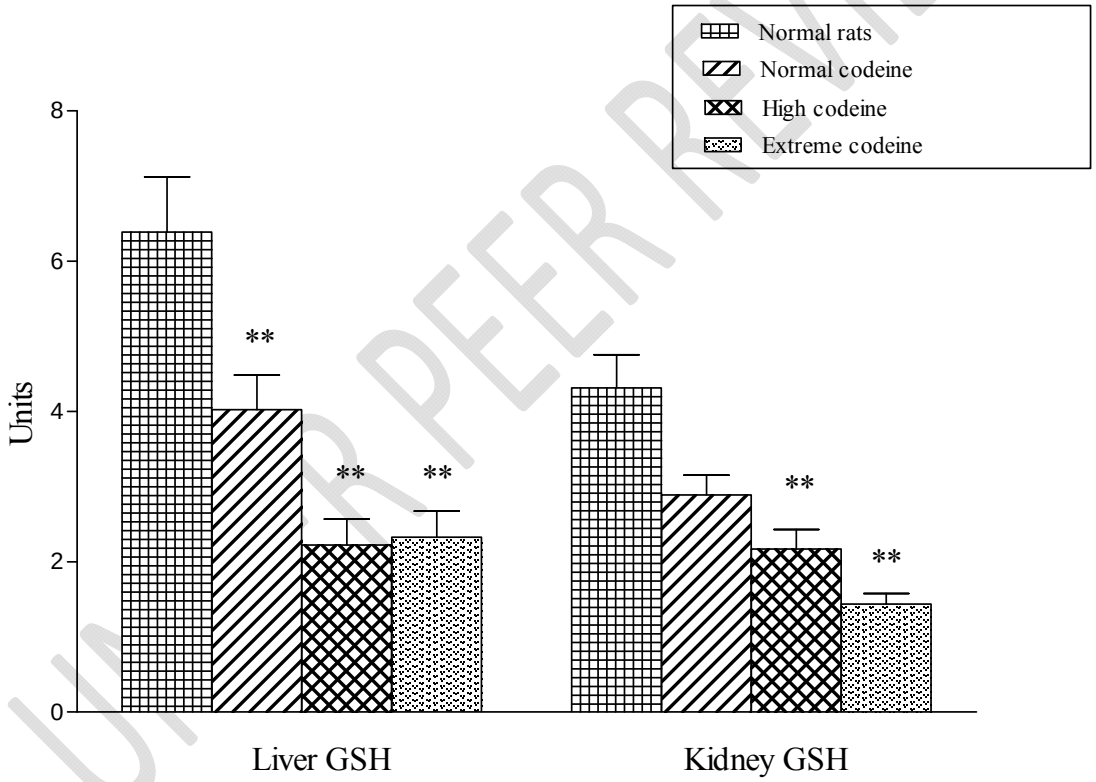

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247 **Figure 4**. Effect of codeine administration on hepatic and renal GSH activity of rats. Values
248 are mean ± SEM (n=5). ** = significantlydifferent from control ($p < 0.05$).
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4. Discussion

Codeine (Opiod) is an analgesic mainly used as anantituitive drug and to manage mild to moderate pain [14,15]. It is,however, a drug of abuse because of its stimulatory effect onCNSamong some adults [16]. Toxic effects of codeine use have been reported, although little is known about codeine toxicity mechanisms [2,17]. In this study, the toxic effects of codeine wereexamined in animal models. Codeine was studied as adrug and not as analgesics because alarming misuse of codeine recently made Nigeria Government ban production and importation of cough syrup that has codeine as an ingredient [8]. Therefore, this study evaluated the toxicity of codeine on systemic body organs because of people use of codeine without doctor prescriptions.

Free radicals and reactive oxygen species are generated by chemicals and pollutants such as factory waste, toxic gases and they are known to disrupt biochemical and haematological parameters in organisms [18]. Disruption ofhaematological parameters could provide valuable information and insight intothe diagnosis of various diseases and pathological conditions. The deviation from normal haematological parameters levels representsthe presence of toxicity or disease conditions [19]. The decrease in red blood cell count (RBC) level could be a result of an imbalance between its production and loss [20]. In this study, codeine administration caused a significant reduction in red blood cell counts (RBC). The observed decrease in the number of RBCssuggest that codeine administration resulted in blood loss due to serious gastrointestinal tract bleeding, red blood cell haemolysis and poor iron absorption in the intestine.

Codeine administration also resulted in a reductionof WBC of experimental animals in this study. White blood cells fight infections, defend the body againstforeign organisms'invasion and produce antibodies in immune response[21]. Animals with low

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WBC are at high risk of disease infection, while high WBC results in high resistance to diseases [21]. Thereduction of WBC by codeine observed in this study agrees with pervious study which revealed that abuse or long-term use of opium supresses the immune system and individuals are more susceptible to infectious disease [22].

Blood platelets are involved in blood clotting and its low level will prolongthe process of clot-formation resulting in excessive blood loss during injury. Although, there was no significant variation in platelet concentration of rats administered with normal and high doses of codeine in this study, however, the extreme dosage of codeine significant reduce platelet concentration. A decreased number of platelet (thrombocytopenia) by codeine in this study is in supports of pervious work which observed that morphine administration induced thrombocytopenia [23]. Codeine administration also resulted in increased in the levels of mean corpuscular haemoglobin (MCH) and mean corpuscular volume (MCV).We strongly believe that disruption in haematological parameters observed in this study may be due to increased population of unquenched free radicals caused by codeine administration.The changes observed in other haematological parameters such as HGB, HCT, RDW%, RDW, MCHC, MPV,PCT, PDW in this study were largely found to be non-significant, an observation that may be different if codeine administration period was much longer than 28 days used in this study.

In this study, many biochemical parameters on liver and kidney functionswere determined in plasma samples to assess damage to metabolizing organs. The increased in the activities of ALT, aspartate aminotransferase (AST), ALP and lactate dehydrogenase (LDH) have been reported in previous studies following exposure to opioids, including morphine and tramadol [24-26]. Administration of codeine in this study significantly increased ALP and AST activities which are in conformity with previous research that revealed that AST. ALP and ALT activities in plasma increased significantly in an addicted patient of opioid[27].The liver

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302 is anorgan that detoxified toxic elements and chemical drugs in the body,the increasein the
303 activities AST and ALP in plasma in this study are indicative of liver damage[28]. The
304 increased secretion of these liver enzymes may be accompanied by acute cell necrosis,
305 therefore, the increased plasma level of these enzymes in rats treated with codeine could be
306 due to necrosis or damage to liver cell membrane which leak the enzymes into the blood
307 circulation[29].

308 The level of plasma creatinine is used to determined glomerular filtration rate and serves
309 as renal function assessment[30]. Codeine administration significantly increased plasma
310 creatinine level of rats in this study and this can be taken as evidence of renal damage
311 because the high level of creatinine in the blood implies a loss of kidney function in ensuring
312 creatinine excretion. Similarly, administration of codeine in this study increased blood urea
313 concentration. Urea is a nitrogenous waste and product of protein and amino acid
314 metabolism, it is eliminated from the body through urinary excretion. It is an important
315 clinical parameter because it can be used to determine the nephrotoxic profile of xenobiotics.
316 The increased in blood urea concentration observed in this study following codeine
317 administration agrees with previous research [31]and it is an indication of renal toxicity
318 which might have instigated decrease in glomerular filtration rate leading to the build-up of
319 creatinine and urea in the blood.

320 There was a decrease in plasma total protein in rats treated with codeine in this study this
321 is in support of previous research finding which showed decrease in plasma total protein
322 levels in opium dependent participants when compared to the control group[32]. The clinical
323 diagnosis has shown that a decrease in plasma concentrations of protein characterized by
324 significant increases in the urinary excretion of protein and albumin are indicators of renal
325 dysfunction[33]. Therefore, the decrease in plasma total protein observed in this study can be
326 taken as an indication of kidney damage.

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Administration of codeine resulted in increased levels of MDA the last metabolite of lipid peroxidation chain, and inhibition of the antioxidant enzymes, superoxide dismutase (SOD), catalase (CAT) and reduced glutathione (GSH) in liver and kidney of rats. Elevated levels of MDA have been reported to be an indication of an increase in free radical generation and it is considered a useful measure of oxidative stress status [34]. SOD, CAT and GSH are important antioxidant enzymes which played a pivotal role in scavenging of oxidative free radicals [35]. The inhibition of these antioxidant enzymes observed in this study could be linked to exhaustion of these enzymes as a result of oxidative stress caused by codeine administration.

The toxic effect of codeine administration leads to a large population of unquenched free radicals leading to the state of oxidative stress. Oxidative stress form when there is an imbalance between free radical generating and scavenging systems has been implicated in the pathogenesis of a wide range of disorders, including neurodegerative disorders, cardiovascular diseases, cancer, and ageing [36].

Conclusion

Our results evidence that codeine administration may cause haematoxicity, hepatotoxicity and nephrotoxicity and as such, its use should be limited to prescription only. Our findings underlined the need to avoid indiscriminately and prolong use of codeine, since prolonged daily use of the drug either at a therapeutic dose or the extreme dose may lead to damage accumulation.

Ethical Approval:

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As per international standard or university standard ethical approval has been collected and preserved by the authors.

Conflict of Interests

Authors have declared that no competing interests exist.

Article type

Original research paper

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