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Effect of classic soft drink Coca-Cola as a solvent in the administration of tramadol and diazepam on biochemical and histological changes in liver and kidney

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Abstract. The objective of this study was to determine the biochemical and morphological changes in the liver and kidney as a result of the acute administration of tramadol and diazepam with classic soft drink Coca-Cola (Coke).

Method: Thirty-six (36) adult male Wistar rats were divided into six groups: Group A-control (distilled water), Group B (Coke), Group C (tramadol, 50 mg/kg), Group D (tramadol dissolved in Coke, 50 mg/kg), Group E (diazepam, 10 mg/kg) and Group F (diazepam dissolved in Coke 10 mg/kg). All administrations were done intraperitoneal. Twenty-four hours after administration, blood samples were collected via cardiac puncture for evaluation of the liver (Aspartate aminotransferase [AST] and Alanine aminotransferase [ALT]), kidney (urea and creatinine [CREA]) function and the organs were excised and processed for histopathological examination.

Result: A significantly increased in AST, creatinine and urea concentrations was observed in Tramadol and Coke Groups compared to control ($P < 0.05$), while diazepam had no significant effect on AST, ALT ($P > 0.05$), though it caused a significant increase in urea and CREA ($P < 0.05$). Dissolving the tramadol in Coke aggravated its hepatotoxicity and nephrotoxicity, while Coke had no significant effect on diazepam. Histological examination also corroborated the biochemical result.

Conclusion: The results showed that mixing drugs with Coke does not improve the toxicity of tramadol and has no significant effect on diazepam.

Keywords: hepatotoxicity, nephrotoxicity, histology, tramadol, diazepam, Coke..

Conflict of interest statement: all the authors declared no competing interests.

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Вплив класичного безалкогольного напою Кока-Коли як розчинника при введенні трамадолу та діазепаму на біохімічні та гістологічні зміни печінки та нирок

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Резюме. Метою цього дослідження було визначити біохімічні та морфологічні зміни в печінці та нирках внаслідок введення трамадолу та діазепаму разом з класичним безалкогольним напоєм Coca-Cola.

Методи. Тридцять шість (36) дорослих самців щурів Вістар були розподілені на шість груп: група А-контроль (дистильована вода), група В (Coca-Cola), група С (трамадол, 50 мг/кг), група D (трамадол, розчинений у Coca-Cola, 50 мг/кг), група E (діазепам, 10 мг/кг) та група F (діазепам, розчинений у Coca-Cola 10 мг/кг). Розчини вводили внутрішньочеревно. Через двадцять чотири години після введення, за допомогою пункції серця, забирали зразки крові для оцінки функції печінки (аспартатамінотрансферази [AST] та аланінаміно-трансферази [ALT]), нирок (сечовини та креатиніну [Cr]). а органи були висічені та оброблені для гістопатологічного дослідження.

Результати. Введення розчину трамадолу з Coca-Cola значно збільшувало активність AST, концентрації Cr та сечовини порівняно з контрольною групою ($P < 0,05$), тоді як діазепам не мав значного впливу на AST, ALT ($P > 0,05$), але призводив до значного збільшення сечовини та Cr ($P < 0,05$). Розчинення трамадолу в Coca-Cola посилювало його гепато- та нефротоксичність, тоді як Coca-Cola не мала значного впливу на діазепам. Гістологічне дослідження також підтвердило результати аналізу біохімічних даних.

Висновки. Змішування наркотиків з Coca-Cola не зменшує токсичність трамадолу та не має істотного впливу на діазепам.

Ключові слова: гепатотоксичність, нефротоксичність, гістологія, трамадол, діазепам, Coca-Cola.

Introduction. Any chemical or substance that affects biological functions positively or negatively can be referred to as a drug [1, 2]. Some of these functions can be physiological (behavioral, mood swing, cognitive, etc.). This is as a result of the ability of drugs to alter various metabolic processes in living organisms, leading to the observed physical changes [2-4]. The ability of any drug to alter macromolecules to an extent is often dependent on the chemical nature, concentration, specific target, solubility and presence of other drugs [5]. The drug alters the body functions either positively or otherwise depending on the body composition of the user, the type of drug used, the amount used and whether used singly or with other drugs at the same time [2]. One of the major global challenges of drug production and circulation is the abuse. Drug abuse can be defined as non-prescript/ nonmedical usage of drugs [6]. In order words, any substance or chemical that is used excessively, without a health practitioners' diagnosis and prescription, which often leads to addiction and dependence on the drug, can be classified as abuse [7]. The idea that the individual is in control of drug usage

is a major challenge in combatting drug abuse, further aggravating the toxicological and physiological implications of abusive drugs [8].

Tramadol is a synthetic opioid used medically to treat pains and its associated pathologies, initially classified as safe with minimal side effects. However, a recent report shows that tramadol can be addictive, with the respiratory problem and other challenges [9, 10]. Tramadol is metabolized to its active component, O-desmethyl tramadol [11]. It acts as a weak μ -opioid receptors agonist as well as prevent the reuptake of neurotransmitters such as serotonin and norepinephrine, which are linked to drug addiction [12]. The concentration of tramadol after oral administration peaked 2-3 h later. It is evenly distributed in the tissue, with half its concentration excreted within 6 h, the rest are demethylated, conjugated and sulfated in the liver [13]. Diazepam is an example of benzodiazepines (BZDs). It is one of the commonest psychotropic drugs that are often prescribed for their sedative and anxiolytic activities. They act on the central nervous system viz the gamma-aminobutyric acid receptors [14-16]. In addition to the central receptors described for BZD, peripheral-type binding sites had been identified in liver cells, endocrine steroidogenic tissues, and immune cells [17]. Tramadol and diazepam are one of common over the counter drugs and due to its abuse and addiction, they are categorized as class IV by the food drug administration (FDA) [18, 19]. Class IV drugs are medicinal drugs that have a

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low potential for addiction, which their abuse can result in drug dependence.

A soft drink is a drink that typically contains carbonated water, a sweetener, and a natural or artificial flavoring. The sweetener may be sugar, high-fructose corn syrup, fruit juice, sugar substitutes, or in the combination of these. Soft drinks may also contain caffeine, colorings, preservatives and other ingredients [20]. Coca-Cola (Coke) is a widely known soft drink that is consumed in arguably every part of the world. Its content includes carbonated water, sugar, carbon-dioxide, caffeine, phosphoric acid, caramel color and flavoring [21]. The sweetness of Coke has made it a suitable solvent for dissolving drugs, for ingestion for purposes such as rape and, in addition, it is generally believed that Coke improves the euphoric effect when taken with some additive drugs [22–24].

The important role of the liver and kidney in drug metabolism makes them prone to the toxic effect of drugs. Depending on drug dosage and route of administration, the most drug can be toxic [25]. Hepatic metabolism is a mechanism that converts drugs and other compounds into products that are more easily excreted [26]. In some cases, a metabolite may have higher activity and/or greater toxicity than the original drug [27]. Liver and kidney are the two major organs involved in drug metabolism, detoxification and excretion of drugs from the body [9]. Thus, their health status is important in the efficient metabolism of drugs.

This study aimed to determine the effect of Coke as a solvent on high dose administration of tramadol and diazepam on the integrity of the liver and kidney.

Materials and methods. Chemicals and Reagents. Sodium dihydrogen phosphate, Disodium hydrogen phosphate, Ethanol, Potassium chloride solution (KCl), Formalin, Tris KCl, Sodium Hydroxide (NaOH), Tramadol hydrochloride, Diazepam.

Animals. The study was conducted on thirty-six male Wistar rats weighing between 170–220g. These rats were obtained from a breeding animal house from the Department of Biochemistry, University of Benin.

They were housed at room temperature in plastic cages and were kept under constant healthy environmental and nutritional conditions. They were fed on rat pellets and water ad libitum. The maintenance of the animals and the experimental procedures were following the guiding principles of animal handling. They were left to acclimatize for 2 weeks before the start of administration.

Experimental Design. Thirty-six (36) rats were divided into six groups of six rats per group as follows: Group I (negative control) administered 1 ml/kg distilled water; Group II (Coke) administered 1 ml/kg of Coca-Cola; Group III (tramadol) administered 50 mg/kg tramadol dissolved in distilled water; Group IV (tramadol+Coke) administered 50 mg/kg tramadol dissolved in Coke; Group V (diazepam) administered 10 mg/kg diazepam dissolved in distilled water; Group VI (diazepam+Coke) administered 10mg/kg diazepam dissolved in Coke.

Serum Biochemistry. Serum Alanine aminotransferase (ALT), aspartate aminotransferase (AST), urea and creatinine kit were assayed spectrophotometrically using commercial kits purchased from Bio Diagnostic Co., according to the manufacturer's protocol.

Histopathological evaluation. Kidney and liver tissues were taken from the eviscerated rats and fixed in 10% formalin for 24 h, and then processed to obtain paraffin blocks. Sections of 4–6 μ m thickness were cut using a microtome and stained with hematoxylin and eosin (H and E) stain by using the method of Stevens and Wilson (Stevens and Wilson [28]).

Statistical Analysis. Data were organized, tabulated, and statistically analyzed using the SPSS software for Windows, Version 16.0. (SPSS Inc., Chicago, USA). For quantitative data, the mean and SD were calculated and were expressed as mean $\pm$ standard deviation, percentage change and were analyzed using Analysis of Variance (ANOVA). For comparison of means of more than two groups, the F-test was used. Statistical significance was taken at a P value of less than 0.05.

Results. The results of blood biochemical measuring are presented in Figures 1–4.

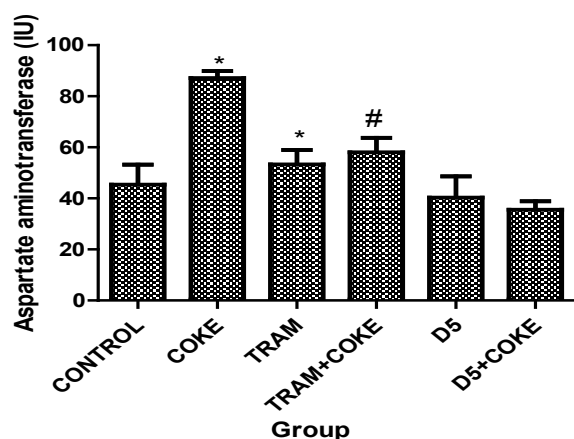


Fig. 1. Effect of tramadol or diazepam dissolved in Coke on Aspartate aminotransferase activity. Results are expressed as M $\pm$ SD (n = 6). *P<0.05 (control vs treatment group), # P<0.05 (TRAM vs TRAM+COKE)

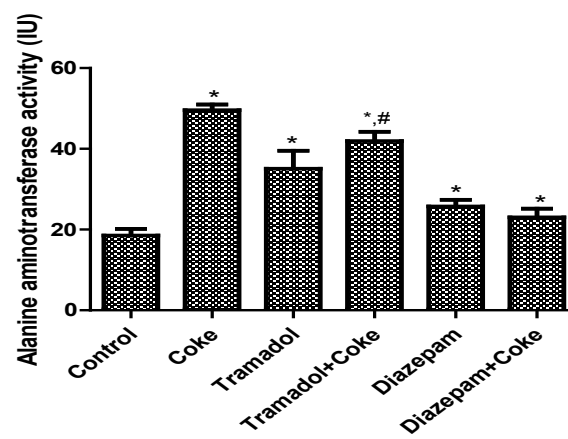


Fig. 2. Effect of tramadol or diazepam dissolved in Coke on Alanine aminotransferase activity. Results are expressed as M $\pm$ SD (n = 6). *P<0.05 (control vs treatment group), # P<0.05 (TRAM vs TRAM+COKE)

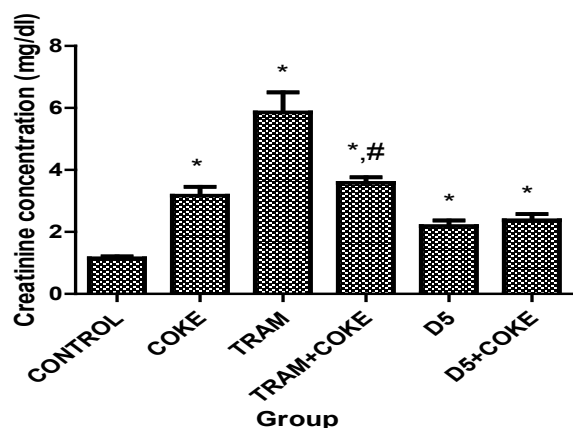


Fig. 3. Serum creatinine level of Wistar rat in control and treatment groups (Coke, D5 (diazepam) and D5 and Coke respectively). Results are expressed as $M \pm SD$ ($n = 6$).
* Significantly different at $P < 0.05$ (control vs treatment group)

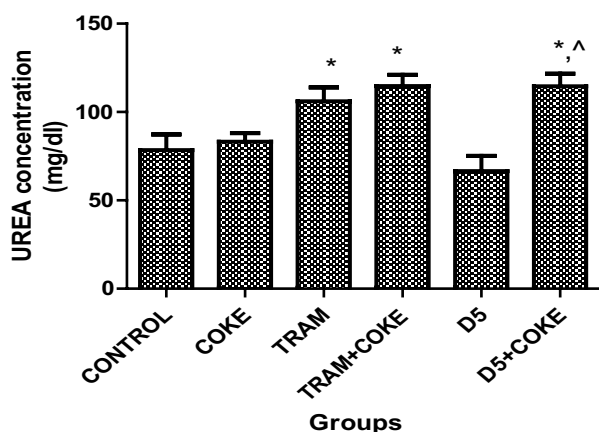


Fig. 4. Blood urea level of Wistar rat in control and treatment groups (coca-cola, diazepam and diazepam and coca-cola respectively). Results are expressed as $M \pm SD$ ($n = 6$).
* Significantly different at $P < 0.05$ (control vs treatment group)

As presented in Figure 5, the Control Group (I) showed normal central venules without congestion (white arrow), the morphology of the hepatocytes appears normal (blue arrow), the sinusoids appear normal without infiltration of the inflammatory cell (slender arrow). No pathological lesion was seen. The Coke Group (II) demonstrated portal tract with mild congestion of portal vein (white arrow) and periportal infiltration of inflammatory cells (black arrow), the morphology of some of the hepatocytes showed mild to moderate infiltration of fat; moderate microvesicular steatosis (blue arrow), the sinusoids appeared normal without infiltration of inflammatory. The Tramadol Group (III) showed central venules with congestion (white arrow), liver parenchyma showed a focal area of mild hepatic necrosis, the morphology of other hepatocytes appeared normal (blue arrow), the sinusoids appeared normal without infiltration of the inflammatory cell (slender arrow). The Tramadol+Coke Group (IV) indicated poor architecture, there was severe congestion of the central venules and portal veins (white arrow), the liver parenchyma showed a focal area of an abscess (black arrow), mild portal triad noted (red arrow), some of the liver plates were destroyed and some necrotized hepatocytes were seen (blue arrow), the moderate showed an attendance of red cells (slender arrow). The Diazepam Group (V) showed normal central venules without congestion (white arrow), the morphology of the hepatocytes were with severe microvesicular steatosis, fat degeneration and necrosis (blue arrow), and the sinusoids showed no infiltration of the inflammatory cell (slender arrow). Lastly, the Diazepam+Coke Group (VI) demonstrated normal central venules without congestion (white arrow), the morphology of the hepatocytes and the sinusoids appeared normal (blue arrow).

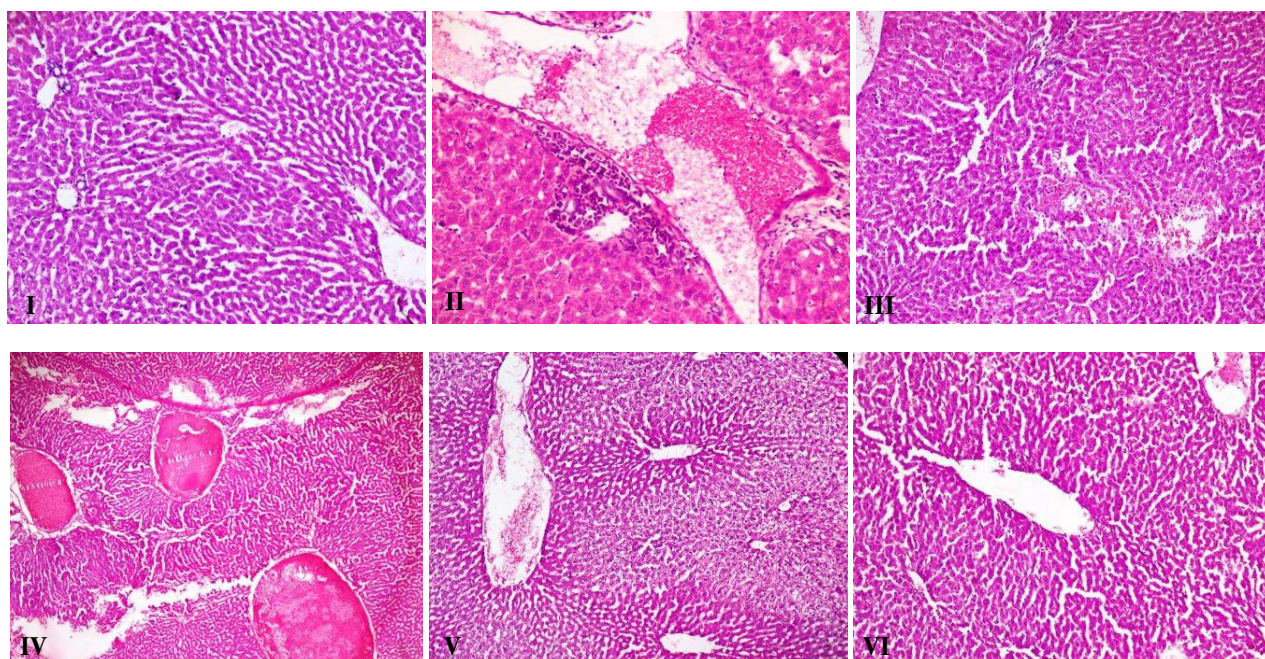


Fig. 5. Photomicrograph of rat liver sections stained with hematoxylin and eosin (X100).

Figure 6 demonstrated of rat kidney sections stained with hematoxylin and eosin. The Control and Coke Groups showed poor architecture, the renal cortex showed some glomeruli with atrophic mesangial cells and wide capsular spaces (white arrow), the renal tubules including Distal convoluted tubules and Proximal convoluted tubules appeared normal (blue arrow), the interstitial spaces showed interstitial dilated vessel with mild congestion (slender arrow). The Tramadol Group (III) indicated poor architecture as seen in lower magnification x100, the renal cortex showed some normal glomeruli (white arrow) and glomeruli with sclerosis (black arrow), the renal tubules appeared normal (blue arrow) but few tubules showed eosinophilic materials within their lumen (red arrow). The interstitial spaces appeared normal (slender arrow). The Tramadol+Coke Group (IV) showed poor architecture as seen in lower magnification x100, the renal cortex showed normal glomeruli with normal mesangial cells and capsular spaces (white arrow), however, the renal

tubules demonstrated tubular epithelial degeneration (blue arrow) as well as the presence of eosinophilic casts within their lumen (red arrow); the interstitial spaces showed the focal area of inflammatory cells aggregate (slender arrow). The Diazepam Group (V) showed normal architecture as seen in lower magnification x100, the renal cortex indicated normal glomeruli with normal mesangial cells and capsular spaces (white arrow), the renal tubules including Distal convoluted tubules appeared normal (blue arrow) while few Proximal convoluted tubules showed attenuation with lack of luminal spaces (red arrow), the interstitial spaces appear normal (slender arrow). The last, Diazepam+Coke Group (VI) showed poor architecture as seen in lower magnification x100, there were some atrophic glomeruli (black arrow) and normal glomeruli with normal mesangial cells in the renal cortex, the renal tubules appeared normal (blue arrow), the interstitial spaces showed moderate vascular congestion (slender arrow).

III

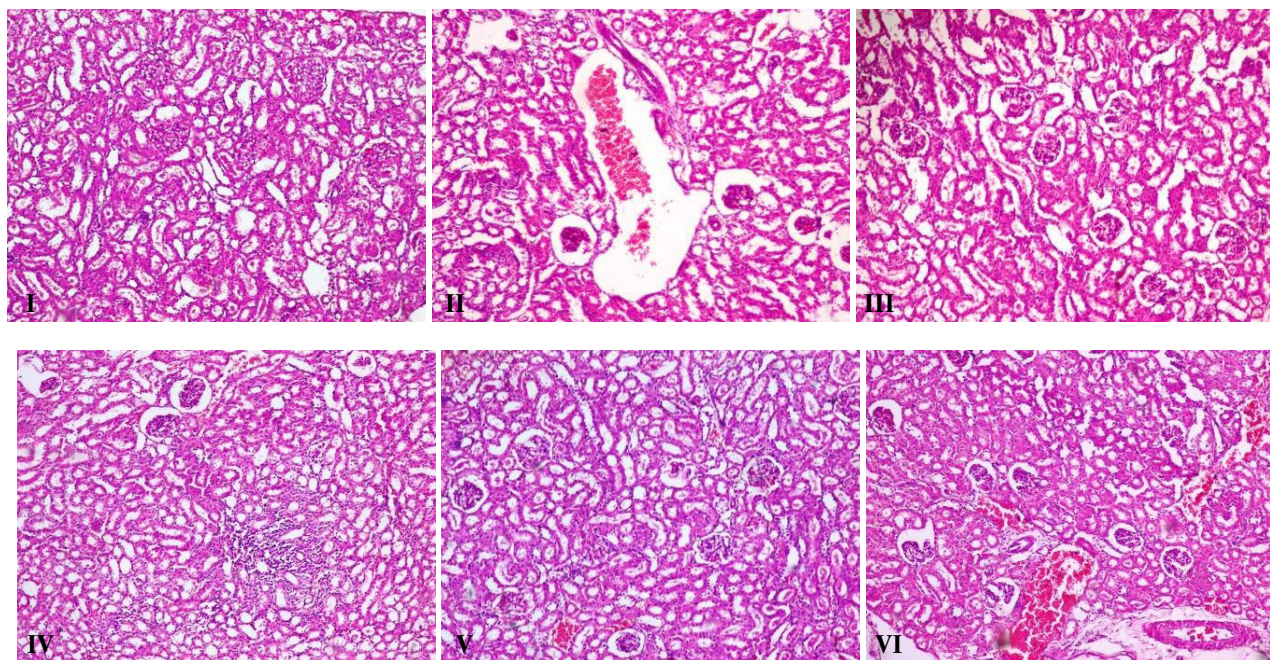


Fig. 6. Photomicrograph of rat kidney sections stained with hematoxylin and eosin. (X100)

Discussion. One of the major challenges among young adults is increasing usage of over the counter drugs for non-medicinal purposes, such as staying awake for a longer time to prepare for the exam, sport or sexual prowess [6]. These drugs are often taken with a soft drink to cover the unfriendly taste, especially when given to an unwilling victim [24, 28]. In recent times, tramadol and diazepam have been among the most commonly abused drugs, especially for non-medicinal purposes [18, 29]. Tramadol is used as an alternative to narcotics due to the difficulty of getting the latter [30], while, diazepam is commonly used by opioid abusers to reduce anxiety, reinforce opioid effects and treat craving and withdrawal symptoms. In addition, to the reported toxicity of the drugs, previous studies have also shown

that high consumption of some of these soft drinks can be unhealthy for consumers [31-34]. The present study was conducted to evaluate the effects of Coke on acute administration of tramadol and diazepam on the integrity of liver and kidneys.

The administration of tramadol and tramadol dissolved in Coke increased AST and ALT activity as compared to control. ALT and AST are compartmentalized enzymes, thus, their increase in the serum as a result of leakage from liver is a strong marker of hepatotoxicity. An increase in AST level can occur in connection with damages of the heart or skeletal muscles as well as of the liver parenchyma. However, liver-specific enzyme ALT is only significantly elevated in hepatobiliary disease [12, 34]. Hepatic metabolism is a mechanism that converts

drugs and other compounds into products that are more easily excreted and that usually have a lower pharmacologic activity than the parent compound. Thus, the toxicity observed might be as a result of tramadol (parent drug) or its metabolite [12, 35]. Coke has been reported to increase serum level of markers of hepatic injury when taken with ibuprofen [36]. However, in the results, Coke decreases the induction of ALT by tramadol. This can be linked to some of the constituents present in Coke. Treatment with diazepam and diazepam dissolved in Coke decrease the activity ALT while it did not affect AST, indicating that Coke did not affect diazepam concerning hepatic function. Benzodiazepines, such as diazepam is rarely associated with serum ALT elevation during therapy, AST might be increased due to high metabolic activities in the body [37-39]. This results further substantiate the hepato-safety of diazepam.

Besides, the levels of AST and ALT activity in diazepam only was seen to be decreased in comparison to the group of diazepam dissolved in Coke. This is in agreement with the study of Mudd, who has stated that co-administration of classes of benzodiazepines and Coke gave antagonistic reactions.

The kidney is responsible for the elimination of a myriad of drugs, non-drug xenobiotics, and endogenous compounds. Renal clearance is normally considered the net result of glomerular filtration, tubular secretion, and reabsorption, and characterization of the contribution of individual transporters expressed on basolateral and apical membranes of the tubule epithelium to drug and chemical excretion has advanced significantly over the last two decades [40].

There has been the contrary result on the safety of Coke consumption on kidney integrity, while some researchers linked chronic consumption of Coke to nephrotoxicity [41], others reported no effect on kidney integrity [42]. Thus, there is no agreed report on the nephrotoxicity of Coke. However, various studies have shown that the nephrotoxicity of Coke is at a chronic and not acute level. The contradictory effect of Coke on the concentration of urea and creatinine showed that Coke might not cause direct damage to kidney but other organs such as the muscle and the likes. Our experiment showed that, while Coke caused an increase in creatinine levels, which might be from various metabolic processes occurring in various organs apart from kidney, the non-effect on the urea concentration showed it does not directly damage the kidney. However, the administration of diazepam with Coke did not cause any significant increase in urea concentration. This indicates that dissolving diazepam with Coke, such as Coke might not have any significant effect on the single administration of diazepam.

However, reduced serum total protein concentration may be a result of the major effect of drug abuse in reducing the defense system of the body (deposition of immune nephrotic syndrome) thus increasing the risk of infection [43-45]. Diazepam metabolites such as oxazepam, desmethyldiazepam, and temazepam have

been shown to exert oxidative stress on the kidney leading to total failure [46].

The histopathological examination of the liver tissue corroborates the findings of the biochemical assays, congestion of the central venules, the hepatic necrosis and abscess in the liver parenchyma, decreased portal triads and necrotized hepatocytes noticed upon acute administration of tramadol and tramadol dissolved in Coke might be explained by direct hepatocellular injury during the metabolism of tramadol or by the effect of its metabolites [47]. The histopathological results were also in agreement with the biochemical findings of AST and ALT enzyme activities on the effect of diazepam and diazepam dissolved in Coke. The observed normal central venules without congestion confirmed that there was no adverse effect of diazepam on the liver tissues [48]. Fat degeneration, necrosis, and the severe microvesicular steatosis observed in the group administered with diazepam alone could be a result of the effect of its metabolites which are known to be more active than the parent drug. This is not found when diazepam is dissolved in Coke. Histology report of this research also follows a similar pattern of nephrotoxicity; these include loss of an architectural unit of the nephron, atrophic mesangial cells, wide capsular spaces, dilated interstitial vessels and mild congestion. All these might be responsible for the leakage of urea and creatinine into the serum [49-52].

Conclusion. The results of the present experiment showed that the toxicity of Coke under acute consumption might be from muscular activities and not necessarily through liver metabolism. Mixing tramadol with Coke further increase the mild hepatotoxic effect of tramadol, while there was no significant effect of mixing diazepam with Coke on kidney metabolism and texture. In conclusion, mixing drugs with Coke did not improve the efficacy of the drug, instead it aggravated the potential toxicity of the drugs.

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Conflict of Interest Statement. The authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but the advancement of knowledge.

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Authors Contribution. Both OI and TO contributed to the design and implementation of the research, to the analysis of the results and the writing of the manuscript.

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