Sub-Chronic Administration of Tramadol, Caffeinated Drink and Alcohol Precipitated Dysfunctions in Health Indices of Male Wistar Rats

E. B. Oyewo[1*], J. B. Oso[2], J. O. Fatoki[3], A. L. Adedeji[1] and G. E. Adeleke[1]

[1]*Department of Biochemistry, Faculty of Basic Medical Sciences, Ladoke Akintola University of Technology, Ogbomoso, Oyo State, Nigeria.*
[2]*Department of Biochemistry, Faculty of Biosciences, McPherson University, Ogbomoso, Oyo State, Nigeria.*
[3]*Department of Medical Biochemistry, Faculty of Basic Medical Sciences, Osun State University, Osogbo, Osun State, Nigeria.*

Authors' contributions

This work was carried out in collaboration among all authors. Author EBO conceived, designed and supervised the study, and drafted the manuscript. Author GEA did the statistical analyses and supervised the study. Authors JBO and JOF provided some materials and reagents used in the study. Author ALA provided some reagents and performed some of the experimental procedures. All authors read and approved the final manuscript.

Original Research Article

ABSTRACT

Tramadol is a popular drug of abuse among adolescent and young adults in many developing African countries due to the opioid agonist properties. We investigated the health implications of the sub-chronic concurrent abuse of tramadol, caffeinated drink and alcohol in adult male Wistar rats. Tramadol was administered at 40 and 20 mg/kg BW respectively, caffeinated drink at 10 ml/kg BW and alcohol at 2 ml/kg BW. The rats were handled such that: group A received distilled water; groups B and C received tramadol and distilled water; groups D and E received tramadol and caffeinated drink; groups F and Greceived tramadol and alcohol; and groups H and I received

---

Corresponding author: E-mail: eboyewo@lautech.edu.ng;

caffeinated drink and alcohol respectively. The concentrations of nitric oxide (NO), reduced glutathione (GSH),malondialdehyde (MDA),protein carbonyl (PC),protein thiol (PT), interleukin 1β (IL-1β), vascular cell adhesion molecule (VCAM-1), monocyte chemotactic protein-1 (MCP-1), oxidized low density lipoprotein cholesterol (ox-LDLC), and activities of paraoxonase (PON-1) and acetylcholine esterase (ACE) were determined. Histo-pathological analysis was performed on the liver, kidney, brain and small intestine. The concentrations of blood nitric oxide, GSH and MDA increased ($p < 0.05$) inconsistently with no alterationsin PC ($p > 0.05$). Inconsistent alterations were obtained in blood PON-1 activities across the groups. Decreases were recorded in the GSH and TPT in the liver and brain with increases in PC and MDA ($p < 0.05$). Inconsistent increases were obtained in the concentrations ox-LDLC, VCAM-1, IL-1β and MCP-1, and ACE activities. Consistent alterations were observed in the photomicrographs of the brain, kidney, intestine and liver of rats co-administered 40 mg/kg BW of tramadol withcaffeinated drink or alcohol. The overall findings indicated that the use of tramadol singly at 40 mg/kg BW or co-administered at both doses with caffeinated drink and alcohol precipitated various dysfunctions to health that could reduce the quality of life.

1. INTRODUCTION

The liability of drug abuse was highly underestimated in developed and developing countries, while the evidence of abuse of drugs and dependence have emerged in many localities, particularly amongst adolescent and young adults. Cannabis tops the chart of the world's most widely used illicit drug, while opiods have been shown to present the worst health implications in users. Opioids are agents that are characterised with dulling of senses as well as relief of pain [1]. Examples of opioids are morphine, heroin, codeine, tramadol etc.Tramadol is an opioid agent clinically used to treat moderate to severe pain that common analgesics failed to relieve [2]. It is a centrally acting analgesic, which possesses opioid agonist properties and do activates monoaminergic spinal inhibition of pain. Tramadol is a synthetic drug agent with two distinct synergistic mechanisms of action. It acts as both a weak opioid agonist with selectivity for the μ-receptors and an inhibitor of monamine neurotransmitter (nor epinephrine, serotonin etc.) re-uptake. It is an effective analgesic acute ureteric spasm, postoperative, musculoskeletal and cancer pain [3]. It is mostly administered orally, but can also be administered intravenously or intramuscularly [4].

Tramadol is extensively metabolized in the liver to O-desmethyl metabolite that have been shown to possess over 200 folds increased affinity for opioid receptors than the un-metabolized tramadol.It was considered as a medicinal drug with low probabilities of dependence relative to morphine. Nonetheless, dependence of tramadol has been recorded when used for a sustained period of time, especially in people with history of substance abuse. Scientific literatures have documented some negative effects of the abuse of tramadol to include seizures, nausea, impaired coordination, opioid withdrawal syndrome, aggressiveness and psychiatric display [5,4].

A recent study in Nigeria reported that 85.2 %of young adults who drive commercial vehicles engaged in the misuse of tramadol [5]. A similar trend in the misuse of tramadol was reported in the south eastern part of Nigeria, where 53.4 % of the student admitted the use of tramadol for various purposes, like feeling of euphoria, intoxicated, induce sleep and as sexual enhancer [6]. The oral use of tramadol by the abusers is mostly with alcoholic beverages or caffeine based drinks and sparingly with water.

The abuse of tramadol with alcohol was alleged by users to work in synergy to depress the central nervous system and the respiratory system, and give the users the drowsy-euphoric feel intended and hallucination. On the other hand, the abuse of tramadol with caffeinated drinks was alleged to stimulate the central nervous system, mobilise energy production and excite the users to engage in strenuous physical activities, sexual act, studying, hyper-activity, recreationetc. However, with the very high index of tramadol abusers among the youths, and the few isolated reported/documented cases of physical display of tramadol intoxication and death, there is the need to scientifically investigate the effect of frequent abuse of tramadol on the health status of chronic and sub-chronic abusers. Therefore, this study

investigated some health implications of the sub-chronic concurrent abuse of tramadol with caffeinated drink and alcohol in adult male Wistar rats.

2. MATERIALS AND METHODS

2.1 Materials

2.1.1 Experimental animals

One hundred and eight apparently healthy male Wistar rats with weights of 150 - 167g were obtained from the animal house facility, Ladoke Akintola University of Technology, Ogbomoso, Oyo State, Nigeria.

2.1.2 Tramadol

Tramadol was obtained locally from Alkol Pharmacy, Ogbomoso, Oyo State, Nigeria.

2.1.3 Caffeinateddrink and alcohol

Coke was the source of caffeinated drink and was obtained from renowned supermarket outlet in Ogbomoso, Oyo State, Nigeria. The number one brand of Whisky (alcohol) locally available was obtained from renowned supermarket outlet in Ogbomoso, Oyo State, Nigeria.

2.1.4 Reagents kits and chemicals

Quantitative assay kits for reduced glutathione and malondialdehyde were products of Fortress Diagnostic Laboratory, Unit 2C, Antrim Technology Park, Belfast Road, Antrim, Northern Ireland, United Kingdom. Interleukin-1b, nitric oxide, vascular adhesion molecule-1 were products of RayBio Technology, inc.U.S.A, while oxidized low-density lipoprotein cholesterol and monocyte chemoattractant protein are products of Bioassay Technology Laboratory, Junjiang Inter. Bldg. 218 Ningguo Rd. Yangpu Dist. Shangai, China.

2.2 Methods

2.2.1 Experimental design

The rats were kept in compartmentalized cages and grouped into nine, comprised of twelve animals each. They were kept under condition of uniform humidity and temperature on a 12 hour light- dark cycle and allowed access to standard pellets and distilled water. The cages were cleaned daily. The rats were allowed to acclimatize for two weeks, during which they were weighed and properly observed for any changes in their behaviour.

The rats were handled such that: Tramadol was administered orally at 20 and 40 mg/kg body weight (BW), while caffeinated drink and alcohol were administered orally at 10 and 2 ml/kg BW respectively to the rats such that: rats in group A received distilled water, groups B and C rats received 40 and 20 mg/kg BW of tramadol in distilled water respectively, groups D and E received 40 and 20 mg/kg BW of tramadol in 10 ml/kg BW of caffeinated drink respectively, groups F and E received 40 and 20 mg/kg BW of tramadol in 2 ml/kg BW of alcohol respectively and groups H and I received 10 and 2 ml/kg BW caffeinated drink and alcohol respectively. The administration lasted 56 days, during which the six (6) rats in each group were sacrificed after twenty eight days (4 weeks), while the remaining six rats were sacrificed after 56 days (8 weeks) of administration. The body weights of the rats were monitored during the administration. Conversion from human dose of tramadol to animal dose was done using the equation by [7].

2.2.2 Collection of plasma and serum

The male rats were subjected to overnight fast and anaesthetized using diethyl ether. Blood was collected via cardiac puncture and plasma, and serum was prepared for each drawn blood. Organs of interest, such as the liver, intestine, kidney and brain were harvested immediately, cleansed of blood, adjoining tissues or metabolic waste and rinsed with normal saline solution, and weighed. The organs were minced and a small part was fixed in 10% formosaline or formocalcium for histopathology examinations.

2.2.3 Determination of health indices

2.2.3.1 Oxidative status

Standard procedures / methods spectrophotometric methods described by [8] for reduced glutathione and total protein thiol, [9] for nitric oxide (NO), [10] for malondialdehyde, [11] for protein carbonyl and [12] for paraoxonase.

2.2.3.2 Immunological proteins

Standard procedures /methods as instructed in the ELISA reagent kit manual were used for the determinations of the concentrations of interleukin 1β, vascular cell adhesion molecule,

monocyte chemotactic protein-1β and oxidized low density lipoprotein cholesterol.

2.2.3.3 Enzymes

Standard procedures /methods as instructed in the ELISA reagent kit manual were used for the determinations of the activity of acetylcholine esterase and paraoxonase.

2.2.4 Histopathology

Histological procedures were performed in the liver and brain accordingto the method of [13] Avwioro, 2010.

2.2.5 Statistical analysis

This research work was a completely randomised design (CRD). Results were expressed as mean ± standard error of mean(S.E.M.). Data generated were subjected to two ways analysis of variance (ANOVA), after which Tukey Test was conducted in other to identify the variation within the treatment group, using Graphpad prism8 statistical software. P-value <0.05 was regarded as statistically significant and denoted by alphabets.

3. RESULTS

3.1 Oxidative Indices

The results obtained in the oxidative indices were presented in Tables 1 and 2. In Table 1, the blood concentration of nitric acid increased across the groups in no duration dependent manner (p<0.05), except in group C (20 mg/kg BW of tramadol) at four weeks of administration. In the liver, the concentration of reduced glutathione (GSH) decreased across the groups, while marked decreases were also recorded in the brain GSH in some of the groups in no definite manner (p<0.05). Surprisingly, the concentration of GSH increased in the blood in a non-consistent and definite manner (p<0.05), except in rats in group D and E at both weeks, and group G at four weeks. Still in Table 1, the concentration malondialdehyde (MDA) increased markedly (p<0.05) in the brain and liver of rats across the groups in a somewhat duration dependent manner, except in the liver of rats in

group C at both weeks, and groups H and I at four weeks (p>0.05). In the blood, MDA concentrations were increased only in rats in group F at both weeks and groups D, E and G at eight weeks (p<0.05).

In Table 2, the total protein thiol (TPT) content decreased in the brain and liver of rats in an inconsistent manner across the groups, except in the brain of rats in groups B, C and H at four weeks of administration (p>0.05). However, marked decreases were obtained only in the TPT content in the blood of rats in groups F, G and H (p<0.05). The protein carbonyl (PC) contents increased in the liver and brain in an inconsistent manner across the groups (p<0.05), except in the liver of rats in group C and brain in groups B and C at both weeks, and groups E and H at eight and four weeks respectively (p>0.05). Interestingly, the concentration of protein carbonyl in the blood were not altered (p>0.05) across the groups (Table 2).

3.2 Inflammatory and Immunological Proteins

Depicted in Table 3 were the concentrations of the acute phase proteins and oxidized low density lipoprotein cholesterol (ox-LDLC) in rats administered tramadol, caffeinated drink and whisky. Marked increases (p<0.05) were recorded in the concentration of ox-LDLC in the plasma of rats in a somewhat duration dependent manner, with exceptions in group B at both weeks, groups H and I at four weeks (p>0.05) and reductions (p<0.05) in rats in group C (Table 3). Similar increases were presented across the groups (p<0.05) in the monocyte chemotactic protein-1 (MCP-1) like that ox-LDLC concentrations, except in rats of groups B and H at both weeks, and group E rats at four weeks (p>0.05), as well as reductions (p<0.05) in rats in group C. The concentrations of interleukin-1β in the serum increased in an inconsistent manner across the groups (p<0.05), except in rats in groups B and C at both weeks, and H, at four weeks (p>0.05). The serum concentrations of vascular cell adhension molecule depicted increases (p<0.05) akin to those of ox-LDLC and MCP-1, but with no alterations (p>0.05) in groups B, C G and I at four weeks, and reduction (p<0.0) at eight weeks in group C (Table 3).

Table 1. Patterns of some oxidative indices in male wistar rats administered with tramadol, caffeinated drink and alcohol

Index	Tissue			Group A	B	C	D	E	F	G	H	I
Malondialdehyde (µM)	Blood	X		1.62 ± 0.05^a	1.78 ± 0.10^{ab}	1.47 ± 0.11^a	1.44 ± 0.13^a	1.54 ± 0.12^a	1.82 ± 0.06^b	1.56 ± 0.08^a	1.56 ± 0.10^a	1.31 ± 0.10^a
		Y		1.48 ± 0.11^a	1.69 ± 0.10^{ab}	1.56 ± 0.10^a	1.80± 0.08^b	1.88 ± 0.09^b	1.96 ± 0.10^b	1.97 ± 0.12^b	1.52 ± 0.12^a	1.44 ± 0.09^a
	Brain	X		13.55 ± 0.78^a	32.58 ± 2.34^d	25.04 ± 1.52^c	40.18 ± 2.34^e	33.87 ± 3.22^d	51.04 ± 2.98^d	42.80 ± 3.62^e	18.56 ± 1.40^b	27.39 ± 1.78^c
		Y		12.87 ± 1.21^a	29.26 ± 1.62^{cd}	22.73 ± 2.48^{bc}	44.67 ± 2.81^e	36.92 ± 2.05^{de}	56.49 ± 3.28^f	44.54 ± 1.75^e	22.40 ± 1.83^{bc}	31.66 ± 2.53^d
	Plasma	X		17.71 ± 1.09^a	22.90 ± 1.46^{ab}	23.20 ± 2.65^{ab}	31.69 ± 2.16^c	39.36 ± 2.51^d	36.95 ± 2.54^d	35.93 ± 2.69^{cd}	22.11 ± 1.40^{ab}	24.63 ± 1.24^{ab}
		Y		20.23 ± 2.50^a	29.03 ± 1.77^c	22.87 ± 0.98^{ab}	38.34 ± 2.06^d	35.85 ± 3.29^{cd}	39.22 ± 1.83^d	35.74 ± 1.80^{cd}	25.58 ± 1.10^b	28.61 ± 2.27^{bc}
	Liver	X		0.36 ± 0.03^{ab}	0.45 ± 0.03^{bc}	0.42 ± 0.00^b	0.40 ± 0.03^{ab}	0.41 ± 0.01^{ab}	0.53± 0.00^{de}	0.47 ± 0.03^c	0.50 ± 0.00^{cd}	0.57 ± 0.02^e
		Y		0.35 ± 0.02^a	0.51 ± 0.02^{cd}	0.44 ± 0.00^{bc}	0.41 ± 0.01^{ab}	0.37 ± 0.03^{ab}	0.57 ± 0.05^e	0.40 ± 0.02^{ab}	0.48± 0.01^c	0.52 ± 0.00^d
Reduced glutathione (mM)	Brain	X		0.42 ±0.04^{ab}	0.40±0.05^{ab}	0.38±0.02^{ab}	0.28±0.02^c	0.35±0.01^b	0.31±0.05^{bc}	0.36±0.07^{ab}	0.28±0.00^c	0.23 ± 0.03^c
		Y		0.44±0.07^a	0.34 ±0.01^b	0.39 ±0.03^{ab}	0.31±0.06^{bc}	0.30±0.03^{bc}	0.28±0.01^c	0.31±0.02^{bc}	0.26±0.04^c	0.22 ± 0.00^c
	Liver	X		1.02 ± 0.08^a	0.68 ± 0.08^{bc}	0.81 ± 0.06^b	0.40 ± 0.08^d	0.53 ± 0.05^{cd}	0.38 ± 0.07^e	0.44 ± 0.05^d	0.77 ± 0.02^b	0.75 ± 0.09^b
		Y		1.02 ± 0.10^a	0.53 ± 0.04^{cd}	0.78 ± 0.10^b	0.45 ± 0.00^d	0.49 ± 0.06^{cd}	0.32 ± 0.10^e	0.45 ± 0.07^d	0.74 ± 0.08^b	0.60 ± 0.05^c
Nitric oxide (ng/ml)		X		167.54 ± 5.89^a	202.78 ±7.71^{bc}	174.33±9.91^{ab}	254.42±8.70^{de}	225.61 ±10.09^c	247.18 ± 9.31^d	252.63±6.46^{de}	200.89 ± 6.62^{bc}	244.89 ± 10.72^d
		Y		159.73 ± 9.03^a	218.65 ± 6.84^c	181.99±7.22^b	271.55±8.13^e	210.85 ± 6.29^c	271.83 ± 9.67^e	231.55 ±7.43^{cd}	187.27 ± 9.66^b	238.76 ±11.36^{cd}

Note: A-Control, B-40 tramadol, C-20 tramadol, D-40 tramadol and caffeinated drink, E-20 tramadol and caffeinated drink, F-40 tramadol and alcohol, G-20 tramadol and alcohol, H-caffeinated drink, I-alcohol.(Tramadol in mg/kg body weight) (Caffeinated drink and alcoholwere 10 and 2 ml/kg body weight respectively). X denotes 4weeks, Y denotes 8 weeks. Values are means ± SEM and mean values bearing different alphabets are significantly different (P<0.05)

Table 2. Protein carbonyl and thiol concentrations in male Wistar rats administered with tramadol, caffeinated drink and alcohol

			Group								
			A	**B**	**C**	**D**	**E**	**F**	**G**	**H**	**I**
Protein Carbonyl (nmol/mg protein)	Blood	X	25.16 ± 2.35^a	27.81 ± 2.04^a	24.76 ± 1.53^a	28.31 ± 2.54^a	26.08 ± 2.17^a	25.83 ± 3.34^a	27.91 ± 3.71^a	24.77 ± 2.00^a	25.43 ± 3.40^a
		Y	22.70 ± 3.48^a	22.39 ± 2.82^a	25.90 ± 2.31^a	27.22 ± 1.96^a	26.40 ± 2.79^a	23.69 ± 1.87^a	25.02 ± 2.57^a	22.58 ± 2.62^a	25.16 ± 1.89^a
	Brain	X	30.53 ± 1.91^a	31.39 ± 2.74^a	27.91 ± 1.42^a	44.69 ± 2.18^d	39.27 ± 1.91^{cd}	57.53 ± 3.74^f	46.83 ± 2.71^{de}	33.67 ± 2.53^{abc}	40.22 ± 2.68^{cd}
		Y	28.57 ± 1.63^a	30.61 ± 1.84^a	30.87 ± 2.11^a	52.54 ± 3.17^f	32.41 ± 2.75^{ab}	55.29 ± 4.03^f	47.90 ± 3.31^{de}	37.42 ± 1.80^{bc}	46.51 ± 1.47^{de}
	Liver	X	41.30 ± 2.71^a	33.19 ± 3.44^b	35.62 ± 2.92^{ab}	62.14 ± 4.05^{de}	57.58 ± 3.47^d	78.41 ± 3.79^f	64.08 ± 5.16^{de}	45.29 ± 2.76^{ac}	32.58 ± 2.09^b
		Y	38.89 ± 1.85^a	50.11 ± 5.74^{cd}	38.27 ± 2.23^a	58.39 ± 2.66^d	57.29 ± 3.62^d	81.53 ± 4.86^f	68.40 ± 3.36^e	50.72 ± 4.41^{cd}	47.38 ± 3.27^c
	Plasma	X	65.03 ± 5.32^a	60.14 ± 6.49^{ab}	63.83 ± 4.28^{ab}	62.67 ± 4.51^{ab}	59.18 ± 5.37^{ab}	52.52 ± 5.28^{bc}	53.65 ± 4.06^b	54.43 ± 3.74^b	59.74 ± 4.61^{ab}
		Y	61.78 ± 4.96^{ab}	61.33 ± 5.26^{ab}	60.76 ± 3.99^{ab}	62.91 ± 6.02^{ab}	61.52 ± 4.74^{ab}	44.47 ± 5.20^c	53.77 ± 5.41^b	51.26 ± 5.39^b	57.65 ± 6.23^{ab}
Protein thiol (µg/mg protein)	Brain	X	82.71 ± 4.66^a	74.53 ± 5.22^{ab}	78.34 ± 6.04^a	55.73 ± 3.16^{cd}	50.81 ± 4.42^d	51.67 ± 5.45^d	56.08 ± 6.05^c	78.61 ± 2.78^a	60.27 ± 3.86^c
		Y	90.84 ± 7.29^a	62.16 ± 7.36^{bc}	71.58 ± 3.78^b	57.22 ± 5.35^c	55.49 ± 3.61^{cd}	50.30 ± 2.67^d	50.44 ± 4.92^{cd}	65.10 ± 7.22^{bc}	52.78 ± 6.41^d
	Liver	X	152.51 ± 8.11^a	95.93 ± 5.42^{bc}	103.65 ± 5.49^b	96.34 ± 6.07^{bc}	99.67 ± 5.81^b	71.64 ± 5.73^d	80.46 ± 4.72^{cd}	91.63 ± 6.01^{bc}	108.90 ± 5.54^b
		Y	146.89 ± 6.73^a	80.66 ± 7.22^c	111.04 ± 6.90^b	94.72 ± 3.21^{bc}	100.56 ± 7.23^b	65.89 ± 7.37^e	74.39 ± 6.52^b	100.44 ± 4.68^b	109.31 ± 7.87^b

Note: A-Control, B-40 tramadol, C-20 tramadol, D-40 tramadol and caffeinated drink, E-20 tramadol and caffeinated drink, F-40 tramadol and alcohol, G-20 tramadol and alcohol, H-caffeinated drink, I-alcohol.(Tramadol in mg/kg body weight) (Caffeinated drink and alcoholwere 10 and 2 ml/kg body weight respectively). X denotes 4weeks, Y denotes 8 weeks. Values are means ± SEM and mean values bearing different alphabets are significantly different (P<0.05)

Table 3. Some cytokines and oxidized low density lipoprotein cholesterol concentrations in Wistar rats administered tramadol, caffeinated drink and alcohol

Parameter	Week	A	B	C	D	E	F	G	H	I
Oxidized LDL-C (ng/ml)	X	88.79 ± 3.81^a	79.59 ± 4.40^{ab}	67.65 ± 5.06^b	128.84 ± 3.91^{ef}	114.99 ± 6.55^e	131.90 ± 6.01^f	100.44 ± 4.61^d	91.55 ± 5.82^{ad}	80.45 ± 3.90^a
	Y	83.50 ± 5.20^a	95.56 ± 3.25^{ad}	60.15 ± 7.00^{bc}	132.35 ± 4.69^f	103.75 ± 4.81^d	142.01 ± 5.60^{fg}	150.06 ± 4.34^g	101.45 ± 4.15^d	110.56 ± 4.48^{de}
Interleukin-1β (ng/ml)	X	96.00 ± 4.10^a	101.45 ±2.61^a	106.55 ± 3.06^a	294.99 ± 5.02^i	205.90 ± 6.00^f	184.45 ± 4.50^e	188.20 ± 4.65^e	115.10 ± 4.01^{ab}	134.65 ± 5.80^c
	Y	104.45 ± 3.29^a	98.50 ± 2.44^a	101.64 ± 3.31^a	255.80 ± 6.89^h	221.76 ± 4.70^g	202.50 ± 5.69^f	172.45 ± 6.10^{de}	128.55 ± 5.00^{bc}	155.15 ± 4.65^d
Monocyte chemotactic protein-1 (pg/ml)	X	185.30 ± 8.11^a	168.60 ±11.04^{ab}	160.55 ± 7.10^b	226.00 ±7.31^{cd}	204.69 ± 6.81^{ac}	242.75 ± 8.90^{de}	244.56 ±9.06^{de}	202.55±11.80^{ac}	255.54 ± 8.69^e
	Y	193.81 ± 6.32^a	180.49 ± 8.31^a	155.84 ± 10.61^b	260.45 ± 8.20^e	220.60 ± 8.15^{cd}	300.04 ± 12.41^f	256.00 ± 9.39^e	191.01 ±7.99^a	260.40 ± 9.22^e
Vascular cell adhesion molecule (ng/ml)	X	23.25 ± 1.22^{ab}	18.60± 1.68^{ac}	18.45 ± 2.42^{ac}	36.10 ± 2.41^e	31.56 ± 2.38^{de}	26.21 ± 1.80^{bd}	22.10± 2.04^{ab}	25.80 ± 2.39^{abd}	25.35 ± 1.01^{ab}
	Y	20.89 ± 2.38^a	26.80± 2.69^{bd}	15.59 ± 1.41^c	39.41 ± 1.89^{ef}	43.74 ± 2.58^{fg}	48.25 ± 3.09^g	27.20 ± 1.45^{bd}	28.20 ± 3.42^{bd}	30.85 ± 2.50^d

Note: A-Control, B-40 tramadol, C-20 tramadol, D-40 tramadol and caffeinated drink, E-20 tramadol and caffeinated drink, F-40 tramadol and alcohol, G-20 tramadol and alcohol, H-caffeinated drink, I-alcohol.(Tramadol in mg/kg body weight) (Caffeinated drink and alcoholwere 10 and 2 ml/kg body weight respectively). X denotes 4weeks, Y denotes 8 weeks. Values are means ± SEM and mean values bearing different alphabets are significantly different ($P<0.05$)

Table 4. Activity of acetylcholine esterase and paraoxonasein wistar rats administered tramadol, caffeinated drink and alcohol

Parameter	Week									
		A	**B**	**C**	**D**	**E**	**F**	**G**	**H**	**I**
Acetylcholine esterase (U/ml)	X	253.45 ± 6.24^{ab}	328.15 ± 10.25^{e}	315.62 ± 8.65^{de}	291.99 ± 9.75^{cd}	300.05 ± 10.61^{d}	328.85 ± 12.56^{d}	301.92 ± 8.45^{d}	260.21 ± 9.85^{b}	274.66 ± 11.51^{bc}
	Y	235.78 ± 8.10^{a}	310.55 ± 7.29^{d}	295.94 ± 6.26^{d}	300.67 ± 5.34^{d}	292.89 ± 8.38^{cd}	294.74 ± 9.81^{cd}	287.45 ± 6.64^{c}	302.34 ± 7.71^{d}	268.20 ± 8.75^{bc}
Paraoxonase (μmol/g tissue)	X	9.56 ± 0.81^{a}	8.04 ± 0.75^{ab}	11.07 ± 0.78^{ae}	5.54 ± 0.30^{bc}	5.97 ± 0.85^{b}	3.02 ± 0.33^{cd}	4.47 ± 1.00^{bc}	5.88 ± 0.80^{b}	14.77 ± 1.46^{e}
	Y	11.31 ± 1.31^{ae}	6.26 ± 0.63^{b}	13.92 ± 0.85^{e}	3.41 ± 0.51^{cd}	5.06 ± 0.77^{bc}	2.51 ± 0.69^{d}	3.55 ± 0.50^{cd}	4.04 ± 0.57^{c}	11.18 ± 1.01^{ae}

Note: A-Control, B-40 tramadol, C-20 tramadol, D-40 tramadol and caffeinated drink, E-20 tramadol and caffeinated drink, F-40 tramadol and alcohol, G-20 tramadol and alcohol, H-caffeinated drink, I-alcohol.(Tramadol in mg/kg body weight) (Caffeinated drink and alcoholwere 10 and 2 ml/kg body weight respectively). X denotes 4weeks, Y denotes 8 weeks. Values are means ± SEM and mean values bearing different alphabets are significantly different (P<0.05)

3.3 Activities of Aetylcholine Esterase and Paraoxonase

The respective activities of acetylcholine esterase (AChE) in the plasma and paraoxonase (PON-1) in the liver homogenate were presented in Table 4. The PON-1 activities presented increases ($p<0.05$) in rats in group C at eight weeks and I at four weeks, but were unchanged ($p>0.05$) at the other weeks in the same rats and group B at four weeks. However, decreases that were partly duration dependent ($p<0.05$) were obtained in the PON-1 activities across the other groups of rats. In Table 4, the activities of AChE increased in no definite pattern across the groups ($p<0.05$).

3.4 Histopathology

The observations revealed in the histopathological analyses of the liver, kidney, small intestine and brain after eight weeks of administration were depicted in Plates 1-28. In the photomicrographs of the liver, Plates 1-9, the liver of six of the rats depicted unaltered histo-architecture with normal sinosoids and central venules without inflammation (Plates 1-3, 5 and 8), while three indicated mild-moderately dilatated sinosoids and normal central venules (Plates 4, 6, 7 and 9).The photomicrographs of the kidney of some of the rats indicated normal nephrons with preserved renal cortex, glomeruli containing normal mesengial cells and normal cuboidal epithelial arrangement (Plates 10, 12 and 17) and mild congestion in the capsular spaces of the glomeruli, but with normal preserved renal cortex (Plates 11, 13, 14 and 18). However, the photomicrographs of the kidney of other groups depicted mild to moderate normal renal cortex, mild-moderate congestion in the capsules, tubular necrosis with severe renal cast with high nucleo-cytoplasmic ratio and lymphocyte infiltration (Plates 15 and 16).

The observations on some of the photomicrographs of the jejunum of the rats indicated mild-moderate mucosa layers with varied grades of inflamed intestinal glands and infiltration of inflammatory cells, involving the lamina propria that were necrotic in some (Plates 20, 22, 24-28), with exceptions in Plates 19, 21 and 23that had normal mucosa layers with good epithelium lining and intestinal glands. Indications in the photomicrographs of the brain of rats revealed that the metabolite(s) of tramadol and whisky, either singly or in synergy had varied effects (mild-moderate-severe) on the neurones (Plate 28). Only photomicrograph H depicted no alterations in the neuronal arrangement, morphology nor neurodegenrative features.
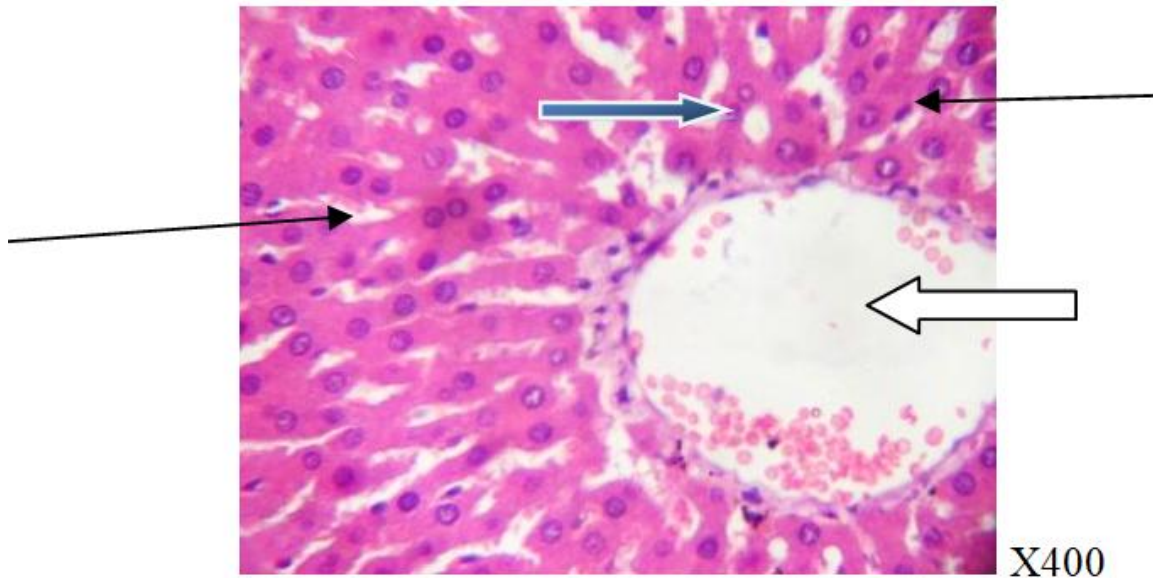
4. DISCUSSION

Consequential liabilities of drug abuse were highly underestimated in developed and developing countries and the evidence of abuse of drugs and dependence have rendered many adolescent and young adults medical unfit,unproductive economically and dead. The trends we reported in the concentrations of nitric oxide (NO*) in rats administered tramadol, caffeinated drink and whisky (Table 1) indicated the activation and or induction of nitric oxide synthase (NOS). The increases in NO* concentration might be to enhance the flow of blood in circulation to meet probable increased demand in metabolic activities or regulate the vascular tone, but these could predispose to the generation of free radicals via oxidative modifications by oxygen-derived reactive species, such as anion superoxide ($O_2\bullet$), hydrogen peroxide (H_2O_2) and hydroxyl radical ($OH\bullet$) to form 'reactive nitrogen species' (RNS) [14]. This is because the continuous production of NO* can be potentially fatal as RNS are formed, and are the main cause of cellular damage. Although, the cytoprotective and cytotoxic capabilities of NO* is the function of the concentration of NO*, biotransformation processes as well as the isoforms of NOS [15].
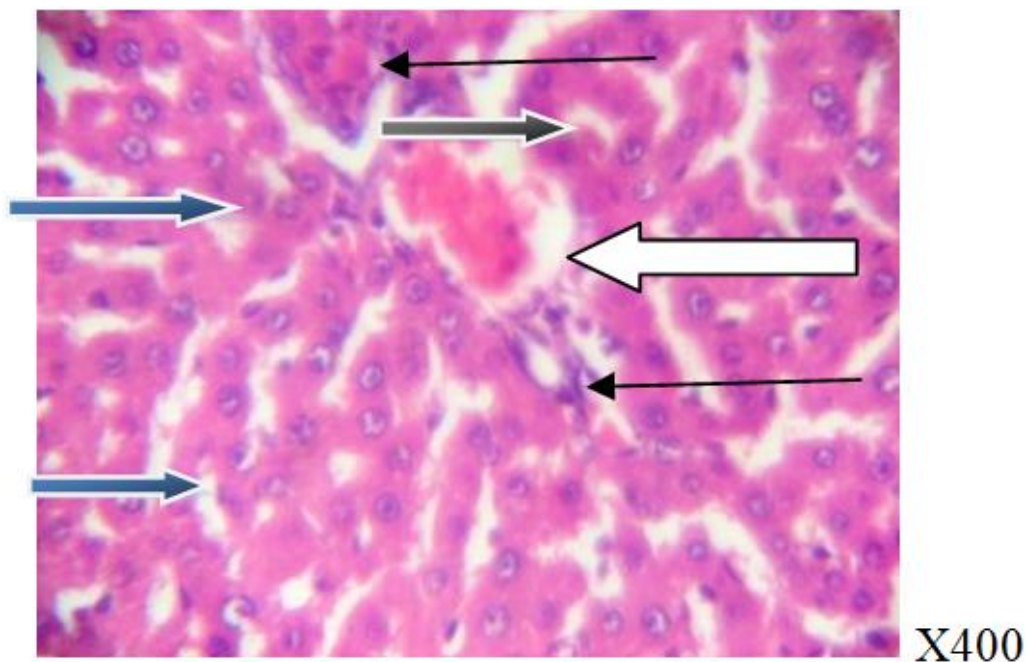
The patterns we reported in the concentrations of reduced glutathione in the brain and liver of rats (Table 1) were pointers to inductions of reactive oxygen species (ROS). This gave credence to the alleged cytotoxicity in the increased NO* concentrations. Reduced glutathione (GSH) has a multifactorial role in the antioxidant defense system, as it is a direct scavenger of free radicals that neutralizeshydrogen peroxide (H_2O_2) and or, indirectly by repairing initial damage to macromolecules inflicted by H_2O_2, as well as, a co-substrate for peroxide detoxification by glutathione peroxides [16,17]. Although, the trends in the GSH concentrations in the plasma (Table 1) did not support directly the induction of ROS indicated in the brain and liver, but might be that the metabolites of tramadol, caffeinated drink and alcohol were not cytotoxic to the blood and or, had little or no effects on the cells of blood. It can also be a biological response to the induction of ROS. This is because scavenging or neutralization of the free radicals generated during cellular metabolism can necessitate the

direct increase in the activities of both enzymatic and non-enzymatic antioxidative systems in order to prevent oxidative cell damage [18]. Although, the former seemed more logical due to the 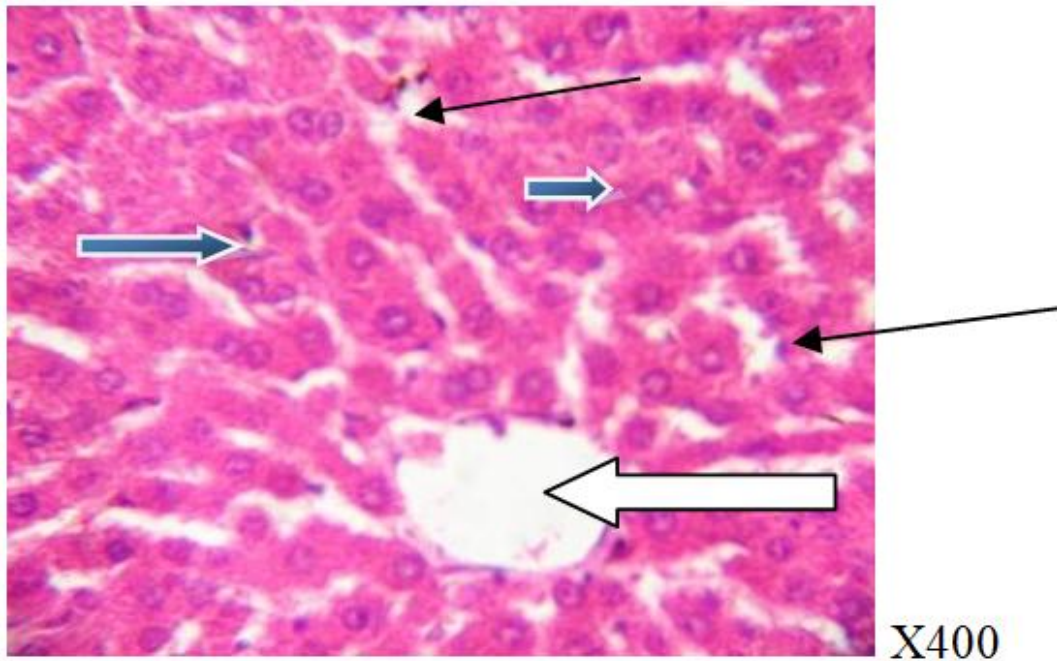insignificant increases (p<0.05) reported in some of the rats (groups D, E and G), but the later was more likely as other groups of rats indicated increases in the plasma GSH levels (Table 1).
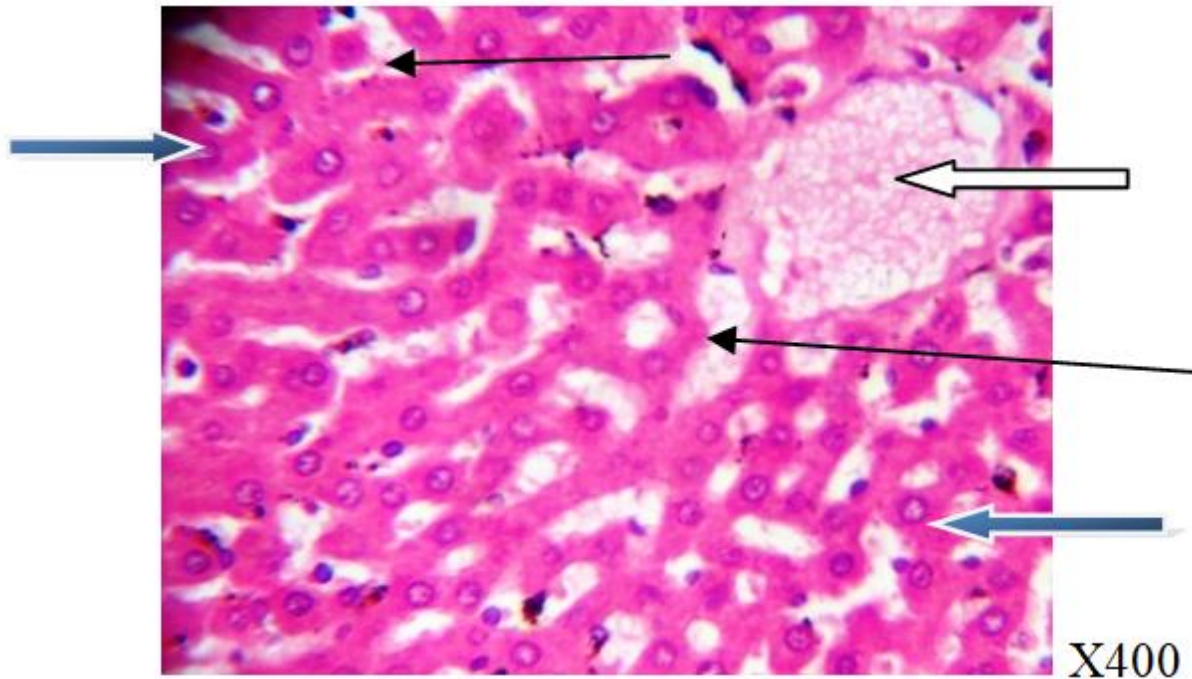

Plate 1. Photomicrograph of the liver section of rat in the control group. The histo-architecture (blue arrow), sinusoids (slender arrow) and central venules appeared normal, not congested (white arrow) andwithout infiltration of inflammatory cells. The hepatocytes were normal.H&E

Plate 2. Photomicrograph of the liver section of rat administered 40 mg/kg body weight of tramadol.The histoarchitexture were moderately normal architecture (blue arrow), the central venules showed mild vascular congestion (black arrow), the sinusoids were normal (slender arrow) without infiltration of inflammatory cells.The hepatocytes were normal. H&E
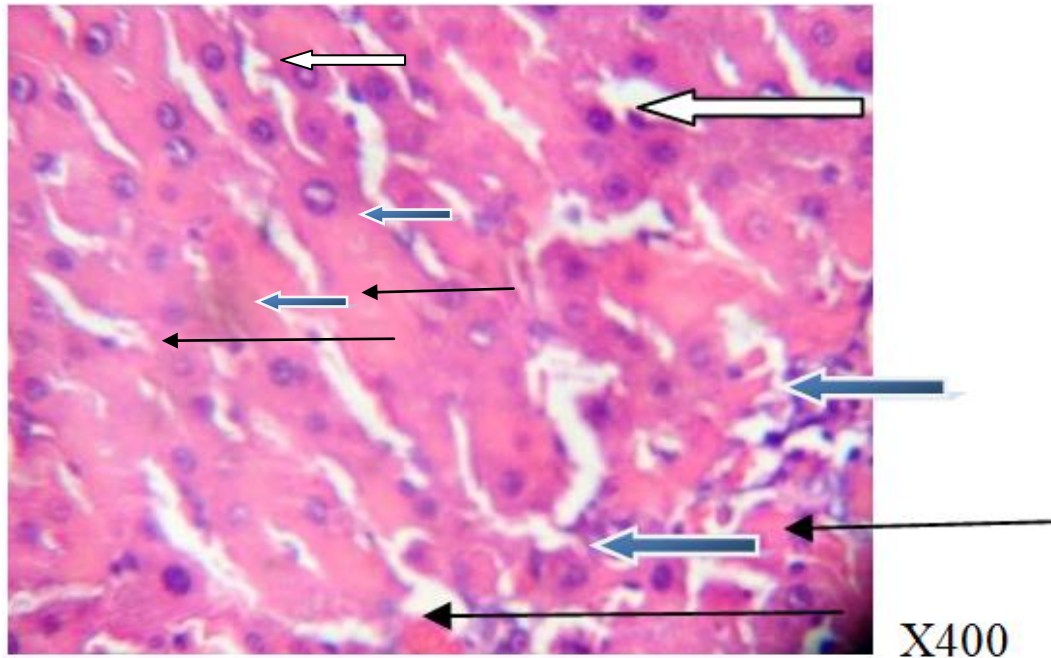
Plate 3. Photomicrograph of the liver section of rat administered 20 mg/kg body weight of tramadol.The histo-architecture (blue arrow), sinusoids (slender arrow) and central venules appeared normal, not congested (white arrow) andwithout infiltration of inflammatory cells.The hepatocytes were normal.H&E
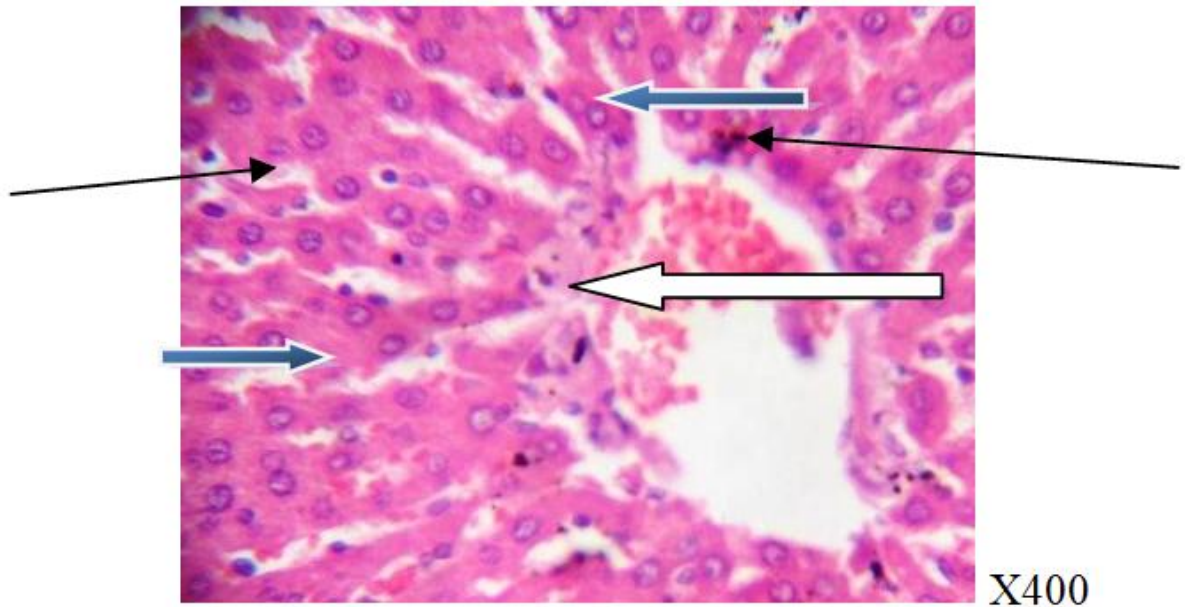

Plate 4. Photomicrograph of the liver section of rat administered 40 mg/kg body weight of tramadol and 10 ml/kg body weight of caffeinated drink.The histo-architecture (blue arrow), sinusoids were mildly dilatatedwith scanty infiltration of inflammatory cells(slender arrow) and central venules appeared normal, not congested (white arrow) andwithout infiltration of inflammatory cells. The hepatocytes were normal. H&E

Plate 5. Photomicrograph of the liver section of rat administered 20 mg/kg body weight of tramadol and 10 ml/kg body weight of caffeinated drink. The histo-architecture appeared normal with the central venules (blue arrow), sinusoids were congestive (while arrow) with blood cells (slender arrows) without infiltration of inflammatory cells. The hepatocytes were normal. H&E
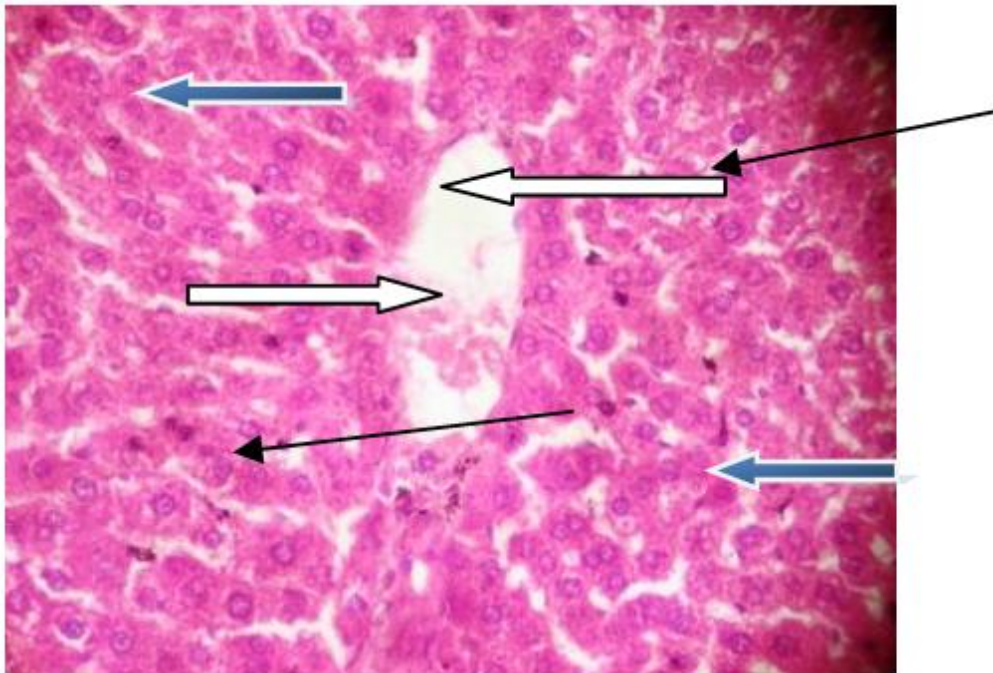

Plate 6. Photomicrograph of the liver section of rat administered 40 mg/kg body weight of tramadol and 2 ml/kg body weight of alcohol. The histo-architecture indicated necrotized hepatocytes with hyperchromatic nuclei (blue arrow), moderately dilatated sinusoidswith infiltration of inflammatory cells and red blood cells(slender arrow) and normal central venules that were not congested (white arrow).The hepatocytes were normal.H&E
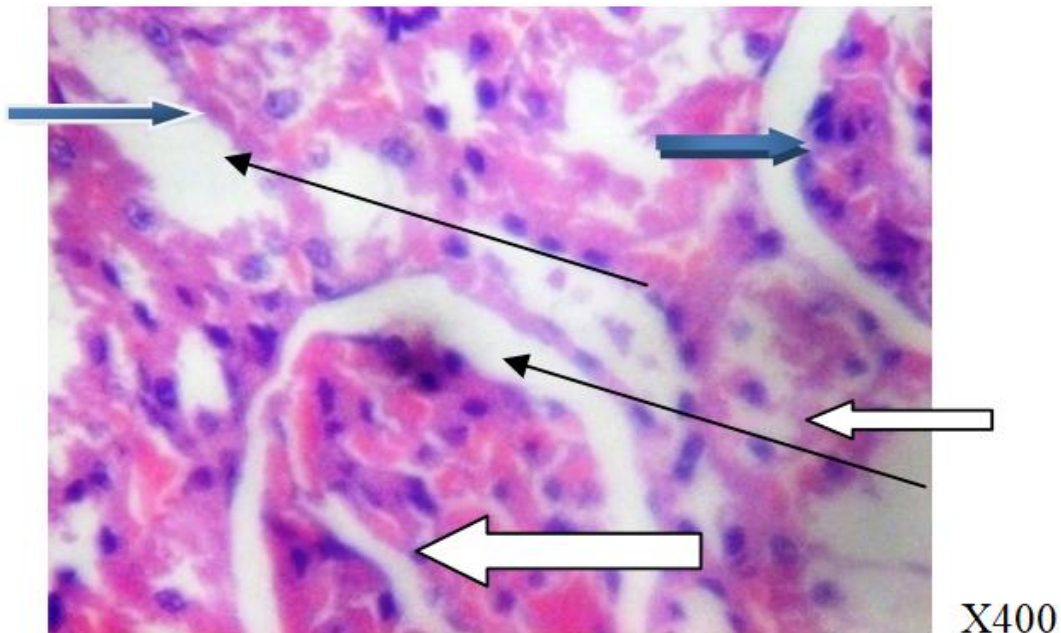
Plate 7. Photomicrograph of the liver section of rat administered 20 mg/kg body weight of tramadol and 2 ml/kg body weight of alcohol. The histo-architecture indicated perivenular necrosis with hyperchromatic nuclei (blue arrow), moderately dilatated sinusoidswith infiltration of inflammatory cells and red blood cells(slender arrow) and normal central venules that were not congested (white arrow).The hepatocytes were normal.H&E
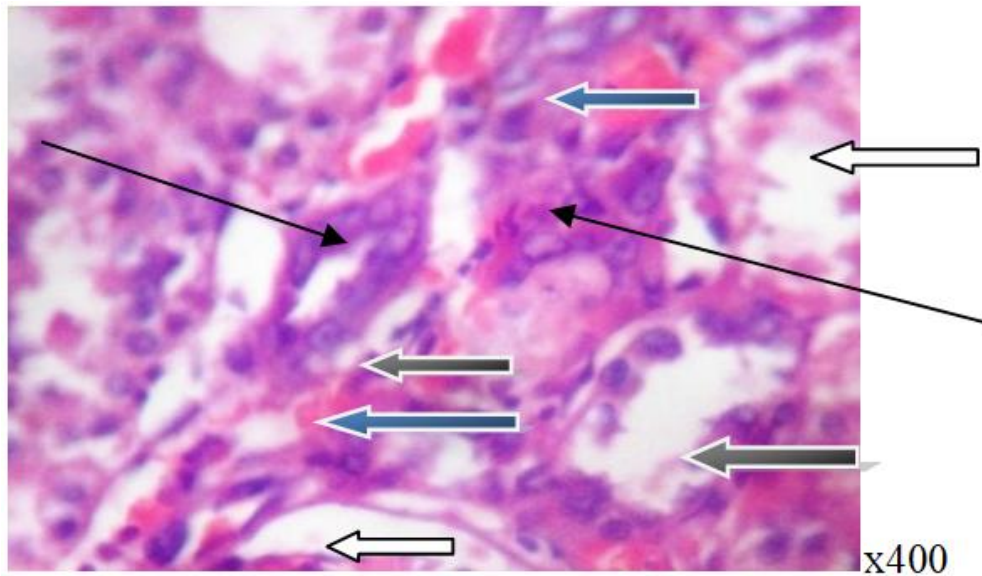

Plate 8. Photomicrograph of the liver section of rat administered 10 ml/kg body weight of caffeinated drink. The histo-architecture (blue arrow), sinusoids (slender arrow) and central venules appeared normal, not congested (white arrow) andwithout infiltration of inflammatory cells. The hepatocytes were normal.H&E
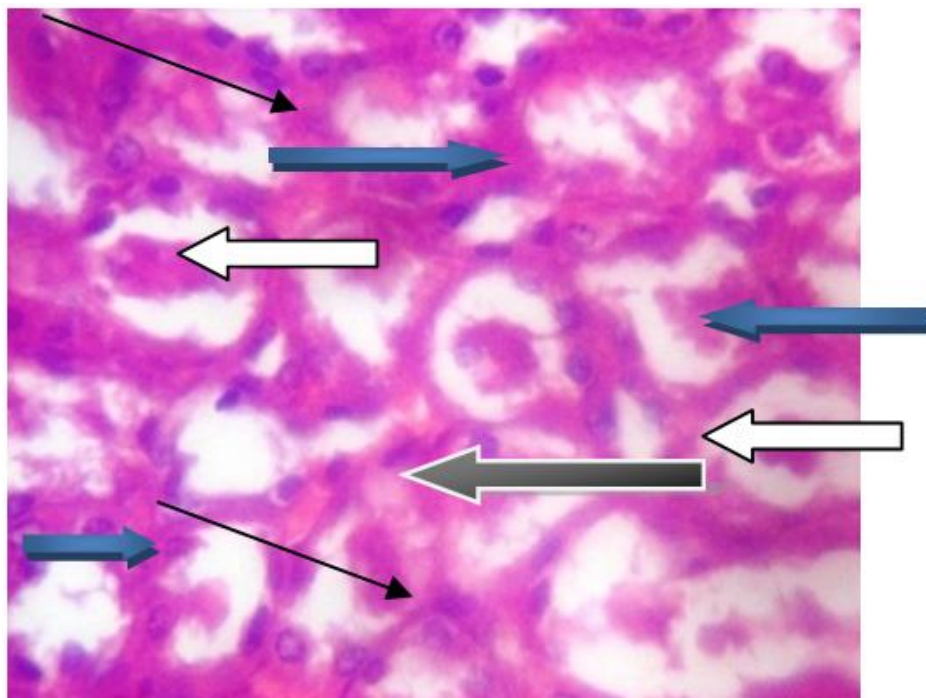
Plate 9. Photomicrograph of the liver section of rat administered 2 ml/kg body weight of alcohol. The histo-architecture was mildly congested (blue arrow), the sinusoids were mildly dilatatedwith scanty infiltration of inflammatory cells (slender arrow) and central venules appeared normal, not congested (white arrow) andwithout infiltration of inflammatory cells. The hepatocytes were normal. H&E
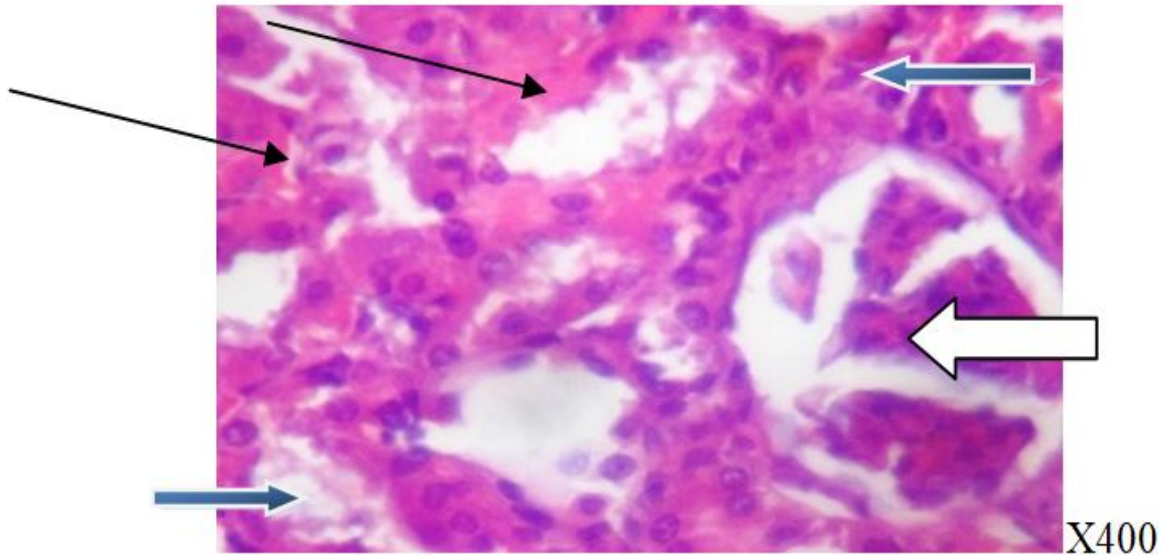

Plate 10. Photomicrograph of the kidney sectionof rat in the control group.The histo-architecture was normal with the renal cortex (white arrow) with the glomeruli containing normal mesengial cells and capsular space andthe renal tubules were preserved with normal cuboidal epithelial arrangement (blue arrow) and no pathological lesion. The interstitial spaces were normal and not infiltrated (slender arrow). H&E
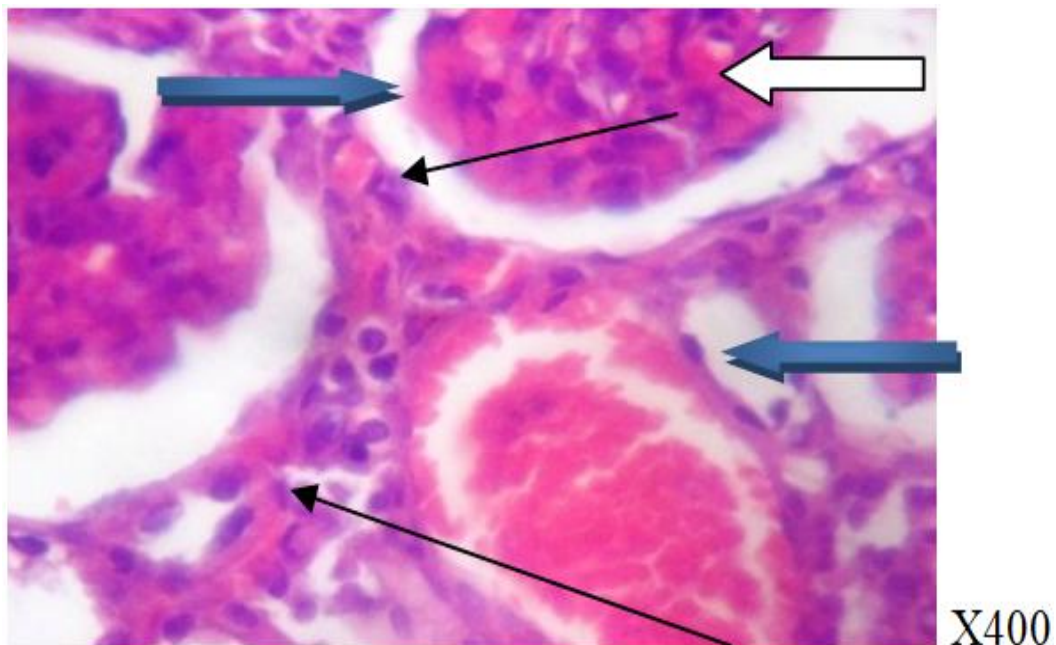
Plate 11. Photomicrograph of the kidney section of rat administered 40mg/kg body weight of tramadol. The histo-architecture was normal with mildly congested renal cortex (white arrow) and the glomeruli containing normal mesengial cells and capsular space (black arrow).The renal tubules were preserved with normal cuboidal epithelial arrangement (blue arrow) and no pathological lesion, and the interstitial spaces were normal and butmildly infiltrated(slender arrow).H&E

Plate 12. Photomicrograph of the kidney sectionof rat administered 20mg/kg body weight of tramadol.The histo-architecture was normal with the renal cortex (white arrow) with the glomeruli containing normal mesengial cells and capsular space andthe renal tubules were preserved with normal cuboidal epithelial arrangement (blue arrow) and no pathological lesion. The interstitial spaces were normal and not infiltrated(slender arrow).H&E
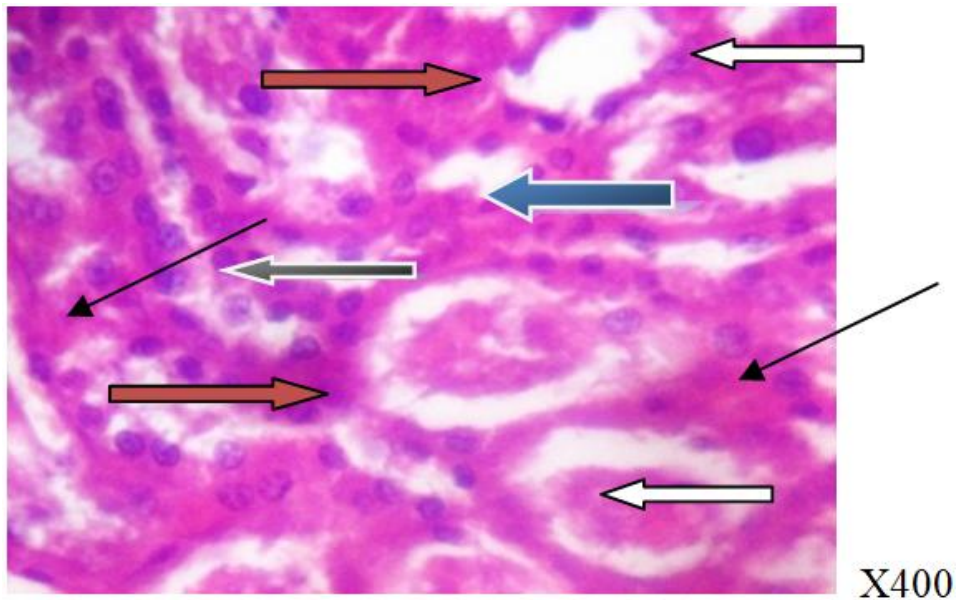
Plate 13. Photomicrograph of the kidney section of rat administered 40 mg/kg body weight of tramadol and 10 ml/kg body weight of caffeinated drink. The histo-architecture indicated mildly normal renal cortex and glomeruli with normal mesengial cells and capsular spaces that were mildly congested (white and blue arrow). The renal tubules were preserved with normal cuboidal epithelial arrangement (black arrow) and no pathological lesion. The interstitial spaces were normal, but mildly infiltrated (slender arrow). H&E
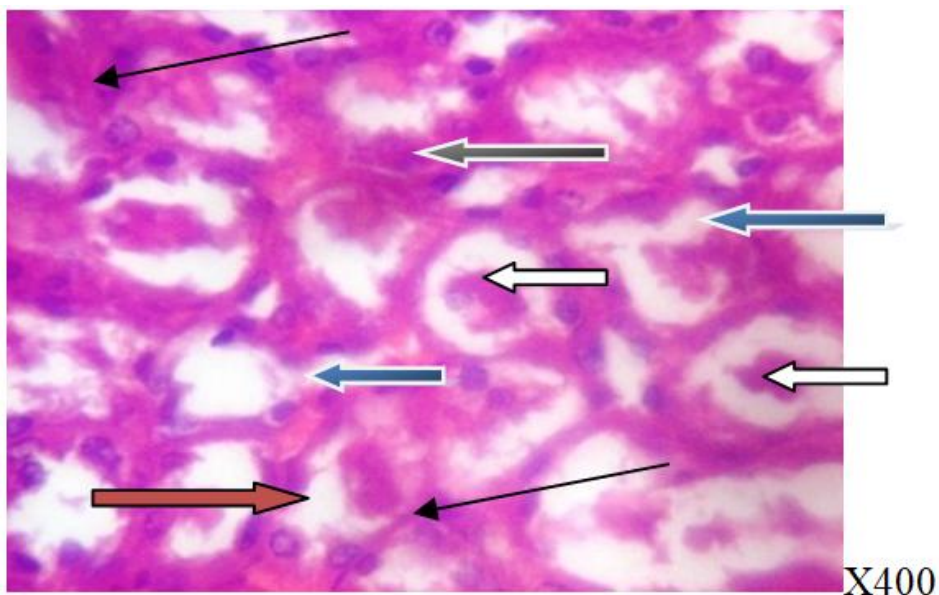

Plate 14. Photomicrograph of the kidney section of rat administered 20 mg/kg body weight of tramadol and 10 ml/kg body weight of caffeinated drink. The histo-architecture was normal with the renal cortex with the glomeruli containing normal mesengialcells in side and between capillary glomerulusand capsular space, but mildly congested(white and blue arrow).The renal tubules were preserved with normal cuboidal epithelial arrangement (black arrow) and no pathological lesion, and the interstitial spaces were normal and butmildly infiltrated(slender arrow). H&E
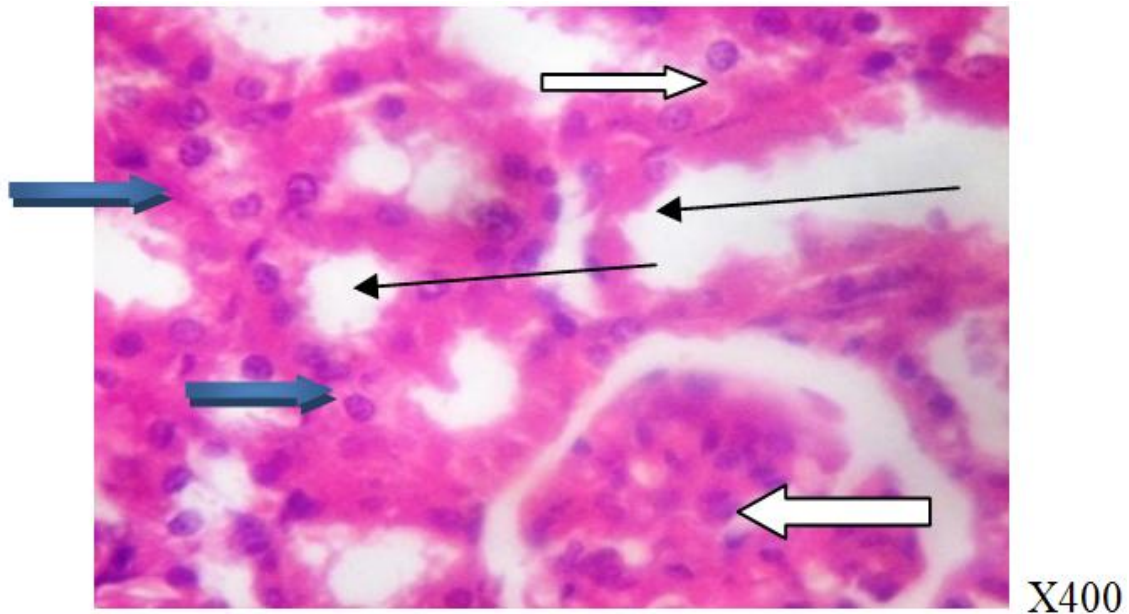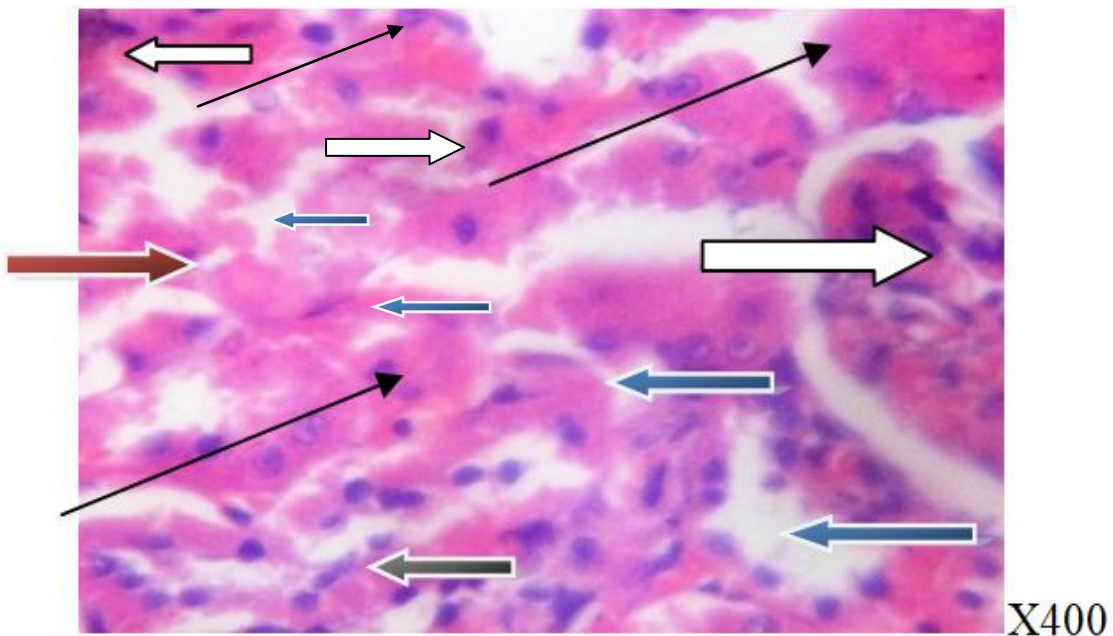
Plate 15. Photomicrograph of the kidney sectionof rat administered 40 mg/kg body weight of tramadol and 2 ml/kg body weight of alcohol. The histo-architecture indicated moderately normal renal cortex (white arrow) and glomeruli with mildly normalglomeruli elements(mesengial cells and capsular spaces) that were mildly congested (black arrow). The renal tubules indicated moderate tubular necrosis (red arrow) with severe renal casts within the tubules luminar spaces (blue arrow) and the epithelia of the tubules had high nucleo-cytoplasmic ratio(slender arrow). The interstitial spaces were normal, but moderately infiltrated. H&E

Plate 16. Photomicrograph of the kidney section of rat administered 20mg/kg body weight of tramadol and 2 ml/kg body weight of alcohol.The histo-architecture indicated mildly normal renal cortex (white arrow) and glomeruli with mildly normalglomeruli elements(mesengial cells and capsular spaces) that were mildly congested (black arrow). The renal tubules indicated moderate tubular necrosis (red arrow) with severe renal casts within the tubules luminar spaces (blue arrow) and the epithelia of the tubules had high nucleo-cytoplasmic ratio(slender arrow). The interstitial spaces were normal, but moderately infiltrated. H&E
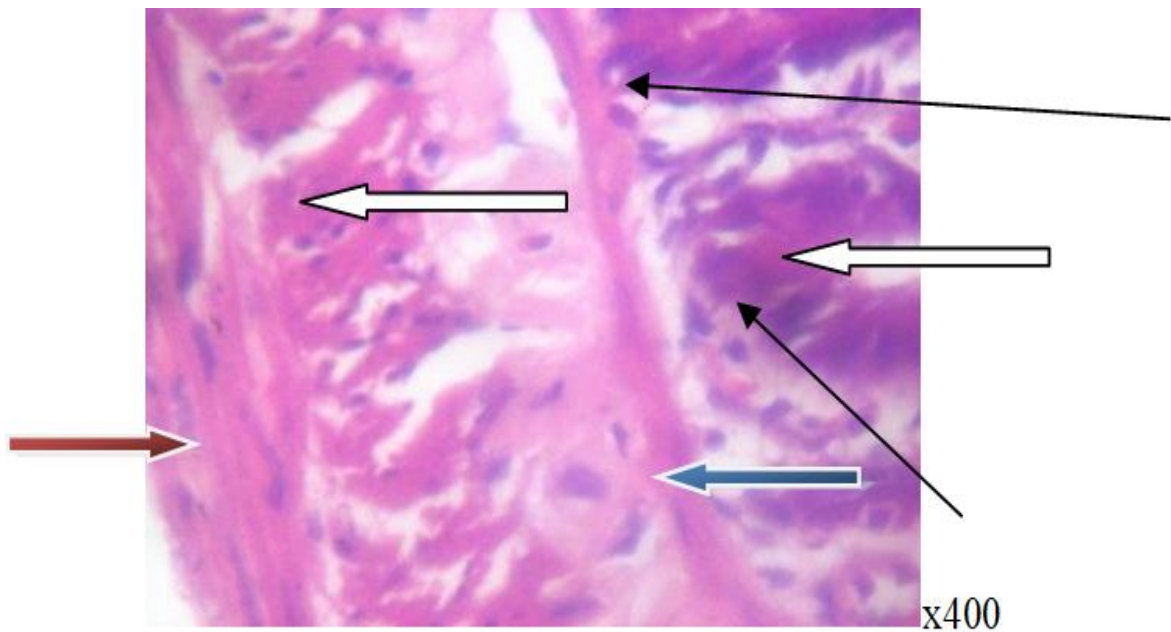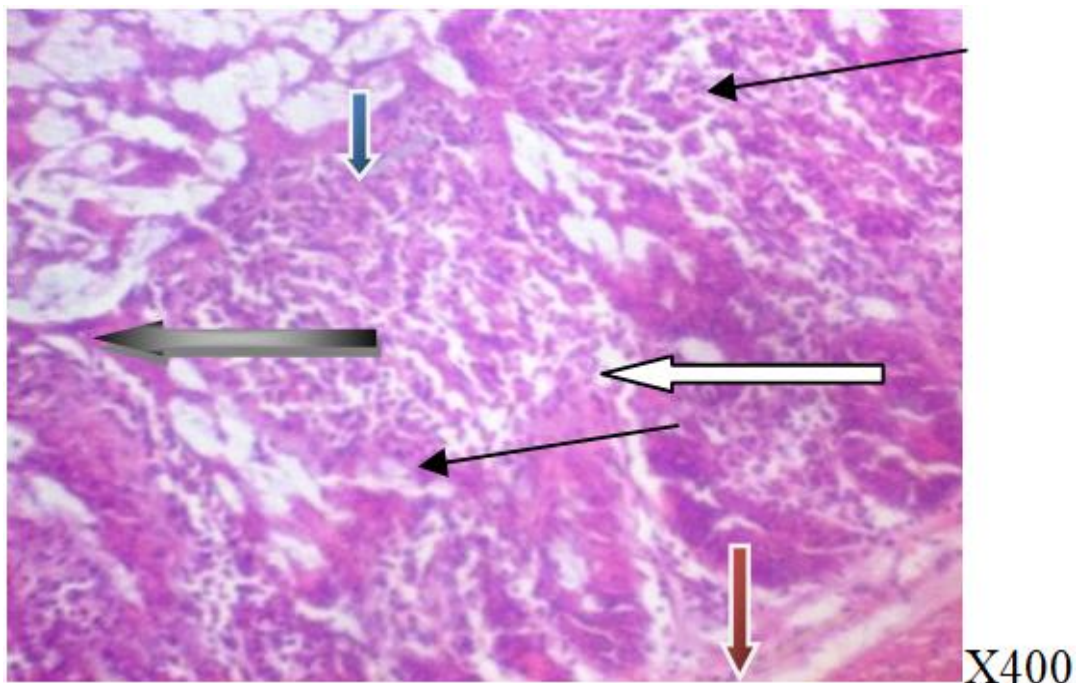
Plate 17. Photomicrograph of the kidney sectionof rat administered 10 ml/kg body weight of caffeinated drink.The histo-architecture was normal with the renal cortex (white arrow) with the glomeruli containing normal mesengial cells and capsular space andthe renal tubules were preserved with normal cuboidal epithelial arrangement (blue arrow) and no pathological lesion. The interstitial spaces were normal and not infiltrated (slender arrow). H&E

Plate 18. Photomicrograph of kidney section of rat administered 2 ml/kg body weight of alcohol. The histo-architecture was mildly normal with the renal cortex (white arrow) with the glomeruli containing normal mesengial cells and capsular space (black arrow).The renal tubules had moderate tubular necrosis (red arrow)with mild vascular congestion (black arrow), but preserved with normal cuboidal epithelial arrangement (blue arrow) and no pathological lesion, and the interstitial spaces were normal and mildly infiltrated(slender arrow). H&E
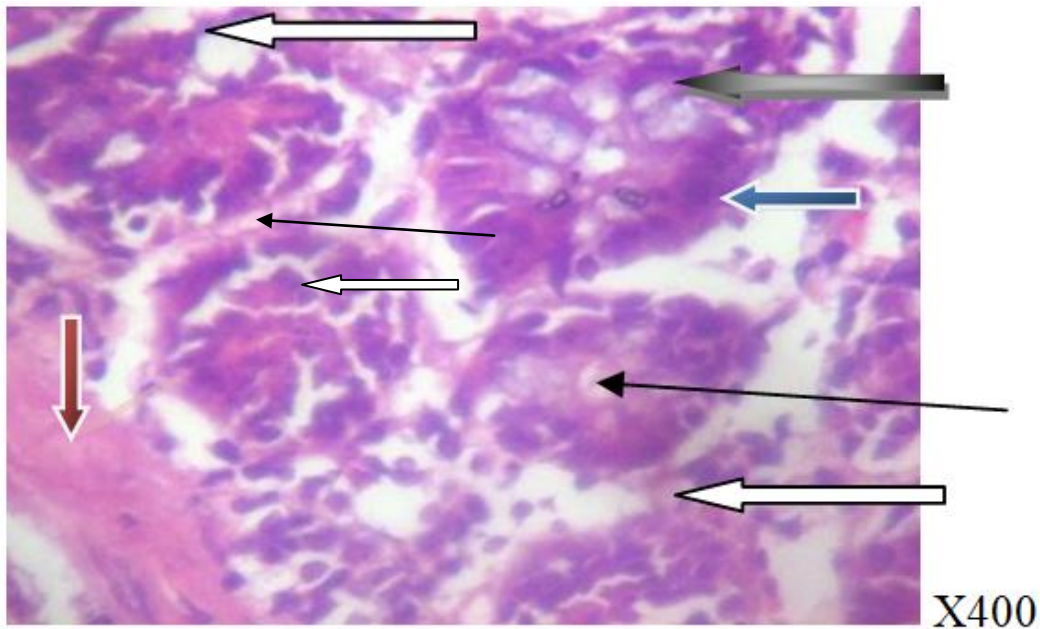
Plate 19. Photomicrograph of the intestinal (jejunum) sectionof rat in the control group.The histo-architecture was normal with the preserved mucosa layer, the epithelium lining through to the villi (white arrow). There were normal intestinal glands (slender arrow) and the muscularis externa containing inner circular (blue arrow) and outer longitudinal muscle (red arrow). H&E

Plate 20. Photomicrographof the intestinal (jejunum) sectionof rat administered 40mg/kg body weight of tramadol. The histo-architecture was normal with the preserved mucosa layer, the lining epithelium through to the villi (white arrow). There were normal intestinal glands (slender arrow) and the muscularis externa containing inner circular (blue arrow) and outer longitudinal muscle (red arrow). H&E

Plate 21. Photomicrographof the intestinal (jejunum) sectionof rat administered 20mg/kg body weight of tramadol. The histo-architecture was normal with the preserved mucosa layer, the lining epithelium through to the villi (white arrow). There were normal intestinal glands (slender arrow) and the muscularis externa containing inner circular (blue arrow) and outer longitudinal muscle (red arrow). H&E
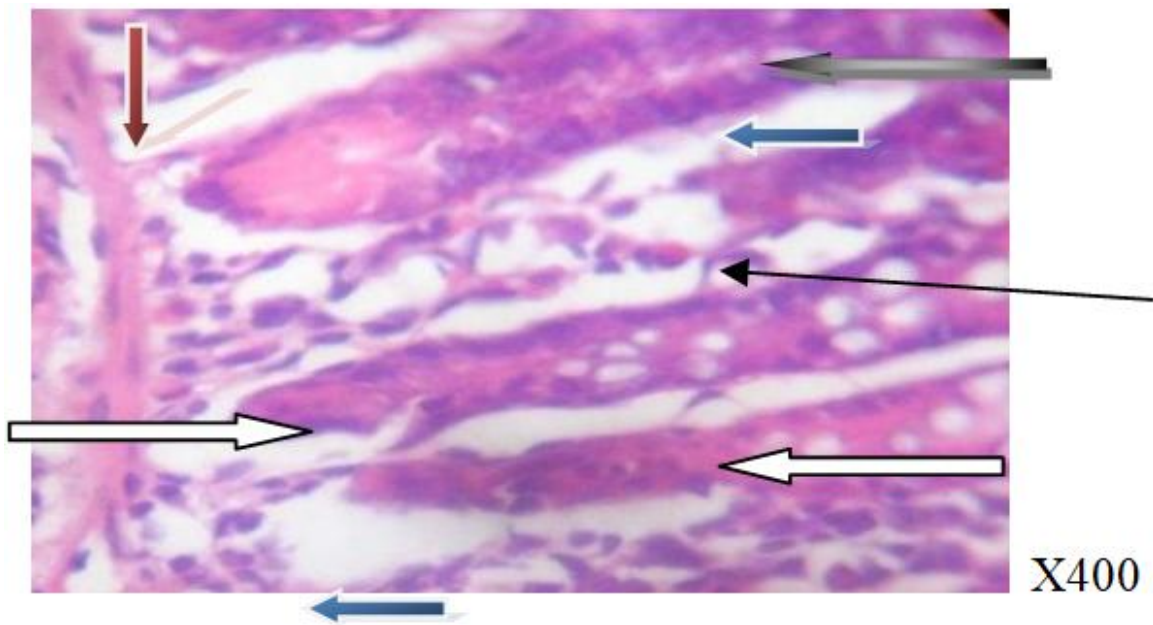

Plate 22. Photomicrographof the intestinal (jejunum) sectionof rat administered 40mg/kg body weight of tramadol. The histo-architecture was moderately normal with mildly sloughed villi and shortened villi (white arrow) of the mucosa layer (black arrow). There are severely inflammed intestinal glands with severe infiltration of inflammatory cells involving the lamina propria(slender arrow). The muscularis externa containing inner circular muscle (blue arrow) and outer longitudinal muscle appear normal (red arrow). H&E
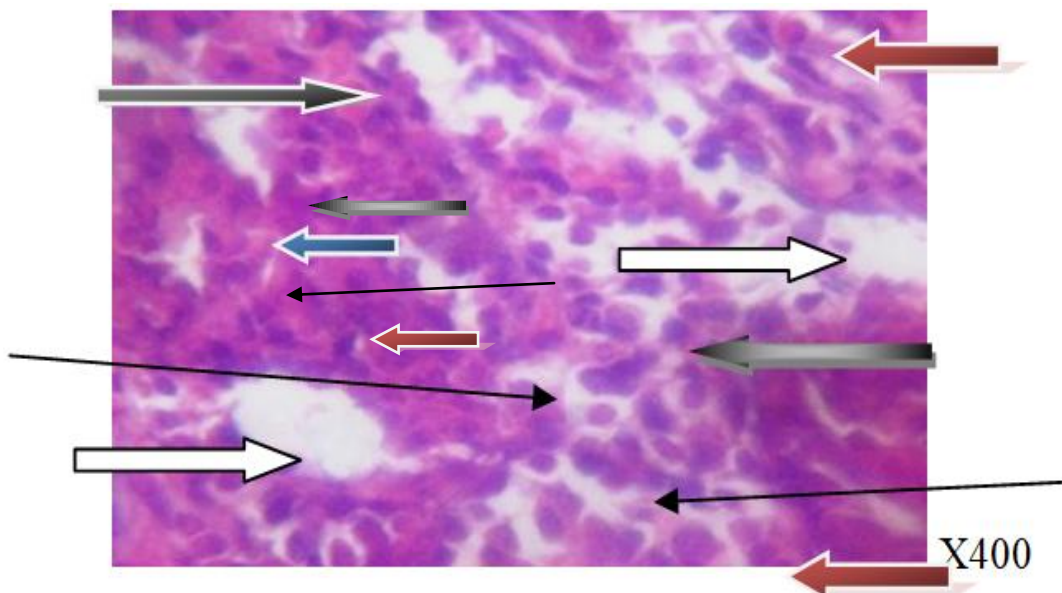
Plate 23. Photomicrograph of the intestinal (jejunum) sectionof rat administered 20 mg/kg body weight of tramadol and 10 ml/kg body weight of caffeinated drink. The histo-architecture was normal with the preserved mucosa layer, the epitheliumlining through to the villi (white arrow). There intestinal glands and lamina propria were mildly infiltrated by inflammatory cells (slender arrow) at lymphatic nodules (black arrow) in the submucosa region, but the muscularis externa containing inner circular muscle (blue arrow)and outer longitudinal muscle (red arrow) were normal.H&E
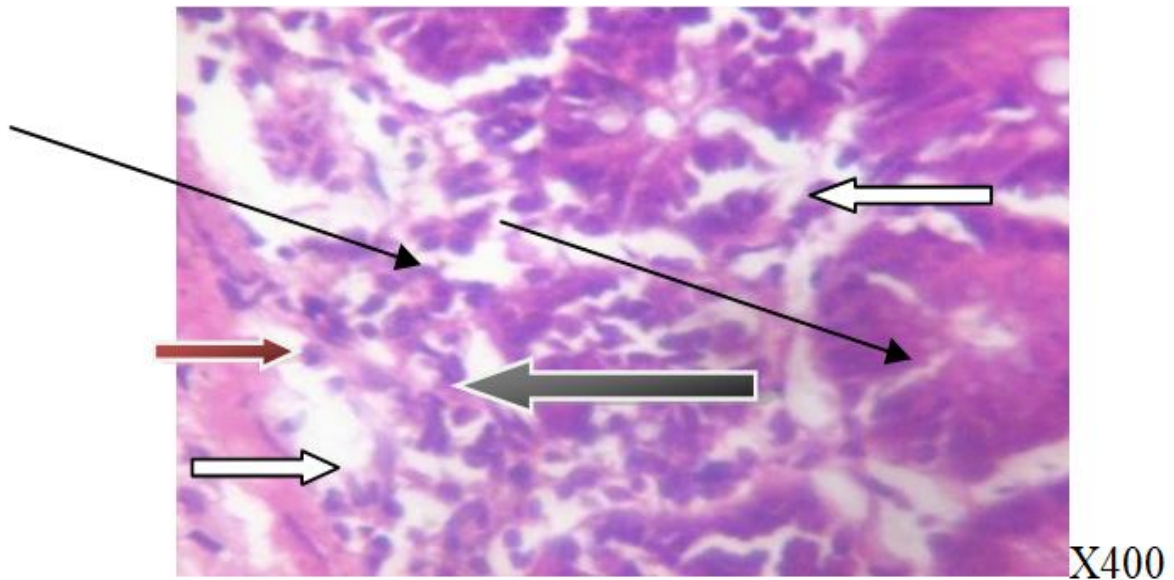

Plate 24. Photomicrograph of the intestinal (jejunum) section of rat administered 40mg/kg body weight of tramadol and 2 ml/kg body weight of alcohol.The histo-architecture was mildly normal with intravilli infiltration, but normal villi (white arrow) of the mucosa layer (black arrow). There are inflammed intestinal glands with moderate infiltration of inflammatory cells, the lamina propria are infiltrated as well (slender arrow). The muscularis externa containing inner circular muscle (blue arrow)and outer longitudinal muscle were normal (black arrow). H&E
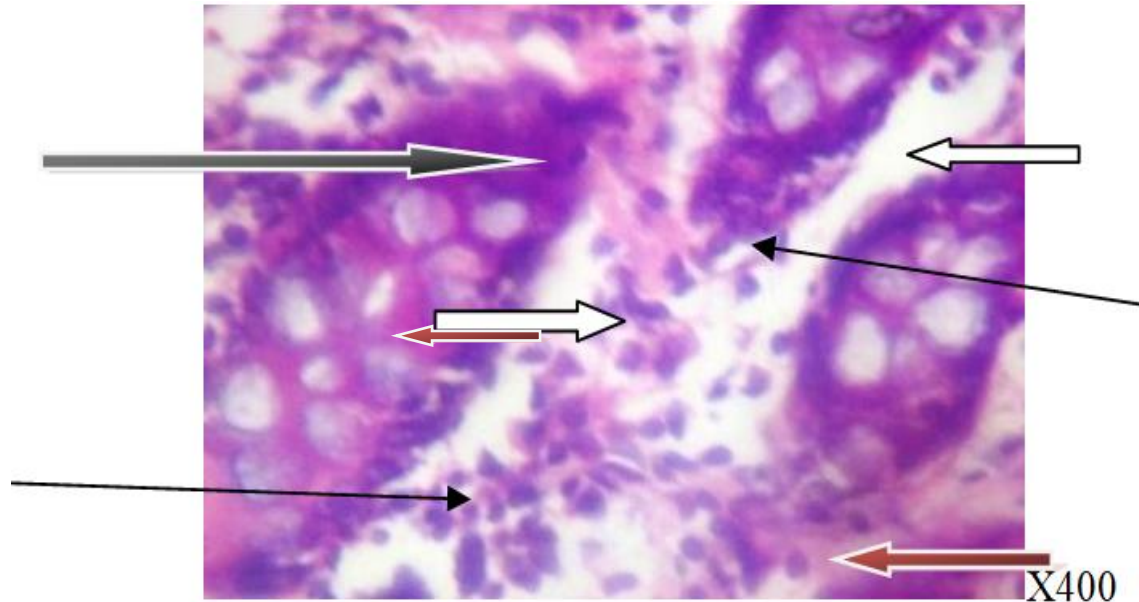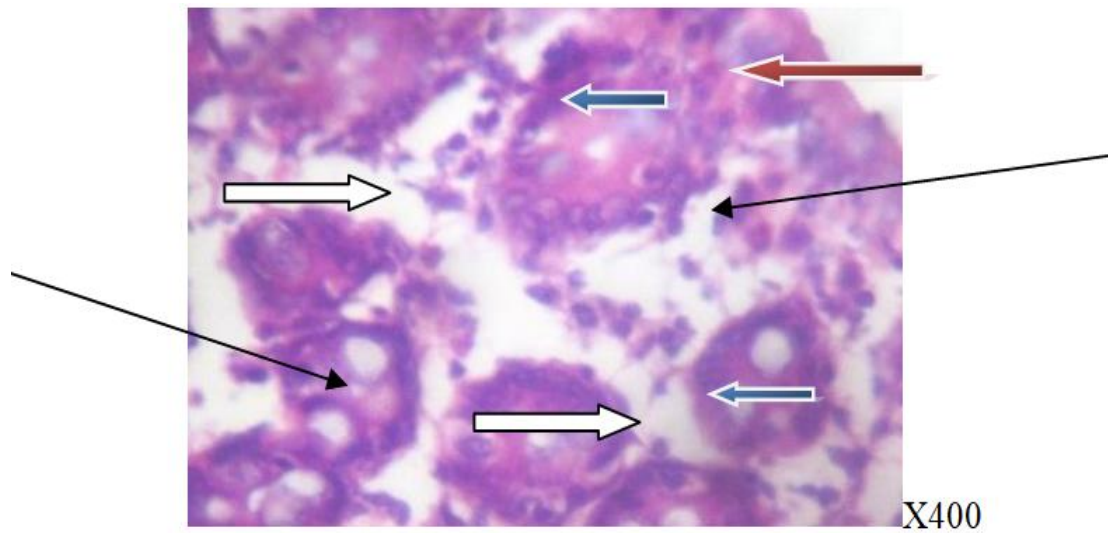
Plate 25. Photomicrograph of the intestinal (jejunum) section of rat administered 20 mg/kg body weight of tramadol and 2 ml/kg body weight of alcohol. The histo-architecture was normal with the preserved mucosa layer, the lining epithelium through to the villi (white arrow). There intestinal glands and lamina propria were mildly infiltrated by inflammatory cells (slender arrow) at lymphatic nodules (black arrow) in the submucosa region, the muscularis externa containing inner circular muscle (blue arrow)and outer longitudinal muscle (red arrow). H&E

Plate 26. Photomicrograph of the intestinal (jejunum) section of rat administered 10 ml/kg body weight of caffeinated drink.The histo-architecture was mildly normal with the preserved mucosa layer,masisive lymphoid aggregate extending from submucosa layer to the mucosa regions(black arrow), the mucosa layer villi were short(white arrow). There intestinal glands are severely infiltrated by inflammatory cells (white arrow), but were normal (slender arrow) with the muscularis externa containing inner circular (blue arrow) and outer longitudinal muscle (red arrow). H&E

Plate 27. Photomicrographof the intestinal (jejunum) section of rat administered 2 ml/kg body weight of alcohol.The histo-architecture was mildly normal with the preserved mucosa layer, the epithelium lining through to the villi (white arrow). There intestinal glands and lamina propria were mildly infiltrated by inflammatory cells (slender arrow) at the lymphatic nodules (black arrow) in the submucosa region, the muscularis externa containing inner circular muscle (blue arrow)and outer longitudinal muscle (red arrow). H&E
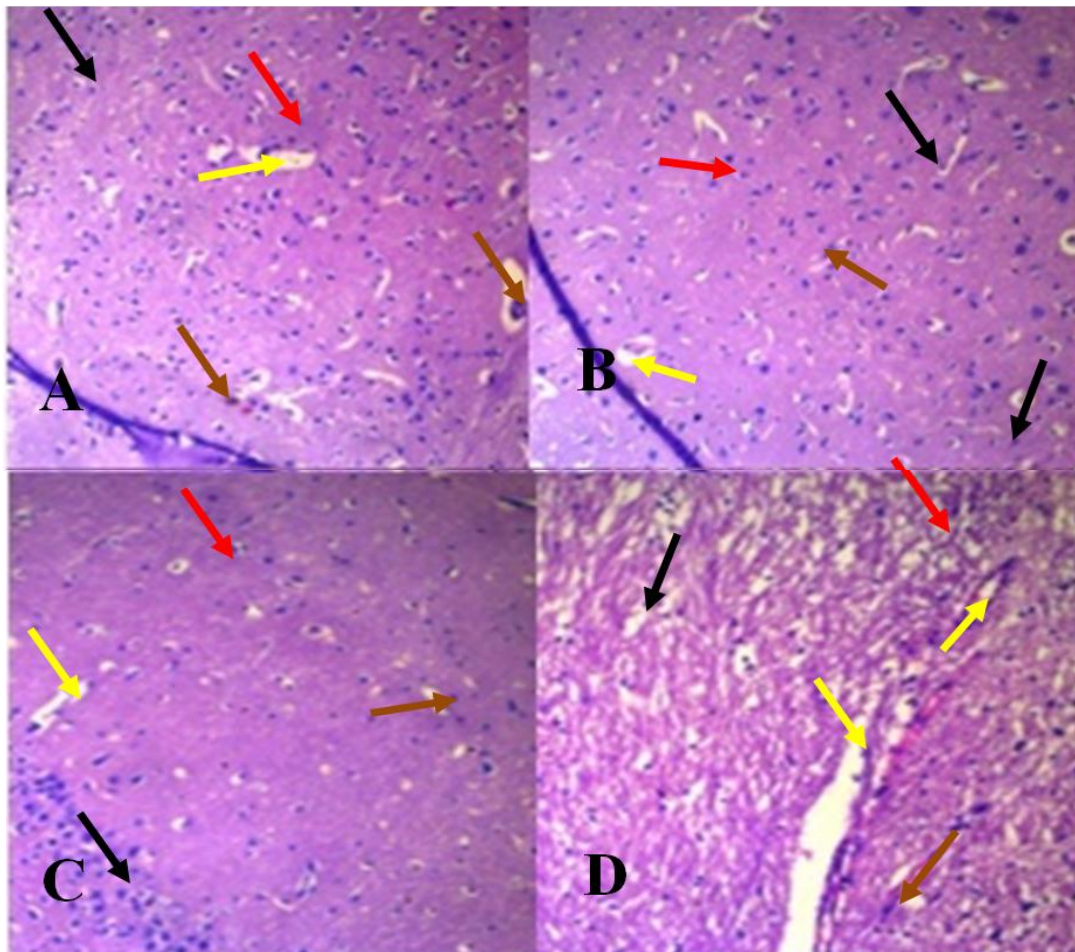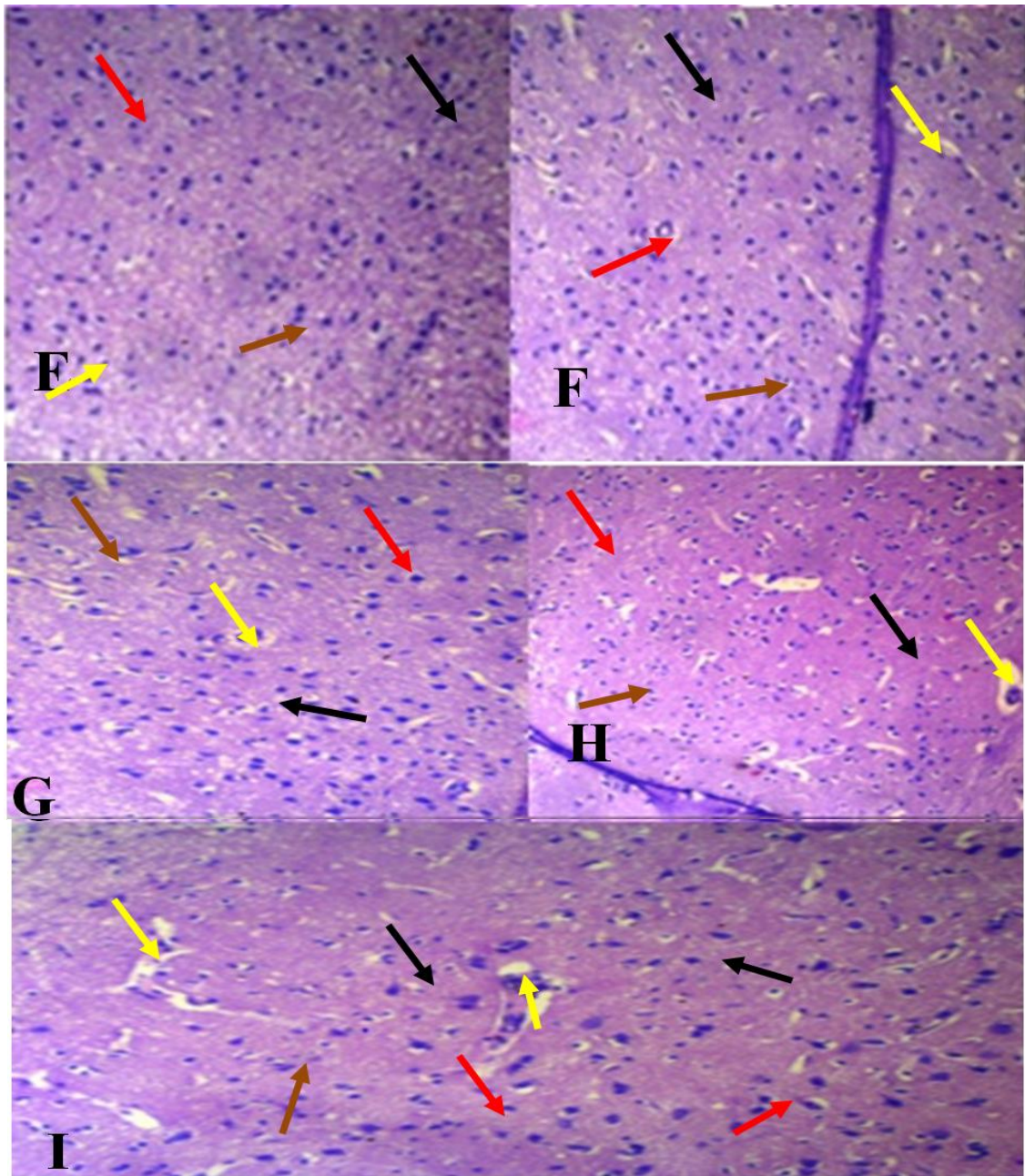
Plate 28. Photomicrographs of the brain of the rats administered tramadol, caffeinated drink and alcohol (A – I). In A, the neuronal cells were well preserved and devoid of neurodegenerative features. In B, C and E, there were abundant blood vessels (yellow arrows) and the morphology of the neurons was mildly distorted (black arrows), but devoid of neurodegenerative features. In D, there were various neurodegenerative features such as chromatolysis, neuronal cavitation, and neuronal perinuclear spacing (red arrows) and vacuolation in the stroma (yellow arrow). In F and G, there are abundance of swollen blood vessels (yellow arrows) and glial cells in the neurophil, neuronal cavitation and vacoulations. In H, neuronal cells were well preserved and devoid of neurodegenerative features. In I, the morphology of the neurons was mildly distorted (black arrows), but devoid of neurodegenerative features

Note: A-Control, B-40 tramadol, C-20 tramadol, D-40 tramadol and caffeinated drink, E-20 tramadol and caffeinated drink, F-40 tramadol and alcohol, G-20 tramadol and alcohol, H-caffeinated drink, I-alcohol. (Tramadol in mg/kg body weight) (Caffeinated drink and alcohol were 10 and 2 ml/kg body weight respectively)

The decreases recorded in the concentration of protein thiol in the liver and brain and supported the alleged induction of ROS by the administration of tramadol, caffeinated drink and alcohol, which were further strengthened when co-administered (Table 2). However, the plasma protein thiol levels weakly supported the alleged induction of ROS in the tramadol and caffeinated drink singly or co-administered rats, but the whisky administered rats supported it strongly. Reversible protein thiol oxidations or modifications have been demonstrated in proteins with a range of functions, including; signal transduction, ion transport and contractility, and protein metabolism [19,20]. The sulfhydryl groups (thiol) in proteins are very susceptible to oxidative damage and are often low in patient suffering from diseases and infections. That is, depletion in levels of protein thiols are linked to many medical disorders, such as chronic renal failure and other kidney disorders, cardiovascular disorders, stroke, and other neurological disorders, diabetes mellitus, alcoholic cirrhosis and several other degenerative or metabolic disorders [21].

The measurement of malondialdehyde (MDA), a lipid peroxidation product, is the mostly used biomarker to investigate the levels of oxidative damage on lipids that are the major target of ROS [22]. The trends we obtained in the concentration of MDA in the liver and brain supported the induction of ROS by the administration of tramadol, caffeinated drink and alcohol (Table 1). Although, the MDA levels in the liver of rats administered tramadol singly did not support the induction of ROS, as well as, single alcohol administration at four weeks. However, the few inconsistent increases recorded in the blood MDA levels in some groups of rats (groups D, E, F and G) supported the cytotoxicity of the co-administration of tramadol, caffeinated drink and whisky.

The increase obtained in the protein carbonyl (PC) concentrations in the liver and brain of the groups co-administered tramadol, caffeinated drink and alcohol (Table 2) supported the induction of oxidative stress indicated by the results of protein thiols, MDA and GSH.This can't be farfetched as MDA do interact with proteins and is itself potentially atherogenic.That is, MDA can react with the free amino group of proteins, phospholipids and nucleic acids leading to structural modification, which could induce dysfunctions of immune systems, which are usually correlated with the pathogenesis of various diseases such as tissue damage, atherosclerosis, stroke, cancer and Graves' disease etc. [22,23]. However, the trend in the brain of the groups of rat singly administered tramadol did not support the induction of oxidative stress. The results of the PC concentrations in the blood were surprising, but somehow in consonance with those of MDA in the blood. It is plausible that the blood was not a good target for bio-assimilation / cytotoxicities of the metabolites of tramadol, caffeinated drink and alcohol. The results of the GSH and PT ambiguously supported this assertion in the blood.

The results obtained in the paraoxonase (PON-1) activities in the liver of rats administered tramadol, caffeinated drink and alcohol (Table 4) indicated the 'use up' in the activities of PON-1, which were partly underlined by the results of PT, PC and MDA liver concentrations when examined critically (Tables 1 and 2). These further strengthened the induction of oxidative stress by the co-administration of tramadol, caffeinated drink and alcohol that were not severe / well established when administered singly. PON-1 is a hydrolytic enzyme that protects against lipid peroxidation in lipoproteins. Paraoxonase in high density lipoprotein cholesterol (HDL-C) prevent the oxidation of cholesterol in low density lipoprotein cholesterol (LDL-C) by prolonging oxidation lag phase, and reducing peroxides and aldehydes contents in HDL-C [24]. Therefore, the decreases in PON-1 activities might increase the probabilities of the oxidation of lipids in LDL-C, which clinically is not a desired metabolic process.

In Table 4, the patterns presented in the concentrations of oxidized low density lipoprotein cholesterol (ox-LDL-C) reflected partly the trends in the PON-1 activities. The increases in the activities of PON-1 in the liver of groups C rats supported the reductions in the concentrations of ox-LDL-C in the same rats, as well as the unaltered PON-1 and ox-LDL-C in the liver of group B rats. In addition, the decreases recorded in PON-1 activities in the liver of other groups of rats resulted in corresponding increases in ox-LDL-C concentrations. Although, there were no scientific associations yet between ox-LDL-C concentrations and the severity of inflammation, as the risks of oxidation of LDL-C did not change after the adjustment of some inflammatory markers [25]. However, oxidative modification of LDL-C and the subsequent engulfment of ox-LDL-C by macrophages in the vascular walls are

the significant steps known to promote fatty-streak formation, the early lesion of atherosclerosis [26]. This is because ox-LDL-C was shown to induce production of monocyte chemotactic protein-1 (MCP-1) and monocyte-colony stimulating factor, which lead to severe inflammatory responses in vascular cells [27].

Monocyte chemotactic protein-1 (MCP-1) is a key chemokine that regulate migration and infiltration of monocytes/macrophages. The migration of monocytes from the blood stream across the vascular endothelium is a routine process involved in immunological surveillance of tissues, to take out infected and or, damaged cells, as a response to inflammation initiation. Therefore, the recorded increases in the concentrations of interleukin-1β (IL-1β) and vascular cell adhesion molecule (VCAM) in this study (Table 3) gave credence to the increased need of monocytes migration from the blood stream across the vascular endothelium to support likely macrophage pick up of ox-LDL-C. VCAM-1 is an endothelial molecule that is expressed on endothelial cells during inflammatory responses, while IL-1β is the first chemokine produced at the onset of inflammatory response to enhance chemotaxis of immune cells [28].

The no alterations reported in the concentrations of ox-LDL-C,MCP-1, VCAM-1 and IL-1b at four weeks in this study (Table 3), might not mean that all was well with the rats, but could be that the progression of the assaults or damage had not accumulated sufficiently to elicit marked alteration in their concentrations. This is because the progression of vascular related disorder is known as to be insidious and is only evident after prolonged accumulation of inflammatory responses. Paradoxically, the increases recorded in the concentrations of the chemokines and cytokines (MCP-1, VCAM-1 and IL-1b) (Table 3), are the confirmation of plausible vascular related disorder that have been implicated in the progression of heart diseases and various metabolic disorders. This is in consonance with the reports of [29,30] that increases in the concentrations of chemokines and cytokines must precede the formation of atherosclerotic plaques, fatty streaks etc. These results supported the trends reported in the oxidative indices (Tables 1, 2 and 4) and might indicate a reduction in the quality of life. The reduction in the quality of life is plausible because the oxidation of the lipid and protein contents in cell membranes increases the polarity of lipid-phase

surface charge, as well as an increase in the formation of protein oligomers, while the number of thiol (SH) groups, molecular mobility of lipids and resistance to thermo-denaturation decreases.

Acetylcholine esterase (AChE) is an enzyme that hydrolyses the neurotransmitter, acetylcholine by terminating the action of acetylcholine at the cholinergic synapses, therebyregulating cholinergic neurotransmission [31]. AChE is present in the erythrocytes, brain, autonomic ganglia, skeletal muscle end-plate etc. The result presented in the acetylcholine esterase activities in rats administered tramadol, caffeinated drink and alcohol (Table 4) suggested decreases in the concentration of acetylcholine, which could result in memory, confusion (coordination) and or, cognitive deficit. This because the donepezil and rivastigmine used in the treatment of cognitive decline, inhibit the action of acetylcholine esterase in the brain, so that intracellular acetylcholine concentration increases to improve learning, memory, language, coordination etc. [31]. Although, these increase recorded in AChE activities might be due also to enzyme induction by the continuous synthesis of acetylcholine for the excitation of nerve cells. This is logical as acetylcholine is always removed at the synapses by AChE and could have led to bad social adaption. This is in agreement with the previous report of [32] that tramadol resulted in increases in AChE activities and recorded bad social adaption, memory and cognitive deficits, depression etc.

The results of the oxidative indices (Tables 1 and 2) in the brain supported the trend in the AChE activities and might be the underlining factors in the AChE activities obtained. This is because the brain has a high index of polyunsaturated fatty acids, thereby making it highly vulnerable to oxidative damage [33]. Thus, the unregulated production of ROS in the brain and the attendant alteration in balance of the antioxidant defense system have been linked to pathological changes in neurodegenerative diseases, such as Parkinson's disease (PD) and Alzheimer's disease (AD) [34,35].

The revelations on the photomicrographs of the liver and kidney (Plates 1-18), indicated that the single and co-administration of caffeinated drink and tramadol at 20 mg/kg body weight did not compromise the histo-architecture of the hepatocytes and nephrons, which connoted that they were not toxic to the organs. Although, the

co-administration of tramadol at 20 mg/kg body weight with alcohol indicated toxicities to hepatocytes and nephrons, but were less to those of the rats administered tramadol at 40 mg/kg body weight.The photomicrographs of the intestine (Plates 19-27) and brain (Plate 28) supported the alleged induction of inflammatory responses (Table 3) following the administration of tramadol at 40 mg/kg body weight and or, co-administration of tramadol and alcohol andcaffeinated drink. The alterations in the photomicrographs of brain gave brain gave credence to the patterns recorded in the oxidative indices (Tables 1, 2 and 4) and could be responsible for the increases reported in the chemokine and cytokine concentrations (Table 3). Although, some photomicrographs of the kidney and intestinealso indicated remarkable alterations, but we did not determine the concentrations of any parameter in them in this study.

It is plausible that the increases in the chemokine and cytokine concentrations (Table 3) were the outcomes of the toxicities of the metabolites of tramadol, caffeinated drink and alcohol to the brain and likely the intestine (necrosis was revealed in some photomicrographs), as the results of the oxidative indices did not indicate major toxicity in the blood, except the concentration of NO* (Tables 1 and 2). The result obtained in the activities of AChE supported this assertion as the increased AChE activities might mean that more cellular communications (signalling) were on-going, which could be as a result of massive cellular activities, metabolism etc. Therefore, the concentration of NO* might be the cause of the few alterations recorded in the other oxidative indices, though were not consistent in the blood.

5. CONCLUSION

The overall patterns of the results presented indicated that the frequent administration of caffeinated drinks, alcohol and high dose of tramadol precipitated dysfunctions in the health indices. Therefore, the indulgence in the use/abuse of tramadolwith or without caffeinated drink or alcohol was highly detrimental to health and could reduce the quality of life.

CONSENT

It is not applicable.

ETHICAL APPROVAL

This study was carried out in accordance with international ethical laws on animal handling, following the Principles of laboratory animal care (NIH publication No. 85-1985, revised 1985).

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely 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 for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

ACKNOWLEDGEMENTS

REFERENCES

1. World Health Organization W.H.O. Tramadol; Expert peer review (2). Geneva p. 3. Crime, U.U/N.O.O.D.A., Criminals rake in $250 million per year in counterfeit goods that pose health and safety risks in unsuspecting public. 2017. 41st ECDD: Tramadol; 2018.
Available:https://www.who.int/medicines/access/controlled-substances/Tramadol.pdf?ua=1
2. Miotto K, Cho AK, Khalil MA, Blanco K, Sasaki JD, Rawson R. Trends in tramadol: Pharmacology, metabolism and misuse. Anesthesia and Analgesia. 2017;124(1): 44-51.
3. Abdel-Hamid, IA, Anderson KE, Waldinger MD, Anis TH. Tramadol abuse and sexual function, Sexual Medicine Review. 2016; 4:235-46.
4. Alkhubaizi EA. Tramadol abuse. International Journal of Advanced Research. 2019;2320-5407:850-863.
5. Yunusa U, Bello UL, Idris M, Haddad MM, Adamu D. Determinants of substance abuse among commercial bus drivers in Kano Metropolis, American Journal of Nursing. 2017;6(2):125-133.

6. Duru C, Oluoha U, Okafor C, Diwe K, Iwu A. Sociodemographic determinants of psychoactive substance use among students of tertiary institutions in Imo State, Nigeria. Journal of Addiction Research and Therapy. 2017;8(345):2.

7. Reagan S. S, Nihal M, Ahmad N. Dose translation from animal to human studies revisited. Federation of American Societies for Experimental Biology Journal. 2008; 22(3):659-661.

8. Ellman GL. Tissue sulfyhydrl groups. Archives of Biochemistry and Biophysics. 1959;82:70.

9. Schmidt H.H.Non-enzymatic Colorimetric Assay for Nitric oxide. Biochemica. 1995; 2:22-23.

10. *Varshney,* R., *Kale,* R.K. Effect of calmodulin antagonists on radiation induced lipid peroxidation in microsomes. Int J Radiat Biol. 1990;58:733-743.

11. Levine RL, Stadtman ER.Oxidative modification of proteins during aging. Exp Gerontol. 2001;36:1495-1502.

12. Furlong CE, Richter R, Seidel SL, Costa LG, Motulsky AG. Spectrophotometer assay for the enzymatic hydrolysis of active metabolites of chlorpyrifos and parathion by plasma paraoxonase/arylesterase. Anal Biochem. 1989;180: 242-247.

13. Avwioro OG. Histochemistry and Tissue Pathology: Principle and Techniques. Claverianum Journal. 2001;15(8):45-77.

14. Lius S, Lezanne O, Scott R. Nitric Oxide: A Regulator of Cellular Function in Health and Disease. Oxidative Medicine and Cellular Longetitity. 2016;9(6):823-46.

15. Pacher P, Beckman J, Liaudet L. Nitric oxide and peroxynitrite in health and dieases. Physiol Rev. 2007;87(1): 315-424.

16. Mahboob M, Shireen KF, Atkinson A, Khan AT. Lipid Peroxidation and Antioxidant Enzyme Activity in Different Organs of Mice Exposed to Low Level of Mercury. Journal of Environmental Science. 2001; 36(5):687-697.

17. Lopez GE, Rodriguez AF, Rexrode KM, Logroscino G, Hu FB. Coffee Consumption and Risk of Stroke in Women. Circulation Research. 2009;119(43):1116-1123.

18. Ighalo EE, Erilibe JE, Ehimigbai AR, EzeukoVC.Protective Properties of Yoyo Cleanser Bitters against Mercury II Chloride (HgCl^2)-Induced Kidney Damage in Adult Wistar Rats. Journal of Biomedical Sciences. 2015;14(4):118-128.

19. Wu WN, Mckown LA, Liao S. Metabolism of the Analgesic drug ULTRAM (Tramadol hydrochloride) in humans: API-MS and MS/MS characterization of metabolites. Xenobiotica. 2002;32(5);411-425.

20. Choudhary AK, Devi RS. Imbalance of the Oxidant-Antioxidant Status by Aspartame in the Organs of Immune System of Wistar Albino Mice.African Journal of Pharmacy and Pharmacology. 2014;8(8):220-230.

21. Mungli P, Mahesh SS, Prasiddha T, Naureen A. Total thiols: Biomedical Importance and their alteration in various diseases. Online Journal of Health and Alllied Sciences. 2009;8(2):509.

22. Edwin H, Keyvan KG, Chia CM, Ravibhind GM, Gemma AF.Biological Markers of Oxidative Stress: Applications to Cardiovascular. Redox Biology. 2013; 65(23):483-491.

23. Terzioglu D, Uslu L, Simsek G, Atukeren P, Erman H, Gelisgen R, Ayvaz S, Aksu B, Uzun, H. The effects of hyperbaric oxygen treatment on total antioxidant capacity and prolidase activity after bile duct ligation in rats. Journal of Investigative Surgery. 2017;30**:**376–382.

24. Litvinov D, Mahini H, Garelnabi M. Antioxidant and antiinflammmatory role of paraoxonase 1: implication inartherosclerosis diseases. North Amer J of Med Scie. 2012;4(11):523-532.

25. Wu MD. Is Plasma Oxidized Low Density Lipoprotein, measured with widely used Antibody 4E6, an independent predictor of coronary heart disease among U.S men and women? Journal of American College of Cadiology. 2006; 48(5):973-979.

26. Lusis AJ. Atherosclerosis. Nature. 2000; 407:233-241.

27. Qiwei W, Jun R, Stephanie M. Monocyte Chemotactic protein-1 Regulates Macrophage Cytotoxicity in Abdominal Aortic Aneurysm. Journal Prone. 2015;13(71):53-56.

28. Joan MI, Cook M, Michelle E. Vascular Cell Adhesion Molecule 1 Expression and Signaling During Disease Regulation by Reactive Oxygen Species and Antioxidants. Antioxidants and Redox Signaling Journal. 2011;15(2):3522-3541.

29. Libby P, Ridker PM, Maseri A. Inflammation and atherosclerosis. Circulation. 2002;105:1135-1143.

30. Zipes DP, Libby P, Bonow RO, Braunwald E. Braunwald's heart disease. A textbook of cardiovascular medicine (7th edition). Pennsylvania, Elsevier Saunders, USA; 2005.

31. Schliebs R, Arendt T. The cholinergic system in aging and neuronal degeneration. BehavBrain Res. 2011; 221(2):555-563.

32. Hosseini SA, Rabbani M, Sharifzadeh M, Bagheri N. Acute and chronic tramadol administration impair spatial memory in rat. Res Pharm Sci. 2016; 11(1):49-57.

33. Butterfield DA, Castegna A, Lauder CM, Drake J. Evidence that amyloid beta-peptide–induced lipid peroxidation and its sequelae in Alzhemimer's disease brain contribute to neuronal death. Neurobiology of Aging. 2002;23:655-664.

34. Von Campenhausen S, Bornschein B, Wick R, Botzel K, Sampaio C, Poewe W, OertelW, Siebert U, Berger K, Dodel R. Prevalence and incidence of Parkinson's disease in Europe. European Neuropsychopharmacology. 2005;15:473-90.

35. Halliwell B. Oxidative stress and neurodegenaration: Where are we now? Journal of Neurochemistry. 2006;97(6): 1634-1658.