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Research Article

EVALUATING THE EFFECT OF TAURINE AND CAFFEINE ON THE BLOOD AND BIOCHEMICAL PARAMETERS OF RATS

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A R T I C L E I N F O

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A B S T R A C T

Background: Energy Drinks (Eds) commonly contain caffeine and taurine in their contents. They are generally consumed by adolescents. The abuse and the excess consumption of such drinks might induce near-fatal ventricular arrhythmia and cause cardiac arrest in young people. The present study aims to address caffeine and taurine's effect on the liver, kidney, and heart tissues of white Wistar Rats and evaluate their toxicity on the biological and hematological parameters. Therefore, 42 male Wistar rats (12w-old) were divided into seven groups; one control group and another six control groups, daily and orally gavaged with different caffeine and taurine concentrations. The hematological and biochemical parameters were recorded every two weeks.
Results: The values of red blood cells RBC and white blood cells WBC, in addition to the MCHC, were higher (P <0.05) than the control group. The activities of ALT, ALP, AST, LDH, and CK and the concentrations of cholesterol, urea, and globulin were higher (P <0.05) than the control group. Albumin concentrations were lower (p <0.05) than the control group. Neither statistically significant change in the other values nor change in body weight gained weekly were observed. Our results suggest that the pathological changesin the liver, kidney, and heart besides the hematological and biochemical changes result from taurine and caffeine's effect on rats. The toxic effects of simultaneous caffeine intake (CAF) and taurine (TA) were more severe to rats than their toxicity separately.
Conclusions: The tissues of the liver, kidney, and heart are sensitive to apotential toxic effect of Taurine and Caffeine. As a result, CAF and TA can lead to the emergence of some diseases in the liver, the kidney, and the heart by inducing changes in the tissues, including congestion in the portal vein and lymphatic infiltration, sinusoidal dilatation, hemorrhage, and necrosis in the liver. The damage can extend to include tubular congestion, congestion of capillary tubes, hemorrhage, glomerular degeneration, and necrosis in the kidney, as well as hemorrhage and congestion in the heart. The toxic effects of concurrent caffeinated and taurine intake were more toxic to rats than their toxicity alone.

INTRODUCTION

Energy drinks (ED) are non-alcoholic beverages containing stimulant compounds, usually caffeine, and marketed to improve energy, stamina, athletic performance, and concentration (Higgins *et al*., 2010). However, they are categorized as "functional beverages" alongside sports drinks and nutraceuticals; the ED industry has grown dramatically in the past 20 years, reaching over $9.7 billion in the United States (U.S.) sales in 2015(Heckman *et al*., 2010). ED's target consumer market is adolescents and young adults (Heckman *et al*., 2010), with one study finding that 51% of college students report consuming at least one ED each month (Malinauskas *et al*., 2007).

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Energy drinks can contain more than 15 ingredients, but the essential components come in five categories (Reissig *et al*., 2009): caffeine, a sweetener of some kind (usually sugar) (Breda *et al*., 2014), kola nuts, guarana, or yerba mate (Seifert *et al*., 2011).

Negative consequences of Ed's ingestion can be due to the toxicity of certain ingredients, mixing of EDs with alcohol and drugs, or accompanying physical exertion (Bigard, 2010; Breda *et al*., 2014). However, many fatal cases have been attributed to the mixing of energy drinks with alcohol. Some accidental and emergency cases were reported in the literature due to abuse of energy drinks and intoxication by caffeine (Bonsignore *et al*., 2014; Cappelletti *et al*., 2018; FitzSimmons & Kidner, 1998; Jabbar & Hanly, 2013; Sepkowitz, 2013; Wolk *et al*., 2012) Since we are focusing on evaluating the effect of specific EDs, we selectively decided to study caffeine

and taurine's impact on biological and hematological parameters.

Caffeine is one of a group of plant alkaloids that occurs naturally in the leaves, seeds, and fruit of more than 60 plant species, of which cocoa-beans, tea, and coffee are the most well-known (CARRILLO & BENITEZ, 1996). Caffeine (1,3,7-trimethylxanthine) is chemicallysimilar to adenosine allowing caffeine to bind efficiently to the adenosine receptors. After consuming high concentrations of a caffeinated beverage, caffeine's mode of actionis to act as a blocker to adenosine receptors in the brain (Pettenuzzo *et al*., 2008). Thus, adenosine's sleep-promoting effectis suppressed by caffeine, which acts as an adenosine-competitor in the neurons, resulting in stimulating the neurons instead of calming down (Ferré, 2008).

Moreover, caffeine is also known to increase epinephrine secretion, leading to various secondary metabolic changes that can positively affect physical or mental performance(Graham, 2001). Another stimulatory action of caffeine is predominantly exerted on the central nervous system, myocardium, and skeletal muscles(Yen & Ewald, 2012). When caffeinated beverages are consumed at average levels, caffeine will act as a non-selective competitive antagonist of adenosine receptors, especially subtypes A1 and A2A. When ingested at higher concentrations, it can induce intracellular calcium release and phosphodiesterase inhibition. At the same time, it has been reported to cause γ-aminobutyric acid inhibition at doses significantly above the limits of typical consumption (Pelchovitz & Goldberger, 2011).

Taurine (2-aminoethane sulphonic acid) is the major free intracellular amino acid found naturally in our bodies, primarily in the retina and skeletal and cardiac muscle tissues (Imagawa *et al*., 2009). It is an essential sulfonated beta-amino acid derived from the metabolism of methionine and cysteine(Stipanuk, 2004). Incorporating taurine into energy drinks and other products has increased much over the past 10 years with taurine also being one of the most extensively used and studied amino acids (Shao & Hathcock, 2008). Taurine is associated with various physiological functions, including neuro-modulation, cellular membrane stability, and modulation of intracellular calcium levels (Brosnan & Brosnan, 2006). The beneficial effect of taurine as an antioxidant in the biological system has been attributed to its ability to stabilize biomembranes (Wright *et al*., 1986), scavenging reactive oxygen species (Wright *et al*., 1985). Steineke and others determined the effect of EDs on blood pressure and heart rate in humans. This study's results reveal that an individual's blood pressure and heart rate increased significantly after 4 hours of EDs consumption. In contrast, diastolic blood pressure increased within 2 hours of ED consumption(Steinke *et al*., 2009).

Several studies suggest that the adverse effects and pharmacological alterations are caused by EDs intake are due to the excess intake of caffeine(Al-Saikhan, 2020). Most of these studies have demonstrated that such adverse effects were observed after along time of consumption or combining the ED with alcohol (Costa-Valle *et al*., 2018; Reis *et al*., 2017; Tsvetkova *et al*., 2015). These findings prompted us to evaluate caffeine and taurine's toxicity on the Westar Rats tissues using similar experimental designs.

METHODS

Animals and management

Forty-two male Wistar rats 12-week-old (150-160g) were obtained from the Animal Care Center, College of Pharmacy, King Saud University, Riyadh, Saudi Arabia. Animals were maintained at a 12 h light/dark in cycle polypropylene cages (six rats in each) at the ambient temperature of 23 ± 2 °C and relative humidity of 50-60% with food and water provided *ad libitum*. The rats were acclimatized for one week before the start of the experiment.

Chemicals and reagents

Biochemical Kits were purchased from Roche, Germany, to perform all analyses of biomarkers, enzymes, and elements in the blood sera. Caffeine (trade name purcaf Caffeine) was purchased in 200mg capsules from Kaged Muscle Company (United States). Taurine (brand name L-Taurine Powder) was purchased as 750 mg powder form from Life Extension Company (US United States).

Experimental design

A sub-acute toxicity study (28-day repeated oral administration) was carried out according to OECD 407 guidelines (OECD, 2006). Rats were randomly divided into seven experimental groups as in table (1).

All rats were observed twice daily for mortality and morbidity until the completion of the experiment. The clinical signs, the time of onset, and the duration of symptoms were observed and recorded. The bodyweight (BW) of all rats was recorded once before starting the oral administration, once weekly during the administration period, and finally, on the day of sacrifice.

Blood samples were collected from overnight fasted rats (only water allowed) after 2 and 4 weeks of treatment by retro-orbital bleeding into heparinized and non-heparinized tubes for hematological and biochemical analyses.

Hematological parameters

The heparinized blood was used for the analysis of hematological parameters such as hemoglobin (Hb) concentration, red blood cell (RBCs) counts, packed cell volume (PCV), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC) and white blood cell (WBC) count using (Hematology Analyzer cellular analysis system DXH 600).

Biochemical Parameters

The serum was separated from nonheparinized blood, and the biochemical parameters including aspartate aminotransferases (AST), alanine aminotransferases (ALT), alkaline phosphatase (ALP), total protein, albumin, cholesterol, urea, and creatinine were analyzed by using biochemistry Analyzer Cobas 6000, Roche.

Histopathology

After 2 and 4 weeks of treatment, three rats from each group were sacrificed under diethyl ether anesthesia to identify gross lesions. Liver, heart, and kidney were collected from all the animals for histopathology. The collected organs were weighed and preserved in 10% neutral buffered formalin,

trimmed, and a 5µ thickness of tissue sections was stained with hematoxylin and eosin for histopathological study.

Statistical Analysis

Results were expressed as mean ± standard error mean (SEM). Experimental data for statistics and correlations analysis were conducted by Student's t-test was used for analysis. Values of $P < 0.05$ were regarded as significant. Data analysis was performed with SPSS version 22 statistical software (SPSS, Chicago, IL, USA). The p-value less than 0.05 was considered statistically significant (Carrillo and Benitez, 1996).

RESULTS

The effects of caffeine intake at different doses 32.2 and 64.4 mg/kg BW/day and taurine at doses 300 and 600 mg/kg BW/dayduring Wistar rats' growth were shown in Table (1). Mainly, no significant change in the weekly gained body weight was observed, and two rats died during the experimental period.

Table 1 The experimental groups of Westar rats that were orally gavaged with different Caffeine and Taurine doses

Rat Group	Label	Caffeine dose mg/kg bw/day	Taurine dose mg/kg bw/day
Control Group	G0	0	0
Group 1	G1	32.2	0
Group 2	G2	64.4	0
Group 3	G3	0	300
Group 4	G4	0	600
Group 5	G5	32.2	300
Group 6	G6	64.4	600

Where the dose mg/kg BW/ day= mg/kg body weight (bw) per day

Histopathological changes

The control group (group 1) showed no significant microscopic changes in the rat tissue after two weeks. Only acute histopathological changes such asliver necrosis, few lymphocytes infiltration, apoptosis, dilated sinusoids, and central artery congestion were observed in the liver. Congestion of glomeruli and tubules in the kidney was remarkable. Besides, congestion and hemorrhage were observed in the heart as well.

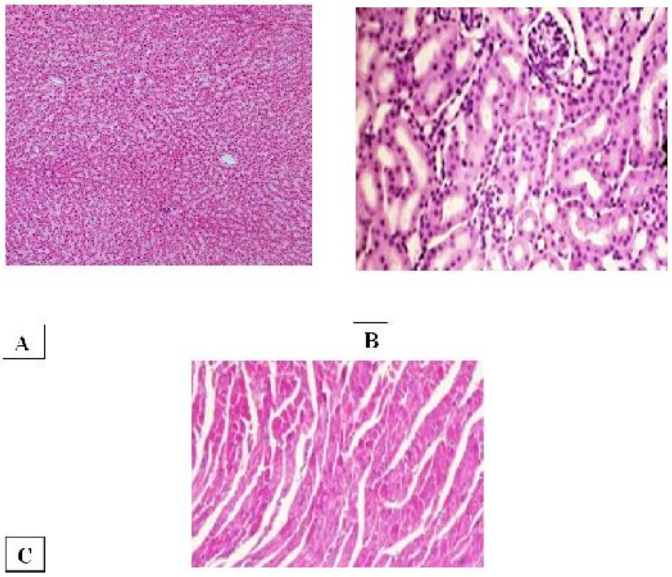

Figure (1) (A) a cross-section of the rat liver of a reference group, (B) a cross-section for the rat kidney of a reference group, and (C) a cross-section of the rat heart of a reference group.

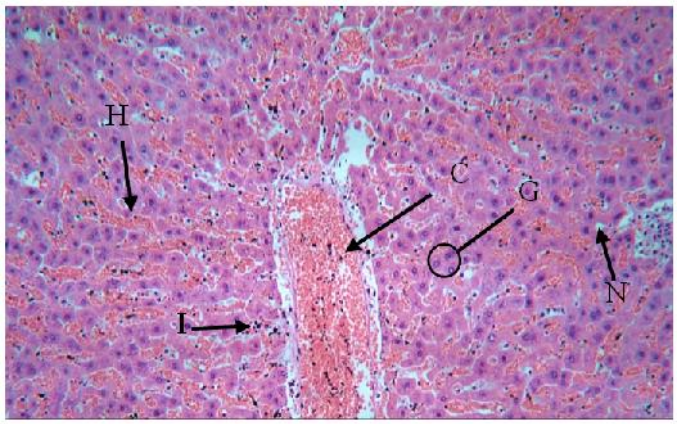

Figure 2 After 2 weeks, the liver tissue in group 1, that was orally gavage with Caffeine 32.2 mg/kg BW/day, shows necrosis N, severe hemorrhage H, congestion C, cytoplasmicgranulation G, and a fewinfiltrating lymphocytesI (x 100 - H&E).

The rats of group 1 and group 2, which were orally and gavaged with Caffeine 32.2 and 64.4 mg/kg BW/day, respectively, have shown lymphocytic infiltration, dilated sinusoids, hemorrhage, congestion, and necrosis in some hepatocyte (Fig 2 and 4). Besides, lymphatic infiltration, hemorrhage, and congestion can be seen in the heart (Fig.3).

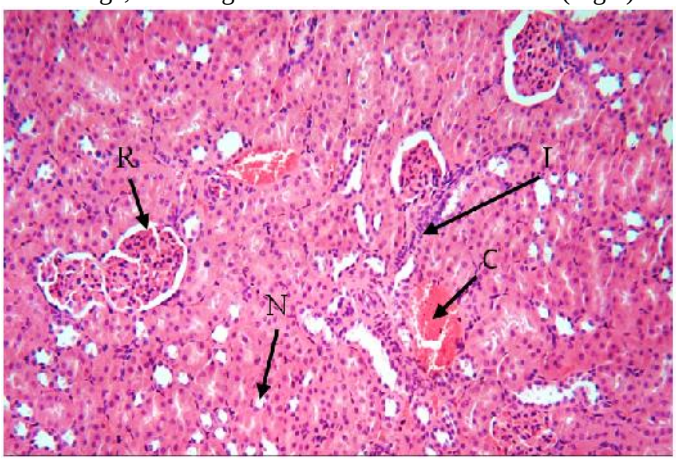

Figure 3 After 2 weeks, the renal tissue in group 1, that was orally gavage with Caffeine 32.2 mg/kg BW/day, showsnecrosis N, glomerular congestionC, infiltrating lymphocytesI, and the glomerulus revealed retraction of capillary tufts and segmental wrinkling of membranesR. (x 100 - H&E).

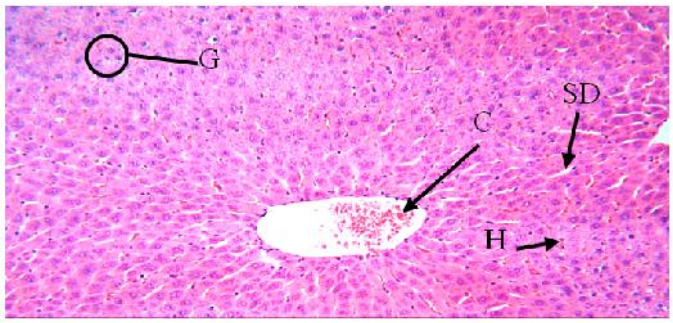

Figure 4 After 2 weeks the liver tissue in group 2, that was orally gavage with Caffeine 64.4 mg/kg BW/day, shows congestion in the portal veinC, hemorrhageH, sinusoidal dilatation SD, and cytoplasmicgranulation G(x 100 - H&E).

The rats of group 3, which were orally gavaged with taurine 300 mg/kg BW/day, have shown acute lymphatic infiltration, hemorrhage, congestion, necrosis, and glomerular mesangial cells proliferation in the kidneys (Fig. 7 and 8). Besides, an expansion of the acute dilated sinusoids, lymphatic infiltration, hepatocytes degeneration, and focal necrosis, hemorrhage, and congestion can be seen in the liver. Moreover, congestion,

hemorrhage, and lymphatic infiltration can be seen in the heart. The rats of group 4, which were orally administered withtaurine600 mg/kg BW/day, have shown a lymphatic infiltration, dilated sinusoids, hemorrhage, congestion, and necrosis in some hepatocytes. In addition to glomerular lymphocytic infiltration, congestion, necrosis, and hemorrhage in the kidneys (Fig.9).

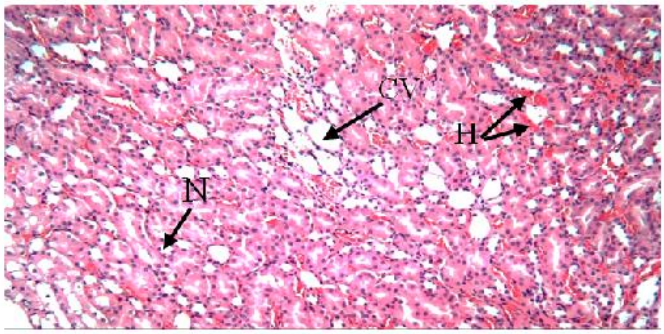

Figure 5 After 2 weeks the renal tissuein group 2, that was orally gavage with Caffeine 64.4 mg/kg BW/day, shows necrosis N, hemorrhageH, and Cytoplasmic vacuolationCV(x 100 - H&E).

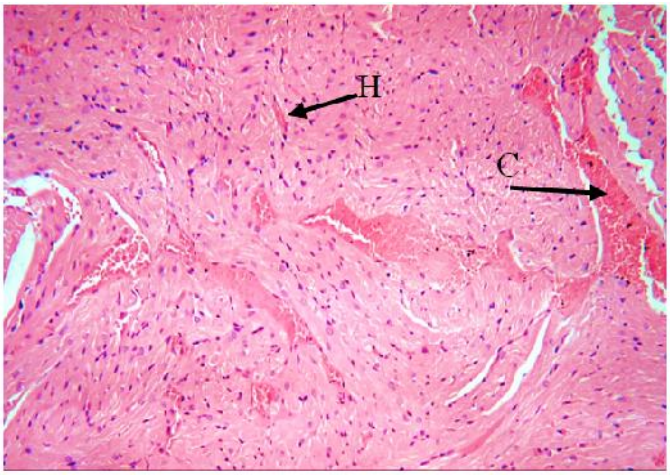

Figure 6 After 2 weeks the heart tissue in group 2, that was orally gavage with Caffeine 64.4 mg/kg BW/day, shows congestion CandhemorrhageH (x 100 - H&E).

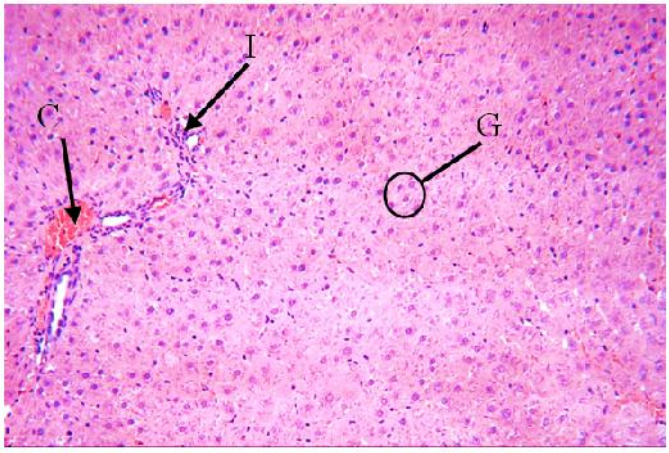

Figure 7 After 2 weeks the liver tissueingroup 3, that was orally gavage with Taurine 300 mg/kg BW/day, shows congestion C, infiltrating lymphocytesI, and cytoplasmicgranulation G (x 100 - H&E).

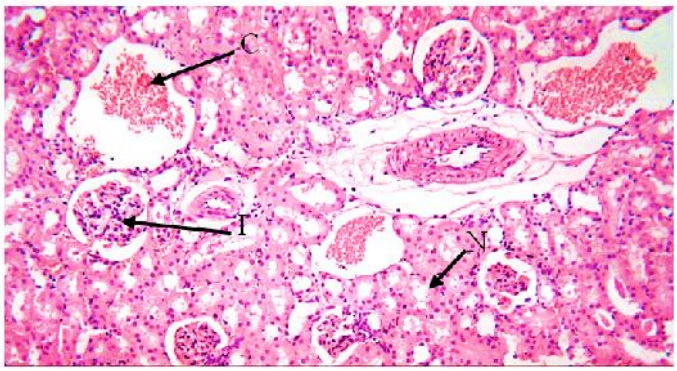

Figure 8 After 2 weeksthe kidney tissueingroup3, that was orally gavage with Taurine 300 mg/kg BW/day, showsnecrosis N, glomerularcongestionC, in addition to a glomerular lymphatic infiltrationI(x 100 - H&E).

Histopathological changes in group 5, which were orally administered withcaffeine32.2 mg/kgBW/day and with taurine300mg/kg BW/day, showed lymphatic infiltration, hemorrhage, congestion, necrosis, and renal glomerulosclerosis (Fig.19). Besides, acute lymphatic infiltration, zonal hepatocytes necrosis, hemorrhage, and congestion can be seen in the liver (Fig.18), in addition to hemorrhage and congestion that can be seen in the heart sections (Fig.20).

Table 2 Changes in mean BW and the gained BW of rats exposed to different doses of caffeine and taurine for 2 and 4 weeks.

Treatment groups	Initial body weight(g)	Body weight (g)	Body weight gain (g)
2 weeks			
Control (normal)	150.6± 1.6 ^{NS}	179.1± 2.7 ^{NS}	12.5 ± 2.1 ^{NS}
Caffeine (32.2mg/ kg/ bw)	155.5± 0.6 ^{NS}	160.8± 3.6 ^{NS}	10.8 ± 4.2 ^{NS}
Caffeine (64.4mg/ kg/ bw)	158.3± 1.8 ^{NS}	166.8± 0.5 ^{NS}	11.5 ± 3.9 ^{NS}
Taurine (300mg/ kg/ bw)	156.5± 0.7 ^{NS}	176.8± 2.7 ^{NS}	19.3 ± 5.3 ^{NS}
Taurine (600mg/ kg/ bw)	150.8± 0.6 ^{NS}	159.1± 4.2 ^{NS}	22.5 ± 4.9 ^{NS}
Caffeine (32.2mg/ kg/ bw) + Taurine (300mg/ kg/ bw)	159.5± 0.6 ^{NS}	172.8± 3.6 ^{NS}	10.3 ± 5.6 ^{NS}
Caffeine (64.4mg/ kg/ bw) + Taurine (600mg/ kg/ bw)	155.3± 2.8 ^{NS}	165.8± 5.5 ^{NS}	11.5 ± 2.6 ^{NS}
4 weeks			
Control (normal)	179.1± 2.7 ^{NS}	212.4± 3.2 ^{NS}	18.5 ± 5.1 ^{NS}
Caffeine (32.2 mg/ kg/ bw)	160.8± 3.6 ^{NS}	185.2± 2.1 ^{NS}	12.6 ± 2.3 ^{NS}
Caffeine (64.4 mg/ kg/ bw)	166.8± 0.5 ^{NS}	187.1± 3.1 ^{NS}	11.5 ±4.3 ^{NS}
Taurine (300 mg/ kg/ bw)	176.8± 2.7 ^{NS}	198.2± 4.7 ^{NS}	12.4 ± 2.1 ^{NS}
Taurine (600 mg/ kg /bw)	159.1± 4.2 ^{NS}	176.8± 2.5 ^{NS}	16.8 ± 7.2 ^{NS}
Caffeine (32.2mg / kg / bw) + Taurine (300mg/ kg/ bw)	172.8± 3.6 ^{NS}	195.6± 6.9 ^{NS}	18.3 ± 8.2 ^{NS}
Caffeine (64.4mg/ kg/ bw) + Taurine (600mg/ kg/ bw)	165.8± 5.5 ^{NS}	180.8± 2.3 ^{NS}	15.5 ± 5.6 ^{NS}

Values are means ± SE; NS= Not Significant

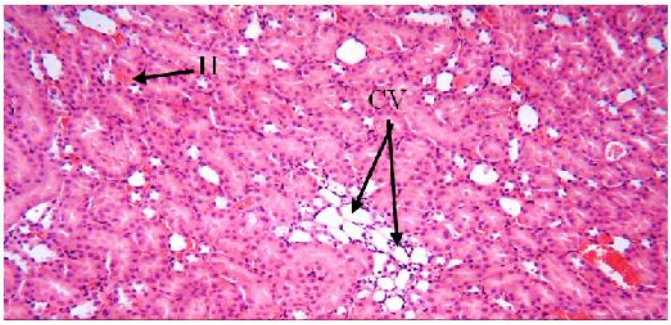

Figure 9 After 2 weeks the kidney tissueingroup 4, that was orally gavage with Taurine 600 mg/kg BW/day, showshemorrhage H, and Cytoplasmic vacuolation CV(x 100 - H&E).

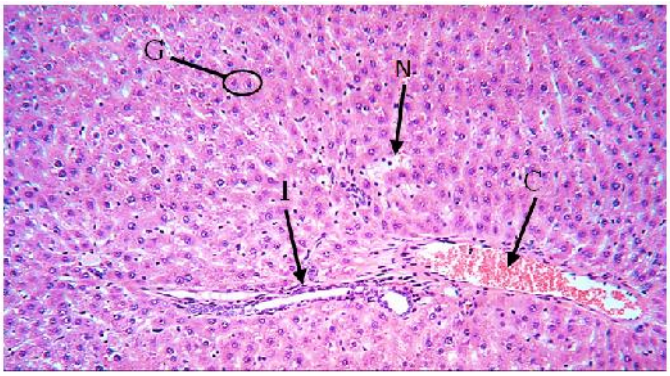

Figure 10 After 2 weeksthe kidney tissueingroup 6, that was orally gavage with Caffeine 64.4 mg/kg BW/day +Taurine 600 mg/kg BW/day, showsnecrosis N, congestionC, cytoplasmicgranulation G, and fewlymphatic infiltrationsI(x 100 - H&E).

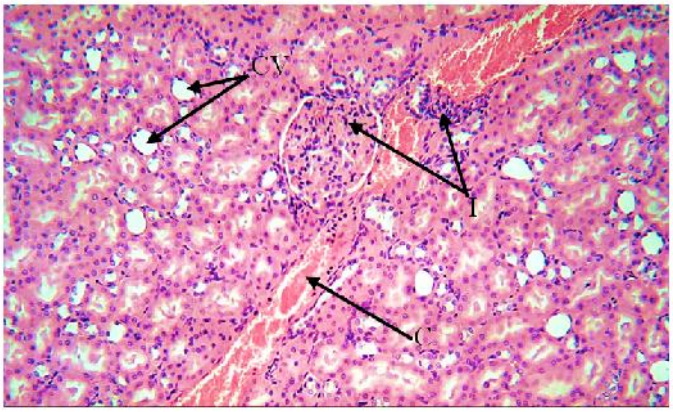

Figure 11 After 2 weeksthe kidney tissueingroup 6, that was orally gavage with Caffeine 64.4 mg/kg BW/day +Taurine 600 mg/kg BW/day, showscongestionC, lymphatic infiltrationsI, and cytoplasmic vacuolation CV(x 100 - H&E).

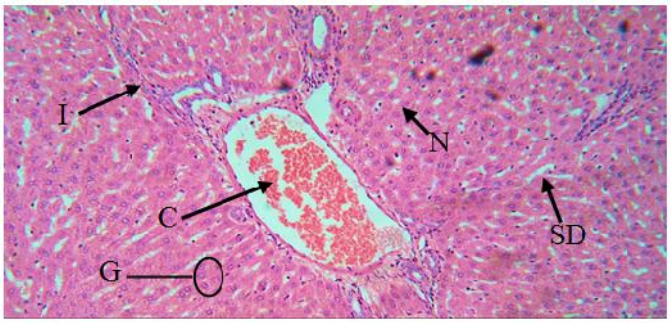

Figure 12 After 4 weeksthe liver tissue in group 1, that was orally gavage with Caffeine 32.2 mg/kg BW/day, shows necrosisN, congestion C, sinusoidal dilatation SD, cytoplasmicgranulation G, and few lymphatic infiltrations I(x 100 - H&E).

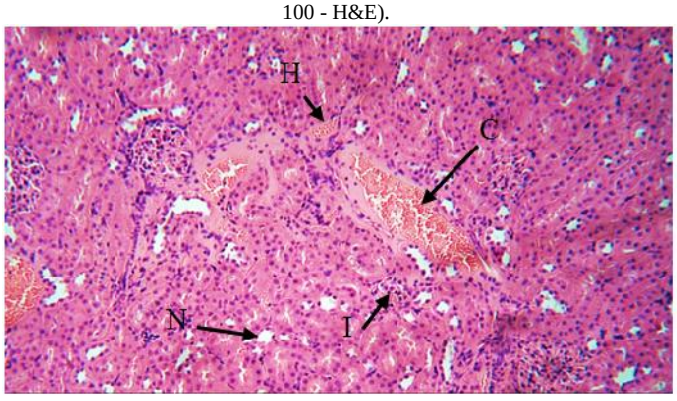

Figure 13 After 4 weeksthe kidney tissue in group 1, that was orally gavage with Caffeine 32.2 mg/kg BW/day, showsnecrosis N, congestion C, hemorrhage H, and lymphatic infiltrations I(x 100 - H&E).

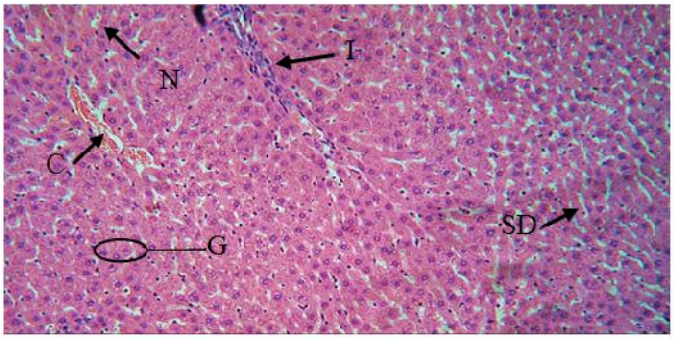

Figure 13A After 4 weeks the liver tissue in group 2, that was orally gavage with Caffeine 64.4 mg/kg BW/day, showsnecrosis N, congestion C, sinusoidal dilatation SD, cytoplasmicgranulation G, and few lymphatic infiltrations I(x 100 - H&E).

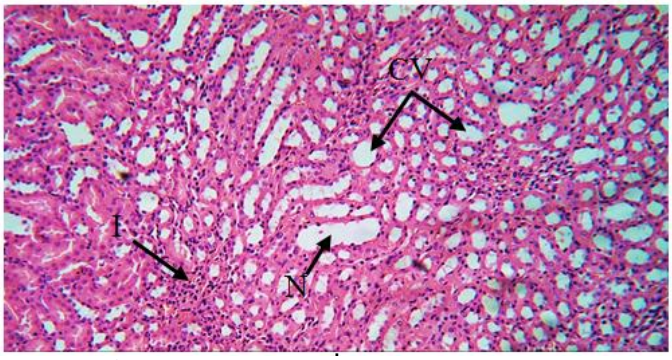

Figure 14 After 4 weeks the kidney tissue in group 2, that was orally gavage with Caffeine 64.4 mg/kg BW/day, showstubular necrosisN, lymphocytic infiltration I, and Cytoplasmic vacuolation CV(x 100 - H&E).

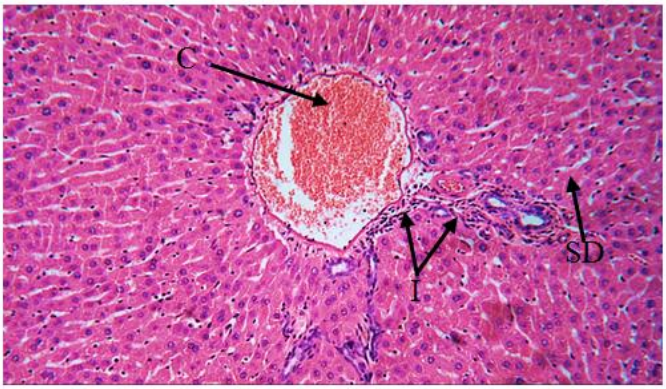

Figure 15 After 4 weeksthe liver tissue in group 3, that was orally gavage with Taurine 300 mg/kg BW/day, showslymphocytic infiltration in the portal region I, congestion in the portal veinC and sinusoidal dilatation SD.(x 100 - H&E).

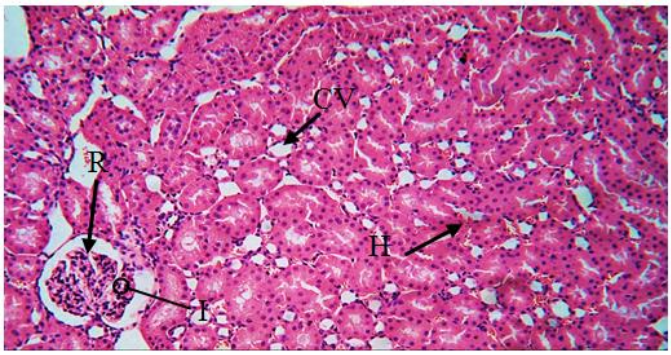

Figure 16 After 4 weeksthe kidney tissue in group 3, that was orally gavage with Taurine 300 mg/kg BW/day, after 4 weeks, shows Glomerular lymphocytic infiltration I, hemorrhage H,Cytoplasmic vacuolation CV, and the glomerulus revealed retraction of capillary tufts and segmental wrinkling of membranes R.(x 100 - H&E).

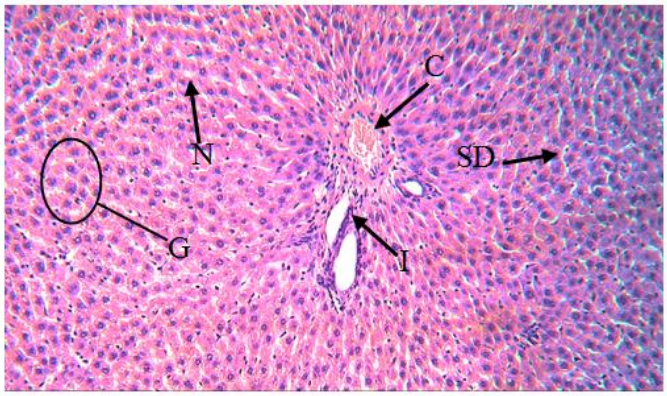

Figure 17 After 4 weeks the liver tissue in group 4, that was orally gavage with Taurine 600 mg/kg BW/day, showsnecrosis N, congestion in the portal vein C, sinusoidal dilatation SD, sever cytoplasmicgranulation G, and few lymphatic infiltrationsin the portal region I.(x 100 - H&E).

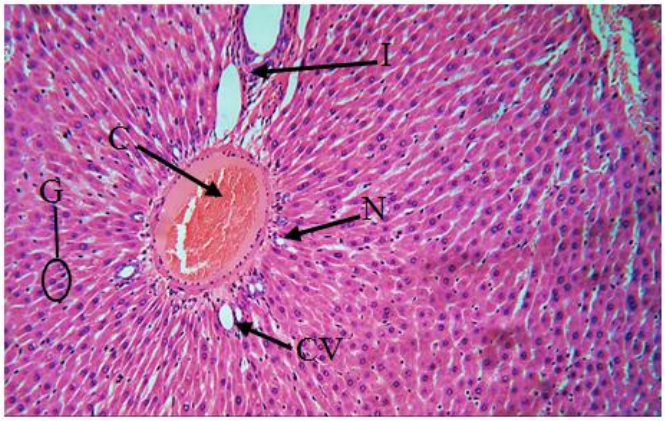

Figure 18 After 4 weeks the liver tissue in group 5, that was orally gavage with Caffeine 32.2 mg/kg BW/day+Taurine 300 mg/kg BW/day, showsnecrosis N, congestion in the portal vein C, sever cytoplasmicgranulation G, Cytoplasmic vacuolation CV and few lymphatic infiltrationsin the portal regionI. (x 100 - H&E).

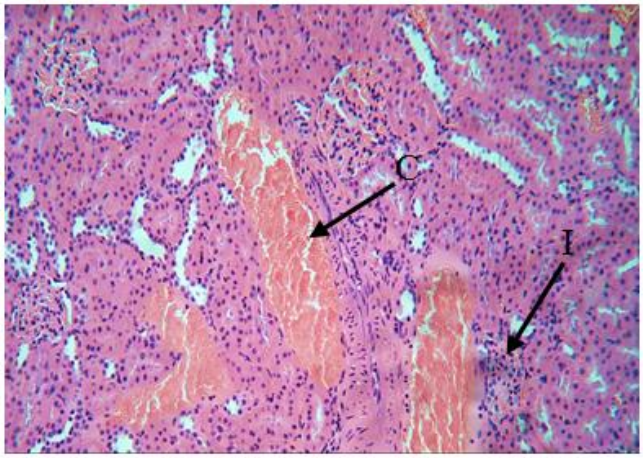

Figure 19 After 4 weeks the kidney tissue in group 5, that was orally gavage with Caffeine 32.2 mg/kg BW/day +Taurine 300 mg/kg BW/day, showssever congestionC and lymphocytic infiltration I(x 100 - H&E).

The rats of group 6, which were dosed with caffeine 64.4 mg/kg BW/day together with taurine 600 mg/kg BW/day, have shown lymphatic infiltration, dilated sinusoids, hemorrhage, congestion, and focal zonal hepatocytes necrosis (Fig.15). Besides, lymphatic infiltration, hemorrhage, and congestion can be seen in the kidneys and heart (Fig.20).

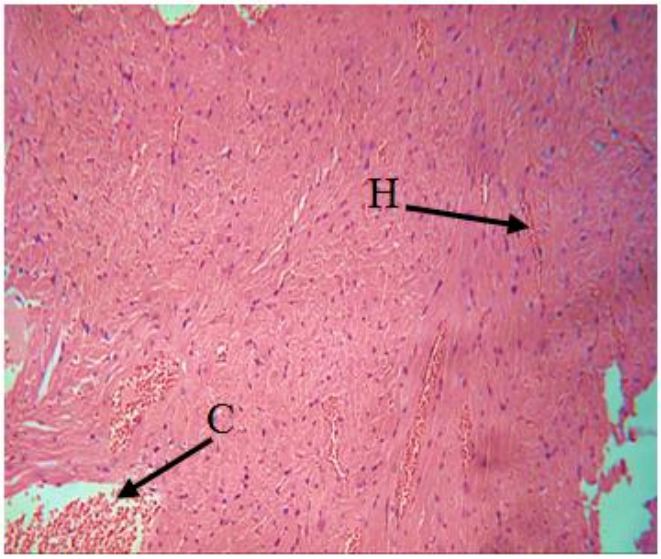

Figure 20 After 4 weeks the heart tissue in group 5, that was orally gavage with Caffeine 32.2 mg/kg BW/day +Taurine 300 mg/kg BW/day, shows congestionC, and hemorrhageH in the cells(x 100 - H&E).

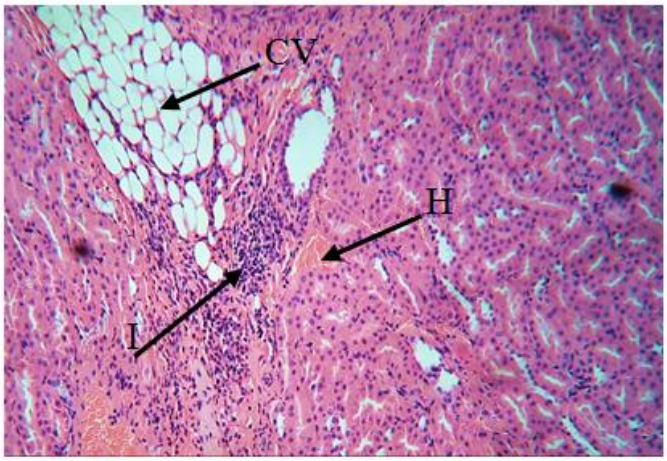

Figure 21 After 4 weeks the kidney tissue in group 6, that was orally gavage with Caffeine 64.4 mg/kg BW/day +Taurine 600 mg/kg BW/day, shows a hemorrhage H, lymphocytic infiltration I, and Cytoplasmic vacuolation CV(x 100 - H&E).

Four weeks after the beginning of the experiment, congestion in the portal vessel, lymphatic infiltration, dilated sinusoids, hemorrhage, and necrosis in some hepatocytes were observed in rats of group 1, which were dosed with caffeine 32.2 mg/kg BW/day (Figure 13A). Kidney sections also showed dilated sinusoids, hemorrhage, congestion, necrosis, and glomerular hemorrhage (Fig 13). The rats of group 2, which were dosed with caffeine 64.4 mg/kg BW/day, have shown lymphatic infiltration, hemorrhage, and congestion in the heart. In addition to severe necrosis, dilated sinusoids, lymphatic infiltration, hemorrhage, congestion, and necrosis in some hepatocytes (Fig.13A). The renal tissue showed glomerular hemorrhage, lymphatic necrosis, and congestion (Fig 14). Furthermore, there were lymphatic infiltration, hemorrhage, and congestion in the heart tissue. Histopathological changes were observed in the rats of group 3, which were dosed with taurine 300mg/ kg / BW. Lymphocytic infiltration, hemorrhage, congestion, macular degeneration of the glomeruli were observed in the kidneys (Fig 16). In addition to dilated sinusoids, lymphatic infiltration and zonal hepatocytes necrosis, hemorrhage, and congestion were observed in the liver (Fig. 15). Moreover, congestion, hemorrhage, and lymphatic infiltration were observed in the heart.

Table 3 Changes in haematological parameters in rats exposed to different doses of caffeine and taurine for 2 and 4 weeks

Parameters	Control	CAF (32.2 mg\kg)	CAF (64.4 mg\kg)	TA (300 mg\kg)	TA (600 mg\kg)	CAF (32.2 mg\kg) + TA (300 mg\kg)	CAF (64.4 mg\kg) + TA (600 mg\kg)
2weeks							
WBCs(10^3mm^3)	2.77± 0.22	3.97 ± 0.49 ^{NS}	3. 6± 0.42 ^{NS}	5.3 ± 1.66*	3.5±0.59*	4.43±2.09*	3.03±0.54*
Hb (g/dL)	13.97±0.38	14.3±0.42 ^{NS}	13.97±0.24 ^{NS}	13.27±0.15 ^{NS}	13.13± 1.22 ^{NS}	14.2± 0.31 ^{NS}	13.93± 0.32 ^{NS}
RBC (10^6mm^3)	7.71±0.22	7.51±0.01 ^{NS}	8.21±0.14*	7.1±0.47 ^{NS}	7.75±0.22 ^{NS}	8.1±0.19*	7.66±0.16 ^{NS}
PCV (%)	45.3±1.42	43.97±0.94 ^{NS}	44.97±0.59 ^{NS}	41.1±2.56 ^{NS}	43.63±2.95 ^{NS}	44.73±0.61 ^{NS}	45.2±0.87 ^{NS}
MCV (m^3)	58.77±0.15	58.53±0.9 ^{NS}	54.77±0.95 ^{NS}	57.9±1.12 ^{NS}	56.23±2.89 ^{NS}	55.27±1.85 ^{NS}	59.03±1.58 ^{NS}
MCH (pg)	18.13±0.13	19.03±0.8 ^{NS}	17.0±0.35^{NS}	18.9±1.14 ^{NS}	16.93±1.26 ^{NS}	17.53±0.72 ^{NS}	18.17±0.2 ^{NS}
MCHC (%)	30.87±0.23	32.57±1.43*	31.07±0.15*	32.57±1.77*	30.03±0.90 ^{NS}	31.67±0.28*	30.77±0.58 ^{NS}
4 weeks							
WBCs(10^3mm^3)	4.49±2.18	5.7± 0.31*	5.41±1.2*	6.35±0.22*	5.67±0.54*	6.32±0.64*	5.9±1.56*
Hb (g/dL)	13.9± 0.31	13.83±0.22 ^{NS}	13.17± 0.66 ^{NS}	14.3±1.0 ^{NS}	14.97±0.47 ^{NS}	14.3± 0.36 ^{NS}	14.17± 0.38 ^{NS}
RBC (10^6mm^3)	7.64±0.08	7.58±0.09 ^{NS}	6.74±0.03 ^{NS}	7.42±0.18 ^{NS}	7.41±0.63 ^{NS}	7.88±0.22 ^{NS}	7.67±0.1 ^{NS}

The rats of group 4, which were dosed with taurine 600 mg/kg BW/day, have shown lymphocytic infiltration, dilated sinusoids, hemorrhage, congestion, and necrosis in some liver cells (Fig. 17), in addition to lymphatic infiltration, hemorrhage, congestion, necrosis, and glomerular nephropathy. Congestion, hemorrhage, and lymphatic infiltration were observed in the heart. Histopathological changes were observed in the rats of group 5, which were dosed with caffeine 32.2 mg/kg / BW together with 300mg/kg / BW/day. These changes include lymphocytic infiltration, hemorrhage, congestion, necrosis, and glomerulosclerosis in kidneys (Fig. 19). The liver tissue changes include portal vessel congestion, lymphocytic infiltration, dilated sinusoids, focal hepatocytes necrosis, hemorrhage, and congestion (Fig. 18). Besides, lymphatic infiltration, hemorrhage, and congestion were observed in the heart tissue (Fig. 20). The rats of group 6, which were dosed with caffeine 64.4 mg/kg BW/daytogether with taurine 600 mg/kg BW/ day, have shown lymphocytic infiltration, dilated sinusoids, hemorrhage, congestion, and focal necrosis hepatocytes. Besides, lymphatic infiltration, hemorrhage, congestion, necrosis, and glomerular hemorrhage can be seen in the kidneys (Fig. 21). Moreover, heart sections showed lymphatic infiltration, hemorrhage, and congestion.

Hematological changes

After two weeks of treatment (Table 2), WBCs in groups (4, 5, 6, 7) and RBC in groups 3 and 6 and MCHC in groups 2, 3, 4, and 6 were higher ($p < 0.05$) than in the control groupand the values of Hb, MCH and MCV, have not changed. After four weeks of the experiment, WBCs in groups 2 - 7 were higher ($P < 0.05$) than the control group, and there was no statistically significant change in the values of HB, PCV, MCV, MCH, MCHC, and RBC.

Serobiochemical changes

These data were presented in Table 3. After two weeks of treatment (Table 3), ALT, ALP, AST, LDH, and CK activities in groups (2-7) ($P < 0.05$) were lower than in the control group, and Cholesterol and Urea concentrations were in groups (2-7) ($P < 0.05$) were lower than in the control group, (group 1). The concentrations of globulin and albumin in groups (3, 4, and 7) ($P < 0.05$) were lower than in the control group (group 1). There was no statistically significant change in total protein concentration.

After four weeks of the experiment, ALT, ALP, AST, LDH, and CK activities in groups (2-7) ($P < 0.05$) were higher than in the control group. Cholesterol and urea in groups (2-7), globulin in groups (3, 4, 5, and 6), and albumin concentrations in groups (4, 5, and 7) ($P < 0.05$) were higher than the control group (group 1). The concentration of albumin in groups (3and 6) ($P < 0.05$) was lower than in the control group. There was no statistically significant change in total protein concentration.

DISCUSSION

In general, no difference was observed in the average body weight between the rat groups during the experiment. This result might be due to the same food supply received by all animals. Caffeine may cause harmful health effects at high levels by altering the cardiovascular system's function, leading to a calcium imbalance and increased risk of cancer and even death (Cappelletti *et al*., 2018; Peacock, 2010; Van Hemelrijck *et al*., 2013).The lack of adequate studies on caffeine intake's long-term effects, caffeine consumption should be considered with caution (Nawrot *et al*., 2003).

Energy drinks have not been evaluated empirically, although many young adults routinely use them. Studies are limited to the individual components of beverages, most of which are in an initial phase, except for caffeine, whose mode of action and operation method has been fully explained. There is the same scarcity of studies for taurine and other components in energy drinks and the cumulative effects of these substances with other products such as alcohol or drugs (Schuchowsky *et al*., 2017). The natural value of total cholesterol can be explained by the presence of taurine, the amino acid whose function is to maintain cholesterol solubility by attaching it to some of the bile salts, thus improving its ability to digest (Chen *et al*., 2016). However, caffeine will induce a dose-dependent increase in total cholesterol, HDL, and LDL(Du *et al*., 2005). According to Onuegbu and others' study on the biochemical profiles of healthy men and women who ingest 2g of coffee daily for 30 days, some biochemical markers have increased, such as AST, ALT, alkaline phosphatase (ALP), and total proteins(Onuegbu *et al*., 2011). Another study shows that energy drinks also affect the concentration of creatinine, uric acid, albumin, and total protein (Worthley *et al*., 2010).

The toxic substances break down the peroxide in the fatty tissue, leading to liver cells' infiltration(Duru, Amadi, *et al*., 2012). It has been reported that tissue damage is usually

associated with the release of specific enzymes to tissues or organs involved in circulation(Duru, Agomuo, *et al*., 2012). According to (Emmanuel and others., 2017), cell-derived enzymes have significant activity in cells and only leak into the plasma when cells are damaged or increased in enzymes' production(Emmanuel *et al*., 2017). According to Ranjna (Ranjna, 1999), the increase of AST and ALT enzymes' activities is an indicator of liver damage caused by liver exposure to toxic substances (Chawla, 1999). However, ALT is the most specific liver enzyme when the hepatic membrane's integrity is compromised(Moss & Henderson, 1996). The increased transaminase levels of test rats can be linked to the control group, as observed in the current study of caffeine consumption. The mechanism behind enzyme leakage can be related to caffeine's oxidative effect in rats' fatty liver tissue, as reported by Dianzaniand others (Dianzani *et al*., 1991). The significant increase in ALT activity among the test groups noted in the current study could be evidence that the cause of the toxicity is caused by caffeine. According to Emmanuel and others, alkaline phosphatase (ALP) is found in the liver besides the bile ducts and the bones(Emmanuel *et al*., 2017). It leaks into the bloodstream like the ALP and AST. It is associated with the prostate (Urgert *et al*., 1995). (Urgertand others., 1995) found that the activity of the alkaline phosphatase enzyme (ALP) in rats that consumed caffeine was not consistent with the current study results.

Liver enzymes were assumed to be the target of caffeine or other components of coffee(Nyblom *et al*., 2006). Different authors have observed that assessing levels of metabolites such as urea and creatinine may be used to evaluate kidney function(Kolawole *et al*., 2014). Urea is the primaryend product of protein destruction (Harper and Mayers, 1975). Amino acid deamination occurs in the liver, which is also the urea cycle site where ammonia is converted to urea and excreted by urine (Kolawole *et al*., 2014). According to (Ranjna, 1999), renal diseases that reduce glomerular filtration led to urea retention. The production of white blood cells, known as leukocytes, this study suggests that the immune system stimulates the immune system through caffeine, which may have protected rats against any chemical or secondary infection. It was observed that increasing the number of leukocytes may be directly proportional to the stress condition's severity (Adebayo *et al*., 2010). This result is contrary to the outcome of the current study.

According to (Adebayo *et al*., 2010) and (Duru *et al*., 2012b), MCV, MCHC, and MCH values are related to individual red blood cells(Adebayo *et al*., 2010; Duru, Agomuo, *et al*., 2012). A significant increase may mean that hemoglobin has been incorporated into red blood cells, morphology, and osmotic fragility of red blood cells. The change in maternal and child health is due to anemia normocytic or hypochromic anemia(Hassan *et al*., 2010; Keele *et al*., 1983). These vulnerabilities can be possible with caffeine consumption, as PCV and MCHC levels for test rats have been significantly altered in the current study.

Taurine (TA) is known to protect the immune system from oxidative stress by preventing DNA damage and apoptosis in lymphocytes(Sokól *et al*., 2009). This process has contributed to maintaining total protein concentrations, albumin, globulin, albumin / Globulin in TA + CPF + LA group. The TA also shows some effects on the liver (El-Sayed *et al*., 2011) and shows nephroprotective results (Das & Sil, 2012). It is worth noting that TA's hepatoprotective property is due to its ability to reduce oxidative stress, Mitochondrial function enhancement, cytoplasmic modification, and Ca2 + balance in biological systems (Asha & Devadasan, 2013). TA protection may also be its ability to become chlorinated with hypochlorous acid, thereby preventing the direct attack of this oxidant on cell membranes in organs, including kidneys(Roy *et al*., 2009). Thus, this is contrary to the result obtained in the present study, where TA activity increased ALT ALP in serum and concentration of total cholesterol, albumin, globulin, and urea.

TA is known to compensate lipid peroxide either by direct ROS scanning or by binding iron or copper ions through the sulfonic acid group. These TA mechanisms may have contributed to the reduction of lipid peroxidation in the liver and kidneys of mice(Hagar, 2004).

Kohashi and others have pointed out that taurine may participate in lipid metabolism by subtracting the daily urine taurine and serum HDL-C(Kohashi *et al*., 1983).Another study by Chen showed no statistically significant change in the concentration of cholesterol and creatine in serum rats, meaning that cholesterol was not significantly affected by taurine(Chen, 1993). For the result of the current study, where total cholesterol rose.Taurine has been shown to act as a direct antioxidant that scrapes or contract oxygen-free radicals, thereby inhibiting lipid peroxidation. As an indirect oxidant inhibitor, it prevents increased membrane permeability caused by oxidative injury in many tissues, including the liver(Chen, 1993). Taurine may stimulate S-nitrosylation production from the GSH S-nitrosoglutathione, which is approximately 100 times stronger than the conventional GSH. Besides, s-nitrosylation of cysteine residues can be produced by caspase-3, thus causing apoptosis (Chiueh & Rauhala, 1999).

Similarly, taurine may reduce cholesterol oxidation by forming chlorine, as more stable and less interacting molecules with HOCl and HOCl-metalloproteins, or by free metal ions such as Fe^{2+} to its sulfonic acid group(Schuller-Levis *et al*., 1994). As an indirect antioxidant, taurine has been proposed as a membrane fixator, where it can maintain membrane regulation, leakage of ions, and water flow, thereby avoiding cell proliferation(Chen, 1993). It was also suggested that the effect of Taurine stability on the cell membrane was assigned to the interaction between taurine and polyunsaturated fatty acids in the membrane, leading to an increase in taurine's affinity for transport and interaction between taurine and sites associated with anion transport and water flow. This characteristic of taurine may be partially explained to protect it from necrosis of oxidized liver cells caused by cholesterol. This result is contrary to the outcome of the current study. The observations in many of these studies are additional proof and support for our findings.

CONCLUSIONS

The study concluded that the liver, kidney, and heart are sensitive organs to the toxic effect of taurine and caffeine due to the generation of reactive oxygen species that cause damage to many components of the cell membrane. (liver, kidney, and heart) changes included in the liver congestion in the portal vein and lymphatic infiltration and sinusoidal dilatation Hemorrhage and necrosis observed in liver, tubular congestion, congestion of capillary tubes, hemorrhage, glomerular degeneration, and necrosis in the kidney,

hemorrhage, and congestion in the heart, as well as minor changes The toxic effects of concurrent caffeinated drinks and taurine intake were more toxic to rats compared to their toxicity alone.

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