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EFFECT OF CAFFEINE ON SOME HEMATOLOGICAL, BIOCHEMICAL PARAMETERS, LIVER AND KIDNEY HISTOLOGY IN WISTAR RATS

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ABSTRACT

Caffeine is likely the most pharmacologically active substance that is frequently consumed in the world. It is commonly found in beverages (like coffee and tea) and products that contains cocoa or chocolate and most widely used in medications. Consumption of caffeine has been reported to cause varying multi systemic effects. This study investigates the effect of caffeine on some liver enzymes and kidney function biomarkers, hematological parameters and histology of liver and kidneys. A total of 24 male Wistar rats aged 6-8 weeks weighing between 120-150 grams were obtained and randomized into 3 groups of 8 animals each, (n=8). Animals in group 1 served as controls and were fed with animal feed and water ad libitum, group 2 and group 3 received caffeine orally alongside their feed and water in 32.2 mg/kg and 64.4 mg/kg respectively for a period of 28 days. Five (5) ml of blood was collected after anesthetizing and sacrificing the animal. Biochemical parameters were assessed using spectrophotometry and hematological parameters were assessed using automated auto analyzer. SPSS version 20 was used for data analysis, values were presented as Mean ±SEM and analyzed using one-way analysis of variance (ANOVA), $p<0.05$ was considered significant. This study observed significant increase in potassium as an indicator of kidney function impairment. There was also an increase in AST, Alkaline phosphatase, Total and Direct bilirubin levels in group 2 animals when compared to group 1 and control group indicating liver derangement in group 2 animals. However, there was no significant change observed in the hematological parameters. The study also observed numerous inflammatory cells in the in the liver histology. Kidney histology showed, vacuolations in the kidney cells with enlargement of bowman's capsule. The collective findings of this study showed that caffeine consumption increases the risks of liver and kidney injuries but has no significant effect on hematological parameters.

Keywords: Caffeine, Histology, Kidney parameters, Liver parameters
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INTRODUCTION

Caffeine is the one of the most widely consumed pharmacologically active substances in the world [1]. It is an alkaloid compound naturally found in tea leaves, cocoa beans, nuts such as bitter kola and kola nut, and fruits of more than sixty plants [2]. Industrially, extract of caffeine is used in the production of soft drinks, coffee, and tea and in some processed foods [3]. Caffeine consumption is mostly associated with socio-cultural factors, because it is not linked with any nutritional values and it is not essential for carrying out any biological function [4]. Due to its wide presence in a variety of foods, drinks, and dietary supplements, the prevalence of daily caffeine consumption can reach up to 80% of the world's population [5].

Caffeine is rapidly absorbed after its intake with a bioavailability of approximately 99% and distributed in most tissues, including the brain, and then metabolized in the liver and excreted via the kidneys, mostly in the form of its metabolites [6].

Although caffeine has been commonly used since ancient times, it is still being studied as a very controversial substance. Scores of studies published in the literature regarding this substance convey both its beneficial and toxic effects on health. In recent years, however, several studies have attempted to establish the link between caffeine and oxidative status, as many of the conditions in which caffeine has been shown to have a beneficial influence can be attributed to a decrease in oxidative stress [7].

Oxidative stress is defined as an imbalance between the production of reactive oxygen species (ROS) and the ability of extracellular and intracellular protection systems to neutralize them. A growing number of systemic effects and medical conditions including but not limited to Liver and Kidney dysfunctions are correlated with the increase in the level of ROS [8].

Caffeine mediates most of its effect by blocking the adenosine A_1 & A_2 receptors [9]. It is known to increase the secretion of epinephrine leading to various metabolic changes that can result to changes in body functions [10]. This study is therefore aimed at investigating the effect of caffeine on some

hematological parameters, serum levels of some liver and kidney functions biomarkers, liver and kidney histology in male Wistar rats.

MATERIALS AND METHODS

Materials

Caffeine sample used in this study was gotten from Ceman scientific limited Kano, Nigeria, centrifuge machine, EDTA bottle, plain bottle, hematology auto-analyzer, using Randox ALT/AST kits (Randox, Crumlin, UK), Randox ALP kit, Semi-automated biochemistry analyzer (Mindray BA-88A, Shenzhen, China), leica DM 750 microscope.

Experimental design

A total of 24 Wistar rats were obtained from the animal house of faculty of pharmaceutical sciences, Bayero University, Kano. The rats were housed in metallic cage (38 × 46 × 24) with saw dust as bedding under standard atmospheric conditions and allowed free access to standard food (rat pallet) and water *ad libitum*. The rats were acclimatized in the laboratory for period of two weeks (2 weeks) before the commencement of study. Caffeine solution was made by dissolving 1 g of the caffeine powder in 2ml of water [11]. Animals were then divided into 3 groups of 8 each. Group 1 served as the control group and was fed only animal feed and water while groups 2 and 3 served as the experimental groups and were administered caffeine orally in low 32.2 mg/kg and high 64.4 mg/kg doses respectively for 28 days [12].

Ethical clearance

The study approval was obtained from Bayero University Animal Care and Use Research Ethics Committee (ACUREC) with animal use protocol: BUK/ACUREC/CAP/UG13. All the experiments were carried out in compliance with the guide for the care and use of laboratory animals.

Blood sample collection

Five (5) ml of blood was collected from dorsal pedal vein in the morning between 8-9 am with 5 ml syringe, 2 ml of the collected blood sample was stored in an EDTA bottle for hematological analysis while 3 ml was stored in plain bottle and allowed to coagulate. After coagulation the sample was centrifuged for 5 minutes at 3000 rpm and the serum was collected and stored at -21 degree Celsius, and was later used for the determination of liver and kidney function biomarkers.

Determination of hematological parameters

The autoanalyzer machine was used for hematological analysis. The blood samples contained in the anticoagulant tubes were swirled/rolled on the blood roller each for five seconds and then opened and put under the probe of the autoanalyzer. The probe then collected the blood from the tubes for about 10 seconds and entered back into the hematology autoanalyzer machine. The result was then printed a few seconds later, giving the parameters. PCV was estimated using micro hematocrit method as described by a previous study [13].

Determination of liver enzymes and kidney function parameters

The serum activities of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) as indexes of liver injury were determined using Randox ALT/AST kits (Randox, Crumlin, UK). The principle of the ALT & AST assays was based on the production of brown-colored hydrazone by reaction of pyruvate (product of ALT/AST activity) with 2,4-ditrophenylhydrazine (DNPH). Additionally, as an indicator of biliary tract injury, the serum activity of alkaline phosphatase (ALP) was determined using the Randox ALP kit. The principle of ALP assay was based on the formation of hydrobenzene from ALP-mediated hydrolysis of phenyl phosphate and the subsequent formation of red-colored complexes by potassium ferrocyanide catalyzed reaction of hydrobenzene with 4- aminophenazone. The concentrations of ALT, AST, and ALP were measured by a semi-automated biochemistry analyzer (Mindray BA-88A, Shenzhen, China) at wavelengths of 550 nm, 546 nm, and 520 nm respectively. Total Biluribin, Direct biluribin and kidney function biomarkers were also assessed using their various Randox kits according to manufacturer's guidelines.

Histological analysis

The Liver and Kidney tissue of rats was fixed with 10% formaline, dehydrated with ascending grade of alcohol, cleared with toluene, infiltrated with molten paraffin wax. The microtome 40 sections were stained with haematoxyline and eosin staining technique and examined with leica DM 750 microscope and photographed with leica ICC 50HD camera [14].

Statistical analysis

Data was analyzed using statistical package for social sciences SPSS version 25. Quantitative variables were summarized using mean and standard error of mean. One-way analysis of variance (ANOVA) and Turkey *post-hoc* were used for multiple comparison. *P* values less than 0.05 were considered to be statistically significant ($p > 0.05$).

RESULTS AND DISCUSSION

Figures 1, 2 and 3 showed the findings of RBC, WBC, and PCV of rats administered caffeine and their control groups. The results observed no significant difference in RBC, WBC and PCV ($p>0.05$). This finding contradicts the result of Emmanuel *et al.* [15], who reported significant increase in the WBC of the caffeine administered groups. The contradiction may be attributed to the difference in the dose of caffeine administered and difference in environmental

conditions which might likely subject the animals used in their study to other foreign organism that may have triggered body's inherent response associated with increase in white blood cells production. The serum levels of liver enzymes and bilirubin were depicted on Table 1.

Findings of the study observed no significant difference in alkaline phosphatase across all the groups. The study also observed significant increase in AST, ALT and TB of group 3, when compared to both groups 1 and 2 ($p<0.05$). However, there was a significant higher value in TB of group 3 when compared to group 2 only ($p<0.05$).

The liver enzymes form the central core of functional markers of liver health. Changes in the levels of the liver enzymes level in the serum serves as a pointer to a short-term or long-term deleterious effect of caffeine consumption. Findings of this study showed that the effect of caffeine is dose dependent, having grater effect at high doses. Findings of this study opposes the report of Shan *et al.* [20], who reported that higher doses of caffeine decrease liver enzymes indicating beneficial effect. And the effect was also dose dependent. The finding of the present study is in tandem with the findings of Emmanuel *et al.* [15] and that of Suman [16].

The significant increase in transaminase levels of test rats as observed in the present study could be linked to consumption of caffeine. The mechanism behind the enzymes leakage from cytosol could be linked to cellular necrosis, indicating cytotoxicity of the liver tissue of rats [17]. It has been hypothesized that liver enzymes are a target for caffeine or other components of coffee [18]. However, Moawed *et al.* [19] reported protective effect of caffeine on liver injury after it was exposed to toxic effect of CCl_4. The liver enzymes were observed to decrease with administration of Arabic caffeine and their finding may not be un connected with the dose and type of caffeine used in their study.

Findings of this study as displayed on Table 2 showed no significant variation in renal function parameters between all the study groups and the control except in the case of potassium where significant increase was observed between the control and study group that were administered 64.4 mg/kg of caffeine. Different authors have noted that assessment of levels of excretory metabolites such as electrolyte, urea, and creatinine can be used to evaluate renal function [20]. From the findings of this study, it could be stated that caffeine has no significant effect on renal function when consumed in moderate doses but can significantly affect the potassium balance by likely impairing the function of potassium pumps in the renal tubule of the kidney. This finding contradicts the findings of Emmanuel *et al*. [15], who reported significant increase in all renal parameters upon administration of caffeine. On the other hand, Afolabi *et al*. [21], reported a nephron protective effect of caffeine against experimental $AlCl_3$-induced renal toxicity in adult male Wistar rats.

CONCLUSION

The findings of this study concluded that caffeine consumption is not associated with alteration in hematologic parameters. However, there was alteration in liver function parameters with associated increase in serum levels of AST, ALP, DB and TB. Caffeine is also associated with induction of liver inflammation and tissue disintegration in liver and kidney histology respectively.

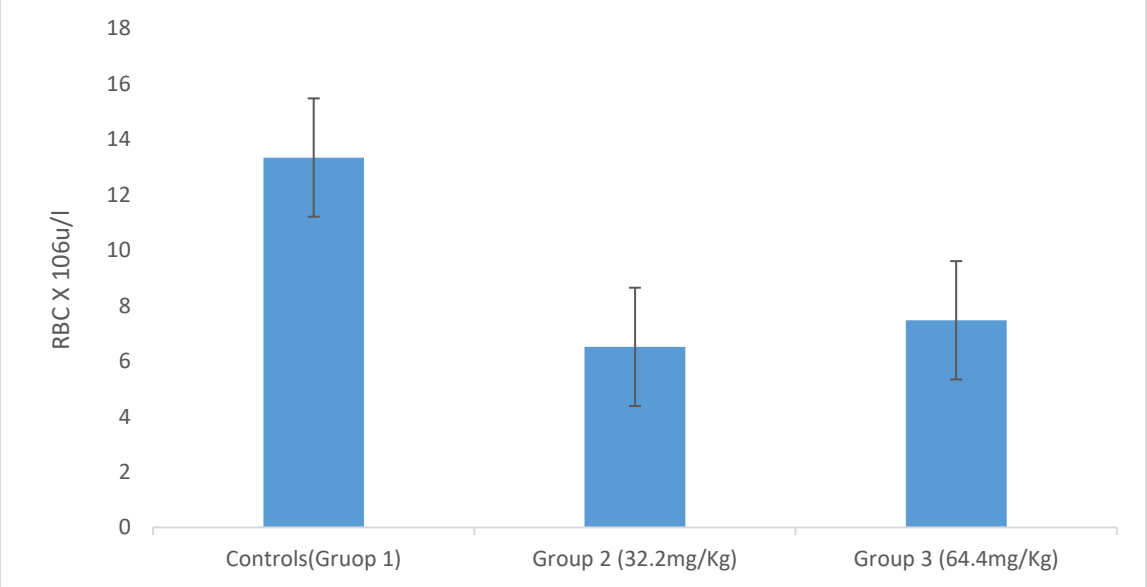

Figure 1: Comparison of red blood cells count (RBC) of rats administered caffeine and control group

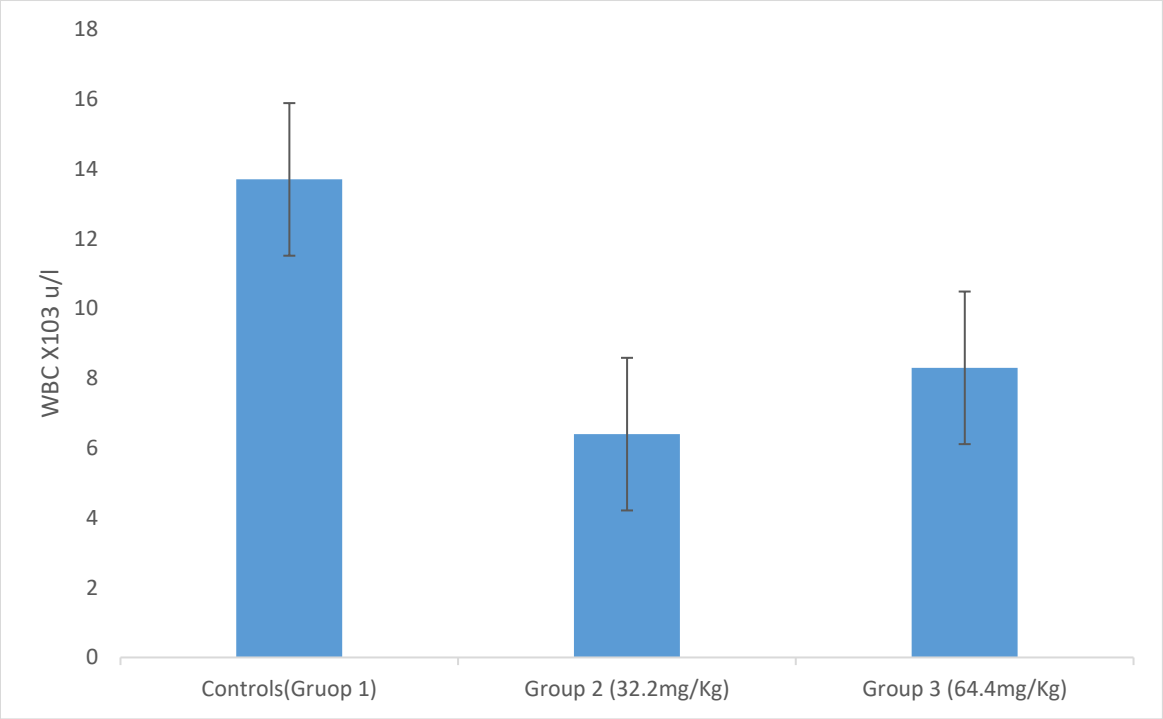

Figure 2: Comparison of white blood cells count (WBC) of rats administered caffeine and control group

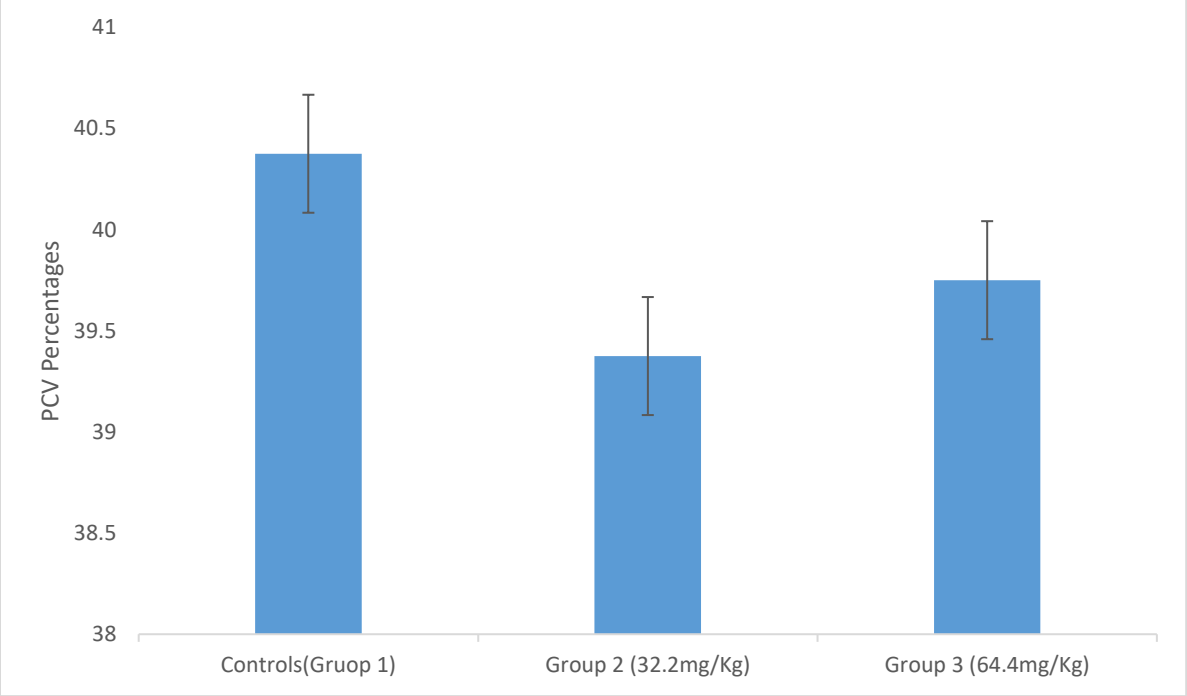

Figure 3: Comparison of packed cells volume (PCV) of rats administered caffeine and control group

Table 1: Comparison of liver enzymes and bilirubin levels of rats administered caffeine and control groups

Parameters (Mean±SEM)	Group 1 (Control)	Group 2 (32.2 mg/kg)	Group 3 (64.4 mg/kg)	F value	P value
ALT(IU/L)	11.37±1.69	10.88±1.90	15.68±2.65	5.664	0.236
AST(IU/L)	20.75±1.780	19.50±2.39	28.13±1.62[a,b]	1.549	0.011
ALP(IU/L)	99.75±2.59	97.63±1.69	112.88±3.77[a,b]	8.603	0.002
TB(IU/L)	0.92±0.07	0.93±0.69	1.23±0.68[a,b]	6.731	0.006
DB(IU/L)	0.44±0.04	0.43±0.31	0.59±0.06[b]	4.260	0.028

ALT-Alanine aminotransferase, AST- Aspartate aminotransferase, ALP-Alkaline phosphatase, TB-Total biluribin, DB - Direct biluribin. [a] when there is significant difference compared to group 1, [b]when there is significant difference compared to group 2.

Table 2: Comparison of renal function parameters between study groups and control

Parameters (Mean±SEM)	**Group 1 (Control)**	**Group 2 (32.2 mg/kg)**	**Group 3 (64.4 mg/kg)**	**F value**	**P value**
Sodium (IU/L)	139.63±1.13	139.50±2.27	136.75±0.96	1.08	0.358
Potassium (IU/L)	4.09±0.07	4.18±0.07	4.36±0.07[a]	4.03	0.033
Chloride (IU/L)	98.75±0.94	97.38±0.96	97.88±0.55	0.68	0.513
Bicarbonate (IU/L)	23.63±1.18	24.00±1.25	23.75±1.67	0.033	0.968
Urea (IU/L)	41.38±1.362	43.50±1.18	41.50±1.49	0.78	0.471
Creatinine (IU/L)	0.70±0.019	0.69±0.035	0.68±0.016	0.60	0.558

$P<0.05$

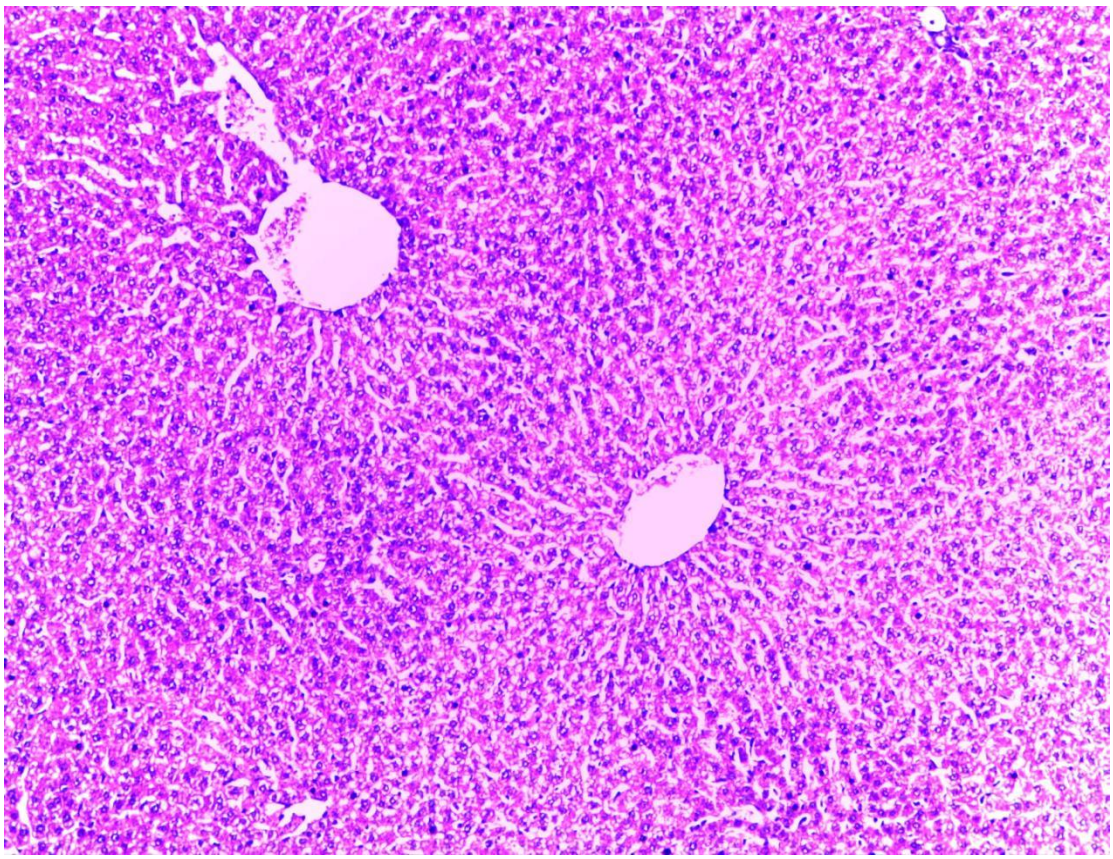

Plate I: Section of unremarkable liver tissue for control

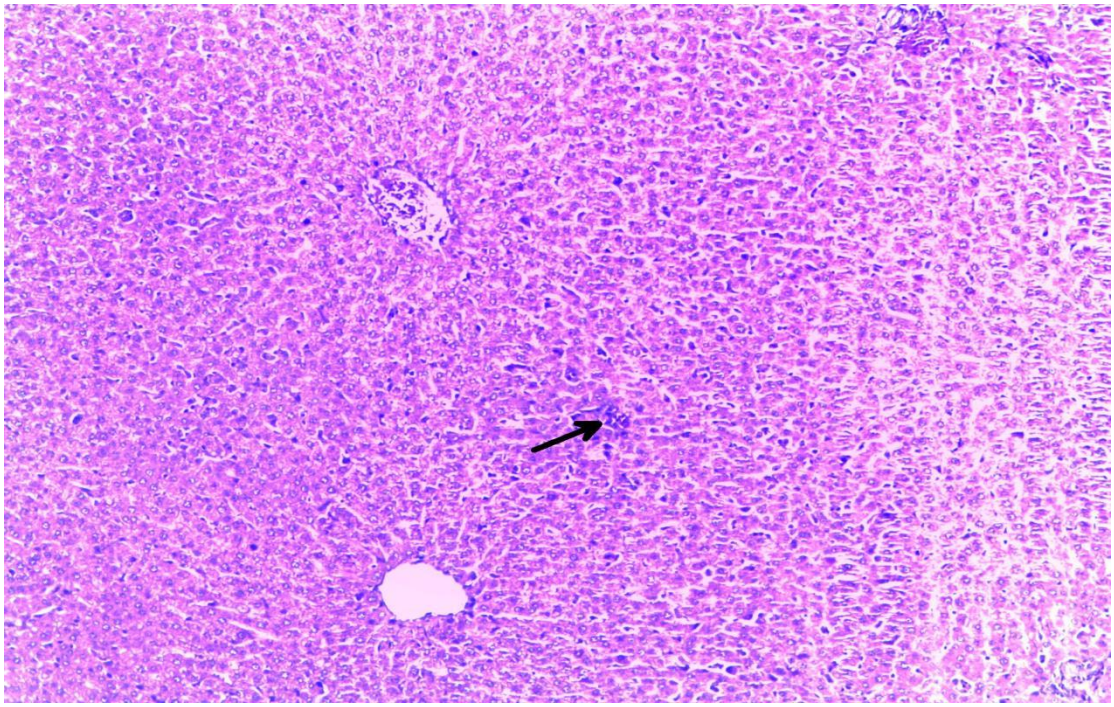

Plate II: This is a section of liver tissue from the study group 1 exposed 32.2 mg/kg of caffeine, arrow showing onset of inflammation in the liver following administration of caffeine.

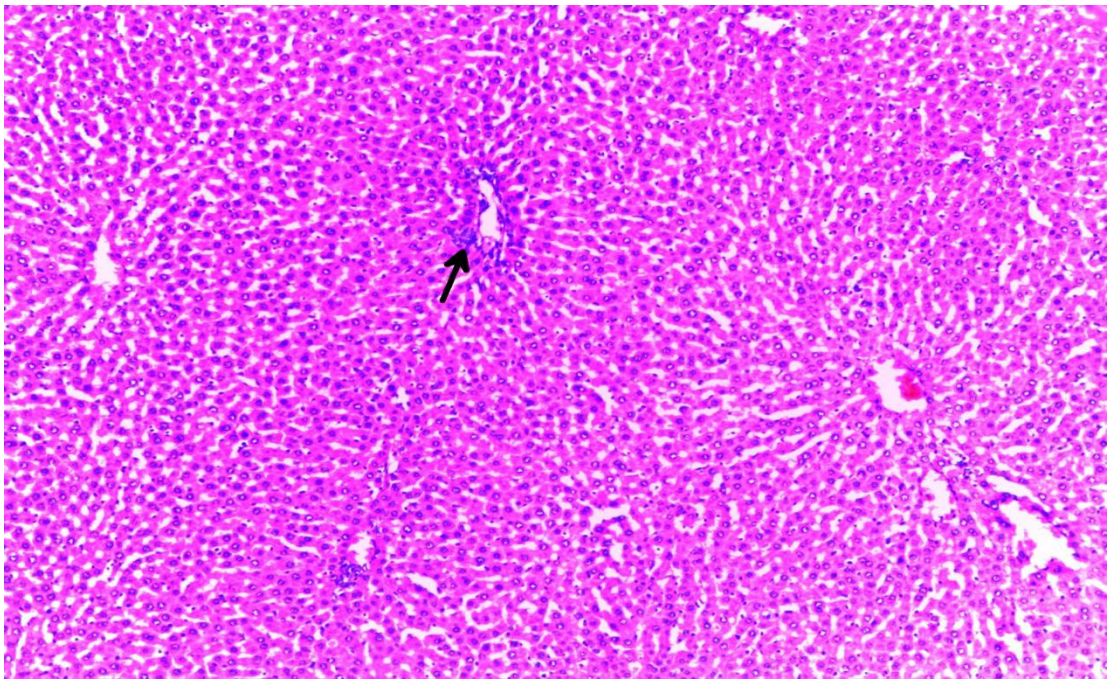

Plate III: This is a section of liver tissue from study group exposed to 64.4 mg/kg of caffeine with arrow showing area more extensive inflammation.

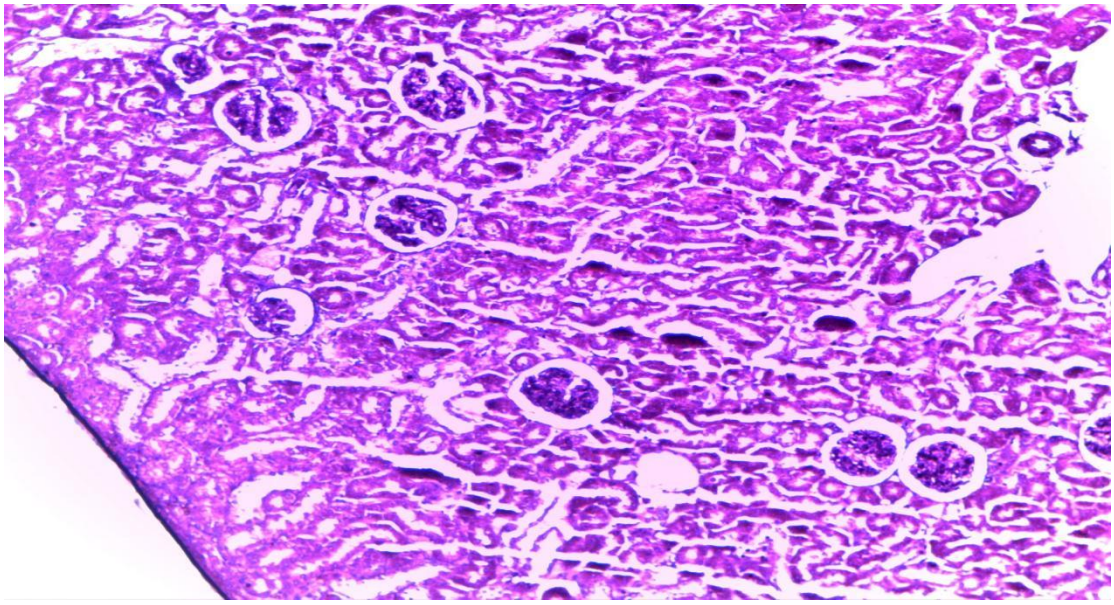

Plate IV: Kidney tissue for the group 1 as control

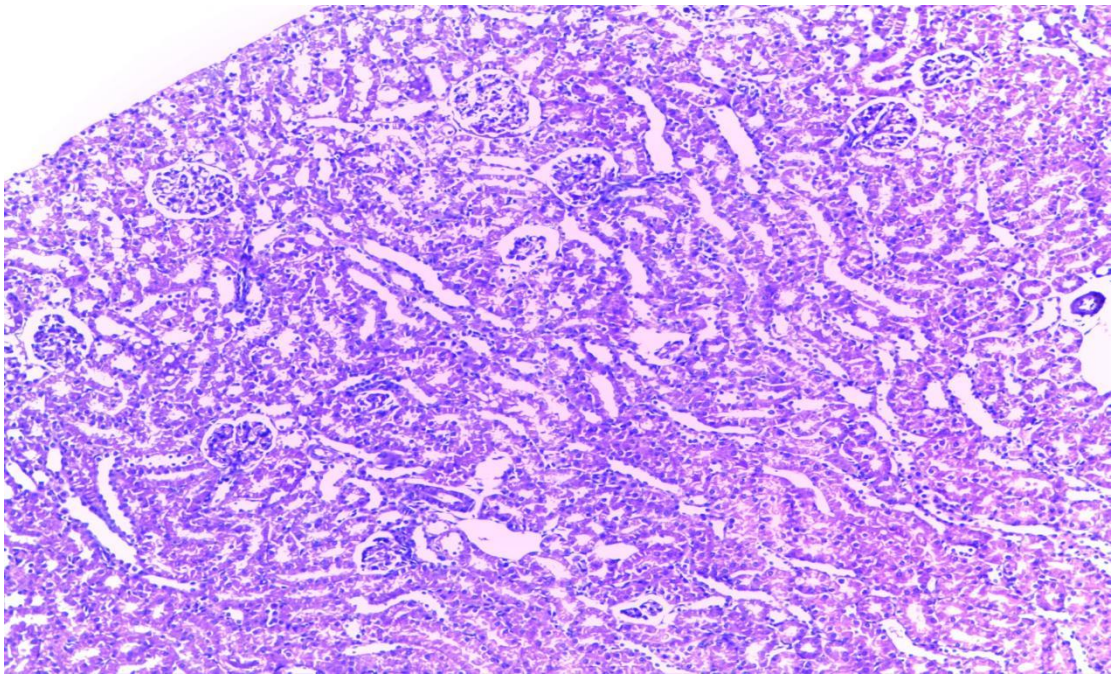

Plate V: Kidney tissue sample for group 1, exposed to 32.2 mg/kg of caffeine. This plate is showing remarkable tissue and cytoplasmic vacuolization

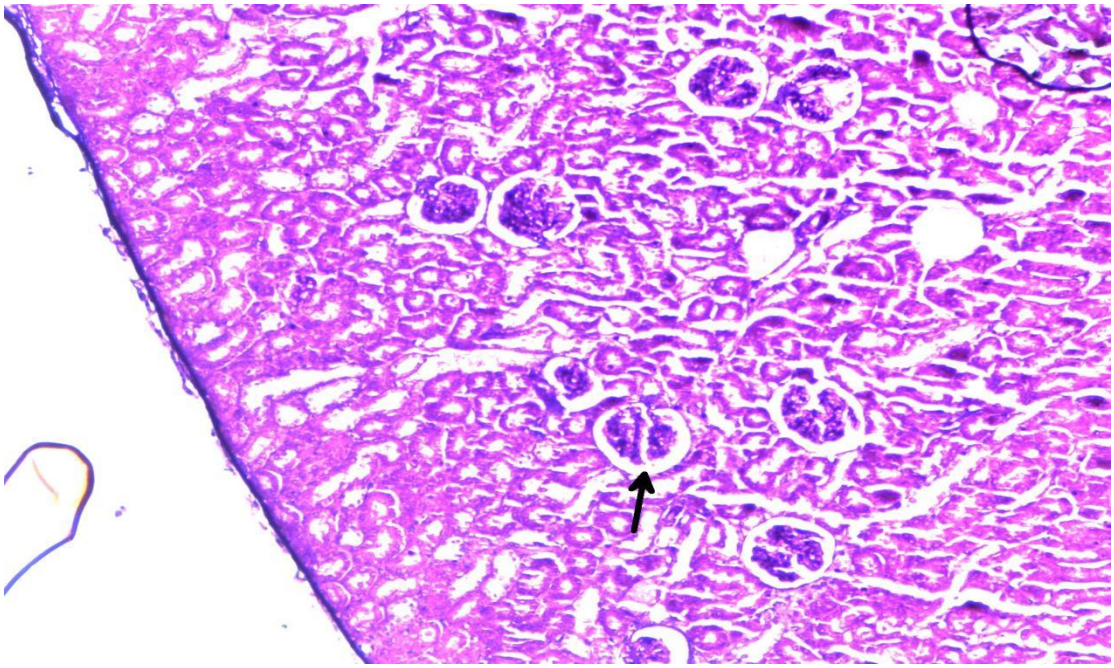

Plate VI: Kidney tissue sample for the group, exposed to 64.4 mg/kg. The arrow in this plate is showing remarkable tissue and dilated Bowman's capsule.

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