ISSN: 2536-7153 SJMLS

Sokoto Journal of Medical Laboratory Science 2024; 9(1): 121 - 125

SJMLS - 9(1) - 013

The Effects of Caffeine Toxicity on The Liver in Wistar Rats

Innih, S.O.*, Calmday-Ombo, D.
Department of Anatomy, School of Basic Medical Sciences, University of Benin, Benin City, Nigeria

Author for Correspondence: silvanus.innih@uniben.edu/+234-703-293-8275/ DOI: 10.4314/sokjmls.v9i1.13

Abstract
Caffeine is a widely used stimulant found in coffee and tea, mostly consumed as a beverage for alertness and enhanced intellectual performance but in excess can lead to dysfunction in normal body metabolism and toxicity on body organs. Studies proving the potential effect of caffeine as a specific liver toxicant are limited hence this study. Caffeine intoxication was induced by oral administration of caffeine in the rats. Twenty rats were randomly divided into four groups. Group A was the normal control group (no caffeine administration). Groups B, C and D were the caffeine intoxicated groups treated with 200, 400, and 800mg/kg body weight of caffeine. Animals in groups B-D received daily oral administration of caffeine for thirty days. Thereafter, we studied the effects of caffeine on liver function enzymes, hematological parameters and liver histomorphology. Caffeine intoxicated groups showed no significant difference ($p<0.05$) in aspartate aminotransferase, alanine aminotransferase and alkaline phosphatase in comparison with the control group yet showed significant increase in organo-somatic index ($p<0.05$) in groups C and D in comparison to control group. Histological slides of caffeine intoxicated rats showed hepatic portal congestion, zonal necrosis, and periportal infiltrates of inflammatory cells, these damages increase in severity with ascending doses of caffeine across the groups. The data obtained from this study suggests that Caffeine induces hepatotoxicity in ascending manner of dose-dependent pattern in adult Wistar rats.

Keywords: Caffeine, hepatoxicity, Wistar rat.

IntroductionCaffeine is a central nervous system (CNS) stimulant of the methylxanthine class (Nehlig *et al.,* 1992). It is mainly used as a mild cognitive enhancer to increase alertness and attentional performance (Wood *et al.,* 2013; Camfield *et al.,* 2014). At normal doses, caffeine has variable effects on learning and memory, but it generally improves reaction time, wakefulness, concentration, and motor coordination (Bolton and Null, 1982; Nehlig, 2010). Its adverse effects range from mild neuropsychiatric symptoms to hemodynamic instability due to malignant dysrhythmias and uncontrolled vasodilation (Pina *et al.,* 2022). Liver is the largest internal organ in most mammals, it carries out metabolism of various substances and acts as a blood detoxifier. Caffeine induced hepatoxicity has not been confirmed yet. In high, toxic doses, caffeine can cause severe effects on brain, heart and muscle function but has not been linked to clinically apparent liver injury. In contrast, there have been several reports of liver injury linked to use of caffeine rich energy drinks. These reports have not been very convincing, and most were not well documented. In many instances, the hepatic injury resembled acute hepatic necrosis or ischemic hepatitis. In other cases, other diagnoses were more likely than liver injury from the energy drinks. Furthermore, it remains unclear whether the hepatic effects were caused by caffeine per se or to other components in typical energy drinks, such as vitamins, herbs or other botanical products (National Institute of Diabetes and Digestive and Kidney Diseases,

2012). The present study seeks to investigate the effects of caffeine toxicity on the Liver in adult Wistar rats.

Materials and Methods

Animals: Twenty (20) adult Wistar rats weighing between 180-250g were bred in the animal house of the Department of Anatomy, University of Benin, Benin City. They were acclimatized under 12 hours light/dark cycle and room temperature at 25°C and they were allowed free access to food and water.

Experimental design: Induction of caffeine intoxication was by daily oral administration with an orogastric tube, the rats were divided into four groups (A, B, C, and D) of five rats each.
Group A served as the normal control rats
Group B were administered low dose of caffeine (200mg/kg body weight).
Group C were administered medium dose of caffeine (400mg/kg body weight)
Group D were administered high dose of caffeine (800mg/kg body weight)

Caffeine was dissolved in distilled water and administered daily for thirty (30) days. At the end of the experimental period, the animals were anaesthetized under chloroform, blood samples were collected through cardiac puncture into plain sample tubes for biochemical investigations (liver function test and antioxidant enzymes) and into EDTA sample bottles for hematological assay. The blood samples for serum biochemical assays were centrifuged at 3000 g for 10 minutes to obtain serum, which was later used for the estimation of biochemical parameters. The liver tissue was also collected for histopathological examinations.

Biochemical parameters: Liver function parameters: Alanine amino transferase (ALT) and aspartate amino transferase (AST) activities were measured by Reitman and Frankel (1957) method while serum ALP was assayed by the method of Englehardt *et al,* (1970). All parameters were assayed using commercially available kits.

Histopathological examinations: Excised liver samples were cleaned with normal saline and fixed for two days in 10 % buffered neutral formalin. Sections (5 µm thick) were paraffin-embedded and stained with hematoxylin and eosin. The sections of the liver were obtained and examined under Leica DM750 research microscope with a digital camera (Leica CC50) attached. Digital photomicrographs of the tissue sections were taken at x400 magnifications.

Statistical Analysis: Data were subjected to statistical analysis using GraphPad prism version 8.1 statistical package and relevant statistical values were obtained. One-way analysis of variance (ANOVA) was carried out and data were presented as mean ± standard error of mean (SEM). Least significant difference (LSD) post-hoc test was used, p values of <0.05 were considered statistically significant. The statistical values obtained were converted into graphical representation in the form of bar charts.

Discussion

Caffeine is a stimulant, enhances physical and mental activity. Caffeine is almost cytochrome P450 enzyme system with 3% or less being excreted unchanged in urine (Begas *et al.,* 2007; Kot and Daniel, 2008). The main route of metabolism in humans (70–80%) is through N-3-demethylation to paraxanthine also known as 1,7-dimethylxanthine or 17X (Benowitz *et al.,* 1995; Begas *et al*., 2007; Kot and Daniel, 2008). This reaction is carried out by CYP1A2 in the liver (Begas *et al.,* 2007). Caffeine itself is not an inhibitor nor an inducer of CYPA12 liver enzyme instead it acts as a substrate. Caffeine pharmacokinetics may be changed by drugs affecting the activity of CYP1A2 (human and rat) or CYP2C (rat), e.g. via autoinduction or by treatment with certain antidepressants or neuroleptics (Kot and Daniel, 2008). Hepatic injuries, cytoplasmic transaminase (ALT and AST) and alkaline phosphatase in circulation can be used as models for screening the extent of caffeine hepatoxicity.

We recorded insignificant changes between initial and body final weights and liver weight across all groups, this clearly shows administration of high caffeine doses does not influence body and liver weights of the intoxicated rats (fig 1 & 2 respectively). A significant increase ($p<0.05$) in organo-somatic

index in the groups with higher doses (400 mg/kg and 800mg/kg body weights) when compared to the control group (fig 3). This shows that administration of high doses of caffeine introduced toxicants into liver soma resulting from excessive substrate to enzyme activity in liver cells.

In this study, injury to the liver was assessed by measuring AST, ALT and ALP levels. Elevations of these enzymes denotes hepatocellular disease (Lala *et al.,* 2023). This was not the case with the enzyme activities in this study as no significant increase in serum ALT, AST, ALP was observed (fig 4), this explains that the caffeine administration caused no severe injury to hepatocytes. There is no indicative loss of functional integrity in liver cells.

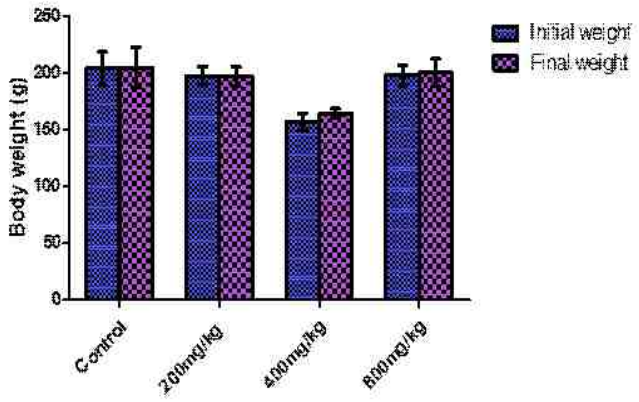

Figure 1: Changes between initial and body final weights across all groups.

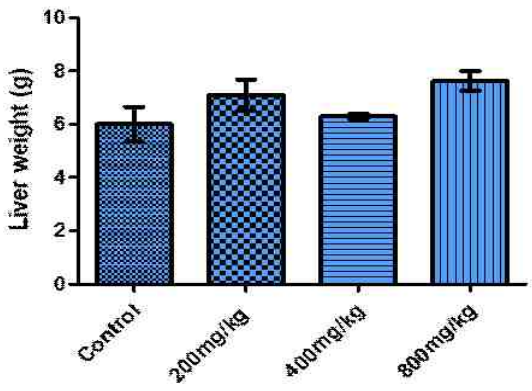

Figure 2: Fin al liver weight across all groups.

Fig 1: Chart showing the initial body weight in comparison to the final weight following administration of graded doses of caffeine. Values is expressed as mean ± SEM, n = 5 rats in each group. P-value < 0.05 as statistical significance, there were no significant differences between initial and final weight across the groups.

In this study, injury to the liver were assessed by measuring AST, ALT and ALP levels. Elevations of these enzymes denotes hepatocellular disease (Lala *et al.,* 2023). This was not the case with the enzyme activities in this study as no significant increase in serum ALT, AST, ALP was observed (fig 4), this explains that the caffeine administration caused no severe injury to hepatocytes. There is no indicative loss of functional integrity in liver cells.

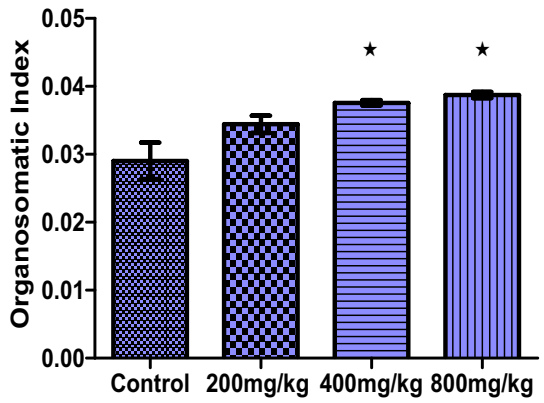

Figure 3: organo-somatic index across all groups.

Fig 2: Chart showing Liver weight following the administration of caffeine at different doses. Wistar rats following graded doses administration of caffeine. p <0.05 as statistical significance. There were no significant differences across the different doses compared with control group.

Table 1: Showing the mean values of initial and final body weights of Wistar rats following the administration of caffeine at difference doses. ˣP<0.05 indicates significant differences in final weight compared with initial weight.

Parameters	Control	200mg/kg	400mg/kg	800mg/kg
Initial Weight(g)	203.7 ± 14.68	197.4 ± 7.99	156.6 ± 7.41	197.8 ± 9.01
Final Weight(g)	204.8 ± 17.69	196.8 ± 8.17	164.3 ± 4.09	200.0 ± 12.12

Fig 3: Chart showing liver organo-somatic index of treated groups following graded doses administration of caffeine in comparison to control group. Mean□±□SEM, n =□5 rats in each group. p <□0.05 as statistical significance, Different superscript indicates statistically significant difference in treated groups compared with the control. There was significant increase in organo-somatic index in groups treated with 400mg/kg and 800mg/kg body weight of caffeine respectively when compared to the control group, there was no significant difference in 200mg/kg dose compared with control.

Fig 4: Investigating serum liver biomarkers in caffeine intoxicated rats. A) Alkaline phosphate (ALP) activity, B) Alanine amino transferase (ALT) activity, C) Aspartate Amino transferase (AST) activity. Values are Mean□±□SEM, n =□5 rats in each group. p <□0.05, a as compared with the normal control group.

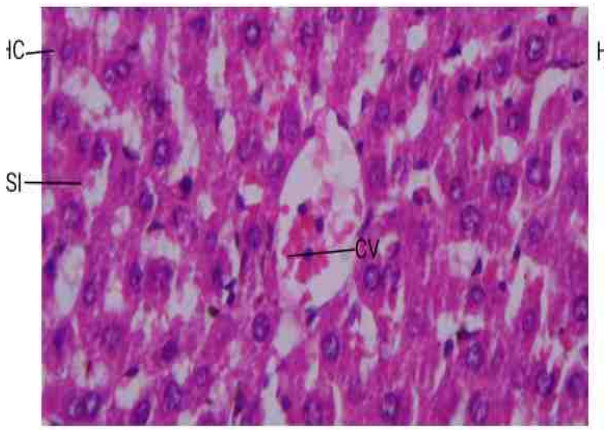

Plate 1. Rat liver. Control. Composed of normal architecture: hepatocytes (HC), sinusoids (SI), central vein (CV): H&E x 400.

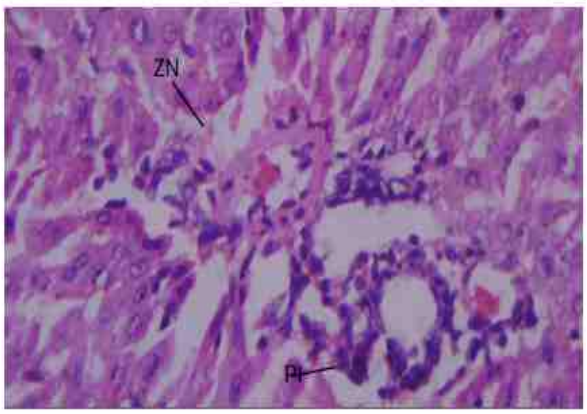

Plate 2. Rat liver given 200mg Caffeine showing: periportal infiltrates of inflammatory cells (PI), zonal necrosis (ZN): H&E x 400.

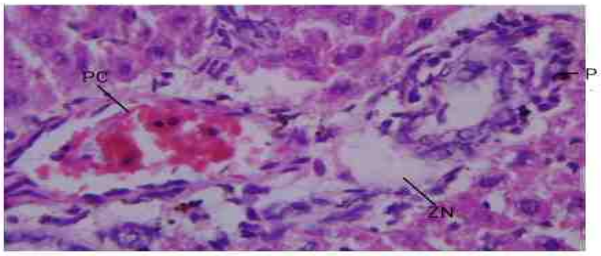

Plate 3. Rat liver given 400mg Caffeine showing: portal congestion (PC), zonal necrosis (ZN), periportal infiltrates of inflammatory cells (PI): H&E x 400.

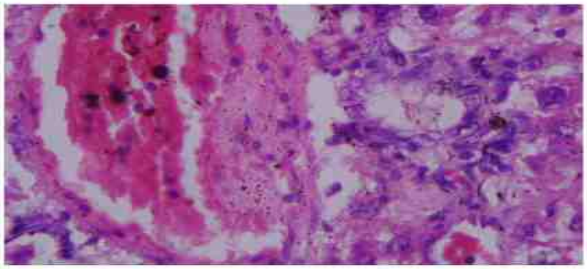

Plate 4. Rat liver given 800mg Caffeine showing portal congestion (PC), periportal infiltrates of inflammatory cells (PI): H&E x 400.

Histological investigation of the liver tissues revealed that the caffeine intoxicated groups (Plates 2 - 4), showed hepatic portal congestion, zonal necrosis, and periportal infiltrates of inflammatory cells in comparison to the control group showed normal hepatocyte architecture. This therefore shows that the organo-somatic index changes observed where corroborated by the histological findings.

Conclusion

Our findings therefore confirm that caffeine at high doses induces hepatoxicity in ascending manner of dose-dependent pattern in adult Wistar rats.

Conflict of interest declaration

The authors declare no conflict of interests.

References

Begas, E., Kouvaras, E., Tsakalof, A., Papakosta, S. and Asprodini, E. K. (2007). 'In vivo evaluation of CYP1A2, CYP2A6, NAT-2 and xanthine oxidase activities in a Greek population sample by the RP-HPLC monitoring of caffeine metabolic ratio's. *Biomedical Chromatography: BMC;* **21(2)**:190–200. https://doi.org/10.1002/bmc.736.

Benowitz, N. L., Jacob, P., Mayan, H. and Denaro, C. (1995). 'Sympathomimetic effects of paraxanthine and caffeine in humans'. *Clinical Pharmacology and Therapeutics;* **58(6)**: 684–691. https://doi.org/10.1016/0009-9236(95) 90025-X.

Bolton S. and Null G. (1981). 'Caffeine: Psychological Effects, Use and Abuse" (PDF)'. *Orthomolecular Psychiatry;* **10 (3)**: 202–211.

Camfield, D. A., Stough, C., Farrimond, J. and Scholey, A. B. (2014). 'Acute effects of tea constituents L-theanine, caffeine, and epigallocatechin gallate on cognitive function and mood: a systematic review and meta-analysis'. *Nutrition Reviews;* **72(8)**: 507–522. https://doi.org/10.1111/nure.12120.

Englehardt A., Adefolaju G., Enaibe B. and Alabi O. (1970). 'Acid and Alkaline Phosphatase activities in the small intestine of the rat (Rattus norvegicus), Bat (Eidolon helvum) and Pangoli (Manis tricuspis)'. *Aerztl Labor;* **16**:42.

Kot, M. and Daniel, W. A. (2008). 'Caffeine as a marker substrate for testing cytochrome P450 activity in human and rat'. *Pharmacological Reports;* **60(6)**: 789–797.

Lala, V., Zubair, M. and Minter, D. A. (2023). 'Liver Function Tests'. *In StatPearls. StatPearls Publishing.*

LiverTox: Clinical and Research Information on Drug-Induced Liver Injury. (2012). 'National Institute of Diabetes and Digestive and Kidney Diseases'. https://pubmed.ncbi.nlm.nih.gov/31643176/. [Accessed 30 December 2023].

Nehlig A. (2010). 'Is caffeine a cognitive enhancer? *Journal of Alzheimer's Disease: JAD;* **20 (1)**:85–94. https://doi.org/10.3233/JAD-2010-091315.

Nehlig, A., Daval, J. L. and Debry, G. (1992). 'Caffeine and the central nervous system: mechanisms of action, biochemical, metabolic and psychostimulant effects'. *Brain Research Reviews;* **17(2)**: 139–170. https://doi.org/10.1016/0165-0173(92) 90012-b.

Pina C. J., Sousa, D. L., Carvalho, C., Girao, A., Pacheco Mendes, A. and Pina, R. (2022). 'Caffeine Intoxication: Unregulated, Over-the-Counter Sale of Potentially Deadly Supplements'. *Cureus;* **14(1)**: e21045. https://doi.org/10.7759/cureus.2104.

Reitman, S. and Frankel, S. (1957). 'A Colorimetric Method for Determination of Serum Glutamate Oxaloacetate and Glutamate Pyruvate Transaminases'. *American Journal of Clinical Pathology;* **28**: 56-63.

Wood, S., Sage, J. R., Shuman, T. and Anagnostaras, S. G. (2013). 'Psychostimulants and cognition: a continuum of behavioral and cognitive activation'. *Pharmacological Reviews*, **66(1)**: 193–221. https://doi.org/10.1124/ pr.112.007054.

Citation: Innih, S.O., Calmday-Ombo, D. The Effects of Caffeine Toxicity on The Liver in Wistar Rats**.** ***Sokoto Journal of Medical Laboratory Science*****; 9(1): 121 - 125.** DOI: 10.4314/sokjmls.v9i1.13