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Effects of Caffeine on Cardiovascular Toxicity in Adult Wistar Rats

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ABSTRACT: Caffeine has been used for thousands of years. When taken in excess, caffeine can have negative effects on the body's overall health, including the cardiovascular system. Hence, the objective of this study was to evaluate the impact of caffeine on the cardiovascular toxicity in adult Wistar rat's using histological techniques. 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. The study's findings demonstrated that caffeine is a somewhat cardio-toxic substance that altered the structure of the Wistar rats' hearts in a dose-dependent manner. Caffeine administration at varying levels resulted in the heart muscle exhibiting distinct separation of neighboring muscle fibers, vascular distortion, and myocardial degeneration accompanied by vascular congestion in all caffeine-treated groups. The group that received greater dosages of caffeine showed more severe insults, indicating that the effects of caffeine were dose-dependent. The data obtained from this study suggests that caffeine induces cardio-toxicity in ascending manner of dose dependent pattern in adult Wistar rats.

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One of the most often utilized psychoactive stimulant substances worldwide, caffeine has been used for thousands of years. Chewing particular plants' seeds, barks, or leaves has been found by several cultures to have the ability to reduce weariness, heighten consciousness, and improve mood (Fredholm *et al*., 2011). Caffeine (1, 3, 7 Trimethylxanthine) is a heterocyclic chemical compound with a purine base that yields xanthine, according to Tarka *et al.* (2019). Caffeine's effects on learning and memory at typical levels vary, but overall, they are better for motor coordination, alertness, reaction time, and concentration (Bolton and Null, 1982; Nehlig, 2010). Malignant dysrhythmias and unregulated vasodilation might cause hemodynamic instability as well as minor neuropsychiatric symptoms (Pina *et al.*, 2022).Caffeine is the most prevalent methylxanthine in food. It can be found in nearly a thousand species across thirteen orders of the plant world. While coffee species is the main source, cocoa, coffee leaves, guarana seeds, and kola nuts are all rich sources (Smith, 2002). It is present in many common commercial soft drinks, energy drinks, and nonalcoholic beverages. While the primary advantage of caffeine consumption is that it prevents fatigue and drowsiness, there are many other benefits as well (McCusker *et al*., 2003). Because caffeine and adenosine have similar structures, caffeine can attach

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to adenosine receptors and act as an imposter, inhibiting adenosine's effects and causing feelings of alertness. This is how caffeine functions as an adenosine receptor antagonist. Research has shown that ingesting significant amounts of caffeine—more than 500 mg per day—for prolonged periods of time can cause the pathological symptoms known as caffeine poisoning (also known as caffeine tension), which can also cause headaches, anxiety, insomnia, and addiction. These adverse effects result from excessive caffeine consumption (Victor, 2012). Reduced brain acetylcholinesterase activity is most likely the reason for the observed effects of caffeine consumption during pregnancy in rodents (Kerrigan and Lindsey, 2005). Some people may find this impact to be disruptive to their sleep, but it may also be beneficial in circumstances where alertness is needed, such as night shift work, long-distance transportation, and prolonged reading. Increased muscle strength, endurance, and workout speed are some of the ways that caffeine might increase sports performance. As such, many athletes and gym aficionados use it as their pre-workout supplement. The majority of research on caffeine's effects on the cardiovascular system has focused mostly on the physiological impacts and, to a lesser extent, on the drastic morphological alterations, with results that have been highly inconsistent and disputed. Studies on the effects of extended and dose-dependent caffeine use on cardiovascular damage are few and far between. According to the majority of epidemiological research on caffeine users, moderate coffeine use may even have preventive effects against cardiovascular disease (Hage and Iskandrian, 2012). So the aim of the study was to identify different histopathological effects caused by different doses of caffeine on the heart of adult Wistar rats.

MATERIALS AND METHODS

Experimental animals: Thirty (20) adult Wistar rats were used as experimental animals in this study. The rats were randomly randomized into six (5) groups of five (5) individuals each. They weighed between 110 and 230 grams when the trial began. The animals were purchased and put in standard cages at the University of Benin's Department of Anatomy's Animal House, where they were cleaned and sanitized. Two (2) weeks were given to the rats to adapt before the start of treatment. All of the animals in the experiment received water and livestock growers' marsh, which was produced by Top Feed Limited in Sapele, Delta State, Nigeria, at their discretion.

Experimental design: An orogastric tube was used to provide caffeine orally to the rats every day until intoxication was achieved. The rats were into four groups (A, B, C, and D), each with five rats.

Group A: serve as normal control rats
Group B was given 200 mg/kg body weight of caffeine
Group C received 400 mg/kg body weight of caffeine
Group D were administered 800 mg/kg body weight of caffeine

Tissue collection, processing and staining: Caffeine was dissolved in distilled water and administered daily for thirty (30) days. The rats were put to sleep with chloroform and then killed. The jugular notch was sliced midline on each rat to sacrifice it. The heart of every rat was quickly removed, preserved in 10% buffered formalin, and then histologically processed and stained with eosin and hematoxylin. Tissues were stained using approved techniques (Avwioro, 2002). The sections of the heart were obtained and examined under Leica DM750 research microscope with a digital camera (Leica CC50) attached. Sections were taken at x400 magnifications

Statistical analyses: The IBM SPSS statistics application (Statistical Package for Social Science) Version 25 (SPSS, inc., Chicago, Illinois, USA) was used to assess all of the data and provide the required statistical values. The values of the treatment groups were compared to those of the control group using a one-way analysis of variance (ANOVA). P-values that were less than 0.05 were deemed noteworthy. A post hoc test using LSD was conducted.

RESULTS AND DISCUSSION

Consuming caffeine has been linked to a number of temporary and reversible physiological effects in general, as well as effects on the heart in particular. This study aims to identify the differences between the effects of caffeine consumption on the cardiovascular system in different subpopulations. The results of this investigation demonstrated that caffeine is a somewhat cardiotoxic substance, causing dose-dependent structural alterations in the Wistar rats' hearts. The control group's observation demonstrated a typical presentation of heart architecture based on the histology (plates 1). The group that received a 200 mg/kg dose of caffeine exhibited perivascular necrosis, perivascular infiltration of inflammatory cells, and stenosis and hypertrophy in the coronary artery. The myocardium also displayed a few localized areas with partial separation of the neighboring cardiac muscle fibers. These findings were consistent with those of (Norian *et al.,* 2014), who discovered that caffeine inhibits the phosphodiesterase enzyme, increases cytosolic calcium concentrations, and acts as a nonspecific competitive antagonist of adenosine receptors at low or moderate doses (less than 100 mg or 100–200 mg, respectively), all of which cause or enhance cardiac conduction and activity. The group

which got 400mg/kg dose of caffeine demonstrated perivascular infiltration of inflammatory cells and vascular deformation alone. The ultra-structural analysis made it even clearer that there were a variety of alterations present in the mitochondria.

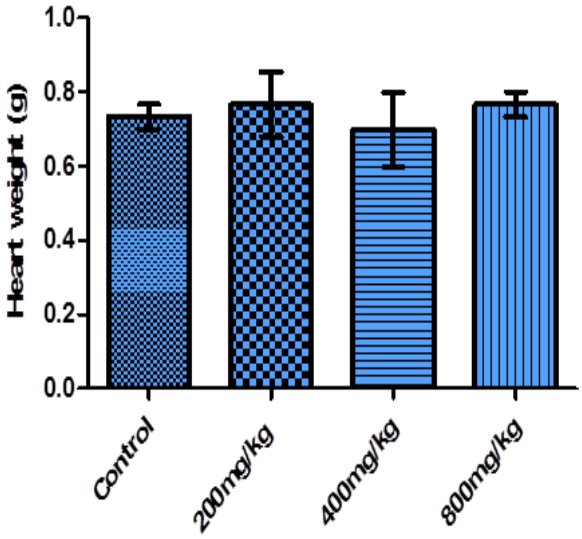

Fig 1: Chart showing heart weight of Wistar rat following graded doses administration of caffeine**.** There were no significant differences across the different doses compared with control respectively.

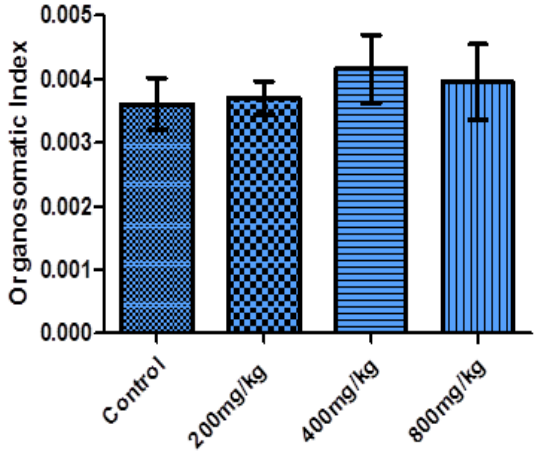

Fig 2: Chart showing Heart organosomatic index of Wistar rat following graded doses administration of caffeine. There were no significant differences across the different doses compared with control respectively.

A portion of the mitochondria experienced swelling and either partial or total loss of mitochondria. In the current investigation, both of the dosages utilized resulted in the observed mitochondrial alterations. Nevertheless, the current study also demonstrated a remarkable proliferation of the sarcoplasmic reticulum with high coffeine dosages, resembling a finger print with few interspersed vacuoles. The current study concurs with Duncan's 1992 findings that ultrastructural alterations in the myofibrils, mitochondria, and sarcoplasmic reticulum caused by caffeine were comparable to those observed following calcium release. It is thought that caffeine works by releasing calcium from intracellular locations such as the mitochondria and the sarcoplasmic reticulum, respectively.

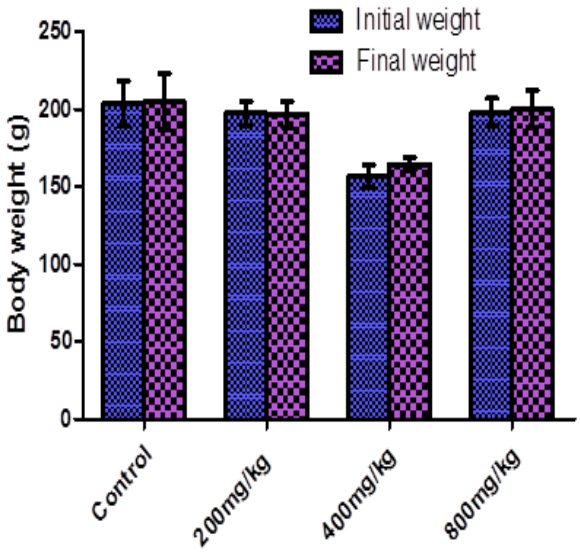

Fig 3: Chart showing body weight of Wistar rat following the administration graded doses of caffeine**.** There were no significant between initial and final weight across the groups.

This raises calcium levels and causes the myofibrils' ultra-structural damage as well as changes in the mitochondria. These alterations are qualitatively comparable to those observed following muscle cell injury brought on by calcium. After consuming coffee, the sarcoplasmic reticulum releases calcium through a complicated procedure. It has been proposed that low dosages of caffeine in ventricular myocytes temporarily activate the sacoplasmic reticulum when applied and temporarily depress it when removed. The sarcoplasmic reticulum's activation and the release of luminal calcium were more pronounced at higher coffee concentrations. The group that received a caffeine dose of 800 mg/kg had myocardial deterioration and vascular congestion in the final photomicrograph. The current investigation showed a correlation between increased degenerative alterations and high caffeine use. The sarcolemmal membrane exhibited severe folding and was unclear. The sarcolemmal membrane's structural alterations make myofibrils more vulnerable to mechanical harm and put them at risk for severe cardiac damage down the road (Duncan, 2011).

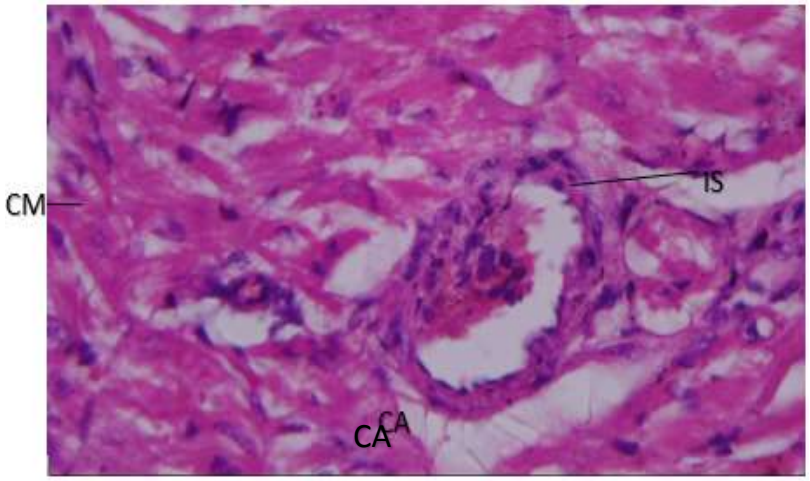

Plate 1. Rat heart. Control. Composed of normal architecture: cardiomyocyte bundles (CM), interstitial space (IS), coronary artery (CA): H&E x 400

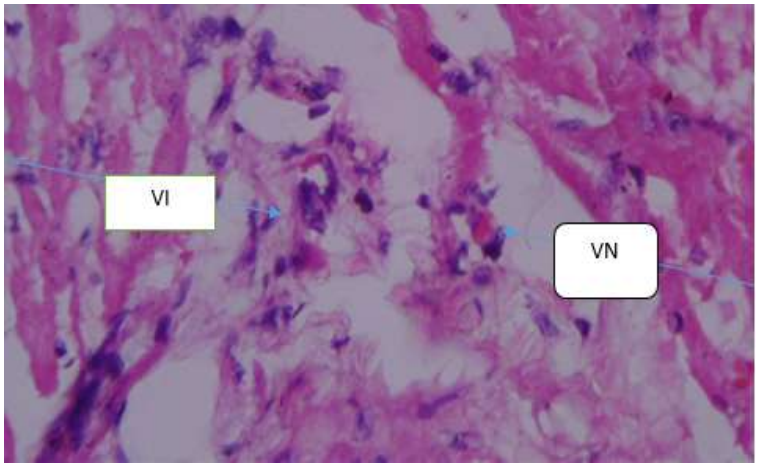

Plate 2. Rat heart given 200mg Caffeine showing: perivascular necrosis (VN), perivascular infiltrates of inflammatory cells (VI): H&E x 400

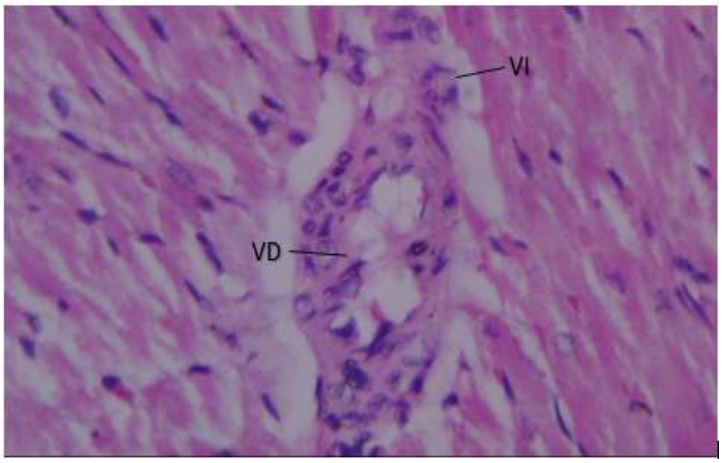

Plate 3. Rat heart given 400mg of caffeine. Showing: vascular distortion (VD), perivascular infiltrates of inflammatory cells (VI): H&E x 400

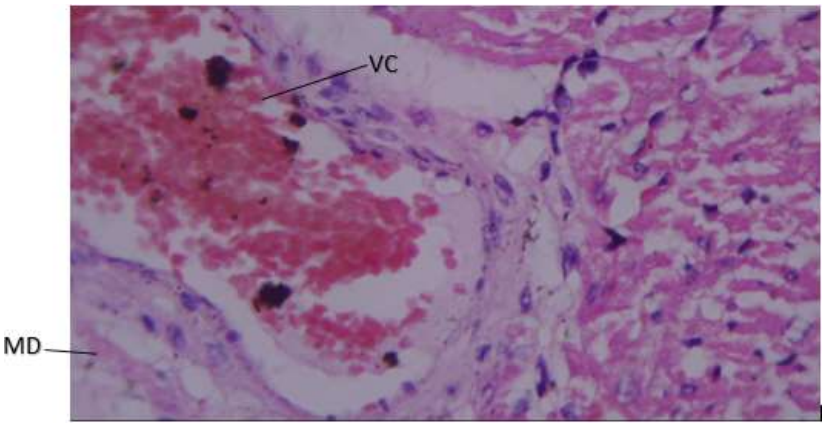

Plate 4. Rat heart given 800mg Caffeine showing: myocardial degeneration (MD), vascular congestion (VC): H&E x 400

Another important method that caffeine damages the body is by the release of cytosolic proteins, primarily creatine kinase, which is enhanced by the disruption to the sarcolemmal membrane. This study supports research conducted in 2012 by the Substance Abuse and Mental Health Services Administration, which found that a high caffeine intake can result in myocardial infarction and cardiac arrest. There was no discernible difference in the heart weight of any group when caffeine was induced when compared to the control, according to the statistical results (Fig 1-3). The experiment also demonstrated that, when caffeine was induced, there was no discernible difference in the organosomatic index between the groups and the control. Additionally, it was noted that, when comparing the group's final weight to its starting weight, there was no discernible variation in body weight when compared to the control. This finding conflicts with a previous study by Ismail *et al*. (2015) that found that induction of caffeine resulted in a significantly less weight increase than the normal control. Other writers' related works: Caffeine and the terpenes found in unfiltered coffee both seem to raise the risk of coronary heart disease, according to a 2007 study by Marilyn and Ahmed. An increased risk of myocardial infarction has been linked to coffee consumption, according to research by MacKenzie et al. (1990). A 2002 study by Corti *et al*. examined the impact of caffeine on hypertension.

Conclusion: The current study's conclusion made it clear that caffeine may be regarded as a cardio-toxic substance. At low doses, it produced few structural alterations. However, it resulted in several ultra-structural alterations at large dosages. Therefore caffeine have deleterious effect on the cardiovascular system in ascending manner in dose dependent pattern.

Declaration of Conflict of Interest: The authors declare no conflict of interest

Data Availability Statement: Data are available upon request from the corresponding author.

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