Content available at Bio-Research

Journal of Biological Research and Biotechnology
Journal Home page: https://bio-research.com.ng/index.php/home

ORIGINAL ARTICLE

Effect of Therapeutic Caffeine Doses on the Histology of Lung Tissue in Experimental Animal Models

[1*]**Benson P. Onyeije**✉**and** [2]**Silvanus O. Innih.**
[1]Department of Human Anatomy University of Delta, Agbor, Nigeria.
[2]Department of Anatomy University of Benin, Nigeria.

ARTICLE INFO

Received: 2nd August 2026
Revised: 21st August 2026
Accepted: 28th August 2026
Published online: 31st August 2026
DOI:https://dx.doi.org/10.4314br.v24i2.34

e-ISSN: 2705-3822
print-ISSN: 1596-7409

Official. Faculty of Biological Sciences, University of Nigeria.

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. The Study evaluated the potential effect of caffeine as a specific pulmonary toxicant on the histological status in adult Wistar rat. Twenty rats were assigned into four groups at random. The standard control group, Group A, did not receive any caffeine. The caffeine-intoxicated groups B, C, and D received doses of 50, 100, and 200 mg/kg body weight respectively. For twenty-one days, animals in groups B–D were given caffeine orally every day. The oral treatment continued for twenty-one days. The rats were humanly sacrificed on day twenty-two, and their lungs were carefully removed, weighed, and preserved in 10% formal saline for histological analysis. Data were presented as mean ± SEM, and one-way ANOVA was used to determine statistical significance at $p < 0.05$. The results showed a significant increase ($p < 0.0$) in body weight and lungs weight when compare to the control group. Histological slides of caffeine intoxicated rats showed histopathological changes, including alveolar congestion, dilated terminal bronchiole, activated mononuclear phagocyte cell, and inflammatory cell infiltration. These damages increase in severity with ascending doses of caffeine across the groups. The results obtained from this study suggest that caffeine induces lungs toxicity in ascending manner of dose-dependent pattern in adult Wistar rats.

Keywords: Caffeine; Lungs; Histomorphology; Toxicity; Wistar rats.

Cite this article: Onyeije, P. B., & Innih, S. O. (2026). Effect of therapeutic caffeine doses on the histology of lung tissue in experimental animal Models. Journal of Biological Research and Biotechnology, 24 (2), 768-772.
✉**Corresponding author**: Onyeije Benson Peter. Email: Benson.onyeije@unidel.edu.ng, bendgreat4us@gmail.com, ORCID: Https://Orcid.Org/0009-0002-3132-0923. Tel: +2348064444184
DOI: https://dx.doi.org/10.4314br.v24i2.34

1.0 INTRODUCTION

Caffeine is one of the most widely consumed psychoactive substances globally, with a history spanning thousands of years across various cultures that discovered its stimulating effects from natural plant sources (Fredholm et al., 2011). Chemically, caffeine (1,3,7-trimethylxanthine) is a heterocyclic organic compound derived from the purine base xanthine, characterized by a pyrimidine ring linked to an imidazole ring (Tarka et al., 1998). As a secondary plant metabolite containing a heterocyclic nitrogen atom, caffeine is classified as an alkaloid, or more specifically, a pseudoalkaloid and this is due to its unique biosynthetic pathway that does not incorporate amino acids (Tarka et al., 1998). The most prevalent methylxanthine, caffeine is present in around a thousand plant species in thirteen orders of the plant kingdom (Ashihara et al., 1999) and can be found in a variety of foods and drinks (Pauwels et al., 2001). Although coffee is the main source, chocolate, tea leaves, guarana seeds, and kola nuts also contain substantial levels of caffeine (Chow et al., 1994). It is a typical component of many soft drinks, energy drinks, and commercial non-alcoholic beverages that are consumed on a daily basis. Although there are numerous other alleged advantages, the main motivation for consuming caffeine is frequently to Figureht weariness and increase alertness Nehlig et al. (1992) because of its structural resemblance to adenosine, caffeine can bind to these receptors and block the actions of adenosine, increasing alertness. This is the main way that caffeine works. This mechanism is a popular pre-workout supplement because it can reduce drowsiness, improve cognitive function for tasks requiring sustained attention (such as long-distance driving, night shifts, and prolonged reading), and possibly improve athletic performance by increasing muscle strength, endurance, and exercise speed (Pauwels et al., 2001). While moderate caffeine consumption is generally considered safe for most adults, high doses, particularly from concentrated sources like energy drinks, have raised health concerns (Hossian et al., 2014). The energy drink market is rapidly expanding, with a projected market value reaching $61 billion by 2021. Statistics indicate significant consumption among younger populations, with approximately 30% of US teenagers (12-17 years old) consuming energy drinks regularly (NCCI, 2014), and nearly 45% of deployed military personnel consuming at least one per day (CDCP, 2017). Despite being marketed for performance and cognitive enhancement, these beverages have been linked to numerous adverse effects, predominantly neurological and cardiovascular issues, due to their high caffeine content (Rottlaender et al., 2012). The number of annual emergency department visits related to energy drinks more than doubled from 2007 to 2011 (SAMSA, 2012), and the Food

and Drug Administration has investigated several deaths attributed to these beverage. Concerns have largely focused on the cardiovascular system, associating energy drink consumption with conditions like cardiac arrest, myocardial infarction, spontaneous coronary dissection, and coronary vasospasm.

However, beyond the widely studied cardiovascular and neurological impacts, the potential effects of chronic or high-dose caffeine consumption on the respiratory system, specifically the lungs, remain less thoroughly investigated, particularly in *in vivo* models. Given caffeine's known bronchodilatory properties and its widespread consumption, understanding its comprehensive physiological effects on all major organ systems is crucial for public health. This project therefore shifts focus to provide a comprehensive overview of caffeine's potential effects, with particular emphasis on the histoarchitecture of the lungs of adult Wistar rats.

2.0 MATERIALS AND METHODS

2.1 Plant collection and identification/chemicals and reagents

The caffeine sample utilized in this research was obtained from the Pyrex chemical store located at First East Circular, Benin City, and produced by Aarti Pharmalabs Company, India.

2.2 Preparation of Caffeine stock solution

Caffeine stock solutions were produced using the procedure outlined by Cappellett et al. (2018). Caffeine was accurately measured and dissolved in a suitable medium to achieve the necessary concentrations for administration. The formulated solutions were utilized to provide caffeine at dosages of 50 mg/kg, 100 mg/kg, and 200 mg/kg of body weight, respectively. The necessary volume of caffeine solution for each Wistar rat was determined according to its body weight and the intended dosage.

Ethical approval

This research was evaluated and sanctioned by the University of Benin Ethics and Research Committee, with approval number CMS/REC/2025/756.

Experimental animals

Twenty (20) adult Wistar rats weighing between 180 g-230 g were procured from the animal houses, Department of Anatomy, University of Benin, Benin City, Edo State, Nigeria, and were utilized for the experimental research. The rats were acclimatized for two (2) weeks before commencement of the experiment. During this period, the animals were allowed free access to standard animal feed (Top grower mash) and clean water. The weights of each animal were checked weekly (so as to get the cumulative weight required for experimental use). Each animal procedure was carried out in accord with approved protocols and in compliance with the recommendations for the proper management and utilization of laboratory animals used research (Buzek & Chastel, 2010).

The dosages of Caffeine

The investigation utilized pure caffeine with a concentration of 99%, at doses of 110 and 130 mg/kg of body weight, prepared by dissolving it in 50 ml of distilled water. These doses were selected according to the lethal dose of pure caffeine, recorded as 127 mg/kg body weight for mice through oral administration (Cappellett et al., 2018, Onyeije & Innih, 2024)

Experimental design

Twenty (20) experimental adult Wistar rats of either sex were randomly assigned into five groups; Groups A-D comprising of five rats per group.
Group A serve as control and received 1ml of distil water
Group B received a low dosage of caffeine (50mg/kg body weight)
Group C received a medium dosage of caffeine (100mg/kg body weight)
Group D received a high dosage of caffeine (200mg/kg body weight)

Histological examination

At the end of the experimental period, the animals were euthanized using chloroform before, the lungs were removed, fixed in buffer 10% formalin for (24- 48) hours. The tissues were trimmed, dehydrated in descending grades of ethanol, cleared, and embedded in paraffin before sectioning at 5 µm thickness using a microtome. The tissue sections were stained with hematoxylin and eosin (Onyeije & Innih, 2024). The stained sections of the lungs were examined under Leica DM750 research microscope with a digital camera (LeicaCC50) attached. Digital photomicrographs of the tissue sections were taken at ×100 objective magnifications.

Statistical analysis

Data were subjected to statistical analysis using the IBM SPSS statistics software (Statistical Package for Social Science) (Version 25) and relevant statistical values were obtained. One-way analysis of variance (ANOVA) was carried out and results were presented as mean ±SEM. Least Significant Difference (LSD) post-hoc test was used. Values of $p < 0.05$ were considered significant. The statistical values obtained were converted into bar charts and tables.

RESULTS

Body weight

The alterations in body weight of the experimental animals are presented in Figure 1. There was a significant increase ($p < 0.05$) in body weight in the control, the low dose (50 mg/kg), and medium dose (100 mg/kg) caffeine-treated groups, as evidenced by the difference between the final and initial body weights. Conversely, the group administered 200 mg/kg of caffeine exhibited no notable rise ($p > 0.05$) in body weight relative to its baseline weight. These results indicate that the normal weight gain observed at the lower doses and in the control group may have been attenuated by caffeine treatment at 200 mg/kg. Relatively, in Figure 2, there was no statistically significant changes in lung weight between the control group and the low dose group administered 50 mg/kg body weight of caffeine ($p > 0.05$). However, when compared to the control group, statistically significant differences were observed between the groups given medium dose (100 mg/kg) and high dose (200 mg/kg) body weight of caffeine ($p < 0.05$). This affirmed that higher caffeine dosages might have had a major effect on lungs weight. In Figure 3, the lung-to-body weight ratio indicated no statistically significant difference in the 50 mg/kg and 100 mg/kg caffeine-treated groups when compared with the control group ($p > 0.05$).

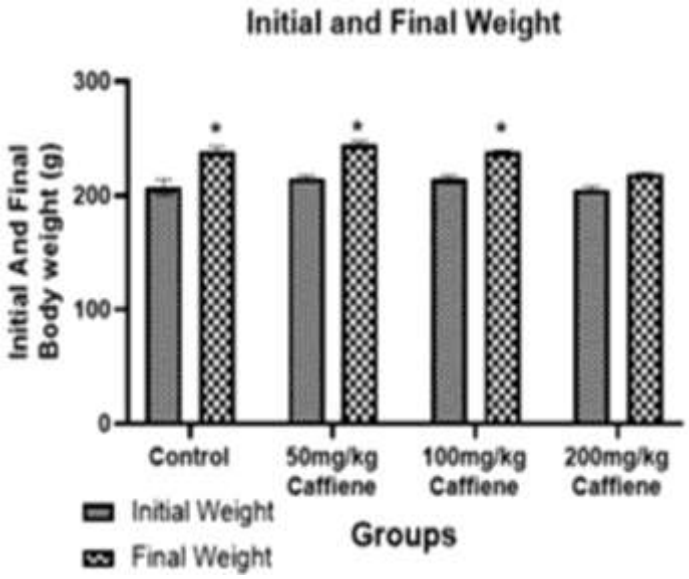

Figure 1: Body weight in each experimental group indicates significant difference compared to initial within the same group. P-value < 0.05.

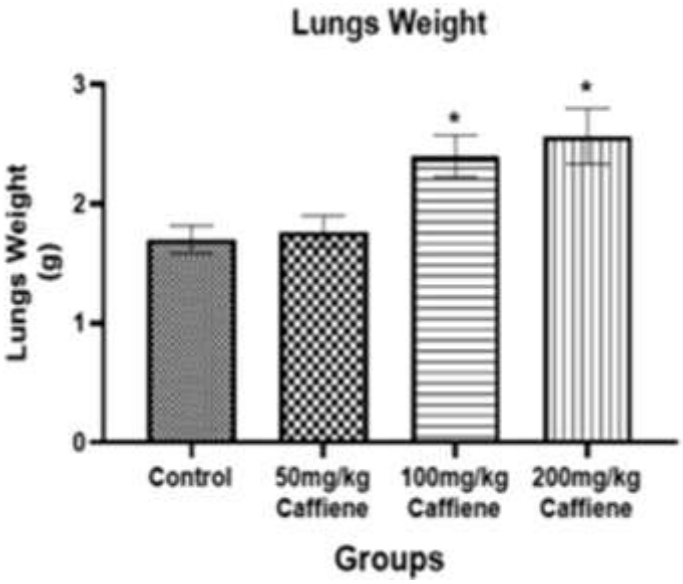

Figure 2. Mean weight changes for each experimental group *indicates significant difference compared to Control. P-value < 0.05.

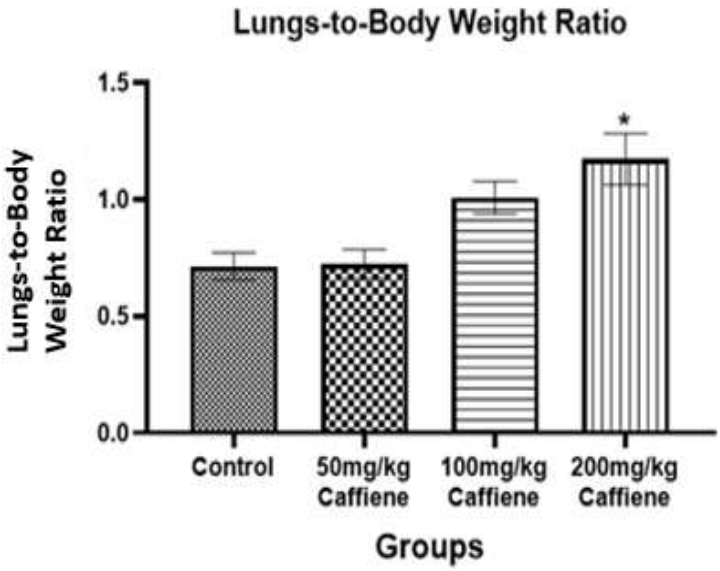

Figure **3.** Organo-somatic index for each experimental group**;** *indicates significant difference when treatment groups are compared to Control. P-value < 0.05

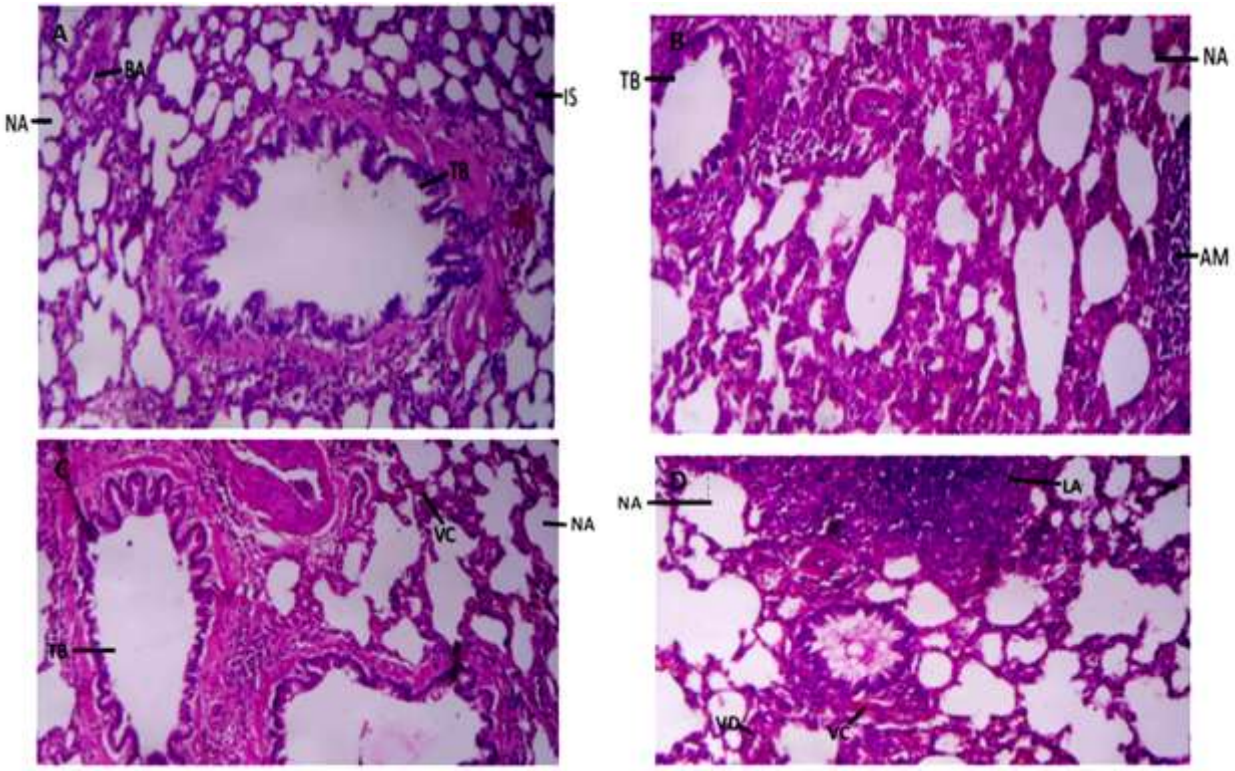

Plate A: Rat lungs control show; normal alveoli (NV), bronchial artery (BA), interstitial space (IS) and terminal bronchiole (TB); **plate B.** Rats lungs given 50mg/kg of caffeine show: normal alveoli (NA), dilated terminal bronchiole (TB) and activated mononuclear phagocyte cell (AM), **plate C.** Rats lungs given 100mg/kg of caffeine show: normal alveoli (NA), interstitial congestion (VC) and dilated terminal bronchioles (TB), **plate D**. Rats lungs given 200mg/kg of caffeine show: normal alveoli (NA), interstitial vasodilation (VD) and congestion (VC), activated bronchio-alveolar lymphoid aggregate, H&E 100X

4.0 DISCUSSION

This study evaluated the effects of caffeine toxicity on the lung tissues of adult Wistar rats, with emphasis on histopathological alterations. The findings demonstrate that graded doses of caffeine produce significant structural and cellular changes in the lungs, indicating its potential to induce pulmonary toxicity. The significant changes between the initial and final body weight with lung weight were noticed in 50mg/kg and 100mg/kg body weight of caffeine. Crucially, the significant increase in absolute lung weight and the Lungs-to-Body Weight Ratio in the high-dose groups 100 mg/kg and 200 mg/kg is a strong indicator of pathological change, most likely due to pulmonary edema and/or inflammation. This effect, mediated by adenosine receptor antagonism, leads to increased energy expenditure and reduced food intake over chronic administration (Fredholm, 1995). Similar toxic effects of caffeine on body tissues have also been reported in experimental studies

(Eyong et al., 2008, Nehlig, 2018). Alveolar congestion, dilated terminal bronchiole, activated mononuclear phagocyte cells, and inflammatory cell infiltration are among the histological alterations that have been noted. These findings imply that caffeine poisoning jeopardises the integrity of lung architecture. These changes could be explained by tissue damage and increased vascular permeability, which are in line with toxic reactions seen in other organ systems exposed to high caffeine levels (Obidoa et al., 2003, Chen et al., 2019, Onyeije & Innih, 2024). The detrimental effects of excessive coffee on pulmonary capillaries are further supported by the existence of significant congestion. Oxidative stress is one of the main processes causing these alterations. It has been demonstrated that consuming too much caffeine increases the production of reactive oxygen species (ROS), which causes lipid peroxidation and cellular membrane damage (Abdel-Daim et al., 2015). According to this study, oxidative damage and compromised antioxidant defence systems may be connected to the morphological deformation of alveolar cells and dilated terminal bronchioles. This is consistent with earlier research showing lower activity of antioxidant enzymes like catalase and superoxide dismutase in caffeine-induced toxicity (Duarte et al., 2012). Another important factor contributing to the lung damage seen was inflammation. The bronchio-alveolar lymphoid aggregation, which includes neutrophils and macrophages in particular, shows that the immune response has been activated. According to Lee et al. (2017), this could be linked to elevated levels of pro-inflammatory cytokines, which cause tissue damage and remodelling. By encouraging fibrosis and decreasing lung flexibility, persistent inflammation in lung tissue might worsen damage even more. According to Wadhwa and Seth (2015), lymphoid aggregates are indicative of the immune system's reaction to long-term irritation or stress and are in line with observations of immune system activation in animal models. Additionally, caffeine's role as an adenosine receptor antagonist may contribute to pulmonary dysfunction. Adenosine normally exerts protective effects in the lungs, including anti-inflammatory actions and regulation of bronchial tone. By blocking these receptors, high levels of caffeine may disrupt normal respiratory function, leading to bronchial irritation and possible congestions (Fredholm et al., 1999; Goldstein et al., 2010). The interstitial vasodilation (VD) and congestion (VC), observed in this study may lead to impairment in gas exchange processes. Damage to these cells reduces the efficiency of oxygen and carbons dioxide exchange, which may lead to hypoxic conditions in severe toxicity. This functional impairment corresponds with the structural abnormalities seen in the lung tissues (Rehfeldt & Aydin, 2017; Nehlig, 2018). It is crucial to note that the effects of caffeine are dose-dependent, according to our research. Due to their antioxidant qualities, low to moderate doses may have negligible or even protective effects, but the large doses utilised in this study unmistakably show harmful results. This emphasises the significance of limiting caffeine intake, particularly in light of its extensive use in drinks and pharmaceuticals.

Some limitations should be considered in spite of these findings. Extrapolating the results to human physiology should be done cautiously because the study was done on animal models. Furthermore, biochemical tests for inflammatory mediators and oxidative stress indicators that might shed more light on the mechanisms of harm were not investigated.

Conclusion

The histological results show that the lungs parenchyma was not overtly destroyed by caffeine administration because the alveolar architecture was mostly unaltered in all treatment groups. However, the dose-dependent occurrence of interstitial vasodilation, vascular congestion, and activation of bronchiole-alveolar lymphoid tissue affirmed that caffeine caused mild inflammatory and lung vascular reactions. These changes were more evident at the higher dosages, indicating that continuous exposure to caffeine may cause histological alterations and inflammatory reactions.

Further studies involving pulmonary inflammatory and oxidative stress biomarkers are needed to clarify the mechanisms underlying these dose-dependent changes and their effects on long-term usage**.**

Authors'contributions

Onyeije Benson conceived and design the study, as well as methodology and data analysis. Histological slides were analysed by Innih Silvanu. He critically reviewed and edited the manuscript. All authors read and approved the final manuscript.

Declaration of Conflict of Interest

There is no conflict of interest disclosed by the writers.

Acknowledgment

The authors express their gratitude to the University of Benin and the technical personnel of the Anatomy Laboratory, especially Mr. Samuel Nwagbada, for their invaluable assistance with the tissue processing, animal care, and experimental protocols. Their technical proficiency, commitment, and readiness in improving the quality of this work.

Data Availability Statement

Data are available upon request from the corresponding author.

Funding

No financial support was obtained from any source for this research project. The study was conducted without external funding or institutional grants.

REFERENCES

Abdel-Daim, M. M., Abuzead, S. M. M. and Halawa, S. M. (2015). Protective role of *Spirulina platensis* against acute caffeine-induced toxicity in rats. *Environmental Toxicology and Pharmacology, 40*(2), 701–709.

Ashihara, H. and Crozier, A. (1999). Distribution and biosynthesis of caffeine in plants. *Plant Physiology, 120*(1), 1–10.

Cappelletti, S., Piacentino, D., Fineschi, V., Frati, P., Cipolloni, L., and Aromatario, M. (2018). Caffeine-related deaths: Manner of deaths and categories at risk. *Nutrients, 10*(5), 611. https://doi.org/10.3390/nu10050611.

Centers for Disease Control and Prevention. (2017). Energy drink consumption among deployed U.S. military personnel. *Morbidity and Mortality Weekly Report, 66*(14), 373–377.

Chen, X., Ghribi, O. and Geiger, J. D. (2019). Caffeine protects against disruptions of the blood-brain barrier in animal models. *Journal of Alzheimer's disease, 20*(Suppl. 1), S127–S141.

Chow, S. H., Yen, C. K. and Chen, J. K. (1994). Isolation and identification of caffeine in coffee, tea, and soft drinks by high-performance liquid chromatography. *Journal of Food Protection, 57*(11), 978–980.

Duarte, J. M. N., Carvalho, R. A., Cunha, R. A. and Gruetter, R. (2012). Caffeine consumption attenuates neurochemical modifications in the hippocampus of diabetic rats. *Journal of Neurochemistry, 111*(2), 368–378.

Eyong, E. U., Ikegbulam, N. C. and Eteng, M. U. (2008). Haematological changes following administration of alcohol and caffeine in albino Wistar rats. Bio-Research, 6(1), 290–292.

Fredholm, B. B. (1995). Adenosine, adenosine receptors and the actions of caffeine. *Pharmacology & Toxicology, 76*(2), 93–101.

Fredholm, B. B., Bättig, K., Holmén, J., Haglund, A. C. and Kaléy, T. E. (2011). Actions of caffeine in the brain with special reference to factors that contribute to the widespread use of caffeine. *Pharmacological Reviews, 63*(4), 1079–1103.

Fredholm, B. B., Bättig, K., Holmén, J., Nehlig, A. and Zvartau, E. E. (1999). Actions of caffeine in the brain with special reference to factors that contribute to its widespread use. *Pharmacological Reviews, 51*(1), 83–133.

Goldstein, E. R., Jacobs, P. L., Whitehurst, G. T. and Arent, S. M. (2010). Caffeine: Physiological effects and clinical use in performance-enhancing dietary supplements. *Current Sports Medicine Reports, 9*(3), 177–184.

Hossain, M. Z., Rashid, M. H. and Alam, A. H. (2017). Toxicological effects of high-dose caffeine administration on liver and kidney of mice. *Bangladesh Pharmaceutical Journal, 20*(2), 195–201.

Lee, C., Song, Y. and Park, J. (2017). Role of inflammation in respiratory diseases. *Journal of Inflammation Research, 10*, 1–12.

National Center for Complementary and Integrative Health. (2014). *Energy drinks: What you need to know* (NIH Publication No. 14-AT-04870).

Nehlig, A. (2018). Interindividual differences in caffeine metabolism and factors driving caffeine consumption. *Pharmacological Reviews, 70*(2), 384–411.

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.

Obidoa, O., Eirewele, E., Eseanyika, L. U. S. and Shoyinka, S. V. O. (2003). Effects of nominal exposure to petrol on organ pathogenesis and histopathology of rats. Bio-Research, 1(1), 75–82

Onyeije, B. P. and Innih, S. O. (2024). Effects of caffeine on cardiovascular toxicity in adult Wistar rats. *Journal of Applied Sciences and Environmental Management, 26*(9), 2797–2802.

Pauwels, B., Neven, F. and Gremmels, C. M. L. (2001). Caffeine: Occurrence in the environment and toxicological effects on aquatic organisms. *Food and Chemical Toxicology, 39*(2), 163–172.

Rehfeldt, A. L. and Aydin, M. A. (2017). Theophylline and caffeine in asthma: Is there a future? *Journal of Asthma, 54*(6), 555–562.

Rottlaender, D., Leon, S. and Paul. J. R. (2012). Cardiac arrest and myocardial infarction in a young male following ingestion of an energy drink: Case report and review of the literature. *The American Journal of Emergency Medicine, 30*(6), 1010.e1–1010.e3.

Substance Abuse and Mental Health Services Administration. (2012). *Update on emergency department visits involving energy drinks: A final report*.

Tarka, S. M., Stacewicz-Sapuntzakis, M. and Theis, C. R. (1998). A review of the toxicological effects of caffeine. Regulatory Toxicology and Pharmacology, 27(2), 157-209.

Wadhwa, P. and Seth, R. K. (2015). Effect of chronic caffeine consumption on immune organs of Wistar rats. *International Journal of Research in Medical Sciences, 3*(11), 3121–3127.