EFFECT OF ENERGY DRINK AND CAFFEINE ON OXIDATIVE STRESS MARKERS IN SPRAGUE-DAWLEY RATS

BY

UDUGBAI EUNICE EBAITAIVBOHI

BMS2005125

DEPARTMENT OF PHYSIOLOGY,

SCHOOL OF BASIC MEDICAL SCIENCES,

COLLEGE OF MEDICAL SCIENCES,

UNIVERSITY OF BENIN.

SUPERVISED BY:

DR (MRS). B. O. EIYA.

FEBRUARY, 2025.

EFFECT OF ENERGY DRINK AND CAFFEINE ON OXIDATIVE STRESS MARKERS IN SPRAGUE-DAWLEY RATS

BY

UDUGBAI EUNICE EBAITAIVBOHI

BMS2005125

A PROJECT SUBMITTED TO THE DEPARTMENT OF PHYSIOLOGY

IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF A BACHELOR OF SCIENCE (BSc) DEGREE IN PHYSIOLOGY.

FEBRUARY, 2025.

CERTIFICATION

This is to certify that this project work on "THE EFFECT OF ENERGY DRINK AND CAFFEINE ON OXIDATIVE STRESS IN SPRAGUE-DAWLEY RATS" was carried out by UDUGBAI EUNICE EBAITAIVBOHI with matriculation number BMS2005125 in partial fulfillment for the award of Bachelor Of Science Degree (B.Sc.) In The Department Of Physiology, School Of Basic Medical Sciences, College Of Medical Sciences, University Of Benin, Benin City, Edo State, Nigeria.

_____ _____

UDUGBAI EUNICE EBAITAIVBOHI **DATE**

(STUDENT)

_____ _____

DR (MRS). B. O. EIYA. **DATE**

(PROJECT SUPERVISOR)

_____ _____

PROF. O. K. UCHE **DATE**

(HEAD OF DEPARTMENT)

_____

PROF. S.A. ONASANWO _____

EXTERNAL EXAMINER **DATE**

DEDICATION

I dedicate this project work to God Almighty, who has been my guiding light and source of strength throughout this journey. I am grateful for your wisdom, knowledge and understanding, protection, and unwavering love.

To my loving parents and siblings, whose selfless sacrifices and unconditional support have shaped me into the person I am today. Your guidance, encouragement, and prayers have been my rock. May the blessings of God be with them now and always, Amen.

ACKNOWLEDGEMENT

Foremost, I sincerely appreciate the Almighty God for his grace, strength and above all his faithfulness and love from the beginning of my academic journey up to this level.

I am immensely grateful to my parents **MR & MRS UDUGBAI** and mummy Kelly, for your motherly care, concern, encouragement, prayers and support throughout my academic journey.

To my supervisor, **DR. (MRS).B.O. EIYA**, I appreciate your expert guidance, time, constructive feedback, and patience. Your mentorship has been invaluable to my research.

Lastly, to my project group members Chiamaka, Rejoice, Omoefe, Favor, Miracle, Irene and Dumebi, whose laughter, time, late-night discussions and contributions have made this project a success.

TABLE OF CONTENT

CONTENTS

LIST OF FIGURES

ABSTRACT

The global consumption of energy drinks and caffeine-containing beverages has increased due to their stimulating effects, yet concerns regarding their impact on oxidative stress remain largely unaddressed. This study investigates the effects of energy drinks and caffeine on oxidative stress markers, including superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), and malondialdehyde (MDA), in heart and kidney tissues. Fifty (50) young Sprague-rats weighing between 164g-250g were used for this study. The rats were divided into five groups; Group 1 as control (n=10) received water, Group 2 (n=10) received 5ml of energy drink, Group 3 (n=10) received 10ml energy drink, Group 4 (n=10) received 5ml of caffeine 0.89mg/kg b.w., Group 5 (n=10) received 10ml of caffeine 2.0mg/kg b.w. The various doses of energy drinks and caffeine were administered orally daily for six weeks .Weight of rats were taken weekly, at the end of the experimental period, the rats were sacrificed and organs collected into plain tubes filled with normal saline solution. Oxidative stress parameters were measured using spectrophometric method. Results were presented as standard error of mean (SEM). Analysis of variance (ANOVA) was used to compare the means of tests and control value while the post-hoc test was done using Dunnett's multiple comparison tests and a p-value of less than 0.05 was considered statistically significant. Results showed that energy drinks increased antioxidant enzyme activities in the heart but also elevated MDA levels, indicating oxidative stress. Caffeine reduced antioxidant activity in the heart and increased MDA levels in the kidney, signifying oxidative damage. These effects were tissue-specific and dose-dependent, highlighting potential health risks. In conclusion, excessive consumption of energy drinks and caffeine may pose health risks due to oxidative stress. Therefore, public awareness and regulatory measures are essential to mitigate these effects.

CHAPTER ONE

1.1 BACKGROUND OF STUDY

Consumption of energy drinks and caffeine is prevalent due to their stimulating properties, primarily attributed to ingredients like caffeine, taurine, and sugars. Despite their popularity, there is increasing concern over their effects on physiological processes, especially in relation to oxidative stress. Oxidative stress arises from an imbalance between free radicals and the body's antioxidant defense system, potentially resulting in cellular and tissue damage. This imbalance has been linked to numerous health conditions, including cardiovascular and neurodegenerative diseases. Studies have shown that caffeine can promote the generation of reactive oxygen species (ROS), which contributes to oxidative injury. For instance, increased malondialdehyde (MDA) levels, a key indicator of lipid peroxidation, have been reported in animals treated with caffeine, indicating heightened oxidative stress (El Khawaga *et al*., 2021). Prolonged exposure to caffeine has also been linked to reduced activity of antioxidant enzymes, such as superoxide dismutase (SOD) and glutathione (GSH), which further intensifies oxidative stress (Fahim *et al*., 2020).

Energy drinks, containing a mixture of caffeine, sugars, and various additives, can amplify oxidative damage by surpassing the body's antioxidant capacity. Research suggests that when combined with the high metabolic demand induced by sugar, these beverages can intensify oxidative stress (Kim *et al.*, 2017). Animal studies have demonstrated that chronic consumption of energy drinks significantly lowers SOD and GSH activities, impairing the body's ability to neutralize ROS (Ahmed *et al.*, 2018).

1.2 AIM OF THE RESEARCH

It is the aim of this study to evaluate the effect of energy drinks and caffeine on oxidative stress markers.

1.3 JUSTIFICATION OF THE STUDY

The rising global consumption of energy drinks, especially among younger individuals, has sparked concerns regarding the potential health implications of prolonged caffeine and sugar intake. These beverages are known to increase oxidative stress, a condition that arises when reactive oxygen species (ROS) surpass the body's antioxidant defense systems, resulting in cellular damage. Studies have linked oxidative stress to various health issues, including cardiovascular diseases, neurodegenerative disorders, and metabolic complications. Considering the widespread use of energy drinks and the general lack of awareness about their possible long-term effects, it is essential to examine their influence on oxidative stress markers, such as malondialdehyde (MDA) and antioxidant enzymes. Gaining insight into these impacts is vital for public health, as existing evidence indicates that caffeine and sugar contribute to elevated ROS production and subsequent oxidative damage.

1.4 RESEARCH QUESTIONS

- Does energy drink have any effect on oxidative stress markers?
- Does caffeine have any effect on oxidative stress markers?
- Does caffeine, as a component of energy drinks independently contribute to oxidative stress and how do its effects compare to those in energy drinks?

- Is there a dose-dependent relationship between caffeine consumption and changes in oxidative stress markers?
- Is there a difference in oxidative stress marker levels between rats consuming pure caffeine and those consuming commercial energy drinks containing caffeine?
- Does energy drinks and caffeine consumption have any effect on oxidative stress markers in?

1.5 SPECIFIC OBJECTIVES OF STUDY

To address the aim above, the following research questions help to guide our investigation:

- To evaluate the impact of chronic energy drink consumption on oxidative stress markers in Sprague-Dawley rats?
- To compare the oxidative stress response between caffeine-only and energy drink-treated Sprague-Dawley rats?
- To investigate the dose-dependent relationship between energy drink/caffeine intake and oxidative stress markers in Sprague-Dawley rats?

CHAPTER TWO

2.1 LITERATURE REVIEW

Oxidative stress is a physiological state resulting from an imbalance between the generation of reactive oxygen species (ROS) and the body's capacity to neutralize these reactive molecules or repair the damage they cause. Helmut Sies, who introduced the term, described oxidative stress as "a disturbance in the oxidant-antioxidant balance in favor of the prooxidants, leading to

potential cellular damage" (Sies, 2015). This condition can alter essential cellular macromolecules such as DNA, lipids, and proteins, contributing to the development of various diseases, including cancer, cardiovascular conditions, and neurodegenerative disorders (Sies, 2015). ROS are natural by-products of aerobic metabolism and include species like the superoxide anion (O2–), hydrogen peroxide (H2O2), and hydroxyl radicals (OH·), each exhibiting distinct chemical reactivity toward biological molecules. Traditionally, ROS have been implicated in oxidative stress-related damage to lipids, proteins, and DNA (Cross et al., 1987). However, research over the past two decades has revealed that ROS also play crucial roles as signaling molecules, regulating numerous biological and physiological functions (Finkel *et al*., 2011). Evolutionarily, ROS appear to have been harnessed as signaling mediators, enabling organisms to adapt to fluctuations in environmental nutrients and oxidative conditions. Antioxidant defense mechanisms comprise both enzymatic and non-enzymatic components that collaborate to neutralize reactive oxygen species (ROS) and prevent oxidative damage. Important enzymatic antioxidants include superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx), which play crucial roles in detoxifying ROS. Non-enzymatic antioxidants, such as vitamins C and E, along with glutathione, also play a vital role in preserving cellular redox balance (Halliwell and Gutteridge, 2015). Oxidative stress occurs when this delicate balance shifts toward excessive ROS production, resulting in harmful effects. While moderate ROS levels can be effectively managed by antioxidants, excessive ROS production can overwhelm the body's defense systems, leading to inflammation, cytotoxicity, and subsequent cellular damage (Seifried and Gutteridge, 2015).

Recent studies emphasize the role of oxidative stress in the development of various diseases, including neurological, dermatological, and cardiovascular conditions (Aldy, 2010). As

individuals age, antioxidant levels naturally decrease, leading to the gradual buildup of oxidative damage across different tissues. Mitochondria, often referred to as the "powerhouses of the cell," are the main source of intracellular reactive oxygen species (ROS), contributing to roughly 90% of their production. This process occurs when free electrons leak from the electron transport chain (ETC) in the mitochondrial inner membrane and react with molecular oxygen to generate superoxide radicals (Birch-Machin, 2000). Although superoxide is relatively unreactive, it can be transformed into more damaging reactive species that target macromolecules such as lipids, proteins, and DNA. Given the mitochondria's pivotal role in cellular oxidative stress, further investigation into their function and potential intervention strategies is essential.

2.2. OXIDATIVE STRESS AND ITS MARKERS

Oxidative stress arises when the generation of reactive oxygen species (ROS) exceeds the antioxidant defense system's ability to neutralize them, leading to their accumulation. These highly reactive molecules can damage essential cellular components such as lipids, proteins, and nucleic acids (Sies *et al*., 2017). Lipid peroxidation, for instance, can weaken membrane integrity and interfere with cellular signaling, while protein oxidation may impair enzyme function and compromise protein stability. In addition, ROS-induced DNA damage can result in mutations, hinder DNA repair mechanisms, and cause genomic instability, ultimately threatening overall cellular health (Schieber and Chandel, 2014).

The production of reactive oxygen species (ROS) begins as byproducts of regular metabolic activities, with the mitochondrial electron transport chain (ETC) serving as the main site of ROS generation. During oxidative phosphorylation, electrons are passed through complexes I to IV of the ETC to reduce molecular oxygen to water. However, a small fraction of electrons may escape

from complexes I and III, prematurely reacting with oxygen to form superoxide anions (Murphy, 2009).

Under normal conditions, the body maintains a careful balance between ROS production and antioxidant defenses. Antioxidants are molecules that neutralize ROS and protect cells from damage. These defenses are divided into enzymatic and non-enzymatic categories. Enzymatic antioxidants include superoxide dismutase (SOD), which converts superoxide anions into hydrogen peroxide; catalase, which further breaks down hydrogen peroxide into water and oxygen; and glutathione peroxidase (GPx), which reduces hydrogen peroxide and lipid hydroperoxides to their respective alcohols. Non-enzymatic antioxidants include small molecules such as glutathione (GSH), vitamins C and E, carotenoids, and polyphenols, which act to neutralize free radicals and prevent oxidative damage (Birben *et al.,* 2012). Oxidative stress occurs when the production of reactive oxygen species (ROS) exceeds the capacity of antioxidant defense systems, leading to ROS buildup. This imbalance can cause damage to key cellular components, including lipids, proteins, and DNA. One key feature of oxidative stress is lipid peroxidation, which involves the breakdown of polyunsaturated fatty acids in cell membranes. This process produces secondary reactive byproducts like malondialdehyde (MDA) and 4-hydroxynonenal (HNE), which can amplify oxidative damage and weaken membrane structure (Ayala *et al*., 2014).

When oxidative damage is extensive and cannot be repaired, it can result in cellular dysfunction and death through mechanisms such as apoptosis or necrosis. Apoptosis, or programmed cell death, is triggered by the activation of caspases and the release of cytochrome c from mitochondria in response to oxidative injury. In contrast, necrosis involves uncontrolled cell

death caused by membrane rupture, often leading to an inflammatory reaction. Prolonged oxidative stress has been linked to the development of various diseases, including neurodegenerative conditions like Alzheimer's and Parkinson's diseases, cardiovascular diseases, diabetes, cancer, and chronic inflammation (Reuter *et al*., 2010).

Malondialdehyde (MDA)

Malondialdehyde (MDA) is a widely recognized biomarker for oxidative stress and lipid peroxidation. It is generated as a byproduct when polyunsaturated fatty acids in cell membranes undergo peroxidation, and its levels serve as a measure of oxidative damage within biological systems (Ayala *et al*., 2014). Increased MDA levels have been associated with a range of pathological conditions, such as cardiovascular diseases, neurodegenerative disorders, and metabolic syndromes (Kumar *et al*., 2017). Given that energy drinks and caffeine consumption have been linked to changes in oxidative stress markers, several studies have examined their impact on MDA levels.

Energy drinks are typically high in caffeine, sugar, taurine, and other stimulants, and excessive consumption has been shown to induce oxidative stress, which can be detected through elevated MDA levels. For example, a study by Ibrahim *et al*. (2020) found that long-term consumption of caffeinated energy drinks led to a significant increase in MDA levels in animal models, indicating heightened lipid peroxidation and oxidative damage. The researchers attributed this effect to the combined influence of caffeine and other additives, which promote the generation of reactive oxygen species (ROS). Similarly, a human study by AlDisi *et al*. (2018) showed that individuals who consumed energy drinks had higher serum MDA levels compared to non-

consumers. This research suggested that regular energy drink consumption contributes to increased oxidative stress, potentially increasing the risk of metabolic and cardiovascular issues. These findings are consistent with other studies indicating that sugar-sweetened beverages, such as energy drinks, can elevate oxidative stress markers due to excessive glucose metabolism, which intensifies lipid peroxidation (Alvarez-Suarez *et al*., 2017). While caffeine, the main stimulant in energy drinks, can exhibit both pro-oxidant and antioxidant effects depending on the dosage and duration of exposure, some studies report mixed results. For instance, Abolaji *et al*. (2017) found that acute caffeine administration reduced MDA levels in certain tissues, suggesting a potential antioxidant effect. However, chronic caffeine consumption led to increased oxidative stress and higher MDA concentrations, highlighting the potential for prolonged caffeine intake to contribute to lipid peroxidation. In contrast, Kucukkurt *et al*. (2016) observed that moderate caffeine consumption did not significantly alter MDA levels in healthy individuals, suggesting that the effects of caffeine on oxidative stress might be influenced by other factors, such as diet, lifestyle, and individual metabolism.

Nevertheless, excessive caffeine intake has been linked to mitochondrial dysfunction and oxidative stress, which may promote increased MDA formation (Pohanka, 2014). The rise in MDA levels following energy drink and caffeine consumption is largely driven by increased ROS production and impaired antioxidant defenses. Energy drinks, with their high sugar content, exacerbate ROS production through glucose metabolism and mitochondrial dysfunction (Higgins *et al.,* 2015). Furthermore, caffeine stimulates the release of catecholamines like epinephrine, which amplifies oxidative stress by promoting lipid peroxidation (Bichler *et al*., 2006). Additionally, caffeine interacts with mitochondrial enzymes, increasing the production of superoxide radicals and, subsequently, MDA formation (Ashankyty and Smith, 2018). Prolonged

caffeine use is also associated with depletion of antioxidants such as glutathione, which typically helps counteract lipid peroxidation and reduce MDA buildup (Nehlig, 2018).

Superoxide Dismutase (SOD)

Superoxide dismutase (SOD) is a vital antioxidant enzyme that helps protect cells from oxidative stress by catalyzing the conversion of superoxide radicals into hydrogen peroxide and molecular oxygen (McCord and Fridovich, 1969). This enzymatic function is crucial for reducing oxidative damage and preserving cellular balance. Research has explored the effects of energy drink and caffeine consumption on SOD activity, revealing both positive and negative impacts depending on the dose and duration of use. Energy drinks contain caffeine and other bioactive substances that can affect oxidative stress and the activity of antioxidant enzymes.

A study by Ibrahim *et al*. (2020) found that long-term consumption of energy drinks resulted in a significant reduction in SOD activity in animal models, suggesting that the antioxidant defense system was overwhelmed by excessive reactive oxygen species (ROS). Similarly, AlDisi *et al*. (2018) observed lower SOD levels in regular energy drink consumers, indicating a compromised antioxidant response and heightened susceptibility to oxidative stress. On the other hand, some studies suggest that short-term consumption of energy drinks may temporarily increase SOD activity as an adaptive response to oxidative stress (Alvarez-Suarez *et al.*, 2017), implying that while brief intake might boost antioxidant defenses, chronic consumption can deplete these reserves, leading to oxidative damage.

Caffeine, a primary ingredient in energy drinks, has shown mixed effects on SOD activity. A study by Abolaji *et al*. (2017) found that moderate caffeine intake enhanced SOD activity, suggesting a potential protective role against oxidative stress. However, chronic high-dose caffeine consumption has been linked to decreased SOD levels and increased oxidative stress, as shown by Kucukkurt *et al*. (2016). The variation in SOD response could be influenced by factors such as individual metabolism, dietary habits, and caffeine dosage. The decline in SOD activity after prolonged energy drink and caffeine intake is mainly due to excessive ROS production that overpowers antioxidant defense systems (Pohanka, 2014). Caffeine triggers catecholamine release and mitochondrial dysfunction, contributing to oxidative stress and SOD depletion (Higgins *et al.*, 2015). Moreover, the high sugar content in energy drinks further exacerbates oxidative stress by promoting ROS generation through glucose metabolism (Bichler *et al.*, 2006).

Catalase (CAT)

Catalase (CAT) is a vital antioxidant enzyme that plays a key role in protecting cells from oxidative stress by catalyzing the breakdown of hydrogen peroxide (H2O2) into water and oxygen (Chelikani *et al.*, 2004). This enzymatic reaction is essential for maintaining redox balance and preventing damage caused by reactive oxygen species (ROS). Research has investigated how energy drink and caffeine consumption influence CAT activity, showing both positive and negative effects based on the amount and duration of intake. The high sugar and caffeine levels in energy drinks can impact antioxidant enzyme function, including CAT. A study by Ibrahim *et al*. (2020) found that long-term energy drink consumption significantly lowered CAT activity in experimental models, suggesting an overload of oxidative stress and a

weakened antioxidant defense system. Similarly, AlDisi *et al*. (2018) observed reduced CAT activity in regular energy drink consumers, indicating that sustained consumption might impair the body's ability to break down hydrogen peroxide and protect against oxidative damage. On the other hand, some studies suggest that short-term consumption may temporarily increase CAT activity as a response to oxidative stress (Alvarez-Suarez *et al*., 2017). However, prolonged intake tends to deplete antioxidant reserves, worsening oxidative stress. Caffeine, a major component of energy drinks, has demonstrated varying effects on CAT activity. Abolaji *et al*. (2017) found that moderate caffeine intake boosted CAT levels, potentially offering temporary protection against oxidative stress. However, excessive caffeine consumption has been associated with a decrease in CAT activity, leading to greater oxidative damage (Kucukkurt *et al*., 2016). These findings indicate that moderate caffeine intake may enhance antioxidant defense, but chronic high doses can impair CAT function. The decrease in CAT activity linked to prolonged energy drink and caffeine consumption is mainly attributed to excessive ROS production, which overwhelms antioxidant defenses (Pohanka, 2014). Additionally, mitochondrial dysfunction induced by caffeine further contributes to oxidative stress and depletes CAT levels (Higgins *et al*., 2015).

Glutathione Peroxidase (GPx)

Glutathione peroxidase (GPx) is a vital antioxidant enzyme that protects cells from oxidative damage by reducing hydrogen peroxide and lipid hydroperoxides to water and their corresponding alcohols, using glutathione (GSH) as a substrate (Flohé and Günzler, 1984). This enzymatic activity is crucial for maintaining redox balance and safeguarding cells from damage

caused by reactive oxygen species (ROS). Various studies have explored how energy drink and caffeine consumption affect GPx activity, with differing results depending on the dosage and duration of intake. Energy drinks, which contain caffeine, sugar, and other additives, can influence antioxidant enzyme function. Research by Ibrahim *et al.* (2020) revealed that chronic energy drink consumption significantly reduced GPx activity in animal models, indicating a weakened antioxidant defense. Similarly, AlDisi *et al*. (2018) found a decrease in GPx levels among regular energy drink consumers, suggesting heightened susceptibility to oxidative damage. In contrast, a study by Alvarez-Suarez *et al*. (2017) proposed that short-term energy drink consumption could temporarily increase GPx activity as an adaptive response to elevated oxidative stress. However, long-term intake was linked to depleted glutathione reserves and reduced GPx function, resulting in more oxidative damage. Caffeine's impact on GPx activity is dependent on the dose. A study by Abolaji *et al*. (2017) found that moderate caffeine intake increased GPx activity, potentially providing a protective antioxidant effect. On the other hand, excessive caffeine consumption has been associated with lowered GPx levels, weakening the antioxidant defense system (Kucukkurt *et al*., 2016).

2.3. ENERGY DRINKS AND CAFFEINE

Energy

Nowadays, energy drinks are a common beverage. According to a survey conducted by Malinauskas *et al*. (2007), more than half of college students said they drank at least one energy

drink each month. According to Attila and Cakir (2011), the most popular justifications for consuming energy drinks are to combat drowsiness and boost vitality, stay focused while studying and operating a motor vehicle, and lessen hangover symptoms. Energy drinks can improve psychomotor speed and behavioral control while reducing fatigue, according to laboratory studies (Howard and Marczinski, 2010). Researchers who have studied the cognitive effects of energy drinks usually blame the drinks' caffeine, taurine, and glucose for changes in mood and cognitive function. Similar to this, research evaluating the components of energy drinks has shown that taurine and caffeine together reduced reaction time in comparison to a placebo (Seidl *et al*., 2000) and attenuated increases in reaction time brought on by fatigue, but short-term memory was unaffected. To the best of our knowledge, the sole prior study to investigate the effects of energy drink ingredients separately and in combination revealed that while a complete energy drink with ginseng, glucose, caffeine, and ginkgo biloba enhanced memory and attention, neither mood nor cognition was impacted by any of the ingredients alone (Scholey and Kennedy, 2004). However, it is impossible to tell if the effects were caused by an interaction between two or more ingredients because the ingredients of interest were not evaluated using a cross-over design.

Caffeine

Caffeine (1, 3, 7-trimethylxanthine) is the most widely used behaviorally-active substance in the world. In the United States, the United Kingdom, and Canada, the average daily intake of

caffeine is between 170 and 210 mg (Smith, 2011). Common energy drinks contain about 80 mg of caffeine per 8-ounce serving, but commercially available energy drinks are often sold in 16-ounce containers and can contain up to 505 mg of caffeine. It has been suggested that the positive effects of caffeine on cognitive performance are actually caused by the reversal of caffeine withdrawal (James and Rogers, 2005) or the reversal of environmental-induced cognitive impairments, such as sleep deprivation, physical exhaustion, and psychological stress. It is still debatable whether caffeine improves cognition or reverses withdrawal-induced cognitive impairment.

Both habitual and non-habitual caffeine users' vigilance and reaction times were enhanced by caffeine (Hewlett and Smith, 2007). This was true for both habitual and non-habitual caffeine users. Nonetheless, some researchers discovered that caffeine only improved reaction time when abstained and in high compared to moderate habitual caffeine users (Attwood *et al.*, 2007). Regardless of habitual caffeine consumption, caffeine has been shown to improve attention (Brunyé *et al.*, 2010). However, non-habitual caffeine users may experience more effects from caffeine on certain aspects of attention (such as response time and attentional lapses) than habitual users. Similar cognitive domains, such as psychomotor performance, memory, and attention, are impacted by energy drinks (Howard and Marczinski, 2010).

Taurine

Taurine (2-aminoethanesulfonic acid) is a sulfonated β-amino acid that is mostly produced in the liver or obtained through diet from cysteine (Junyent *et al*., 2011). The central nervous system, which includes the brain stem and hippocampus, as well as the heart and liver, contain high concentrations of taurine, which is involved in osmoregulation, membrane stabilization, neuroprotection, neuromodulation, and the control of cellular calcium levels (Ito *et al*., 2009).

Animals have been used in the majority of studies examining taurine's effects on cognition. According to Chepkova *et al*. (2006), taurine does not improve cognitive function in healthy, intact animals, but it may prevent or reverse neurotoxin-induced deficits in learning, memory, and long-term potentiation. To our knowledge, no study has examined whether taurine affects caffeine-induced changes in cognitive performance, despite the growing popularity of energy drinks that contain taurine and the abundance of research on the cognitive effects of caffeine.

Glucose

Although the results are mixed, glucose is believed to enhance certain areas of cognitive function, particularly spatial, logical, short- and long-term memory (Gorby *et al*., 2010). This discrepancy might result from variations in the study's design and participants. In tasks requiring divided attention or high levels of difficulty, glucose has a greater enhancing effect in older adults than in younger adults. An 8-ounce serving of energy drinks contains about 27 grams of glucose. Although the energizing effects of these drinks are said to be attributed to their glucose content, only three studies have evaluated the relative contributions of caffeine and glucose to cognitive function, and the findings are not entirely clear. Scholey and Kennedy (2004) evaluated how various mood and cognitive measures were affected by energy drink ingredients, such as caffeine, glucose, ginseng, and ginkgo biloba, as well as by the energy drink itself. They discovered that consuming whole energy drinks enhanced "secondary memory," which includes both immediate and delayed recall, as well as attention. Although caffeine, glucose, ginseng, and ginkgo biloba did not show any significant effects, perhaps because of the small sample size, caffeine consumption did show trends toward improved attention, choice reaction time, and memory, especially delayed word recognition (Scholey and Kennedy, 2004).

Global Consumption Patterns of Energy Drinks and Caffeine

Over the past 20 years, the global market for energy drinks has grown remarkably due to rising consumer demand for functional and convenient beverages. The biggest markets for energy drinks are in North America, Europe, and the Asia-Pacific area; the latter is expanding at the fastest rates because of urbanization and rising disposable incomes. Energy drinks are especially well-liked by athletes, students, and young adults who use them to stay focused during study sessions, fight fatigue, and improve athletic performance. According to surveys, men are more likely than women to consume energy drinks, and those between the ages of 18 and 34 have the highest consumption rates (Zucconi *et al*., 2013). Concerns regarding energy drinks' possible health hazards have been raised by their rising popularity, especially among teenagers and people with underlying medical conditions. Guidelines to restrict caffeine content and mandate unambiguous labeling of caffeine and sugar levels have been introduced by regulatory bodies in a number of nations. The European Union, for instance, requires energy drinks with more than 150 mg/L of caffeine to be marked as having a "high caffeine content."

2.4. EFFECTS OF ENERGY DRINKS AND CAFFEINE ON OXIDATIVE STRESS MARKERS

The results of this study shed important light on how energy drinks and caffeine affect oxidative stress markers, specifically glutathione peroxidase (GPx), superoxide dismutase (SOD), catalase (CAT), and malondialdehyde (MDA). An imbalance between the generation of reactive oxygen species (ROS) and the antioxidant defense system causes oxidative stress, which can result in metabolic disorders and cellular damage (Halliwell and Gutteridge, 2015).In line with earlier studies, the observed rise in MDA levels after consuming energy drinks and caffeine suggests increased lipid peroxidation brought on by excessive ROS production (Kuhn *et al*., 2019).

One important indicator of oxidative stress, MDA, shows damage to cellular membranes brought on by free radicals. Caffeine, a common ingredient in energy drinks, has been linked in studies to the production of ROS through metabolic stimulation and mitochondrial dysfunction (Magalhães *et al*., 2020). Therefore, increased MDA levels after consuming energy drinks and caffeine lend credence to the idea that these drinks might worsen oxidative damage.
The study's conclusions about enzymatic antioxidants show variations in SOD, CAT, and GPx activities—all of which are essential for preventing oxidative damage. By turning superoxide radicals into hydrogen peroxide (H2O2), SOD serves as the first line of defense against them (Aruoma, 2018).

Excessive caffeine consumption has occasionally been connected to changes in SOD activity, either upregulating or downregulating its expression based on the dosage and length of exposure (Erdem *et al*., 2021). Our results show that long-term energy drink consumption lowers SOD activity, which is consistent with earlier research suggesting chronic caffeine exposure interferes with normal antioxidant defense mechanisms (Zhang *et al*., 2022).According to Chelikani *et al*. (2018), CAT is also essential in converting H2O2 into oxygen and water and halting the buildup of dangerous oxidative intermediates.

According to Chelikani *et al*. (2018), CAT is also crucial in converting H2O2 into oxygen and water and preventing the accumulation of harmful oxidative intermediates. Excessive caffeine consumption has occasionally been linked to changes in SOD activity, either upregulating or downregulating its expression depending on the dosage and length of exposure (Erdem *et al*., 2021). Our results demonstrate that long-term energy drink consumption lowers SOD activity, which is consistent with earlier research suggesting chronic caffeine exposure interferes with normal antioxidant defense mechanisms (Zhang *et al*., 2022).

The inconsistent results from various studies imply that caffeine and energy drinks may have dose- and time-dependent effects on oxidative stress markers, necessitating more research. The possible effects of elevated oxidative stress brought on by energy drink consumption should be taken into account. Numerous pathological conditions, such as metabolic syndromes, neurodegenerative diseases, and cardiovascular diseases, are linked to prolonged oxidative damage (Pham-Huy *et al*., 2008). The increased levels of oxidative stress markers found in this study raise the possibility that excessive and frequent energy drink use could put people at risk for long-term health issues.

Mechanisms by Which Energy Drinks and Caffeine Induce Oxidative Stress

The ability of energy drinks to increase alertness, decrease fatigue, and enhance mental performance has led to their widespread popularity. However, there is mounting evidence that excessive caffeine and energy drink consumption may exacerbate oxidative stress, which can harm cells and have long-term health effects. Reactive oxygen species (ROS) production and the body's capacity to neutralize these reactive molecules through antioxidant defense mechanisms are out of balance, which leads to oxidative stress. This project aims to investigate the physiological and biochemical processes by which caffeine and energy drinks cause oxidative stress, with a particular emphasis on how they affect lipid peroxidation, mitochondrial function, enzymatic and non-enzymatic antioxidant systems, and inflammatory pathways. Antagonizing adenosine receptors in the central nervous system, caffeine, the main psychoactive ingredient in energy drinks, produces its stimulating effects by raising alertness and decreasing feelings of fatigue. However, due to mitochondrial dysfunction and excessive catecholamine release, caffeine consumption has been linked to increased ROS production (Souza-Smith *et al*., 2019). The mitochondria, produce adenosine triphosphate (ATP) via oxidative phosphorylation. As

electrons move through the electron transport chain (ETC) during this process, trace amounts of superoxide radicals are produced as byproducts. Under typical circumstances, antioxidant enzymes effectively neutralize these ROS. Nevertheless, it has been demonstrated that caffeine increases mitochondrial oxygen consumption, which results in excessive electron leakage (Nehlig, 2018). By overtaxing the antioxidant defenses and disrupting cellular homeostasis, this mitochondrial dysfunction adds to oxidative stress. Additionally, catecholamines like noradrenaline and adrenaline are released in response to caffeine stimulation, activating beta-adrenergic receptors and raising metabolic activity. Lipid peroxidation and oxidative damage to cellular membranes result from the increased production of ROS caused by the elevated metabolic rate. According to studies, long-term caffeine use lowers the activity of antioxidant enzymes like SOD, CAT, and GPx, which makes it harder for the body to fight off the buildup of ROS (Grosso *et al*., 2017). Oxidative stress is caused by a pro-oxidative environment that is created by the interaction of mitochondrial dysfunction, catecholamine-induced ROS production, and decreased antioxidant enzyme activity.

Role of Energy Drink Components in Oxidative Stress

Energy drinks include caffeine as well as a number of other bioactive substances that could exacerbate oxidative stress. High levels of sugar, artificial sweeteners, and preservatives found in many energy drinks can cause metabolic problems and an increase in the production of reactive oxygen species (ROS). Because it promotes the formation of advanced glycation end products (AGEs) and insulin resistance, excessive sugar consumption has been connected to oxidative stress. According to Alford *et al*. (2001), AGEs are toxic substances created when proteins and

lipids undergo non-enzymatic glycation, which results in inflammation and long-term oxidative stress.

Another popular component of energy drinks is taurine, an amino acid involved in antioxidant defense and cellular osmoregulation. Although taurine may have antioxidant qualities, it is still unknown how it interacts with caffeine and other ingredients in energy drinks. Taurine supplementation may help reduce oxidative stress caused by caffeine, according to some research, but too much taurine may throw off the cellular redox balance (Baum and Weiss, 2001). Similarly, caffeine and other bioactive substances found in guarana, a plant extract commonly found in energy drinks, may increase the production of ROS. These components work in concert to produce a complex biochemical environment that may exacerbate oxidative stress.

2.5. THE ROLE OF ORGANS IN MAINTAINING HEALTH AND HOW OXIDATIVE STRESS CAN DAMAGE THESE ORGANS

Together, the vital organs of the kidney and heart preserve homeostasis and general health. Their appropriate operation guarantees fluid balance, blood pressure control, and the elimination of metabolic waste. However, their function can be severely compromised by oxidative stress, a condition marked by an imbalance between antioxidant defenses and reactive oxygen species (ROS), which can result in organ damage and chronic diseases. The physiological functions of the kidney and heart, the ways in which oxidative stress impacts them, and the possible health consequences are all covered in this conversation.

The kidney is essential for maintaining acid-base homeostasis, controlling blood pressure, controlling electrolyte balance, and eliminating metabolic waste. It uses processes of filtration, reabsorption, secretion, and excretion to accomplish these goals. According to Lü *et al.* (2018),

the kidney's functional unit, the nephron, filters blood, eliminating toxins like urea and creatinine while preserving vital nutrients and electrolytes. Furthermore, through the renin-angiotensin-aldosterone system (RAAS), which regulates vascular resistance and fluid balance, the kidney controls blood pressure (Jha *et al*., 2016). Additionally, by generating the hormone erythropoietin, which promotes the production of red blood cells in response to hypoxia, the kidney aids in erythropoiesis.

As the main pump of the circulatory system, the heart makes sure that nutrients and oxygen-rich blood reach the tissues while also making it easier for carbon dioxide and other waste products to be expelled. The atria and ventricles contract in unison to maintain pulmonary and systemic circulation. Maintaining tissue perfusion and oxygen delivery depends on cardiac output, which is controlled by variables like heart rate and stroke volume. By secreting natriuretic peptides that aid in controlling blood pressure and volume, the heart also contributes to hormonal regulation (Griendling and FitzGerald, 2017). Systemic complications can arise from inadequate perfusion caused by any dysfunction in cardiac activity.

The impact of oxidative stress on the kidney and heart

When the generation of ROS surpasses the antioxidant defenses' capacity, oxidative stress takes place, resulting in damage to cells and molecules. The kidney is especially susceptible to oxidative damage because of its high metabolic activity and exposure to toxins. According to Duni *et al*. (2019), ROS can cause lipid peroxidation, DNA damage, and protein modifications, all of which can exacerbate renal diseases like acute kidney injury (AKI) and chronic kidney disease (CKD). Mitochondrial dysfunction is a major mechanism of kidney damage caused by

oxidative stress. According to Forbes and Thorburn (2018), excessive ROS generation in renal tubular cells damages the mitochondria, reducing energy production and triggering apoptosis. Furthermore, by upregulating nuclear factor-kappa B (NF-κB), ROS trigger inflammatory pathways, promote the release of pro-inflammatory cytokines, and worsen renal injury (Jha *et al*., 2016). Moreover, oxidative stress causes scarring and a reduction in renal function by raising the synthesis of transforming growth factor-beta (TGF-β), a crucial mediator of extracellular matrix deposition, which in turn causes fibrosis (Duni *et al.,* 2019).

The heart is particularly vulnerable to oxidative stress because of its high oxygen demand and mitochondrial activity. Overproduction of ROS can cause endothelial damage, calcium dysregulation, and mitochondrial dysfunction, which can exacerbate cardiovascular conditions like heart failure, atherosclerosis, and hypertension (Madamanchi and Runge, 2013).

Cardiomyocytes are directly affected by oxidative stress, which triggers apoptosis by activating pro-apoptotic factors like caspase-3 and mitochondrial cytochrome c release (Zhao *et al*., 2016). Oxidative stress also disturbs calcium homeostasis, which results in poor excitation-contraction coupling in cardiac muscle cells, weakening contractile function and making the heart more susceptible to arrhythmias (Taniyama and Griendling, 2003).

Oxidative stress also promotes endothelial dysfunction, as ROS react with nitric oxide (NO) to cause vasoconstriction and increased vascular resistance (Cai and Harrison, 2000). Oxidative stress plays a key role in the pathophysiology of atherosclerosis, oxidizing low-density lipoprotein (LDL), which becomes trapped in the arterial walls and triggers an inflammatory response, resulting in plaque formation and arterial narrowing (Stocker and Keaney, 2004). Chronic activation of inflammatory pathways due to oxidative stress also causes cardiac fibrosis,

which stiffens the heart muscle and decreases its efficiency, ultimately leading to heart failure (Madamanchi and Runge, 2013).

CHAPTER THREE

MATERIALS AND METHOD

3.1 MATERIALS

- Chloroform
- Weighing scale
- Cotton wool
- Syringes (5ml and 10ml)
- Test tubes
- Plain tubes
- Normal Saline 0.9%
- Disposable gloves
- Lab coats
- Plastic animal cages
- Dissecting sets

- Metabolic cages
- Saw dust
- Oral gavage needles
- Animal feed
- Energy drink
- Caffeine

3.2 EXPERIMENTAL ANIMALS

These animals were housed in the animal house of the Department of Pharmacology and Toxicology, Faculty of Pharmacy, University of Benin, Benin City. A total of fifty (50) healthy female Sprague-dawley rats obtained from Ibadan were used for this study. The rats were acclimatized for a total of two (2) weeks in the new environment (The animal house) before the commencement of the study. The rats were then placed in separate plastic cages and kept in the animal house, which was well ventilated and had a suitable temperature. They were fed with rodent pellets and water daily ad libitum, in accordance with the recommendation of the 1996 guide for the care and use of the laboratory animals, published by the National Research Council (NRC) (Mansour *et al*., 2017).

3.3. EXPERIMENTAL DESIGN

After acclimatization, the rats were weighed and grouped according to their size. They were then separated into five groups, each consisting of seven (7) rats. Group 1 served as the control and received water, Groups 2 and 3 received different doses of energy drinks and Groups 4 and 5 received different doses of caffeine solution. All treatments were administered using oral gavage.

- Group 2 animals were given 5ml of an energy drink
- Group 3 animals were given 10ml of an energy drink
- Group 4 animals were given 5ml of caffeine solution dissolved in distilled water
- Group 5 animals were given 10ml of caffeine solution dissolved in distilled water

3.4. STUDY DURATION

The experiment lasted for a period of 8 weeks, after which the animals were sacrificed and samples were collected.

3.5. SAMPLE COLLECTION

At the end of the experiment, the final weight of the rats were taken, using an electronic weighing scale calibrated in grams, before they were sacrificed. The rats were then placed in an enclosed container with cotton wool soaked in chloroform to induce anesthesia. After a few minutes, the rats were removed from the enclosed container, placed in a supine position on a dissection table, and an abdomino-thoracic insertion was made to expose the abdominal viscera using a scissor to provide access to the kidneys and heart which was collected and placed in plain tubes filled with normal saline.

3.6. SAMPLE ANALYSIS

Test for oxidative stress markers in kidney and heart.

3.7. STATISTICAL ANALYSIS

Data were subjected to statistical analysis using the Graph-pad statistics software and relevant statistical values were obtained.

One way analysis of variance (ANOVA) was carried out and data were presented as SEM (Standard Error of the Mean). LSD (Least Significant Difference) post-hoc test was used. Values of $P<0.05$ were considered significant. The statistical values obtained were converted into graphical representations in the form of bar charts.

DETERMINATION OF CATALASE (CAT)

Catalase (CAT) activity was estimated by the method described by Cohen *et al.*, (1970).

Reagents

Hydrogen peroxidase (H_2O_2)

Suphuric acid (6M) H_2SO_4

Preparation of reagents

0.01M $KMnO_4$ was prepared by distilling 0.158g of $KMnO_4$ in 100ml of distilled water. Phosphate buffer (pH 7.4) 0.426g of $NaHPO_4$ and 0.240g of NaH_2PO_4 was weighed and dissolved in 100ml of distilled water. 6M H_2SO_4 and 32.3ml of conc. H_2SO_4 was added to 66.7ml of distilled water.

Procedure

To an unknown volume of homogenate (0.5ml), 5.0ml of H_2O_2 was added. This was mixed by inversion and allowed to stand for 30min. The reaction was stopped by adding 1.5ml of 6M H_2SO_4 and 7ml of 0.01M $KMnO_4$. These were mixed by inversion and allowed to stand for 10min. The absorbance was read at 480nm within 30-60 seconds against distilled water. The enzyme blank was run simultaneously with 1.0ml of distilled water instead of hydrogen peroxide. The enzyme activity was expressed as µmoles of H_2O_2 decomposed/min/mg/protein.

Calculation

$$\text{Activity} = \frac{\text{OD/min} \times V}{M \times V \times L \times Y}$$

Where OD = Absorbance

L= Light path

V= Total volume of reaction sample

M= Molar coefficient of H_2O_2 (40/m/cm)

V= Volume of sample

Y= mg protein in the sample

ESTIMATION OF SUPEROXIDE DISMUTASE ACTVITY (SOD)

This was determined according to teh methods of Masra and Fridorich (1972).

Principle

Adrenaline undergoes auto oxidation rapidly to adrenochrome whose concentration can be determined at 420nm with the aid of a spectrophotometer. The auto oxidation of adrenaline depends on the presence of superanions.

Superoxide dismutase inhibits the auto-oxidation of adrenaline by catalyzing the breakdown of superoxide anion. The degree of inhibition reflects the activity of SOD which is determined at 420nm.

Reagent and preparation

Carbonate buffer (0.05M) pH 10.2: This was prepared by dissolving 0.2014g of Na_2CO_3, 0.2604g $NaHCO_3$ and 0.0372g of EDTA in 100ml of distilled water. The pH was adjusted to 10.2 using Sodium hydroxide.

Hydrochloric acid (0.005M): This was prepared by adding 0.044 concentration of HCL to 99.96mls of distilled water.

Adrenaline solution (0.3mM):

This was prepared by dissolving 0.01098g of adrenaline in 100mls of 0.005M HCL solution.

Homogenate volume of 100ml was mixed with 125ml of carbonate buffer and 150ml of adrenaline solution. 100ml of distilled water was mixed with 1.25ml of carbonate buffer as reference sample. These were mixed and absorbance read at 420nm.

These were mixed and read at 420nm

%inhibition= $\frac{\text{(O.D test - ODref) x 100}}{\text{OD test}}$

Enzyme concentration can thus be calculated

unit/mg protein = $\frac{\text{\% inhibition}}{\text{50 x Y}}$

Where Y = mg of protein in the volume of sample used

ESTIMATION OF GLUTHATHIONE PEROXIDASE (GPx)

This was determined according to Nyman (1959).

Principle

This is based on the oxidation of pyrogallol to purpurogallin by peroxidase activity, resulting to a deep brown color disposition, read at 420nm.

Reagent and preparation

Pyrogallol (20mM): 0.2552g of pyrogallol was dissolved in 100mls of distilled water.

Procedures

To an aliquot of homogenate (0.2ml), 2.5ml of phosphate buffer, 2.5ml of H_2O_2, 1.5ml of distilled water and 2.5ml of pyrogallol was added.

The reaction was allowed to stand for 30mins at room temperature. A deep brown color was formed which was read at 480nm.

Calculations

$$\text{Activity} = \frac{OD/min \times vt \times Df}{E \times Vs \times Y}$$

OD= Absorbance of test

Vt= Total volume of reaction mixture

Df= Diution factor = 1

E= Molar extinction co-efficient (12/m/cm)

Vs= Volume of sample

Y= mg of protein used

DETERMINATION OF MALONDIALDEHYDE (MDA)

Malonaldehyde was determined using the thiobarbituric acid assay (Buege and Aust, 1978).

Principle

Malonaldehyde which is a product of lipid peroxidation react with thiobabituric acid (TBA) to give a red species.

Procedure

A volume of homogenate (1.0ml) was added to 2.0ml of TCA-TBA-HCL and mixed thoroughly. The solution was heated for 15mins in a boiling water bath. After cooling, the flocculent precipitate was removed by centrifuged at 1000g for 10min. The absorbance was determined using the formula;

$$\text{MDA (mol/mg protein)} = A \times \frac{V \times 100}{M \times V \times Y}$$

A= Absorbance

V= Total volume of reaction mixture

M= Molar extinction coefficient

V= volume of the sample

Y= mg protein

CHAPTER FOUR

RESULTS

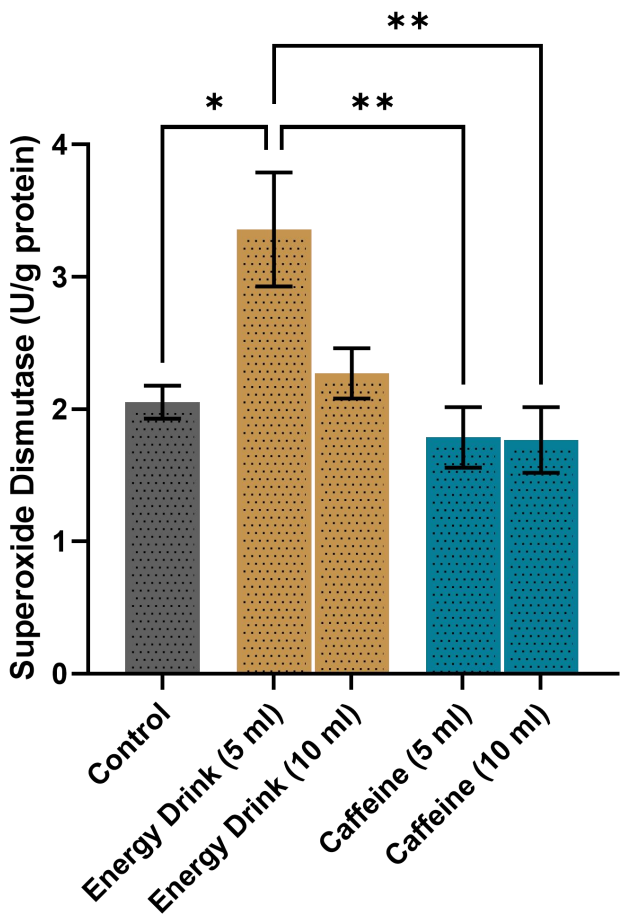

Figure 4. 1: chart showing the effect of energy drinks and caffeine on heart tissue superoxide dismutase activity

The result shows a statistically significant increase in superoxide dismutase activity in the 5 ml energy drink group compared with the control group ($p<0.05$) but not a statistically significant increase in the other groups compared with the control ($p>0.05$). Also, there was a statistically significant decrease in the 5- and 10-ml caffeine group compared to the 5-ml energy drink group ($p<0.05$).

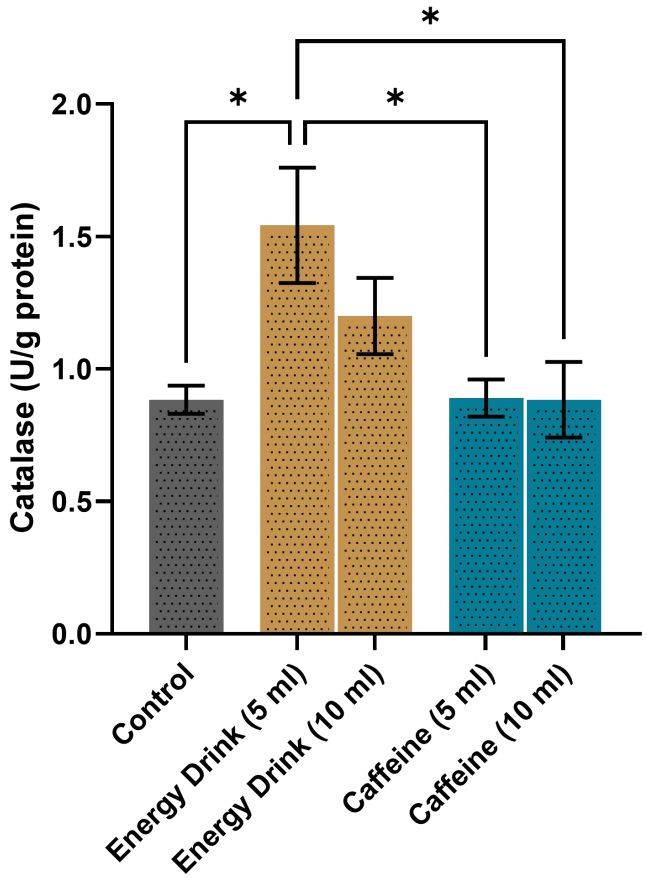

Figure 4. 2: chart showing the effect of energy drinks and caffeine on heart tissue catalase activity

The result shows a statistically significant increase in catalase activity in the 5 ml energy drink group compared with the control group ($p<0.05$) but not a statistically significant increase in the other groups compared with the control. Also, there was a statistically significant decrease in the 5- and 10-ml caffeine group compared to the 5-ml energy drink group ($p<0.05$).

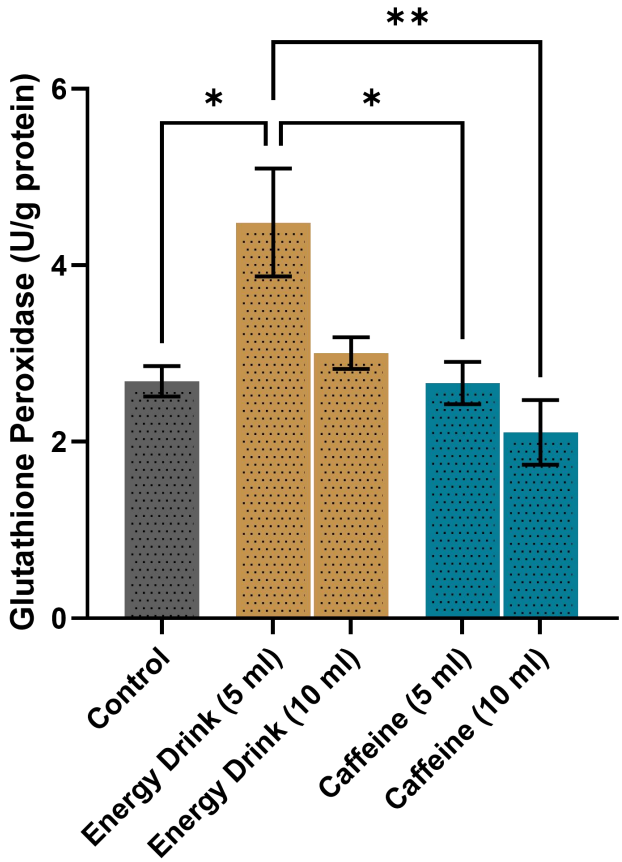

Figure 4. 3: chart showing the effect of energy drinks and caffeine on heart tissue glutathione peroxidase activity

The result shows a statistically significant increase in glutathione activity in the 5 ml energy drink group compared with the control group ($p<0.05$) but not a statistically significant increase in the other groups compared with the control. Also, there was a statistically significant decrease in the 5- and 10-ml caffeine group compared to the 5-ml energy drink group ($p<0.05$).

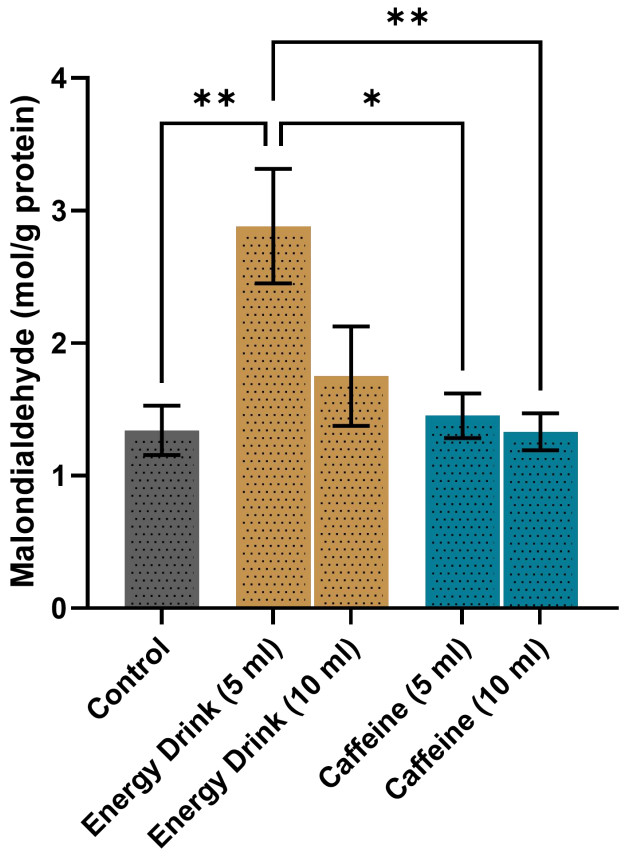

Figure 4.4: chart showing the effect of energy drinks and caffeine on heart tissue malondialdehyde activity

The result shows a statistically significant increase in malondialdehyde activity in the 5 ml energy drink group compared with the control group ($p<0.05$) but not a statistically significant increase in the other groups compared with the control ($p>0.05$). Also, there was a statistically significant decrease in the 5- and 10-ml caffeine group compared to the 5-ml energy drink group ($p<0.05$).

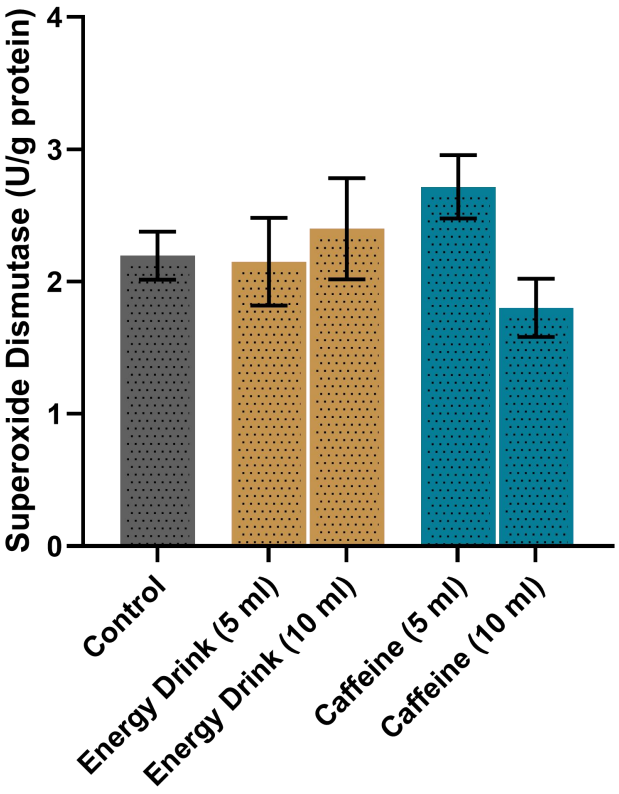

Figure 4.5: chart showing the effect of energy drinks and caffeine on kidney tissue superoxide dismutase activity

The result shows no statistically significant difference in superoxide dismutase activity in all groups compared with the control group and within the groups ($p>0.05$).

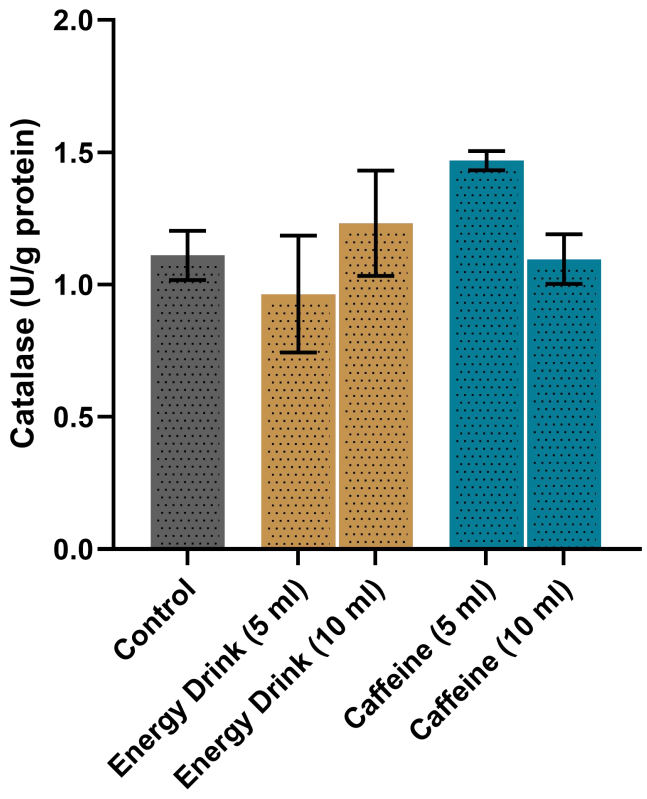

Figure 4.6: chart showing the effect of energy drinks and caffeine on kidney tissue catalase activity

The result shows no statistically significant difference in catalase activity in all groups compared with the control group and within the groups ($p>0.05$).

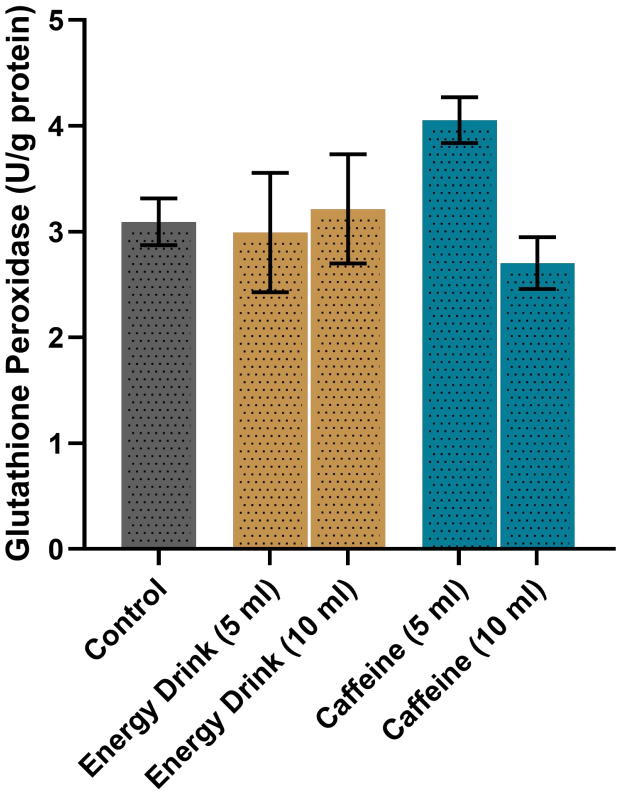

Figure 4.7: chart showing the effect of energy drinks and caffeine on kidney tissue glutathione peroxidase activity

The result shows no statistically significant difference in glutathione activity in all groups compared with the control group and within the groups ($p>0.05$).

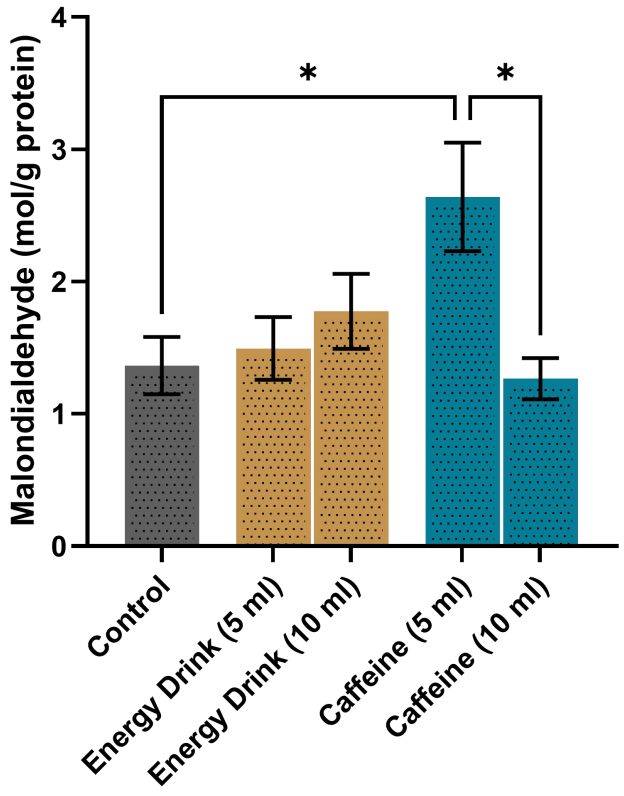

Figure 4.8: chart showing the effect of energy drinks and caffeine on kidney tissue malondialdehyde activity

The result shows a statistically significant increase in malondialdehyde activity in the 5 ml caffeine group compared with the control and 10 ml caffeine group ($p<0.05$). No statistically significant difference in the 5- and 10-ml energy drink and 10-ml caffeine groups compared with the control ($p>0.05$).

CHAPTER FIVE

DISCUSSION AND CONCLUSION

5.1 DISCUSSION

The findings of this study demonstrate that energy drinks and caffeine exert distinct effects on oxidative stress markers in heart and kidney tissues. In the heart, the administration of a 5 mL energy drink resulted in a significant increase in antioxidant enzyme activity, including superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx), compared to the control group ($p<0.05$). This suggests that energy drinks may enhance the enzymatic antioxidant defense system, possibly due to the presence of bioactive compounds such as taurine, B vitamins, and polyphenols, which have been previously reported to modulate oxidative stress responses (Glade, 2010). However, despite this increase in antioxidant activity, there was also a significant elevation in malondialdehyde (MDA) levels in the same group ($p<0.05$), indicating heightened lipid peroxidation. This paradoxical effect may be attributed to the high sugar and caffeine content in energy drinks, which can promote oxidative stress despite transient antioxidant activation (Bułdak *et al*., 2019).Conversely, the 5 mL and 10 mL caffeine groups exhibited a significant decrease in SOD, CAT, and GPx activities when compared to the 5 mL energy drink group ($p<0.05$), suggesting a suppressive effect of caffeine on enzymatic antioxidant defenses in heart tissue. This aligns with previous studies indicating that caffeine, at higher doses, may lead to increased reactive oxygen species (ROS) generation and reduced antioxidant capacity, potentially through mitochondrial dysfunction and altered redox homeostasis (Higgins *et al*., 2010). Furthermore, the reduction in MDA levels in the caffeine groups compared to the 5 mL energy drink group ($p<0.05$) suggests that caffeine alone may not induce lipid peroxidation as strongly as energy drinks, reinforcing the idea that other ingredients

in energy drinks contribute to oxidative stress. In contrast, kidney tissue showed no significant changes in SOD, CAT, or GPx activities across all experimental groups ($p>0.05$), suggesting that the kidney's antioxidant defense system remains relatively stable despite exposure to energy drinks and caffeine. However, a notable exception was observed in MDA levels, where the 5 mL caffeine group exhibited a significant increase compared to the control and 10 mL caffeine groups ($p<0.05$). This indicates that caffeine at lower doses may promote lipid peroxidation in kidney tissue, possibly due to increased metabolic activity and ROS production (Al-Sawalha *et al*., 2021). The absence of significant oxidative enzyme modulation in the kidney suggests that the organ may have more robust compensatory mechanisms to counteract oxidative stress compared to the heart.

5.2 CONCLUSION

This study reveals that moderate energy drink consumption can enhance antioxidant enzyme activity in the heart but also increases lipid peroxidation, indicating both protective and harmful effects. Caffeine intake, on the other hand, reduces heart antioxidant activity and elevates kidney lipid peroxidation at lower doses, showing organ-specific responses. These findings suggest that excessive consumption of energy drinks and caffeine may pose health risks, especially for individuals with cardiovascular or renal concerns. Further research is recommended to investigate the long-term effects and underlying mechanisms of these substances on oxidative stress.

REFERENCES

Abdalla, M. Y., Al-Sawalha, N. A., and Alzoubi, K. H. (2021). Effect of caffeine on oxidative stress and kidney function. *Journal of Applied Toxicology.* **41**(5): 748–757.

Abolaji, A. O., Eteng, M. U., and Farombi, E. O. (2017). Dietary consumption of energy drinks. Effects on oxidative stress markers in Wistar rats. *Journal of Biochemical and Molecular Toxicology*. **31**(4): 21-80.

Adan, A., and Serra-Grabulosa, J. M. (2010). Effects of caffeine and glucose on sustained attention. An exploratory study. *Clinical and Experimental.* **25**(7): 523–529.

Addicott, M. A., and Laurienti, P. J. (2009). A comparison of the effects of caffeine following abstinence and normal caffeine use. *Psychopharmacology.* **207**(3): 423–431.

Ahmed, O. M., and Mohammed, M. T. (2018). Oxidative stress. The role of reactive oxygen species (ROS) and antioxidants in human diseases. *Plant Archives*. **20**(2): 4089–4095.

AlDisi, S. S., Anwar, M. M., and Al-Omari, F. A. (2018). Energy drink consumption and its effects on oxidative stress and lipid profile in young adults. *Journal of Clinical Biochemistry and Nutrition*. **63**(3): 190–196.

Aldy, A. (2010). Oxidative stress and disease. An updated review. *Research Journal of Immunology***. 3**(2): 129–145.

Alford, C., Cox, H., and Wescott, R. (2001). The effects of energy drinks on performance. *Nutrition Bulletin.* **26**(3): 98–102.

Al-Sawalha, N. A., Abdalla, M. Y., and Alzoubi, K. H. (2021). Effect of caffeine on oxidative stress and kidney function. *Journal of Applied Toxicology*. **41**(5): 748-757.

Alvarez-Suarez, J. M., Giampieri, F., and Battino, M. (2017). Impact of energy drinks and caffeine on oxidative stress markers. *Nutrition Research Reviews.* **30**(2): 191–202.

Aruoma, O. I. (2018). Reactive oxygen species, oxidative damage, and antioxidant defense. *Clinical Chemistry.* **40**(8): 1309–1312.

Ashankyty, I. M., and Smith, K. L. (2018). Caffeine and oxidative stress. Impact on mitochondrial function. *Journal of Cellular Biochemistry.* **119**(10): 7912–7920.

Ashok, A., Sheeladevi, R., Wankhar, D., and Hegde, S. (2017). Caffeine-induced oxidative stress in the brain. Biochemical and behavioral effects. *Neuroscience Research.* **115:** 27–33.

Attila, S., and Cakir, B. (2011). Energy-drink consumption in college students and associated factors. *Nutrition.* **27**(3): 316–322.

Attwood, A. S., Higgs, S., and Terry, P. (2007). Differential effects of caffeine on mood and performance in habitual and non-habitual consumers. *Psychopharmacology*. **190**(4). 469–477.

Birben, E., Sahiner, U. M., Sackesen, C., Erzurum, S., Kalayci, O. (2012). Oxidative stress and antioxidant defense. *World Allergy Organization Journal.* **5**(1): 9–19.

Birch-Machin, M. A. (2000). Mitochondria and skin disease. *Clinical and Experimental Dermatology.* **25**(2): 141–146.

Brunyé, T. T., Mahoney, C. R., Lieberman, H. R., Taylor, H. A. (2010). Caffeine modulates attention network function. *Brain and Cognition*. **72**(2): 181–188.

Buege, J. A., and Aust, S. D. (1978). Microsomal lipid peroxidation. *Methods in Enzymology*. **52**: 302–310.

Cai, H., and Harrison, D. G. (2000). Endothelial dysfunction in cardiovascular diseases. The role of oxidant stress. *Circulation Research.* **87**(10): 840–844.

Chelikani, P., Fita, I., and Loewen, P. C. (2004). Diversity of structures and properties among catalases. *Cellular and Molecular Life Sciences.* **61**(2): 192–208.

Chepkova, A. N., Sergeeva, O. A., and Haas, H. L. (2006). Taurine improves long-term potentiation in hippocampal slices from aged rats. *Journal of Pharmacology and Experimental Therapeutics*. **319**(2): 520–528.

Cohen, G., Dembiec, D., and Marcus, J. (1970). Measurement of catalase activity in tissue extracts. *Analytical Biochemistry*. **34**(1): 30–38

Cross, C. E., Halliwell, B., Borish, E. T., Pryor, W. A., Ames, B. N., Saul, R. L., McCord, J. M., and Harman, D. (1987). Oxygen radicals and human disease. *Annals of Internal Medicine.* **107**(4): 526–545.

Duni, A., Liakopoulos, V., Roumeliotis, S., Peschos, D., and Dounousi, E. (2019).Oxidative stress in the pathogenesis and evolution of chronic kidney disease. *International Journal of Molecular Sciences.* **20**(15): 3711.

El khawaga, O. A., Fahim, H. I., and Ahmed, R. S. (2021). Caffeine-induced oxidative stress and antioxidant response in rats. *Toxicology Reports.* ***8*:** 177–186.

Erdem, B., Koca, C., Yılmaz, T. (2021). The effect of caffeine on oxidative stress markers. *International Journal of Biochemistry and Cell Biology.* **134**: 105.

Fahim, R. A., Abdel-Rahman, M. A., Al-Farraj, E. (2020). Impact of energy drink consumption on oxidative stress biomarkers and liver function in rats. *Toxicology Reports. 7***:** 245–253.

Finkel, T., and Holbrook, N. J. (2000). Oxidants, oxidative stress and the biology of ageing. *Nature.* **408**(6): 239–247.

Flohé, L., and Günzler, W. A. (1984). Assays of glutathione peroxidase. *Methods in Enzymology*. **105**: 114–120.

Forbes, J. M., and Thorburn, D. R. (2018). Oxidative stress in diabetic kidney disease. A complex interaction of glucose, mitochondria, and NADPH oxidases. *Diabetes.* **67**(9): 1699–1701.

Glade, M. J. (2010). Caffeine. Not just a stimulant. *Nutrition.* **26**(10): 932–938.

Gorby, H. E., Brownawell, A. M., Falk, M. C. (2010). Do the nutrition claims about glucose and cognitive performance hold up? *Nutrition Bulletin*. **35**(4): 301–309.

Griendling, K. K., and FitzGerald, G. A. (2017). Oxidative stress and cardiovascular disease. *Circulation Research.* **120**(4): 715–735.

Hewlett, P., and Smith, A. (2007). Effects of repeated doses of caffeine on performance and alertness: New insights from research studies. *Clinical and Experimental*. **22**(6): 339–350.

Higgins, J. P., Babu, K. M., Deuster, P. A., Shearer, J. (2018). Energy drinks. A contemporary issues paper. *Current Sports Medicine Reports.* **17**(2): 65-72.

Higgins, J. P., Tuttle, T. D., Higgins, C. L. (2015). Energy beverages. Content and safety. *Mayo Clinic Proceedings.* **85**(11): 1033-1041.

Howard, M. A., and Marczinski, C. A. (2010). Acute effects of a glucose energy drink on behavioral control. *Experimental and Clinical Psychopharmacology*. 18(6): 553–561.

Ibrahim, S. A., Alaaeldin, R., Abdelgawad, H. S. (2020). Impact of chronic consumption of energy drinks on oxidative stress biomarkers in experimental animals. *Nutrition and Metabolic Insights.* **13**: 1-9.

Ito, T., Yoshikawa, M., and Nakanishi, Y. (2009). Taurine and the central nervous system. Mechanisms and actions. *Journal of Biomedical Science*. **16**(1): 79.

James, J. E., and Rogers, P. J. (2005). Effects of caffeine on performance and mood. Withdrawal reversal is the most plausible explanation. *Psychopharmacology.* **182**(1): 1–8.

Jha, J. C., Banal, C., Chow, B. S. M., Cooper, M. E., and Jandeleit-Dahm, K. (2016). Diabetes and kidney disease. Role of oxidative stress. *Antioxidants and Redox Signaling.* **25**(12): 657–684.

Junyent, F., Folch, J., Verdaguer, E., Beas-Zarate, C., Pallàs, M., Camins, A., Auladell, C. (2011). Neuroprotective effects of taurine against oxidative stress. *Journal of Neuroscience Research*. **89**(10): 1520–1531.

Kim, J., Lee, H. J., and Park, Y. (2017). Sugar-induced oxidative stress and the role of antioxidants. *Journal of Clinical Biochemistry and Nutrition.* **61**(1): 12–18.

Kucukkurt, I., Ince, S., Demirel, H. H., Yilmaz, E. (2016). Effects of chronic caffeine consumption on oxidative stress and antioxidant enzyme activities in rats. *Biological Trace Element Research*. **170**(2): 375–382.

Kuhn, C., Brodersen, P., Hesse, J. (2019). Lipid peroxidation and its implications for energy drink consumption. *Nutritional Biochemistry.* **64**: 117–123.

Kumar, S., Pandey, A. K., and Sharma, M. (2017). Malondialdehyde as a biomarker of oxidative stress. *Journal of Medical Research and Practice*. **6**(1): 13–18.

Lushchak, V. I. (2014). Free radicals, reactive oxygen species, oxidative stress, and its classification. *Chemico-Biological Interactions.* **224**: 164–175

Madamanchi, N. R., and Runge, M. S. (2013). Redox signaling in cardiovascular health and disease. *Free Radical Biology and Medicine.* **61**: 473–501.

Magalhães, P. J., Carvalho, F., and Bastos, M. L. (2020). Caffeine. Oxidative stress. *Trends in Molecular Medicine.* **26**(3): 246–258.

Malinauskas, B. M., Aeby, V. G., Overton, R. F., Carpenter-Aeby, T., Barber-Heidal, K. (2007). A survey of energy drink consumption patterns among college students. *Nutrition Journal*. **6**(1): 3-5.

Mansour, S. A., Mossa, A. H., Heikal, T. M. (2017). Haematobiochemical and oxidative stress effects of two neonicotinoid insecticides in male rats. *African Journal of Traditional, Complementary and Alternative Medicines*. **14**(4): 104–112.

McCord, J. M., and Fridovich, I. (1969). Superoxide dismutase. An enzymic function for erythrocuprein (hemocuprein). *Journal of Biological Chemistry*. **244**(22): 6049–6055.

Misra, H. P., and Fridovich, I. (1972). The role of superoxide anion in the autoxidation of epinephrine and a simple assay for superoxide dismutase. *The Journal of Biological Chemistry.* **247**(10): 3170–3175.

Murphy, M. P. (2009). How mitochondria produce reactive oxygen species. *Biochemical Journal.* **417**(1): 1–13.

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

Nyman, K. (1959). Investigations on oxidative stress and therapeutical implications in dementia. *European Archives of Psychiatry and Clinical Neuroscience*. **199**: 299–304.

Pham-Huy, L. A., He, H., and Pham-Huy, C. (2008). Free radicals, antioxidants in disease and health. *International Journal of Biomedical Science.* **4**(2): 89–96.

Reid, J. L., Hammond, D., McCrory, C., Dubin, J. A. (2017). Use of caffeinated energy drinks among secondary school students in Ontario. *Canadian Journal of Public Health.* **108**(3): 303-309.

Reissig, C. J., Strain, E. C., and Griffiths, R. R. (2009). Caffeinated energy drinks. A growing problem. *Drug and Alcohol Dependence.* **99**(1): 1–10.

Reyes, C. M., and Cornelis, M. C. (2018). Caffeine in the diet. Country-level consumption and guidelines. *Nutrients*. **10**(11): 17-72.

Reyes-Irisarri, E., Sierra-Palacios, M. O., Lopez-Beltran, M. (2020). Caffeine consumption and oxidative stress: Implications for cardiovascular health. *Frontiers in Cardiovascular Medicine.* **7**: 36-37.

Sanchez, C. A., Levin, J. M., McKinney, K. J. (2013). Cardiovascular and metabolic effects of energy drink consumption in college-aged adults. *American Journal of Health Promotion.* **27**(5): 324-332.

Schieber, M., and Chandel, N. S. (2014). ROS function in redox signaling and oxidative stress. *Current Biology*. **24**(10): 453-462.

Scholey, A. B., and Kennedy, D. O. (2004). Cognitive and physiological effects of energy drink. *Psychopharmacology*. **176**(3): 320–330.

Seidl, R., Peyrl, A., Nicham, R., Hauser, E. (2000). A taurine and caffeine-containing drink stimulates cognitive performance and well-being. *Amino Acids*. **19**(3): 635–642.

Seifert, S. M., Schaechter, J. L., Hershorin, E. R., Lipshultz, S. E. (2011). Health effects of energy drinks on children, adolescents, and young adults. *Pediatrics.* **127**(3): 511-528.

Seifried, H. E., Anderson, D. E., Fisher, E. I., Milner, J. A. (2007). A review of the interaction among dietary antioxidants and reactive oxygen species. *The Journal of Nutritional Biochemistry.* **18**(9): 567–579.

Sies, H., Berndt, C., and Jones, D. P. (2017). Oxidative stress. *Annual Review of Biochemistry.* **86:** 715–748.

Smith, A. (2011). Effects of caffeine on human behavior. *Food and Chemical Toxicology*. **49**(9): 2930–2937.

Stocker, R., and Keaney, J. F. (2004). Role of oxidative modifications in atherosclerosis. *Physiological Reviews.* **84**(4): 1381–1478.

Taniyama, Y., and Griendling, K. K. (2003). Reactive oxygen species in the vasculature: Molecular and cellular mechanisms. *Hypertension.* **42**(6): 1075–1081.

Zhang, W., Cheng, Y., and Wang, T. (2022). The impact of chronic caffeine consumption on oxidative stress in different tissues. *Biochemical Pharmacology.* **193**: 11-47.

Zhao, H., Alam, A., Soo, A. P., George, A. J., Ma, D. (2016). Ischemia-reperfusion injury reduces long-term renal graft survival. *EBioMedicine.* **10:** 97–105.

Zhu, J., Wang, H., and Zhang, X. (2021). The role of glutathione peroxidase in oxidative stress and disease. *Redox Biology.* **46**: 102-109.

Zucconi, S., Volpato, C., Adinolfi, F., Gandini, E., Gentile, E., Loi, A., Fioriti, L. (2013). Gathering consumption data on specific consumer groups of energy drinks. *EFSA Supporting Publications*. **10**(3): 394.

.