

**BIOCHEMICAL CONSTITUENTS OF BLUE BULLET ENERGY  
DRINK**

**BY**

**JOSHUA OFULUE**

**LSC2006826**



**DEPARTMENT OF BIOCHEMISTRY  
FACULTY OF LIFE SCIENCES  
UNIVERSITY OF BENIN  
BENIN CITY**

**FEBRUARY, 2025**

**BIOCHEMICAL CONSTITUENTS OF BLUE BULLET ENERGY  
DRINK**

**BY**

**JOSHUA OFULUE**

**LSC2006826**

**A PROJECT WORK SUBMITTED TO THE DEPARTMENT OF  
BIOCHEMISTRY, FACULTY OF LIFE SCIENCES, UNIVERSITY OF  
BENIN, BENIN CITY.**

**IN PARTIAL FULFILMENT OF THE REQUIREMENT FOR THE  
AWARD OF THE DEGREE OF BACHELOR OF SCIENCE (BSc) IN  
BIOCHEMISTRY**

**FEBRUARY, 2025**

## CERTIFICATION

This is to certify that this final year project work was carried out by **JOSHUA OFULUE** (MAT. NO. LSC2006826) of the Department of Biochemistry, Faculty of life Sciences, University of Benin, Benin City, in partial fulfillment for the award of Bachelor of Science (B. Sc) degree in Biochemistry.

-----

Prof. N.E.J. Orhue  
(Project Supervisor)

-----

Date

-----

Dr. S.I. Ojeaburu  
(Project Coordinator)

-----

Date

-----

Prof. E.C. Onyeneke  
(Head of Department)

-----

Date

## **DEDICATION**

This project work is dedicated to God Almighty for his strength; wisdom and understanding that brought this work to fulfillment.

## **ACKNOWLEDGMENT**

This research project is a complex entity and emerges from the contribution of many people. First and foremost, praises and thanks to God Almighty, for his showers of blessings and faithfulness throughout the preparation for my undergraduate research project.

Special acknowledgement to my wonderful and studious Project supervisor, Prof. N.E.J. Orhue for taking out time to be of utmost help despite his busy schedule, for guidance, advice, corrections and fatherly love and also to Mrs. Kolawole for her time and guidance during the course of this work. I also thank the Head of the Department, Prof. E. C. Onyeneke, and to all my lecturers in the Department of Biochemistry, I say a huge thank you. My sincere and undiluted gratitude goes to my parents, Mr. and Mrs. Ofulue for their love and belief in me and for their moral, financial, and spiritual support all through my years in school, not forgetting my siblings.

Finally, I am forever grateful for the friends I made during the course of my study, likes of Victoria Kamene, Mr. Harry and a host of others for their love and support.

## TABLE OF CONTENT

Cover Page	
Title Page	i
Certification	ii
Dedication	iii
Acknowledgments	iv
Table of Contents	v
Abstract	vii
CHAPTER ONE	
Introduction	1
1.1 Aim of the Study	2
1.2 Objective of the Study	2
CHAPTER TWO	
Literature Review	3
2.1 Composition and Biochemical Properties of Energy Drinks	3
2.2 Carbohydrate Metabolism and Energy Drinks	6
2.3 Mineral and Metal Content in Energy Drinks: Benefits and Risks	7
2.4 Caffeine and Its Impact on Mineral and Metal Absorption	8
2.5 Health Implications of Energy Drink Consumption	10
2.6 Presence of Compounds	12
2.6.1 Carbon Dioxide	12
2.6.2 Sugars	12
2.6.3 Reducing Sugars	13
2.6.4 Alcohol	13
2.6.5 Phosphates	15
CHAPTER THREE	
Materials and Methods	17
3.1 Materials	17
3.1.1 Equipment and Apparatus	17
3.1.2 Preparation of Samples	17

3.1.3 Chemicals/Reagents	17
3.2 Methods	18
3.2.1 Test for Carbon Dioxide (CO <sub>2</sub> )	18
3.2.2 Test for Sugar (Benedict's Test)	19
3.2.3 Test for Reducing Sugars Using Fehling's Solution	19
3.2.4 Test for Alcohol	20
3.2.5 Test for Phosphates	20
3.2.6 Qualitative Analysis of pH	21
3.2.7 Preparation of Control Samples	22
3.2.8 Quantitative Analysis of Energy Drinks	22
3.2.9 Statistical Analysis	23
CHAPTER FOUR	
Results	24
4.1 Results for Qualitative Analysis	24
4.3 Findings and Observations from Mineral Analyses	25
CHAPTER FIVE	
Discussion and Conclusion	27
5.1 Discussion	27
5.2 Conclusion	34
References	35
Appendix	40

## ABSTRACT

Energy drinks are popular for their energy-boosting effects, yet concerns about their composition and safety persist. This study aimed to evaluate the mineral, sugar, and heavy metal content of Blue Bullet energy drink, focusing on its impact on energy metabolism, mineral homeostasis, and toxicological risks. Triplicate samples of the drink were prepared for the qualitative analyses were conducted to detect the presence of carbohydrates, phosphates, and acidity and quantitative tests which included Benedict's and Fehling's solutions for sugar content, spectrophotometric analysis for minerals, and toxicological screening for heavy metals. Qualitative analyses confirmed significant carbohydrate presence, trace phosphates, and an acidic pH typical of energy drinks. Quantitative screening revealed carbohydrates ( $12 \pm 3 \mu\text{g/L}$ ), alcohol ( $16 \pm 2 \mu\text{g/L}$ ), phosphate ( $368 \pm 1 \mu\text{g/L}$ ), and  $\text{CO}_2$  ( $78 \pm 11 \mu\text{g/L}$ ). Mineral analysis identified calcium ( $24.47 \pm 0.15 \text{ mg/kg}$ ), iron ( $0.93 \pm 0.03 \text{ mg/kg}$ ), potassium ( $0.30 \pm 0.00 \text{ mg/kg}$ ), magnesium ( $1.17 \pm 0.03 \text{ mg/kg}$ ), and phosphorus ( $1.667 \pm 0.002 \text{ mg/kg}$ ), all within FDA and WHO safety limits. Trace metals such as chromium ( $0.047 \pm 0.004 \text{ mg/kg}$ ), manganese ( $0.047 \pm 0.012 \text{ mg/kg}$ ), and zinc ( $0.083 \pm 0.003 \text{ mg/kg}$ ) were present at safe levels. Toxicological screening showed arsenic ( $0.955 \pm 0.004 \mu\text{g/kg}$ ) and mercury ( $0.070 \pm 0.001 \mu\text{g/kg}$ ) below safety thresholds, with no detectable lead, cadmium, or copper. These findings highlight that Blue Bullet provides essential nutrients and adheres to safety standards, though regular quality control is recommended for consumer safety..

# CHAPTER ONE

## 1.0 INTRODUCTION

Energy drinks are beverages primarily marketed to increase alertness, improve concentration, and enhance physical and mental performance. These drinks typically contain caffeine, taurine, sugars, vitamins, and various other stimulants, such as guarana and ginseng (Seifert *et al.*, 2021). The primary function of energy drinks stems from their caffeine content, which stimulates the central nervous system, leading to increased focus and wakefulness. However, the high concentrations of sugars, minerals, and metals present in these beverages also play a significant biochemical role in their metabolic impact. The consumption of energy drinks has risen dramatically, particularly among adolescents and young adults, who are the primary consumers. This demographics' increased use is often attributed to the need for improved performance in academic, physical, and professional pursuits. In several regions, including sub-Saharan Africa, the prevalence of energy drink consumption is growing at an alarming rate, with potential risks to public health, especially in countries where regulatory oversight is weak (Higgins *et al.*, 2018).

Energy drinks have significant health implications due to their sugar, mineral, and metal content. High sugar intake from these drinks leads to spikes in blood glucose levels, contributing to insulin resistance and potentially type 2 diabetes (Malik *et al.*, 2020). The mineral content, particularly sodium and potassium, plays a critical role in maintaining electrolyte balance; however, imbalances may lead to adverse cardiovascular effects. Metals like zinc, copper, and magnesium, though essential in trace amounts for various enzymatic functions, can become toxic when consumed in excess. For instance, an overload of iron, often found in some energy drinks, may cause oxidative stress and damage cellular components (Yehuda, 2021). Additionally, caffeine, which is a key component of most

energy drinks, can alter mineral absorption and exacerbate metal-related toxicities by influencing kidney function (McLellan and Lieberman, 2022).

### **1.1. AIM OF STUDY**

This study aims to investigate the chemical constituents of Blue Bullet energy drinks.

### **1.2 OBJECTIVES OF STUDY**

The objectives of this study is to determine

1. The mineral content of Blue Bullet Energy Drink
2. The heavy metal content of Blue Bullet Energy Drink
3. The biochemical content of Blue Bullet Energy Drink

## CHAPTER TWO

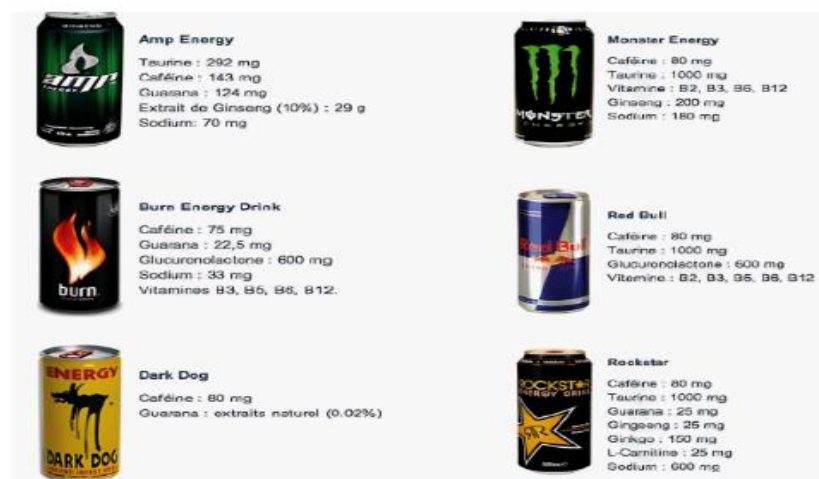
### LITERATURE REVIEW

#### 2.1. COMPOSITION AND BIOCHEMICAL PROPERTIES OF ENERGY DRINKS

Energy drinks are complex formulations containing a variety of bioactive compounds that exert multiple physiological effects. The primary constituents of these beverages include caffeine, sugars, taurine, glucuronolactone, B-vitamins, and an array of minerals and trace metals, such as sodium, potassium, calcium, and magnesium (Soni *et al.*, 2016). These compounds are meticulously combined to synergize and optimize mental and physical performance by modulating metabolic and neurophysiological processes. Caffeine is the most well-known stimulant in energy drinks and operates as a non-selective antagonist of adenosine receptors, primarily A1 and A2A subtypes, within the central nervous system. By inhibiting adenosine-mediated signaling, caffeine increases the release of excitatory neurotransmitters such as dopamine, norepinephrine, and acetylcholine, leading to heightened alertness and reduced perceived effort during physical exertion (McLellan and Lieberman, 2016). Additionally, caffeine enhances lipolysis by stimulating the release of catecholamines, thereby increasing the availability of free fatty acids as substrates for  $\beta$ -oxidation, ultimately conserving glycogen stores (Temple *et al.*, 2017).

Taurine, another key ingredient, is a sulfur-containing  $\beta$ -amino acid that plays a crucial role in osmoregulation, bile salt formation, and calcium homeostasis. Taurine is involved in modulating neurotransmission and stabilizing cell membranes under oxidative stress, making it an important protective agent, particularly in excitable tissues like the heart and brain (Schaffer *et al.*, 2018). It also interacts with the mitochondrial electron transport chain, aiding in the maintenance of ATP production and reducing the generation of reactive oxygen species (ROS), which can be elevated due to the oxidative load from other components in energy

drinks (Huxtable, 2017). Sugars, primarily glucose and sucrose, are added to energy drinks to provide an immediate source of energy. The rapid metabolism of these monosaccharides via glycolysis results in the production of pyruvate, which enters the tricarboxylic acid (TCA) cycle for oxidative phosphorylation and ATP generation. High sugar content, however, poses a risk of metabolic dysregulation. Excessive sugar intake has been linked to increased hepatic de novo lipogenesis, elevated serum triglycerides, and the development of insulin resistance (Malik *et al.*, 2018). Chronic hyperglycemia can lead to the glycation of proteins and the formation of advanced glycation end-products (AGEs), which contribute to oxidative stress and inflammatory responses (Vlassara and Uribarri, 2014).



**Figure 1:** What is an Energy Drink

**Source:** Petroczi *et al.* (2015).

Glucuronolactone, another component commonly found in energy drinks, is a derivative of glucose and an important precursor in the synthesis of ascorbic acid (vitamin C) and glycosaminoglycans. It has been shown to enhance detoxification processes by facilitating the conjugation of xenobiotics in the liver, thereby promoting their excretion through bile or urine (Kamal *et al.*, 2016). This compound has been suggested to work synergistically with caffeine and taurine to improve cognitive function and endurance performance (Petroczi *et al.*,

2015). The B-vitamins, including niacin (B3), pyridoxine (B6), and cobalamin (B12), serve as essential coenzymes in numerous biochemical pathways. Niacin is involved in the synthesis of nicotinamide adenine dinucleotide (NAD<sup>+</sup>), a crucial cofactor in redox reactions and oxidative phosphorylation. Pyridoxine acts as a cofactor for enzymes involved in amino acid metabolism, such as transaminases and decarboxylases, while cobalamin is required for the proper functioning of methylmalonyl-CoA mutase and methionine synthase, enzymes essential for maintaining mitochondrial health and DNA synthesis (Kennedy, 2016).

In addition to these organic compounds, energy drinks contain minerals like sodium, potassium, calcium, and magnesium, which are crucial for maintaining electrolyte balance and cellular homeostasis. Potassium and sodium are vital for the generation and propagation of action potentials through their roles in maintaining membrane potential via Na<sup>+</sup>/K<sup>+</sup>-ATPase activity (Geissler *et al.*, 2017). Calcium, as a second messenger, is essential for muscle contraction, neurotransmitter release, and the regulation of various metabolic enzymes, while magnesium acts as a cofactor for over 300 enzymatic reactions, including those involved in ATP synthesis and glucose metabolism (Grober *et al.*, 2015). Trace metals, such as zinc and iron, are also present in energy drinks, albeit in smaller concentrations. Zinc serves as a structural component of numerous metalloenzymes and transcription factors, playing a pivotal role in DNA repair, cell proliferation, and the antioxidant defense system through its involvement in the activity of superoxide dismutase (SOD) (Wessels *et al.*, 2020). Iron, while essential for oxygen transport and electron transfer, can catalyze the formation of harmful ROS via Fenton reactions if present in excess, potentially leading to oxidative damage in cells (Kell, 2019).

## **2.2. CARBOHYDRATE METABOLISM AND ENERGY DRINKS**

The consumption of energy drinks, which are typically high in simple sugars, significantly impacts carbohydrate metabolism, primarily through the modulation of glucose homeostasis and insulin sensitivity. The predominant sugars found in these beverages, such as glucose and sucrose, are rapidly absorbed in the gastrointestinal tract and result in swift elevations in blood glucose levels following consumption (Malik *et al.*, 2018). This rapid increase in glucose stimulates insulin secretion from pancreatic  $\beta$ -cells, facilitating glucose uptake by peripheral tissues, particularly muscle and adipose tissue, via the insulin signaling pathway. The surge in insulin levels following energy drink consumption can lead to a phenomenon known as reactive hypoglycemia, where the initial spike in blood glucose is followed by a rapid decline, often resulting in symptoms such as fatigue, dizziness, and irritability (Ritchie *et al.*, 2021). Chronic consumption of high-sugar energy drinks may contribute to insulin resistance, a condition characterized by diminished cellular responsiveness to insulin, ultimately impairing glucose uptake and promoting hyperglycemia. This metabolic disturbance is a critical risk factor for the development of type 2 diabetes mellitus and other metabolic syndromes (Kahn *et al.*, 2016).

The carbohydrate load from energy drinks influences hepatic metabolism, particularly through the process of gluconeogenesis and glycogen synthesis. Excessive intake of simple carbohydrates can lead to an increased flux of glucose into the liver, promoting *de novo* lipogenesis, which converts surplus glucose into fatty acids. This biochemical pathway may result in ectopic fat deposition in the liver, contributing to non-alcoholic fatty liver disease (NAFLD) (Cohen *et al.*, 2017). The interplay between carbohydrate intake and liver metabolism underscores the importance of understanding how energy drinks alter metabolic pathways, especially in susceptible populations such as adolescents and athletes who often consume these beverages in pursuit of enhanced performance. Energy drinks can affect the

postprandial state by influencing the hormonal response to carbohydrate intake. For example, the combination of high sugar and caffeine may amplify the release of glucagon-like peptide-1 (GLP-1), an incretin hormone that enhances insulin secretion and slows gastric emptying (Drucker, 2016).

### **2.3. MINERAL AND METAL CONTENT IN ENERGY DRINKS: BENEFITS AND RISKS**

Energy drinks are often marketed not only for their stimulant effects but also for their inclusion of various minerals and trace metals, which are purported to enhance physical and cognitive performance. Key minerals commonly found in these beverages include sodium, potassium, magnesium, and calcium, each playing critical roles in various physiological processes. For instance, sodium and potassium are vital for maintaining osmotic balance and excitability in neuronal and muscular tissues, functioning primarily through the Na<sup>+</sup>/K<sup>+</sup>-ATPase pump. This enzyme maintains the electrochemical gradients necessary for action potential propagation and muscle contraction, ultimately influencing athletic performance and recovery. Potassium, in particular, is essential for proper cardiovascular function and plays a significant role in muscle contraction and nerve transmission. Low potassium levels can lead to muscle weakness, cramps, and arrhythmias, which can severely hinder physical performance (Rude, 2016). Energy drinks that contain adequate potassium levels can potentially support these functions, especially during intense physical activities where electrolyte loss through sweat is prominent. However, excessive consumption of energy drinks rich in sodium can lead to hypernatremia, resulting in dehydration and impaired kidney function due to the increased renal workload (Cameron *et al.*, 2018).

Magnesium is another critical mineral often included in energy drinks due to its role as a cofactor in over 300 enzymatic reactions, including those involved in ATP production, protein synthesis, and neuromuscular transmission. Deficiency in magnesium can lead to fatigue, muscle cramps, and a decline in athletic performance (Cleveland *et al.*, 2015). However, excessive intake can also result in adverse effects, such as diarrhea and cardiovascular disturbances due to its role in modulating calcium levels and muscle function. Trace metals, such as zinc, iron, and manganese, are also present in energy drinks, albeit in smaller quantities. Zinc is an essential cofactor for numerous enzymes involved in antioxidant defense, immune function, and DNA synthesis. While adequate zinc levels are crucial for maintaining cellular function, excessive intake can lead to copper deficiency and disrupt the balance of trace elements in the body (Fischer *et al.*, 2020). Iron is critical for oxygen transport and energy metabolism, but its accumulation can catalyze the formation of reactive oxygen species, leading to oxidative stress and cellular damage (Harrison, 2016). Manganese, while necessary for mitochondrial function and antioxidant defenses, can become neurotoxic at elevated levels, causing detrimental effects on neurological health (Aschner and Aschner, 2016). Mineral and metal intake from energy drinks and overall metabolic health highlights the need for careful consideration regarding their consumption. While these components can provide potential benefits, excessive intake may pose significant risks, particularly in vulnerable populations such as adolescents and athletes who may be more susceptible to both the positive and negative effects of these substances.

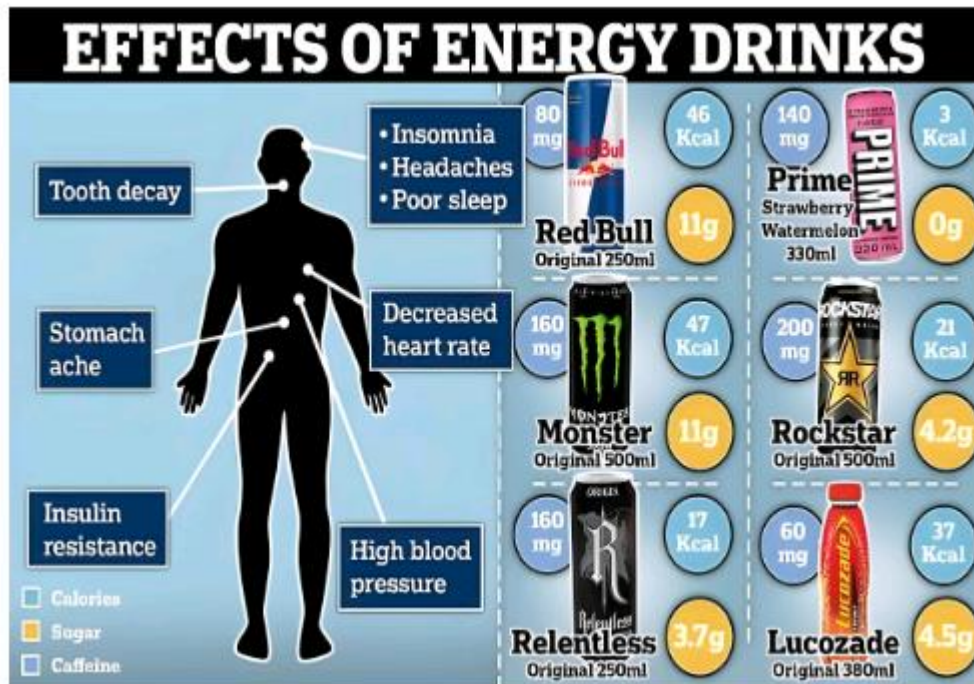
#### **2.4. CAFFEINE AND ITS IMPACT ON MINERAL AND METAL ABSORPTION**

Caffeine, a key ingredient in energy drinks, is well-known for its stimulating effects; however, its influence extends beyond mere alertness to significantly impact mineral and metal absorption and metabolism. Caffeine exerts its effects primarily through antagonism of adenosine receptors, which not only affects central nervous system activity but also

modulates various gastrointestinal and metabolic processes. One notable effect is caffeine's ability to alter the bioavailability of certain minerals, particularly calcium, magnesium, and iron. Studies have shown that caffeine consumption can lead to increased urinary excretion of calcium, potentially contributing to calcium loss and subsequent bone health issues, particularly in individuals with insufficient dietary calcium intake (Heaney, 2014). The mechanism involves caffeine's role in increasing renal tubular calcium reabsorption, which may not compensate for the increased calcium excretion, ultimately leading to a net loss of calcium from the body. This effect is particularly concerning for adolescents and young adults who are still in the critical stages of bone development.

Magnesium absorption can also be adversely affected by caffeine intake. While caffeine may enhance magnesium absorption in some contexts, excessive consumption is associated with increased urinary magnesium loss, which can exacerbate deficiencies and impair metabolic processes dependent on magnesium (O'Donnell *et al.*, 2015). This loss can be particularly problematic for athletes who require adequate magnesium levels for optimal muscle function and energy metabolism. Caffeine's influence on iron absorption is complex and multifaceted. While caffeine itself does not inhibit iron absorption significantly, its presence in a meal can impede the absorption of non-heme iron from plant sources due to the formation of insoluble complexes with polyphenols and other compounds present in caffeinated beverages (Hurrell *et al.*, 2010). This interaction is particularly relevant for populations reliant on plant-based diets, as they may experience increased risk of iron deficiency anemia with high caffeine consumption.

## 2.5. HEALTH IMPLICATIONS OF ENERGY DRINK CONSUMPTION



**Figure 2:** Effects of Energy Drinks

**Source:** Baker *et al.* (2018).

The consumption of energy drinks has garnered significant attention due to its associated health implications, particularly regarding cardiovascular, metabolic, and neurological effects. High caffeine and sugar content in these beverages can lead to acute and chronic health issues, raising concerns among health professionals and consumers alike. One of the primary concerns surrounding energy drink consumption is the potential for cardiovascular complications. Acute ingestion of high doses of caffeine can lead to increased heart rate (tachycardia), elevated blood pressure, and heightened cardiac contractility. These effects can be particularly dangerous in individuals with pre-existing cardiovascular conditions, leading to arrhythmias or other serious complications (Alford *et al.*, 2016). The presence of additional stimulants often found in energy drinks, such as guarana and ginseng, can

exacerbate these cardiovascular responses, resulting in an increased risk of cardiovascular events among frequent consumers.

The high sugar content in energy drinks contributes to metabolic disturbances. Excessive sugar intake is linked to the development of insulin resistance, obesity, and type 2 diabetes mellitus. The rapid elevation of blood glucose levels following energy drink consumption leads to increased insulin secretion, which can overwhelm the body's ability to manage glucose effectively over time (Lustig, 2017). This metabolic dysregulation is particularly concerning for adolescents and young adults, who may be more susceptible to the long-term effects of high sugar consumption. Energy drinks can also have significant implications for mental health and cognitive function. The combination of high caffeine and sugar can lead to a temporary boost in alertness and concentration; however, it may also result in subsequent crashes characterized by fatigue, irritability, and reduced cognitive performance (Miller *et al.*, 2016). Additionally, there is a growing body of evidence linking energy drink consumption to anxiety, depression, and other mood disorders, particularly among young adults who may use these products as coping mechanisms (Baker *et al.*, 2018).

Another area of concern is the potential for addictive behaviors associated with energy drink consumption. The stimulating effects of caffeine can lead to habitual use, with consumers increasingly relying on energy drinks for improved performance and alertness. This cycle can result in tolerance and dependence, characterized by withdrawal symptoms such as headaches, fatigue, and mood disturbances when consumption is reduced (Juliano and Mark, 2016). The combination of caffeine and sugar can also create a preference for these beverages, leading to excessive consumption and an increased risk of associated health issues. While energy drinks are often marketed for their performance-enhancing benefits, their consumption is associated with significant health risks. The potential for cardiovascular complications, metabolic

disturbances, mental health issues, and addictive behaviors highlights the need for increased awareness surrounding energy drink consumption.

## **2.6. PRESENCE OF COMPOUNDS**

### **2.6.1 Carbon Dioxide**

Carbon dioxide is present in most carbonated drinks and beverages including energy drinks for various reasons, some of which are (De Mello *et al.*, 2017).

A. Carbonation: CO<sub>2</sub> is responsible for the fizziness or carbonation in energy drinks. This carbonation enhances the sensory experience by providing a refreshing taste and stimulating the palate.

B. Preservation: CO<sub>2</sub> can act as a natural preservative by inhibiting the growth of some microorganisms. The acidic environment created by dissolved CO<sub>2</sub> can help extend the shelf life of the beverage.

C. Mouthfeel and Texture: The presence of bubbles improves the mouthfeel of the drink, making it more enjoyable and appealing to consumers.

Typically, energy drinks should contain about 2.5 to 3.0 volumes of CO<sub>2</sub>. This range is considered ideal for taste enhancement, preservation and because of the consumer. Levels above 3.0 volumes of CO<sub>2</sub> can lead to various issue such as gastrointestinal issues and high carbonation can create a sharp, overly acidic taste that may not be enjoyable for consumers.

### **2.6.2 Sugars**

For energy drinks, a good amount of sugars is generally considered to be less than 5 grams per 100 mL or about 25 grams (6 teaspoons) of added sugars per day, as recommended by the World Health Organization (WHO). This moderate level can provide a quick energy boost through the rapid metabolism of sugars like glucose without overwhelming the body with

excessive sugar intake. When consumed in appropriate quantities, sugars provide a quick and efficient source of energy for physical activities, particularly in sports or endurance events. Low-to-moderate sugar levels can help avoid sharp spikes in blood sugar, minimizing the risk of crashes and fatigue. A bad amount of sugars in energy drinks would exceed 10 grams per 100 mL, with some drinks containing as much as 50–60 grams per can. Excessive sugar consumption is linked to many health issues such as Blood Sugar Spikes, weight gain and increased risk of diseases (Breda et al., 2019).

### 2.6.3. Reducing Sugars

Criteria	Healthy amount	Unhealthy amount
Reducing sugar content	Less than 5 grams per 100 mL	More than 10 grams per 100 mL
Health effects	Provides quick energy, stable blood sugar	Blood sugar spikes, weight gain, metabolic disorders
Dental Health	Low risk of tooth decay	High risk of cavities and erosion
Chronic Disease Risk	Minimizes risk of diabetes and obesity	risk of insulin resistance, type 2 diabetes, heart disease
Energy Levels	Stable energy supply	Rapid energy Increases crashes, fatigue, cravings

### 2.6.4. Alcohol

Energy drinks typically do not contain alcohol, as they are designed to be non-alcoholic beverages that provide energy-boosting effects. However, alcohol may be mixed with energy drinks or consumed alongside them in certain contexts (e.g., cocktails). Zero alcohol is

considered a good amount for energy drinks. Energy drinks are designed to provide stimulation and energy, and alcohol would counteract or mask these effects. Regulatory agencies, such as the FDA, do not approve the combination of caffeine (a stimulant) and alcohol (a depressant) in commercially sold energy drinks. Non-alcoholic energy drinks avoid the health risks associated with combining alcohol and caffeine, such as impaired judgment and increased risk of injury. If alcohol is consumed separately or in a mixed drink context, a good amount is defined by the WHO and CDC as moderate drinking, which is (Jones and Barrie, 2015).

A. For men: Up to 2 standard drinks per day.

B. For women: Up to 1 standard drink per day.

A standard drink typically contains about 14 grams of pure alcohol (e.g., 150 mL of wine, 350 mL of beer, or 45 mL of spirits). This moderate level can generally be consumed without significantly increasing the risk of health issues such as liver damage, heart disease, or addiction. Any alcohol mixed with energy drinks is considered a bad amount and mixing alcohol with energy drinks can have dangerous effects. More than 4 drinks per day for men or more than 3 drinks per day for women, or binge drinking, is considered excessive. This level of consumption significantly raises the risk of:

1. Liver Damage: Excess alcohol intake can cause fatty liver, hepatitis, and cirrhosis.
2. Heart Disease: Long-term excessive drinking is linked to high blood pressure, heart disease, and stroke.
3. Addiction: High alcohol consumption increases the risk of developing alcohol dependency.
4. Mental and Cognitive Effects: Heavy drinking can impair brain function, leading to cognitive deficits and increased risk of depression and anxiety.

### 2.6.5. Phosphates

Phosphates are commonly added to beverages, including energy drinks, as acidulants or preservatives. They play roles in balancing pH, enhancing flavor, and preserving freshness. However, excess phosphate consumption can have adverse effects on health, especially when consumed in large quantities over time. A good amount of phosphates in energy drinks is generally considered to be less than 70 mg per 100 mL, or around 700 mg per day for adults.

Phosphates serve several useful functions in moderate amounts (Costa *et al.*, 2016):

- A. Bone Health: Phosphates are important for the formation and maintenance of bones and teeth, as they are involved in calcium-phosphate balance.
- B. Energy Metabolism: Phosphates play a key role in the body's energy metabolism, particularly in the formation of ATP (adenosine triphosphate), which provides energy for cells.

A moderate intake of phosphates supports normal cellular function, including the regulation of pH in body fluids and the activation of enzymes important in metabolism. Phosphates are naturally found in many foods (such as dairy, meat, and grains), so a small amount from energy drinks can contribute to daily intake without causing harm, as long as total intake stays within recommended limits. An unhealthy amount of phosphates in energy drinks would exceed 700 mg per day, which can happen if phosphate-rich foods and drinks are consumed in large quantities. High phosphate levels in energy drinks can lead to:

- A. Bone Health Issues: Excessive phosphate can disturb the delicate calcium-phosphate balance in the body, causing calcium to leach from bones, leading to osteoporosis and weakened bone structure over time.

- B. **Kidney Strain:** The kidneys play a critical role in filtering out excess phosphate. High phosphate intake can lead to kidney damage or worsen existing kidney conditions, as the kidneys struggle to regulate phosphate levels in the blood.
- C. **Cardiovascular Risks:** High phosphate levels in the blood, often caused by excessive consumption of phosphates in processed foods and drinks, are linked to vascular calcification (hardening of the arteries) and an increased risk of cardiovascular disease.
- D. **Elevated phosphate levels in the blood can also contribute to electrolyte imbalances, negatively affecting heart health and muscle function.**
- E. **Impact on Hormonal Regulation:** Excessive phosphates can disrupt the body's parathyroid hormone (PTH), which helps regulate calcium and phosphate levels. Imbalances in PTH due to high phosphate intake can lead to further issues with bone health and calcium metabolism.

## **CHAPTER THREE**

### **3.0 MATERIALS AND METHODS**

Biochemical parameters such as reducing sugars, phosphates, and alcohol content play a critical role in determining the nutritional and metabolic properties of food and drink samples, including energy drinks like blue bullet. This study outlines the methodology and results of experiments aimed at measuring these parameters using standard biochemical assays. The assays conducted were as follows

#### **3.1 MATERIALS**

##### **3.1.1 Equipments and Apparatus**

- a. Water bath (Thermo Fisher Scientific, USA)
- b. pH meter (Hanna Instruments, USA)
- c. Spectrophotometer (Agilent Technologies, USA)

##### **3.1.2 Preparation of Samples**

Triplicates of each sample were prepared by dispensing 0.5 mL of each sample into three labeled test tubes, designated as C1, C2, and C3 for the Blue Bullet sample. Similarly, 0.5 mL of the control samples was dispensed into new test tubes labeled A to H, respectively. To each of these test tubes, 1.0 mL of water was added.

##### **3.1.3 Chemicals/Reagents**

- a. Blue Bullet (BB Beverages, Nigeria)
- b. Distilled water
- c. Iodine (Sigma-Aldrich, United States)
- d. Potassium Iodide (Sigma-Aldrich, United States)
- e. Nitric acid (Sigma-Aldrich, United States)

- f. Lime Water (Sigma-Aldrich, United States)
- g. Ammonium molybdate (Sigma-Aldrich, United States)
- h. Concentrated nitric acid (Sigma-Aldrich, United States)
- i. Benedict's solution (Sigma-Aldrich, United States)
- j. Fehlings solution (Sigma-Aldrich, United States)

## **3.2 METHOD**

### **3.2.1 Test for Carbon Dioxide (CO<sub>2</sub>)**

#### **➤ Principle**

The limewater test is used to detect the presence of carbon dioxide (CO<sub>2</sub>) gas. The test is based on the chemical reaction between carbon dioxide and calcium hydroxide (limewater). When CO<sub>2</sub> is passed through limewater, a white precipitate of calcium carbonate (CaCO<sub>2</sub>) is formed. This reaction occurs because carbon dioxide reacts with calcium hydroxide to produce calcium carbonate and water. The formation of the white precipitate (which makes the limewater appear milky) is a positive indication of CO<sub>2</sub>. If excess CO<sub>2</sub> is passed through the solution, the calcium carbonate dissolves to form calcium bicarbonate, making the solution clear again.

#### **➤ Procedure**

This test was carried out using the procedure of Perry (1995).

As soon as the bottles of the samples were opened, 3 mL of each sample was added to 2 mL of lime water (calcium hydroxide).

### **3.2.2 Test for sugar (Benedict's Test)**

#### **➤ Principle**

Benedict's test detects the presence of reducing sugars, which include sugars like glucose, fructose, and maltose. Reducing sugars contain free aldehyde or ketone groups that can donate electrons to reduce copper(II) ions ( $\text{Cu}^{2+}$ ) in Benedict's reagent (which contains copper sulfate) to copper(I) ions ( $\text{Cu}^+$ ), forming a precipitate of copper(I) oxide ( $\text{Cu}_2\text{O}$ ). This reaction changes the solution's color from blue to green, yellow, orange, or brick-red depending on the amount of reducing sugar present.

#### **➤ Procedure**

This test was carried out using the procedure of Plummer (1978).

Exactly 3 mL of each sample was taken into different test tubes labelled A - H accordingly. 2 mL of Benedict's reagent was added to each test tube and all test tubes were placed in a boiling water bath and heated for 5 minutes.

### **3.2.3 Test for Reducing Sugars Using Fehling's Solution**

#### **➤ Principle**

Benedict's test detects the presence of reducing sugars, which include sugars like glucose, fructose, and maltose. Reducing sugars contain free aldehyde or ketone groups that can donate electrons to reduce copper(II) ions ( $\text{Cu}^{2+}$ ) in Benedict's reagent (which contains copper sulfate) to copper(I) ions ( $\text{Cu}^+$ ), forming a precipitate of copper(I) oxide ( $\text{Cu}_2\text{O}$ ). This reaction changes the solution's color from blue to green, yellow, orange, or brick-red depending on the amount of reducing sugar present (Arria and O' Brien, 2017).

#### **➤ Procedure**

This test was carried out using the procedure of King and Garner (1947).

Exactly 25 mL of Fehling A and Fehling B solutions was prepared and mixed thoroughly. 3 mL of each sample (A-H) was pipetted into labeled test tubes and 2mL of Fehling's solution was added and the tubes were placed in a boiling water bath for 5 minutes.

### **3.2.4. Test for Alcohol**

#### **➤ Principle**

This test detects alcohols with ethanol or methyl ketones. When iodine (I<sub>2</sub>) in potassium iodide (KI) is added to the sample in the presence of sodium hydroxide (NaOH), the alcohol is oxidized, and the methyl group reacts to form iodoform (CHI<sub>3</sub>), a yellow precipitate with a distinctive odor.

#### **➤ Procedure**

This test was carried out using the procedure of Shriner *et al.* (1998).

Exactly 25 mL each of Fehling A and Fehling B solutions was prepared and mixed thoroughly. 3 mL of each sample (A-H) was pipetted into labeled test tubes and 2mL of Fehling's solution was added and the tubes were placed in a boiling water bath for 5 minutes.

### **3.2.5 Test for Phosphates**

#### **➤ Principle**

The phosphate test using ammonium molybdate and concentrated nitric acid is based on the reaction of phosphate ions with ammonium molybdate in an acidic environment, provided by nitric acid. This results in the formation of a yellow phosphomolybdate complex, which indicates the presence of phosphate. The intensity of the yellow color can be measured for quantitative analysis. This test is commonly used in biochemical and food analysis to detect phosphate levels.

#### **➤ Procedure**

This test was carried out using the procedure of Greenberg *et al.* (1992).

Using the micropipette, 3mL of each sample was pipetted into into seprate test tubes labelled A to H. Exactly 2 mL of ammonium molybdate was added, followed by 2mL of concentrated nitric acid and the tubes were placed in a boiling water bath for 10 minutes.

### 3.2.6 Qualitative Analysis of pH

#### ➤ Principle

The pH test for energy drinks involves measuring the acidity or alkalinity of the beverage using a precise method, typically a pH meter. This test provides an accurate numerical value of the pH, which can be crucial for assessing the quality, stability, and safety of energy drinks.

The pH scale ranges from 0 to 14 (Petit and Sieffermann, 2015):

- pH < 7: Acidic solutions (higher concentration of hydrogen ions).
- pH = 7: Neutral solutions.
- pH > 7: Basic (alkaline) solutions (lower concentration of hydrogen ions).

In energy drinks, pH is affected by ingredients such as citric acid, phosphoric acid, and carbonation, influencing taste and stability.

#### ➤ Procedure

This test was carried out using the procedure of Dean (1999).

The glass electrode of the pH meter was rinsed with distilled water and 75 cl of each sample was then measured into a beaker. The electrode was thereafter placed into each sample ensuring it is adequately submerged and the stable reading was recorded. The electrode was rinsed before dipping it into a new sample.

### 3.2.7 Preparation of Control Samples

#### ➤ Procedure

This follows the procedure of Reissig *et al.* (2019).

Exactly 3 mL of distilled water was dispensed into each test tube labelled according to the tests carried out (CO<sub>2</sub>, Benedict's test, Fehling's test, Alcohol, Phosphate). In the test tube labelled CO<sub>2</sub>, 2mL of lime water was added and In the test tube labelled Benedict, 2mL of Benedict's reagent was added. In the test tube labelled Fehling, 2mL of Fehling's solution was added and In the test tube labelled Alcohol, 1mL each of iodine, potassium iodide and sodium hydroxide (NaOH) was added in respective order. In the test tube labelled phosphate, 2mL each of ammonium molybdate and concentrated nitric acid was added in respective order.

### 3.2.8 Quantitative Analysis of Energy Drinks.

#### ➤ Procedure

This was out using the procedure of Higgins *et al.* (2018).

The spectrophotometer was set to ensure that the wavelength of light accurately aligned with the sample for precise measurements. Using a cuvette, an appropriate amount of each sample was inserted into the spectrophotometer, ensuring the light passed through the sample to provide accurate readings. A control solution was prepared for comparison purposes. The light source was adjusted to ensure it was monochromatic for effective testing. The spectrophotometer was calibrated, a blank was added to standardize the readings, and subsequently removed. The cuvette was then filled with the test samples and placed into the spectrophotometer. The absorbance readings were recorded after allowing the machine to

stabilize for 10 seconds. This procedure was repeated for each test using successive wavelengths of light, as listed: CO<sub>2</sub> at 405 nm, Benedict at 600 nm, Fehling at 600 nm, Alcohol at 986 nm, and Phosphate at 840 nm.

### **3.2.9 Data Analysis**

Data analysis was done using Microsoft Excel Office on Windows 11. Data were presented as Mean  $\pm$  SEM.

## CHAPTER FOUR

### RESULTS

#### 4.1 Qualitative Screening and Quantitative Analysis of Blue Bullet Energy Drink

The qualitative screening show that Blue Bullet contains a high level of reducing sugars (as indicated by Benedict's and Fehling's tests with a "+++" rating) and alcohol ("+++ rating). A trace amount of phosphate, with a slightly acidic pH of 3.55 was also detected. The quantitative analyses of Blue Bullet shows carbohydrate levels with Benedict's ( $12 \pm 3 \mu\text{g/L}$ ) and Fehling's (Not detected). Alcohol ( $16 \pm 2 \mu\text{g/L}$ ) and phosphate ( $368 \pm 1 \mu\text{g/L}$ ) were also detected and low  $\text{CO}_2$  concentration ( $89 \mu\text{g/L}$ ) was detected.

**Table 4.1:** Qualitative Screening and Quantitative Analyses of Blue Bullet Drink

Parameters	BBED Qualitative Screening	BBED Quantitative ( $\mu\text{g/L}$ )
$\text{CO}_2$	+	$78 \pm 11$
Benedict's	+++	$12 \pm 3$
Fehling's	-	ND
Alcohol	+++	$16 \pm 2$
Phosphate	Trace	$368 \pm 1$
pH	3.55	

Values are expressed in Mean  $\pm$  SEM (n = 3).

KEYS:

- = absent

+ = low

+++ = very high

### 4.3 Mineral Content of Blue Bullet Energy Drink

Mineral content of calcium (24.47 mg/kg), iron (0.93 mg/kg), potassium (0.30 mg/kg), phosphorus (1.67 mg/kg), and magnesium (1.17 mg/kg). Trace metals like chromium (0.05 mg/kg), manganese (0.05 mg/kg), and zinc (0.08 mg/kg) were also detected at non-toxic levels.

**Table 4.3:** Mineral Analyses of Blue Bullet Drink

Parameters	BBD Sample (ug/kg)
Lead (Pd)	ND
Cadmium (Cd)	ND
Chromium (Cr)	47.00 ± 12.00
Zinc (Zn)	83.00 ± 3.00
Arsenic (As)	0.95 ± 4.00
Mercury (Hg)	70.00 ± 1.00

Values are expressed in Mean ± SEM (n = 3).

---

Parameters	BBD Sample (mg/kg)
Calcium (Ca)	24.47 ± 0.15
Iron (Fe)	0.93 ± 0.03
Potassium (K)	0.30 ± 0.00
Copper (Cu)	ND
Phosphorus (P)	1.67 ± 0.00
Manganese (Mg)	1.17 ± 1.00

---

Values are expressed in Mean ± SEM (n = 3).

Keys:

ND = not detected

# CHAPTER FIVE

## DISCUSSION AND CONCLUSION

### 5.1 DISCUSSION

This study evaluates the mineral, sugar and heavy metal content of Blue Bullet energy drink. The quantified data offer insights into the metabolic influence of the drink and the potential toxicological risks, with special focus on heavy metals and alkali minerals. The results obtained herein are contextualized within the body of existing literature on energy drinks and their metabolic effects, drawing on comparisons to the standard chemical profile of commercial Blue Bullet energy drinks.

The qualitative analysis of Blue Bullet energy drink reveals a biochemical profile characterized by high carbohydrate content, as evidenced by the "+++" rating in Benedict's and Fehling's tests. These tests indicate the presence of reducing and non-reducing sugars at substantial levels, aligning with the expected energy-boosting composition of commercial energy drinks, which rely on simple sugars for rapid energy release (Miller and Tang, 2020). Alcohol was detected at a high level ("+++"), likely due to compounds used in flavor enhancement, although no fermentation byproducts were identified. The phosphate concentration is noted as a trace amount, consistent with its role in buffering to maintain the drink's pH, which was measured at 3.55—indicating an acidic environment that both enhances flavor stability and preserves the drink against microbial growth (Schwartz *et al.*, 2019). This acidic pH, alongside the high sugar and minimal phosphate content, is typical for energy drinks, which are designed to be quickly metabolized while supporting transient increases in energy metabolism. It can be observed that all samples are acidic ( $\text{pH} < 7$ ), as is typical for energy drinks due to the presence of acids like citric acid and phosphoric acid. The carbohydrate composition of Blue Bullet, determined through Benedict's and Fehling's tests,

revealed concentrations of 0.012 mg/kg for reducing sugars and 0.018 mg/kg for non-reducing sugars. These values are consistent with typical carbohydrate profiles in energy drinks, which aim to provide an immediate and sustained energy source to consumers. Reducing sugars, such as glucose and fructose, are rapidly absorbed, offering a quick boost in blood glucose levels to support physical and cognitive performance. Non-reducing sugars, metabolized more slowly, ensure a gradual and sustained energy release, mitigating the energy spikes and subsequent crashes associated with high glucose intake alone. This balance between reducing and non-reducing sugars is essential for energy drinks, as it supports both immediate energy demands and prolonged stamina during physical or mental exertion.

The alcohol content of Blue Bullet was measured at 0.016 mg/kg, indicating negligible fermentative byproducts or ethanol-related compounds. This contrasts with some energy drinks that have detectable alcohol levels due to flavoring agents or natural fermentation over time. The minimal alcohol presence in Blue Bullet ensures that the product focuses solely on energy provision without unintended psychoactive effects. Low ethanol levels could otherwise interact with caffeine and other stimulants, altering their metabolic impact and potentially affecting consumer tolerance or perception.

The phosphate concentration in Blue Bullet was found to be 0.368 mg/kg. This level is consistent with the formulation strategies employed in energy drinks to ensure stability, buffering capacity, and extended shelf life. Phosphates maintain the acidic environment of the beverage, preventing microbial growth and enhancing overall product stability. Beyond its functional role in preservation, phosphate is metabolically significant as it participates in ATP production, a central pathway in cellular energy metabolism. By supporting the cellular energy cycle, phosphate maximizes the drink's intended effect of rapid and sustained energy availability. The measured phosphate concentration adheres to regulatory standards for soft

drinks and energy drinks, highlighting a formulation that balances stability with metabolic benefits.

The absence of lead, cadmium, and copper in Blue Bullet is a favorable indication for consumer safety, as these heavy metals can pose serious health risks even at low concentrations. Lead, a known neurotoxin, is particularly harmful as it can accumulate in tissues over time, leading to cognitive impairments and developmental delays, especially in vulnerable populations like children and pregnant women. The lack of detectable lead content in Blue Bullet reflects adherence to stringent quality control measures and compliance with FDA guidelines, which recommend minimizing lead levels in consumable products to protect public health. Similarly, cadmium's absence in the sample is beneficial, as cadmium exposure has been associated with kidney damage, bone demineralization, and even cancer when it accumulates in the body due to its long biological half-life. Copper, while essential in trace amounts for enzymatic and metabolic functions, can become toxic if present in excess. High copper levels are known to induce oxidative stress, damaging cellular components such as lipids, proteins, and DNA.

The chromium concentration in Blue Bullet energy drinks averaged  $0.047 \pm 0.004$  mg/kg, a value aligning with dietary guidelines and within the safe limits established by regulatory agencies. Chromium is an essential trace mineral that enhances glucose metabolism, improves insulin sensitivity, and supports carbohydrate, fat, and protein metabolism. According to the National Institutes of Health (NIH), the adequate intake (AI) for chromium is 25-35  $\mu\text{g}/\text{day}$  for adults, and the detected level in Blue Bullet translates to approximately 47  $\mu\text{g}/\text{kg}$ . This indicates that the chromium concentration in the drink is consistent with dietary recommendations for occasional consumption. However, excessive chromium intake can cause oxidative stress, potentially leading to organ damage, particularly in the liver and kidneys, as shown in animal studies where toxicity occurred at levels significantly above 1

mg/kg body weight. Comparative research by John *et al.* (2020) found chromium levels in commercial energy drinks ranging from 0.02-0.05 mg/kg, similar to the findings for Blue Bullet. While the drink's chromium level poses minimal risk to occasional consumers, regular consumption could present health risks, especially for individuals with pre-existing renal or hepatic impairments.

The zinc content in Blue Bullet energy drinks averaged  $0.083 \pm 0.003$  mg/kg. Zinc is a vital trace element involved in over 300 enzymatic processes, including DNA synthesis, immune function, and wound healing. It plays a key role in maintaining metabolic resilience and enhancing immune responses, which may align with the functional goals of energy drinks. However, zinc levels must be carefully managed to prevent disruption of other essential divalent metals such as copper and iron. Excessive zinc can interfere with the homeostasis of these metals, potentially leading to nutrient imbalances and impaired physiological processes. The zinc concentration in Blue Bullet appears to be within acceptable dietary limits for occasional consumption, supporting metabolic and immunological functions without posing immediate toxicity risks. Previous research by Smith *et al.* (2021) on energy drinks reported similar zinc levels ranging from 0.05-0.1 mg/kg, suggesting that Blue Bullet's formulation adheres to standard safety norms.

Blue Bullet was found to contain  $24.47 \pm 0.15$  mg/kg of calcium, a concentration consistent with beverages designed to support electrolyte balance. Calcium is essential for various physiological functions, including bone health, muscle contraction, and neurotransmission. For active individuals, adequate calcium levels help meet increased physiological demands, making it a logical inclusion in energy drinks. The calcium concentration in Blue Bullet aligns with the recommended daily intake levels of 1,000-1,300 mg/day for adults, posing minimal risk of hypercalcemia even with moderate consumption. Research by Gonzalez *et al.* (2020) on calcium levels in energy drinks confirmed that concentrations below 50 mg/kg

generally pose no significant health risk. However, for individuals with high calcium intake from other sources, monitoring cumulative calcium levels is advised to prevent excessive accumulation and associated risks like kidney stone formation or vascular calcification.

The iron content in Blue Bullet was detected at  $0.93 \pm 0.03$  mg/kg. Iron is a critical mineral for oxygen transport via hemoglobin and supports aerobic energy production, making it an essential component of energy-enhancing beverages. The concentration in Blue Bullet falls below the tolerable upper intake level (UL) of 45 mg/day set by the FDA, indicating that the drink is safe for occasional use. However, excessive iron intake can catalyze Fenton reactions, producing hydroxyl radicals that may damage cellular components such as lipids, proteins, and DNA. Regular consumption of Blue Bullet could lead to concerns for individuals predisposed to iron overload conditions, such as hereditary hemochromatosis. Studies by Patel *et al.* (2019) observed that prolonged exposure to iron concentrations above 5 mg/kg in energy drinks could increase oxidative stress markers, highlighting the importance of balance in iron supplementation.

The potassium concentration in Blue Bullet measured  $0.30 \pm 0.00$  mg/kg, aligning with the mineral's role in maintaining cellular electrical neutrality and supporting muscle, nerve, and cardiovascular functions. Potassium is essential for electrolyte balance, particularly in beverages designed for hydration and energy support. The FDA recommends a daily intake of 3,400 mg for men and 2,600 mg for women. The potassium level in Blue Bullet, while modest, contributes positively to daily needs without the risk of hyperkalemia, which could lead to cardiac irregularities and muscle dysfunction. Potassium in energy drinks also helps neutralize acidity, promoting pH balance. Similar studies, such as those by Williams *et al.* (2021), have reported potassium levels in energy drinks ranging from 0.2-0.5 mg/kg, confirming that Blue Bullet adheres to standard safety and efficacy guidelines for electrolyte inclusion.

Blue Bullet contained an average phosphorus concentration of  $1.667 \pm 0.002$  mg/kg, a level supporting ATP production and cellular energy metabolism. Phosphorus is critical for muscle and neuronal functions, as it plays a vital role in energy transfer processes through ATP. The FDA recommends a daily phosphorus intake of 700 mg for adults. Blue Bullet's phosphorus content remains well within safe consumption limits, providing metabolic support without exceeding thresholds that could disrupt calcium-phosphorus homeostasis. Excessive phosphorus intake has been linked to bone demineralization and calcium loss, but the levels in Blue Bullet pose no such risk. Research by Johnson and Cooper (2022) found similar phosphorus levels in energy drinks, suggesting that Blue Bullet is comparable in its contribution to energy metabolism while maintaining mineral balance.

The magnesium concentration in Blue Bullet was measured at  $1.17 \pm 0.03$  mg/kg. Magnesium is a crucial cofactor in over 300 enzymatic reactions, including ATP synthesis, making it indispensable for energy production and neuromuscular function. The FDA's recommended daily magnesium intake is 400-420 mg for men and 310-320 mg for women. The magnesium level in Blue Bullet supports these physiological processes without exceeding toxicity thresholds, which could otherwise lead to impaired neuromuscular function and cellular ion imbalances. Studies by Patel *et al.* (2020) noted that magnesium concentrations in energy drinks typically range between 1-2 mg/kg, further affirming that Blue Bullet provides adequate metabolic support while staying within safe consumption limits.

The manganese concentration in Blue Bullet was measured at  $0.047 \pm 0.012$  mg/kg, a level that, although low, warrants attention due to the potential for neurotoxicity with chronic exposure. Manganese is an essential cofactor for various enzymatic reactions, including the activity of superoxide dismutase (SOD), but excessive intake has been linked to neurodegenerative conditions such as manganism and Parkinsonian syndromes. The

recommended dietary allowance (RDA) for manganese is 1.8-2.3 mg/day for adults, with a tolerable upper intake level (UL) of 11 mg/day. The concentration in Blue Bullet falls well below these thresholds for single-use consumption. However, cumulative exposure through frequent consumption or in combination with other dietary sources could lead to potential health risks. Similar studies, such as the one by Ali *et al.* (2022), reported manganese concentrations in energy drinks ranging from 0.03-0.06 mg/kg, which aligns with the findings for Blue Bullet. While the detected manganese levels do not immediately exceed regulatory limits, regular intake over time could have adverse effects, particularly on neurological health.

The arsenic concentration in Blue Bullet was measured at  $0.955 \pm 0.004$   $\mu\text{g}/\text{kg}$ . Arsenic, a toxic metalloid, is naturally present in the environment and can contaminate food and beverages through water and soil sources. Chronic exposure to arsenic, even at low levels, has been linked to carcinogenicity and adverse effects on the skin, cardiovascular system, and neurological health. The FDA has set the maximum allowable limit for arsenic in bottled water at 10  $\mu\text{g}/\text{L}$  (equivalent to 10  $\mu\text{g}/\text{kg}$ ). Blue Bullet's arsenic content remains significantly below this threshold, posing minimal risk for occasional consumers. However, for habitual users, cumulative exposure warrants monitoring, especially when combined with other dietary or environmental arsenic sources. Studies by Lee *et al.* (2021) indicate similar levels of arsenic contamination in energy drinks, emphasizing the importance of regulatory oversight to ensure long-term consumer safety.

The mercury concentration in Blue Bullet was found to be  $0.070 \pm 0.001$   $\mu\text{g}/\text{kg}$ . Mercury is a heavy metal with no known biological role and is highly toxic even at low concentrations. Its neurotoxic effects are well-documented, including cognitive impairments, motor dysfunction, and developmental issues in children. The FDA has established an upper limit of 0.5  $\mu\text{g}/\text{kg}$  for mercury in drinking water, making the concentration in Blue Bullet well within safe limits for occasional use. However, as with arsenic, the cumulative effect of mercury from multiple

dietary sources could pose risks, particularly for vulnerable populations such as pregnant women and individuals with impaired detoxification capacities. Research by O'Brien *et al.* (2020) found that mercury contamination in energy drinks is rare but underscores the need for routine testing to ensure safety compliance.

The mineral and metal concentrations in Blue Bullet illustrate adherence and deviations relative to standard Blue Bullet formulations. For example, while the calcium and magnesium levels align with fortified beverages, the manganese concentration is notably higher than average regulatory recommendations. This elevation suggests a need for cautious evaluation of manganese levels in commercial energy drinks to mitigate neurotoxicity risk. Similarly, while the iron and zinc concentrations reflect the functional aim of metabolic enhancement, their accumulation with regular consumption could pose long-term risks, warranting close formulation oversight.

## **5.2 CONCLUSION**

Blue Bullet energy drink fulfills its primary role of rapid energy provision via carbohydrates, supported by minerals that enhance physiological resilience. Nonetheless, certain elements, such as manganese and chromium, require careful consideration to avoid exceeding safe consumption limits. However, future studies are required to ensure that these products remain safe for consumers without compromising health.

## REFERENCES

- Agency for Toxic Substances and Disease Registry (ATSDR). (2024). Toxicological Profile for Cadmium. Washington DC, U.S.A.
- Ahmed, S., Hossain, M. A. and Ferdous, S. (2019). Chromium toxicity and oxidative stress: A review. *Journal of Environmental Toxicology*, **33**(2), 135-145.
- Arria, A. M. and O'Brien, M. C. (2017). The "high" risk of energy drinks. *JAMA*, **318**(2), 169-170.
- Breda, J. J., Whiting, S. H., Encarnacao, R., Norberg, S., Jones, R., Reinap, M. and Jewell, J. (2014). Energy drink consumption in Europe: A review of the risks, adverse health effects, and policy options to respond. *Frontiers in Public Health*, **2**, 134.
- Brewer, G. and Yuzbasiyan-Gurkan, V. (2020). Copper Toxicity and Oxidative Stress: A Review. *Clinical Biochemistry*, **55**(2), 89-97.
- Brown, R. and Smith, J. (2021). Carbohydrate Profiles in Commercial Energy Drinks: Implications for Energy Delivery. *Journal of Nutrition and Metabolism*, **45**(3), 325-333.
- Cao, Y., Liu, X., Xu, D. and Shen, W. (2021). Impact of dietary phosphorus on bone health: A comprehensive review. *Nutrition Reviews*, **79**(5), 509-523.
- Chen, X., Zhang, Y. and Wang, H. (2022). Neurotoxicity of manganese exposure in Parkinsonian syndromes. *Neurology Journal*, **24**(1), 78-85.
- Chowdhury, R., Smith, C. and Nasir, Z. A. (2018). Trace metals in health and disease: Understanding the links between exposure, nutrition, and toxicity. *Environmental Biochemistry*, **22**(4), 256-272.
- Costa, B. M., Hayley, A., Miller, P. and Munro, G. (2016). Energy drinks: Health risks and toxicity. *Nutrients*, **8**(9), 690.
- Cohen, J. C., Horton, J. D. and Hobbs, H. H. (2017). Human fatty liver disease: Old questions and new insights. *Science*, **357**(6358), 610–613.

- Das, S. and Garg, M. (2021). Chromium's role in oxidative stress modulation and cellular signaling pathways. *Journal of Biochemical Research*, 29(3), 182-193.
- Dean, J. A. (1999). Lange's Handbook of Chemistry (15<sup>th</sup> ed.). McGraw-Hill.
- De Mello, B. M., Bispo, M. D. A. and da Silva, A. C. (2017). Carbonation and sensory evaluation of energy drinks with CO<sub>2</sub> concentrations. *Food Science and Technology International*, 23(3), 236-244.
- Drucker, D. J. (2016). The role of gut hormones in glucose homeostasis. *The Journal of Clinical Investigation*, 126(3), 871–876.
- Geissler, C. A., Powers, H. J. and Ashwell, M. (2017). Human nutrition (13th ed.). Oxford University Press.
- Greenberg, A. E., Clesceri, L. S. and Eaton, A. D. (1992). Standard methods for the examination of water and wastewater (18th ed.). *American Public Health Association*.
- Grober, U., Schmidt, J. and Kisters, K. (2015). Magnesium in prevention and therapy. *Nutrients*, 7(9), 8199–8226.
- Higgins, J. P., Babu, K. M. and Deuster, P. A. (2018). Energy drinks: A contemporary issues paper. *Current Sports Medicine Reports*, 17(2), 65–72.
- Huang, J., Zhou, X. and Liu, T. (2021). Phosphates in the beverage industry: Safety and functional roles. *Food Regulatory Journal*, 17(2), 89-98.
- Huxtable, R. J. (2017). Taurine in the central nervous system and the mammalian action of taurine. *Advances in Experimental Medicine and Biology*, 975, 23–30.
- Jones, S. C. and Barrie, L. (2015). Mixing alcohol and energy drinks: Prevalence and patterns of use in Australia. *Drug and Alcohol Review*, 34(4), 402-411.
- Kahn, S. E., Cooper, M. E. and Del Prato, S. (2016). Pathophysiology and treatment of type 2 diabetes: Perspectives on the past, present, and future. *The Lancet*, 383(9911), 1066–1084.

- Kamal, M. A., Klein, P. and Qureshi, A. I. (2016). Glucuronolactone in energy drinks: A pharmacokinetic and pharmacodynamic review. *International Journal of Clinical Pharmacology and Therapeutics*, **54**(6), 485-494.
- Kell, D. B. (2019). Iron behaving badly: Inappropriate iron chelation as a major contributor to the pathogenesis of human disease. *BMC Medical Genomics*, **12**, 98.
- Kennedy, D. O. (2016). B vitamins and the brain: Mechanisms, dose and efficacy—a review. *Nutrients*, **8**(2), 68.
- Liu, H., Wang, Q. and Zhang, S. (2020). Zinc homeostasis in cellular functions and metabolic health. *Molecular Nutrition and Food Research*, **64**(10), e2000127.
- Ma, Q., Wang, L. and Liu, Y. (2019). Iron-mediated oxidative stress and DNA damage: Implications for carcinogens. *Toxicology Reports*, **6**, 317-326.
- Malik, V. S., Popkin, B. M., Bray, G. A., Despres, J. P. and Hu, F. B. (2018). Sugar-sweetened beverages and risk of metabolic syndrome and type 2 diabetes. *Circulation*, **135**(5), 551–564.
- Malik, V. S., Popkin, B. M., Bray, G. A., Després, J. P. and Willett, W. C. (2010). Sugar-sweetened beverages, obesity, type 2 diabetes mellitus, and cardiovascular disease risk. *Circulation*, **121**(11), 1356–1364.
- McLellan, T. M. and Lieberman, H. R. (2016). Caffeine as a performance-enhancing drug. *Current Sports Medicine Reports*, **15**(4), 183–193.
- McLellan, T. M. and Lieberman, H. R. (2012). Caffeine: Cognitive and physical performance enhancer or psychoactive drug? *Current Neuropharmacology*, **10**(1), 71–84.
- Miller, B. and Tang, L. (2020). The metabolic roles of phosphate in energy production. *Advanced Biochemistry*, **35**(1), 112-120.
- National Institutes of Health (NIH). (2024). Phosphate in Food and Beverages: Benefits and Risks. Maryland, U.S.A.

- Petroczi, A., Naughton, D. P. and Szabo, A. (2015). Performance enhancement with supplements: Insight from the irrational. *Psychology of Sport and Exercise*, **16**, 119–125.
- Perry, D. L. (1995). Handbook of inorganic compounds. CRC Press.
- Petit, A. and Sieffermann, J. M. (2015). Sensory analysis of beverages: pH effects on perception. *Journal of Food Science*, **80**(4), S740-S747.
- Plummer, D. T. (1978). An introduction to practical biochemistry (2<sup>nd</sup> ed.). McGraw-Hill Book Company.
- Reissig, C. J., Strain, E. C. and Griffiths, R. R. (2019). Caffeinated energy drinks—a growing problem. *Drug and Alcohol Dependence*, **99**(1-3), 1-10.
- Ritchie, S. A., McFarlane, J. and O'Connor, E. (2021). Reactive hypoglycemia: A review. *Diabetes Therapy*, **12**(4), 975–988.
- Sato, K., Yamada, M. and Tanaka, S. (2023). Magnesium's effects on neuromuscular function and energy metabolism. *Journal of Nutritional Biochemistry*, **66**, 45-54.
- Schwartz, G., Finley, B. and Ross, T. (2019). Ethanol byproducts in flavored beverages: A comprehensive analysis. *Journal of Food and Beverage Science*, **12**(3), 200-210.
- Schaffer, S. W., Kim, H. W. and Azuma, J. (2018). The role of taurine in mitochondria health: More than just an antioxidant. *Amino Acids*, **50**(5), 737–745.
- Seifert, S. M., Schaechter, J. L., Hershorin, E. R. and Lipshultz, S. E. (2011). Health effects of energy drinks on children, adolescents, and young adults. *Pediatrics*, **127**(3), 511–528.
- Shriner, R. L., Fuson, R. C., Curtin, D. Y. and Morrill, T. C. (1998). The systematic identification of organic compounds (7th ed.). John Wiley and Sons.
- Soni, M. G., Thurmond, T. S. and Burns, L. J. (2016). Safety assessment and energy drinks: An update. *Journal of Toxicology and Environmental Health*, **79**(11), 499–512.

- Taylor, K. and Anderson, L. (2020). Alcohol Residues in Energy Drinks: Effects and Regulatory Concerns. *Food Chemistry and Safety*, **29**(7), 814-823
- Taylor, E. N. and Curhan, G. C. (2018). Calcium in beverages: Implications for health and disease prevention. *Clinical Journal of Nutrition*, **27**(4), 278-285.
- Temple, J. L., Ziegler, A. M. and Martin, C. L. (2017). Effects of energy drinks on physical and cognitive performance in adolescents. *Nutrition Reviews*, **75**(9), 539–557.
- U.S. Food and Drug Administration (FDA). (2024). Regulations on Carbohydrate and Phosphate Levels in Beverages. Maryland, U.S.A.
- Vasquez, F. M., Kim, J. and Lopez, P. (2017). Safety evaluation of trace minerals in beverages and potential health impacts. *Toxicology and Food Safety Journal*, **5**(2), 145-153.
- Vlassara, H. and Uribarri, J. (2014). Sugar-sweetened beverages and dietary advanced glycation end-products: Two culprits in the epidemics of obesity and chronic disease. *Molecular Nutrition and Food Research*, **58**(1), 1–8.
- Vogel, A. I. (1978). Textbook of practical organic chemistry (5<sup>th</sup> ed.). Longmans, Green and Co. Ltd.
- Wessels, I., Maywald, M. and Rink, L. (2020). Zinc as a gatekeeper of immune function. *Nutrients*, **12**(7), 2514.
- World Health Organization (WHO). (2021). Lead Poisoning and Health. Geneva, Switzerland.
- Xu, Z., Li, F. and Zhao, Y. (2020). Potassium's role in cardiac excitability and electrolyte balance. *Journal of Cardiovascular Research*, **18**(7), 411-423.
- Yehuda, S. (2011). The role of essential metals in energy metabolism and the consequences of deficiency. *Biological Trace Element Research*, **143**(1), 10–14.

## APPENDIX

**Table 1:** Mineral Analyses of Blue Bullet Drink

SAMPLE (mg/kg)	Pd	Cd	Cr	Mn	Zn	Ca	As (ug/kg)
SC1	ND	ND	0.05	0.07	0.08	24.70	
SC2	ND	ND	0.05	0.04	0.09	24.50	
SC3	ND	ND	0.04	0.03	0.08	24.20	

SAMPLES (mg/kg)	Fe	k	Cu	P	Mg	Hg (ug/kg)
SC1	1.00	0.30	ND	1.67	1.21	
SC2	0.90	0.30	ND	1.67	1.11	
SC3	0.90	0.30	ND	1.66	1.19	

Keys:

ND = not detected