INVESTIGATION ON THE EFFECTS OF ETHANOLIC EXTRACT OF BEETROOT ON SOME HAEMATOLOGICAL PARAMETERS AND HISTOLOGY OF THE LUNGS ON ADULT MALE WISTAR RATS EXPOSED TO HEAT AND BIOMASS SMOKE

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A PROJECT WORK WRITTEN AND SUBMITTED IN PARTIAL FULFILMENT FOR THE REQUIREMENT FOR THE AWARD OF BACHELOR OF SCIENCE (B.Sc) DEGREE IN THE DEPARTMENT OF PHYSIOLOGY, SCHOOL OF BASIC MEDICAL SCIENCES, COLLEGE OF MEDICICAL SCIENCE, UNIVERSITY OF BENIN, BENIN CITY.

OCTOBER, 2025.

CERTIFICATION

This is to certify that this project work on "**Investigation on The Effects Of Ethanolic Extract of Beetroot on Some Haematological Parameters and Histology of The Lungs on Adult Male Wistar Rats Exposed to Heat and Biomass Smoke**" was carried out by Sarah Chimerem EZEABUCHUKWU, with the Matriculation Number: **BMS2102450**; in partial fulfilment for the Award of Bachelor of Science (B.Sc). Degree in the Department of Physiology, School of Basic Medical Sciences, College of Medical Sciences, University of Benin, Benin City.

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DEDICATION

I dedicate this project work to God Almighty who has been my source of inspiration and strength, who in his infinite mercies have seen me through this work.

ACKNOWLEDGEMENTS

I hereby appreciate God Almighty for giving me the Grace, Opportunity, Inspiration and Strength to complete this undergraduate project work an also write this report.

I also like to specifically thank my supervisor, Dr. (Mrs.) M. I. Omigie, for her support, guidance, encouragement and parental dispositions towards me and also for the constructive criticisms that made this project a reality. Also, my appreciation to the Head of Department, my Lecturers and the entire staff of the Department of Physiology, Basic Medical Sciences.

My profound gratitude goes to My Parents and My Siblings their countless support both financially, spiritually, physically and emotionally towards my educational pursuit.

And to My Friends and Course Mates and all others who assisted me at one point or the other. God bless you all.

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ABSTRACT

Heat is the transfer of thermal energy from a hotter system to a cooler system through conduction, convection and radiation. Biomass smoke, produced from the combustion of wood, charcoal, crop residues, and animal dung, is a complex mixture of harmful pollutants including fine particulate matter, carbon monoxide (CO), nitrogen oxides (NOx), polycyclic aromatic hydrocarbons (PAHs), and volatile organic compounds (VOCs). Beetroot (Beta vulgaris L.), is a traditional and popular vegetable in many parts of the world regularly consumed as part of the normal diet, either fresh or after processing for its nutritional benefits. This study aims to investigate the histopathological and hematological protective effects of beetroot extract in heat and biomass smoke-induced pulmonary damage in Wistar Rats. Forty-nine (49) adult female rats were divided into seven groups (n=7), including control, heat-only, smoke-only, smoke + heat, heat + beetroot, smoke + beetroot and heat + ~~trea~~smoke + treatment. The hematological results indicate that exposure to smoke alone caused a significant reduction in total white blood cell count, while other groups showed no such decline. Red blood cell and hemoglobin levels dropped notably in rats exposed to smoke and combined heat and smoke, but these values improved with beetroot ~~pre~~treatment. Most red cell and platelet indices remained unchanged, though MPV and PLCR increased in the heat-only group and were normalized by beetroot extract. Overall, beetroot treatment effectively mitigated hematological disruptions induced by heat and smoke exposure. Histologically, Vascular and bronchiolar ulceration and interstitial inflammation were seen in the lung tissue of the smoke only, heat only and smoke + treatment respectively. Animals in the beetroot treated groups had normal alveoli, bronchiolar dilation and bronchiolar ulceration (heat + beetroot, smoke + beetroot and heat + smoke + beetroot respectively. Beetroot treatment markedly restored WBC, RBC, HGB, and HCT levels and normalized platelet indices, demonstrating its hematoprotective and anti-inflammatory effects. These protective actions are attributed to beetroot's rich composition of betalains, polyphenols, flavonoids, and dietary nitrates, which enhance nitric oxide bioavailability, scavenge reactive oxygen species, and suppress pro-inflammatory cytokines. Beetroot extract therefore shows promise as a natural, affordable antioxidant remedy against heat and biomass smoke-induced hematological toxicity.

CHAPTER ONE

INTRODUCTION

1.1 Background of Study

Hematology is the branch of medical science that deals with the study of blood, blood-forming organs, and blood diseases. It encompasses the understanding of the morphology, physiology, pathology, and treatment of disorders involving blood cells (red cells, white cells, and platelets), hemoglobin, blood proteins, bone marrow, and the coagulation process. It plays a critical role in diagnosing conditions such as anemia, leukemia, lymphomas, hemophilia, clotting disorders, infections and immune deficiencies (Hoffbrand and Moss, 2016). Histology is the study and examination of the microscopic structure of tissues and cells which is crucial for understanding function and diseases (Gurina and Simms, 2023). Heat is the transfer of thermal energy from a hotter system to a cooler system through conduction, convection and radiation (Kenny and Jay, 2013). In developing countries, more than 3 billion people depend on biomass fuels such as wood, charcoal, crop residues, and animal dung, for cooking and heating. This widespread use results in prolonged exposure to harmful combustion byproducts. Consequently, respiratory health is increasingly jeopardized by both environmental pollutants and extreme climate conditions, especially in regions where traditional biomass fuel use remains widespread (Gordon *et al*., 2014). Biomass smoke, produced from the combustion of wood, charcoal, crop residues, and animal dung, is a complex mixture of

harmful pollutants including fine particulate matter (PM2.5 and PM10), carbon monoxide (CO), nitrogen oxides (NOx), polycyclic aromatic hydrocarbons (PAHs), and volatile organic compounds (VOCs) (Torres-Duque *et al*., 2021; Hu *et al*., 2023). Inhalation of these toxic components triggers, pulmonary inflammation, and tissue remodeling, which are key mechanisms underlying respiratory conditions such as chronic obstructive pulmonary disease (COPD), asthma, bronchitis, and lung cancer (Kurmi *et al*., 2012; Bruce *et al*., 2015). Prolonged or recurrent exposure to high temperatures can lead to hyperthermia, systemic inflammation, and even multi-organ failure. These effects are mediated by mechanisms such as the upregulation of heat shock proteins, mitochondrial dysfunction, and the release of pro-inflammatory cytokines (Zhang *et al*., 2024; Bouchama *et al*., 2022). The lungs, due to their extensive vascularization and high metabolic activity, are particularly susceptible to heat-induced damage. The two main respiratory organs, the lungs, are situated on either side of the mediastinum in the thoracic cavity. The lungs are highly specialized respiratory organs designed for efficient gas exchange between the external environment and the bloodstream (Pandey *et al*., 2025). Histologically, the lungs are composed of:

- Conducting airways: including the trachea, bronchi, and bronchioles, lined primarily with pseudostratified ciliated columnar epithelium (Patwa *et al*., 2015).
- Respiratory units**:** comprising respiratory bronchioles, alveolar ducts, and alveoli. Trachea and Bronchi: These structures are lined with pseudostratified ciliated columnar epithelium containing goblet cells that secrete mucus to trap inhaled

particles. The underlying lamina propria contains smooth muscle, cartilage rings (in the trachea and bronchi), and submucosal glands (Junqueira and Carneiro, 2015).

- Bronchioles: Smaller airways lack cartilage and are lined by ciliated simple columnar or cuboidal epithelium, with Clara cells playing a role in detoxification and regeneration. (Krstic, 2012)
- Alveoli: The functional units of the lungs, alveoli are thin-walled sacs lined by type I pneumocytes (for gas exchange) and type II pneumocytes (which secrete surfactant to reduce surface tension). Alveolar macrophages patrol the airspaces, phagocytosing inhaled particulates and pathogens (Krstic, 2012).

Interspersed within the alveolar walls are pulmonary capillaries**,** interstitial fibroblasts**,** and alveolar macrophages, all of which help maintain alveolar function and defend against inhaled pathogens and particles (West, 2012). According to Nemmar *et al*. (2013), the respiratory system is highly susceptible to environmental stressors, a vulnerability that is significantly heightened when biomass smoke exposure occurs alongside heat stress-conditions intensified by ongoing climate change. Heat stress increases pulmonary vascular permeability and promotes inflammatory cell infiltration, while biomass smoke compounds the damage by impairing alveolar structure, surfactant function, and immune responses. The combined effects result in marked pulmonary histopathology, including alveolar septal thickening, edema, hemorrhage, and fibrosis (Mukherjee and Agrawal, 2017; Fiorin *et al*., 2021). These pathological changes are primarily driven by excessive

generation of reactive oxygen species (ROS), depletion of antioxidant defenses, and heightened expression of pro-inflammatory cytokines such as interleukin-6 (IL-6), interleukin-1β (IL-1β), and tumor necrosis factor-alpha (TNF-α) (Yang *et al*., 2022). If left unaddressed, these processes can lead to chronic and irreversible respiratory diseases such as chronic obstructive pulmonary disease (COPD), asthma, and pulmonary hypertension (Po *et al*., 2011). Given that beetroot (*Beta vulgaris* L.) contains a lot of dietary nitrates, iron, folate, and antioxidants, it has shown substantial hematological benefits. According to studies, eating it can raise hemoglobin levels and improve the production of red blood cells, especially in anemic people (Adji *et al.,* 2022). For cardiovascular and hematologic health, beetroot's nitrates increase nitric oxide (NO) bioavailability, which promotes vasodilation and improved oxygen transport to tissues (Clifford *et al.,* 2015). Furthermore, beetroot's antioxidant compounds support its function in preserving hematological stability and enhancing blood quality by shielding erythrocytes from oxidative stress (Ninfali and Angelino, 2013). Nutrient-dense beetroot contains powerful bioactive substances like ascorbic acid, flavonoids, polyphenols, and betalains (betacyanins and betaxanthins), which have strong anti-inflammatory and antioxidant qualities (Clifford *et al*., 2015). It has been demonstrated that betalains, in particular, inhibit lipid peroxidation and suppress NF-κB-mediated inflammation while upregulating endogenous antioxidant enzymes, such as glutathione peroxidase, catalase, and superoxide dismutase (SOD) (Krajka-Kuźniak *et al*., 2017). Beetroot's therapeutic potential is further supported by experimental studies that show it can scavenge reactive oxygen species (ROS), lower protein nitration, and prevent oxidative and inflammatory

damage in a variety of tissues, including the brain, liver, heart, and lungs (Kujawska *et al*., 2009; Kapadia *et al*., 2013). However, there are few studies examining beetroot's protective effectiveness in models involving combined exposure to biomass smoke and heat-induced stress, despite a wealth of evidence supporting its antioxidant activity. This is especially important in tropical areas like sub-Saharan Africa, where people are regularly exposed to indoor biomass smoke while working or residing in hot climates.

1.2 Statement of problem

Thermal stress and biomass smoke exposure in the environment pose a dual risk to systemic and respiratory health. Alveolar damage, oxidative stress, pulmonary inflammation, and impaired hematological balance are the outcomes of either acute or chronic exposure. Finding easily accessible, non-toxic agents with protective qualities is therefore crucial. The effectiveness of beetroot in reducing the simultaneous pulmonary and systemic effects of biomass smoke and heat stress has not been thoroughly determined, despite its well-established antioxidant and anti-inflammatory properties. Comprehending its impact on hematological indices and lung histology may facilitate its application as an inexpensive, natural therapeutic agent in settings with limited resources.

1.3 Justification of study

The rising prevalence of respiratory illnesses brought on by heat exposure and biomass smoke emphasizes the need for practical and cost-effective preventive measures, particularly in environments with limited resources. Interest in natural alternatives has grown as a result of conventional treatments' frequent failure to address the underlying

oxidative and inflammatory processes. Due to its extensive exposure among vulnerable populations, especially women and children in rural areas (WHO, 2021), beetroot may be a cheap, easily accessible dietary intervention with substantial scientific, economic, and public health benefits.

1.4 Aim and Objectives

1.4.1 Aim

To investigate the histopathological and hematological protective effects of beetroot extract in heat and biomass smoke-induced pulmonary damage in Wistar Rats.

1.4.2 Objectives

I. To determine the histopathological effects/changes in lung tissues of rats exposed to heat and biomass smoke.

II. To assess changes in key hematological parameters (e.g., RBC count, WBC count, hemoglobin, hematocrit) after exposure and treatment.

III. To compare the protective efficacy of different doses as well as between treated and untreated of beetroot extract against pulmonary injury.

1.5 Scope of Study

Adult Wistar rats are used in the study and are split up into treatment, control, and exposed groups. Over a predetermined time period, the animals will be exposed to regulated temperatures and biomass smoke. Hematological parameters will be examined in blood samples, and lung tissue will be gathered for histological examination. Protocols

for humane treatment and ethical approval will be adhered to in compliance with institutional guidelines.

CHAPTER TWO

LITERATURE REVIEW

2.1

The respiratory system is particularly vulnerable to environmental contaminants, especially those caused on by excessive heat and smoke. Many developing countries use bio-fuels, like wood, crop remains, and animal dung, for cooking and heating, which causes chronic exposure to harmful emissions and prolonged indoor air pollution. Respiratory conditions like asthma, bronchitis, pulmonary fibrosis, chronic obstructive pulmonary disease (COPD), and an increased risk of lung infections have all been closely associated with this exposure. Exposures to harmful substances in the workplace and the environment, such as smoke and high temperatures, are important risk factors for lung damage because they cause inflammation, oxidative stress, and structural changes in lung tissue (Po *et al*., 2020). Toxic components found in biomass smoke, such as particulate matter, carbon monoxide (CO), and polycyclic aromatic hydrocarbons (PAHs), cause lung disease through inflammatory and oxidative processes (Kumar *et al*., 2021).

2.2 Biomass Smoke and Pulmonary Damage

Nearly 3 billion people worldwide utilize solid organic materials as the principal fuel for indoor cooking and heating, particularly in poor countries, resulting in biomass smoke and household air pollution (HAP) (WHO, 2024). When organic materials like wood, crop wastes, twigs, dried animal dung, and charcoal are not completely burned in crude

stoves or open flames, biomass smoke is produced. In addition to chemical volatiles that are detrimental to human health (Rehfuess and Eva, 2006; Naeher *et al*., 2007; Smith *et al.,* 2004), such as sulfurs, carbon monoxide, and polycyclic aromatic hydrocarbons, this biomass smoke has large amounts of dangerous inhalable particulate matter (Shupler *et al.,* 2020).

2.3 Composition of Biomass Smoke

Biomass smoke is a complex mixture of toxic pollutants:

i. Particulate Matter (PM10, PM2.5, PM0.1)
ii. Carbon monoxide (CO), nitrogen oxides (NOx), sulfur dioxide (SO2)
iii. Volatile organic compounds (e.g., benzene, formaldehyde)
iv. Polycyclic aromatic hydrocarbons (PAHs)

Particulate matter penetrates deep into the lungs and can induce oxidative stress by generating ROS, leading to lipid peroxidation, protein denaturation, and DNA damage (Ghio *et al*., 2012). These pollutants have harmful respiratory and systemic effects due to their small particle size and ability to induce oxidative stress and inflammation (IARC, 2010; Camp *et al*., 2014).

2.4 Pulmonary Damage Induced by Heat and Biomass Smoke

The lungs are a vital organ for the body's gas exchange, and different respiratory system conditions can result in varying degrees of lung damage (Maldonado *et al.,* 2020). Studies shows that a number of factors, including stress, hypoxia, bacterial infection, and

exposure to toxins, are linked to lung injury (Chen, 2011; Dushianthan *et al*., 2011; Looney *et al.,* 2014). In order to disperse heat and maintain body temperature balance, mammals generally rely on sweat glands (Yahav, 2015). In a heat-stressed environment, high-frequency breathing increases the risk of lung tissue damage. Prolonged exposure to biomass smoke is closely linked to an increased incidence of respiratory symptoms, particularly among women who are more frequently exposed during cooking. Compared to those using cleaner fuels like LPG, individuals relying on biomass reported significantly higher rates of wheezing and breathlessness (Shrestha and Shrestha 2005; Kurmi *et al.,* 2010).

Additional factors that also intensifies respiratory risks include tobacco use, especially among biomass-exposed men, significantly increased the likelihood of respiratory symptoms. Socioeconomic variables such as low income and education levels were also linked to greater exposure and worse health outcomes. (Kurmi *et al.,* 2013).

2.5 Beet root (Beta vulgaris)

The well-documented health benefits of diets rich in fruits and vegetables have spurred growing interest in "functional foods" and their roles in health and disease. The discovery that dietary nitrate sources may significantly influence cardiovascular health has recently intensified interest in beetroot (L.) (Lundberg and Weitzberg, 2022). The beet, Beta vulgaris is a plant in the Chenopodiaceae family. It is best known in its numerous cultivated varieties, the most well-known of which is the purple root vegetable known as beetroot or table garden beet. However, other cultivated strains include the leafy

vegetables chard and spinach beets, as well as the root vegetable sugar beet, which is important in sugar production (Babarykin *et al*., 2019). In addition to its high nitrate content, beetroot contains a diverse array of bioactive compounds such as betalains (betacyanins and betaxanthins), flavonoids, phenolic acids, ascorbic acid, and dietary nitrates that contribute to its potential as a health-promoting and disease-preventing functional food (Guldiken *et al.,* 2016). These constituents possess strong antioxidant and anti-inflammatory properties, with betalains in particular demonstrating the ability to scavenge free radicals and inhibit lipid peroxidation (Kujala *et al*., 2002; Clifford *et al*., 2015).

Red beetroot, also known as beet, garden beet, or table beet, is a traditional and popular vegetable in many parts of the world. It is the red root vegetable most commonly associated with the word "beet" and is particularly popular in Eastern and Central Europe, where it is primarily used in pickled cabbage with beetroot, borscht, vinaigrette salad, and Russian "herring under fur" salad. it is regularly consumed as part of the normal diet, either fresh or after thermal processing or fermentation, and commonly used in manufacturing as a food colouring agent known as E162 (Neelwarne and Halagur, 2013; Jasmitha *et al*., 2018; Sawicki and Wiczkowski, 2018).

Recent studies have provided compelling evidence that beetroot ingestion offers beneficial physiological effects that may translate to improved clinical outcomes for several pathologies, such as; hypertension, atherosclerosis, type 2 diabetes and dementia (Gilchrist *et al.,* 2014; Presley *et al.,* 2011; Wotton-Beard *et al*., 2014;Vanhatalo *et al.,*

2010). Hypertension in particular has been the target of many therapeutic interventions and there are numerous studies that show beetroot, delivered acutely as a juice supplement (Jaija *et al*., 2014) or in bread significantly reduce systolic and diastolic blood pressure (Hobbs *et al*., 2013; Kapil *et al*., 2014).

Plate 2.1: Beet root bulb and cross section (Beta vulgaris) (Kabir, Fariha. 2020)

Table 2.1: Bioactive Components of BeetRoot

Bioactive Component	**Description**	**References**
Betalains	Water-soluble pigments divided into betacyanins (red-violet) and betaxanthins (yellow). High antioxidant and anti-inflammatory properties.	Kale *et al*., 2018.; Neelwarne *et al*., 2013
Polyphenols	Phenolic compounds with antioxidant activity, contributing to disease prevention.	Kale *et al*., 2018.
Inorganic Nitrates	Converted to nitric oxide (NO) in the body, supporting cardiovascular health and reducing blood pressure.	Clifford *et al*., 2015; Ugrinovic*et al*., 2012
Vitamins	Includes vitamins C, A, E, K, and B-complex (B_1, B_2, B_3, B_6, B_{12}, folate). Support immune function and metabolism.	Kale *et al*., 2018.; Hamedi and Honarvar, 2018
Minerals	Rich in manganese, potassium, magnesium, iron, zinc, and calcium. Essential for bone health	

	and enzyme functions.	
Carotenoids	Includes β-carotene and lutein, though present in smaller quantities compared to other vegetables. Antioxidant properties.	Jeyanthi *et al*., 2014
Flavonoids	Compounds like kaempferol and rhamnetin with antioxidant and anti-inflammatory effects.	Neelwarne *et al*., 2013
Fibers	Dietary fibers aiding digestion and gut health.	
Amino Acids	Includes threonine, valine, leucine, and others, supporting protein synthesis and metabolic functions.	Neelwarne *et al*., 2013
Saponins	Phytochemicals with potential immune-boosting and cholesterol-lowering effects.	

2.6 Ethnomedicinal uses

Beets have been used in traditional medicine for hundreds of years to treat constipation, gut and joint pain, dandruff (Hamedi and Honarvar, 2018).

2.7 Pharmaceutical Properties of Beetroot

2.7.1 Antioxidant Activity

Beetroot is rich in betalains (betacyanins and betaxanthins), phenolic acids, flavonoids, and vitamin C, all of which exhibit potent antioxidant properties. These compounds protect against oxidative stress by scavenging reactive oxygen species and reducing lipid peroxidation (Babarykin *et al*., 2019).

2.7.2 Anti-inflammatory Effects

Betanin and other betalains have been shown to inhibit pro-inflammatory pathways, including NF-κB and COX-2 expression, reducing cytokines like TNF-α and IL-6. These effects are comparable to some NSAIDs in vitro.

2.7.3 Cardiovascular Protection

High dietary nitrate in beetroot increases nitric oxide (NO) bioavailability, which improves endothelial function and reduces blood pressure. This effect is well-documented in both acute and chronic supplementation trials.

2.7.4 Anti-cancer and Chemopreventive Effects

Betalains and phenolics in beetroot inhibit cancer cell proliferation in vitro (e.g., cervical, liver, and colon cancer). Beetroot extract has shown synergistic effects when used with chemotherapeutic agents like doxorubicin.

2.7.5 Neuroprotective and Cognitive Benefits

Through enhanced NO production and antioxidant action, beetroot has been linked to improved cerebral blood flow and cognitive function, with implications for dementia and neurodegenerative disorders. (Neelwarne *et al*., 2013)

2.7.6 Hepatoprotective Properties

Beetroot extract protects against chemically induced liver damage in animal models by maintaining antioxidant enzyme levels and reducing lipid peroxidation markers.

2.7.7 Antimicrobial and Antifungal Activities

Beetroot has shown antimicrobial, antifungal, and antiviral effects attributed to its diverse phytochemical composition.

2.7.8 Radioprotective and Anti-mutagenic Activity

Betalains exhibit protection against radiation-induced oxidative damage and mutagenesis, showing promise in radioprotective therapies.

CHAPTER THREE

MATERIALS AND METHODOLOGY

3.1 Materials

Materials used for this study include

- Animal feed
- Sterilized water
- Beetroot (roots)
- Dissecting set
- Cotton swab
- Rat cages
- Syringes
- Sample bottles
- Alcohol
- Wire gauze
- Charcoal
- Binding wire
- Plastic containers and pipes

3.2 Methodology

3.2.1 Beetroot Extract Preparation and Administration

Beetroot (*Beta vulgaris*) roots will be cleaned, sliced, and oven-dried. The dried material will be powdered and extracted in 70% methanol. The extract will be filtered, concentrated using a rotary evaporator, and stored at 4 °C.

A dose of 400 mg/kg will be administered to the treatment groups via oral gavage daily for 28 days (Nugraha *et al.,* 2021).

3.2.2 Experimental Animals

Forty-Nine adult female Wistar rats weighing 180–220 g were procured from the animal house of the Department of Anatomy and housed in the same location. They will acclimatized for 14 days under controlled laboratory conditions (25 ± 2 °C, 12-hour light/dark cycle, ad libitum access to food and water).

3.2.3 Experimental Design

The animals were randomly divided into seven groups (n = 7 per group):

Group 1: Control (no treatment)

Group 2: Heat exposure only

Group 3: Biomass smoke exposure only

Group 4: Heat + Biomass smoke exposure

Group 5: Heat + Smoke + Beetroot Extract (400 mg/kg) (Sony *et al.,* 2021)

Group 6: Heat exposure only + Beetroot Extract (400 mg/kg)

Group 7: Biomass smoke exposure only + Beetroot Extract (400 mg/kg).

3.2.4 Exposure Protocols

Heat Exposure: Rats were placed in a controlled environmental chamber at 40 ± 1 °C for 1 hour daily (9:00 am – 10:00 am).

Biomass Smoke Exposure: Biomass smoke was generated using dried wood. Rats were placed in a sealed exposure chamber for 1 hour daily immediately following heat exposure (10:00 am – 11:00 am) (Kanter *et al*., 2004).

Smoke chamber: To perform the biomass smoke exposure, an improvised rectangular exposure chamber (90 cm length, 45 cm wide and 45 cm high) were used. Experimental rats were placed to move freely in this exposure chamber and a lit wood was placed in the inlet, which was left open for air to carry the smoke into the inhalation chamber. This chamber had an outlet made up of a pipe fitted at the top to act as an outlet channel to direct smoke out of the chamber during the exposure period (Ujaddughe *et al.*, 2024).

Heat Chamber: Heat stress (HS) was be simulated using a perforated heated wooden chamber (30 cm x 50 cm x 25 cm). The chamber, was fitted with a digital thermometer, heated using a non-light heat emitter ceramic bulb and regulated using a heat switch. The chamber will be maintained at 40 ± 1 C (Umeh and Bruno, 2023).

3.2.5 Sample Collection

At the end of the 28-day exposure period, animals were anesthetized. 5 ml of blood samples was collected via cardiac puncture for into EDTA bottles (Full blood count). Lung tissues were excised washed in sample bottles containing formaldehyde and taken to the histopathological laboratory of the Department of Anatomy, University of Benin for histological preparation and analyses.

3.3 Ethical Considerations

All procedures complied with animal care guidelines. Ethical clearance was obtained from the Ethics Committee of the College of Medical Sciences, University of Benin, prior to the commencement of the experiment.

3.4 Statistical Analysis

The mean and standard error of mean was determined using Graphpad prism version 8.2.2. The one-way ANOVA followed by Tukey's post hoc analysis was used to determine the difference in means among the groups. The difference in means was considered significant at $p<0.05$.

CHAPTER FOUR

RESULTS

4.1 HEMATOLOGICAL FINDINGS

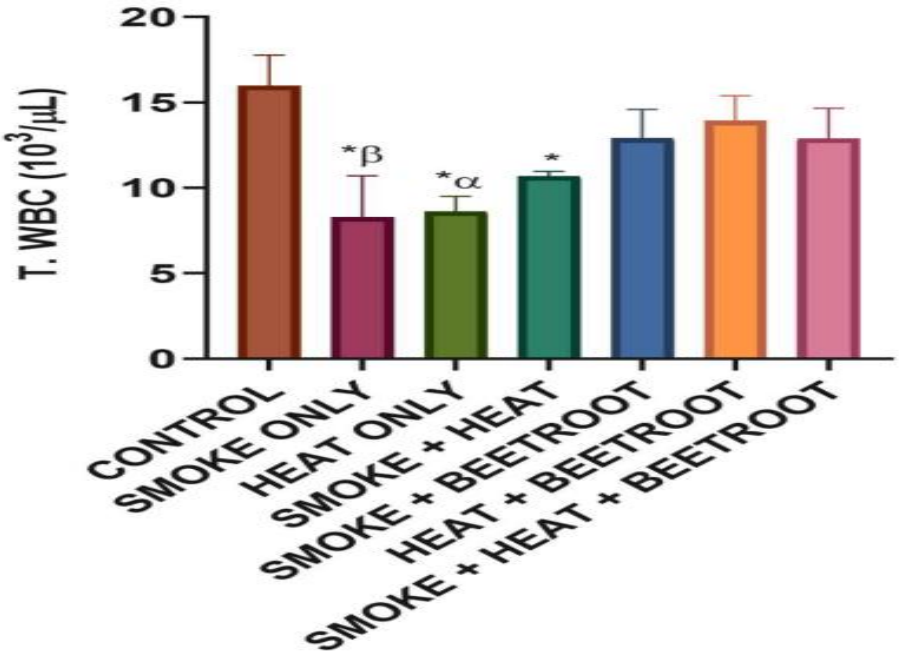

Figure 4.1: Chart showing the effect of smoke, heat and beetroot on Total WBC of wistar rats after 28 days.

There was a significant decrease in T. WBC in the smoke only, heat only and smoke + heat groups of wistar rats compared to control ($p<0.05$, respectively). But no significant difference in the other treatment groups compared to control ($p<0.05$, respectively). There was a significant decrease in the smoke only group compared to the smoke + beetroot. There was also a significant decrease in the heat only group compared to the heat + beetroot group ($p<0.05$).

*$P<0.05$ Indicates statistical difference when treatment group(s) are compared to control

β$p<0.05$ Indicates statistical difference when smoke only group is compared to smoke + beetroot group

αp<0.05 Indicates statistical difference when heat only group is compared to heat + beetroot group.

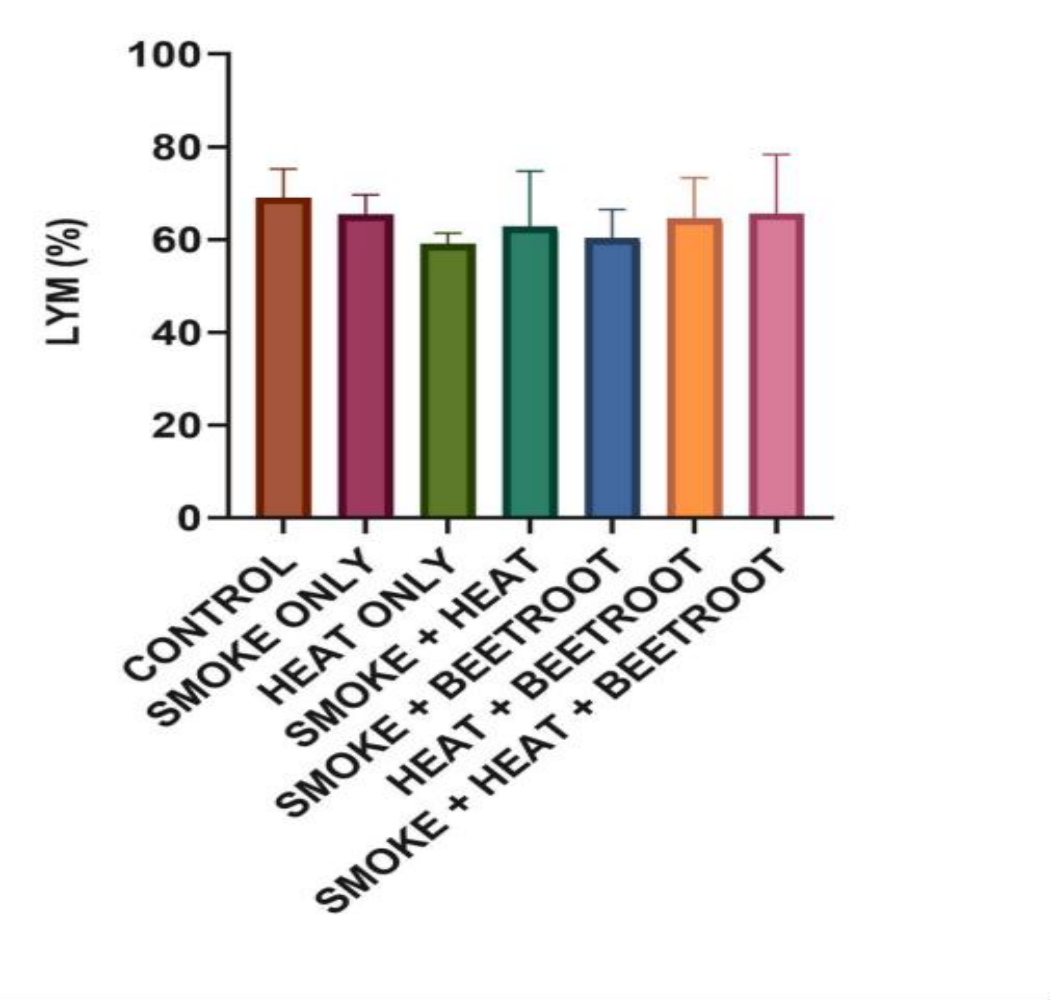

Figure 4.2: Chart showing the effect of smoke, heat and beetroot on LYM count of wistar rats after 28 days.

The result shows no statistically significant difference in LYM count in Wistar rats across all treatment groups compared with control (p>0.05, respectively).

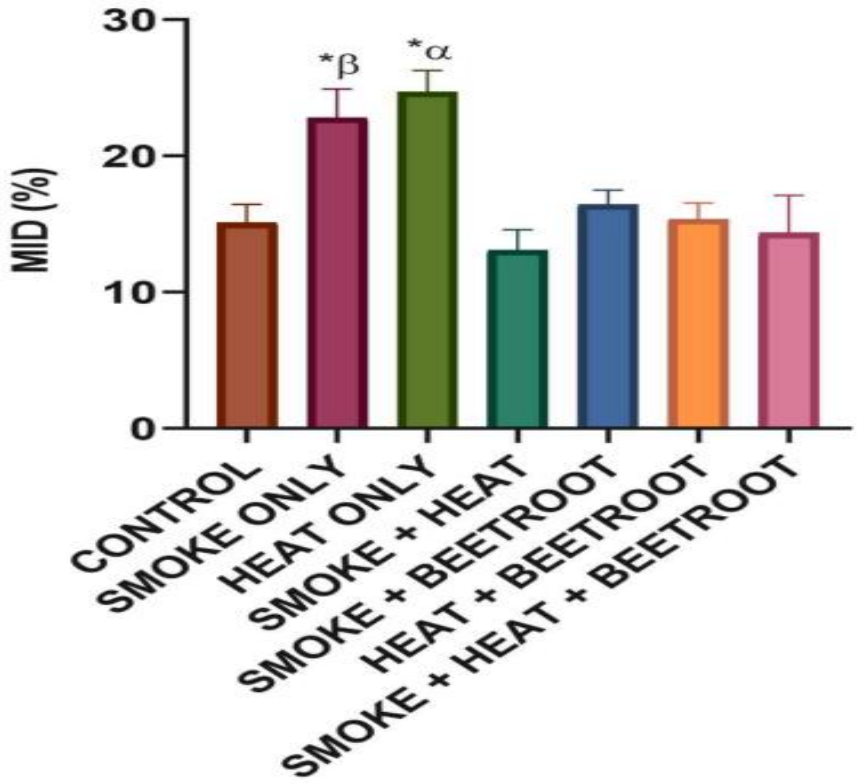

Figure 4.3: Chart showing the effect of smoke, heat and beetroot on MID count of wistar rats after 28 days.

There was a significant increase in MID in the smoke only and heat only groups of wistar rats compared to control ($p<0.05$, respectively), but no significant difference in the other treatment groups compared to control ($p<0.05$, respectively). There was a significant increase in the smoke only group compared to the smoke + beetroot. There was also a significant decrease in the heat only group compared to the heat + beetroot group ($p<0.05$).

*$P<0.05$ Indicates statistical difference when treatment group(s) are compared to control

β$p<0.05$ Indicates statistical difference when smoke only group is compared to smoke + beetroot group.

α$p<0.05$ Indicates statistical difference when heat only group is compared to heat + beetroot group.

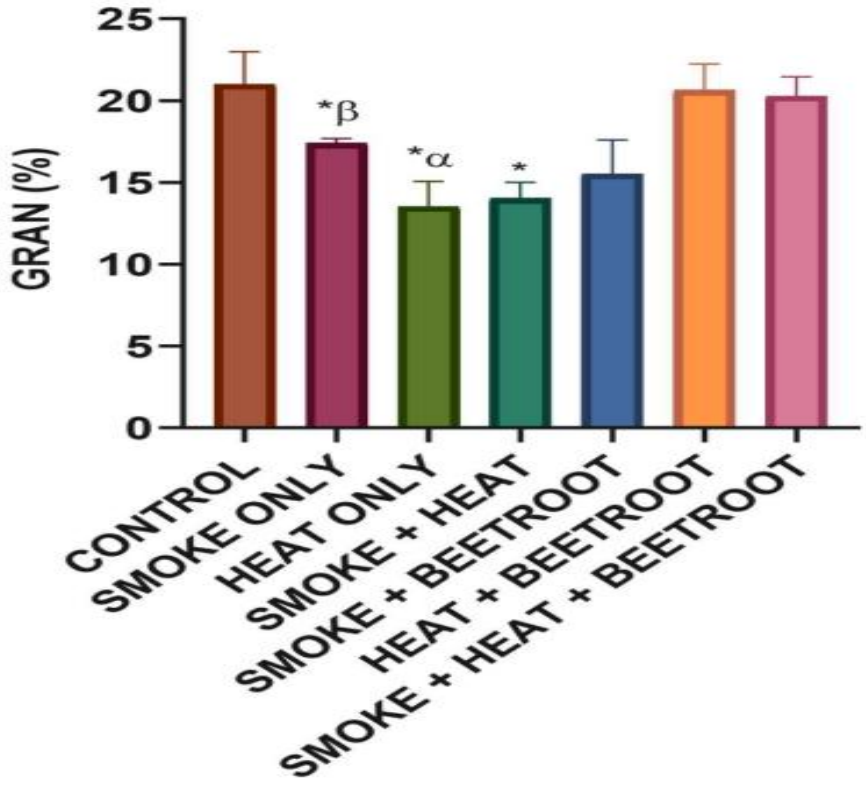

Figure 4.4: Chart showing the effect of smoke, heat and beetroot on GRAN count of wistar rats after 28 days.

There was a significant decrease in GRAN in the smoke only, heat only and smoke + heat groups of wistar rats compared to control ($p<0.05$, respectively), but no significant difference in the other treatment groups compared to control ($p<0.05$, respectively). There was a significant decrease in the smoke only group compared to the smoke + beetroot. There was also a significant decrease in the heat only group compared to the heat + beetroot group ($p<0.05$).

*$P<0.05$ Indicates statistical difference when treatment group(s) are compared to control

β$p<0.05$ Indicates statistical difference when smoke only group is compared to smoke + beetroot group

α$p<0.05$ Indicates statistical difference when heat only group is compared to heat + beetroot group

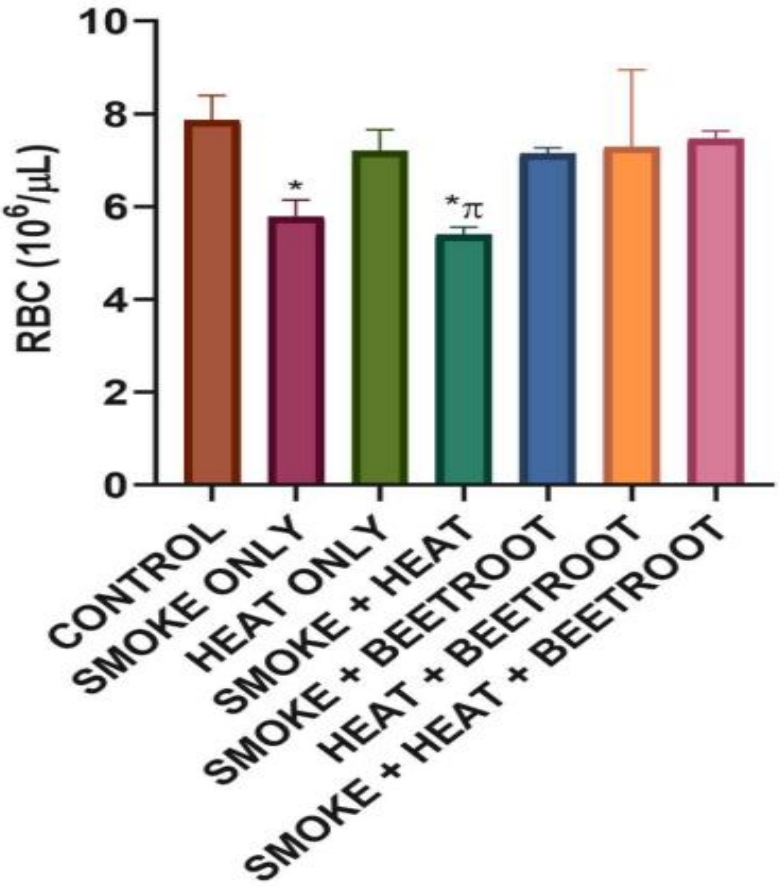

Figure 4.5: Chart showing the effect of smoke, heat and beetroot on RBC count of wistar rats after 28 days.

There was a statistically significant decrease in RBC of the smoke only and smoke + heat groups when compared to control ($p<0.05$, respectively), but no statistically significant difference in the other treatment groups ($p<0.05$, respectively). There was also a statistically significant decrease in the smoke + heat group compared to the smoke + heat + beetroot group ($p<0.05$).

*$P<0.05$ Indicates statistical difference when treatment group(s) are compared to control

π$p<0.05$ Indicates statistical difference when smoke + heat group is compared to smoke + heat + beetroot group.

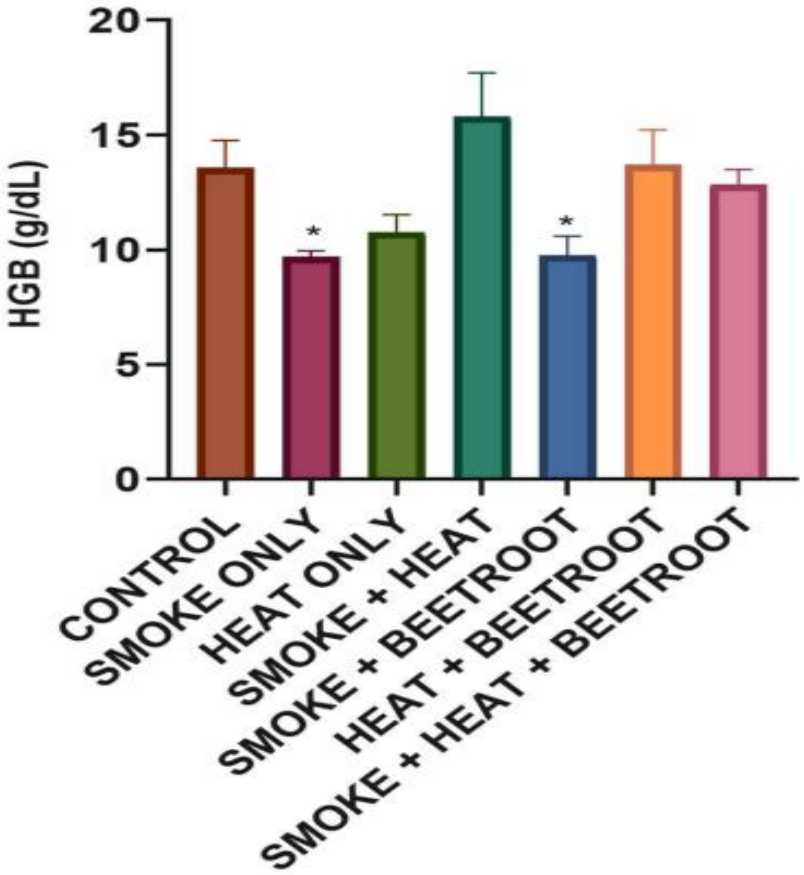

Figure 4.6: Chart showing the effect of smoke, heat and beetroot on HGB of wistar rats after 28 days.

There was a statistically significant decrease in HGB in the smoke only, heat only and smoke + beetroot groups compared to control ($p<0.05$), but no statistically significant difference in the other treatment groups ($p<0.05$, respectively).

*$P<0.05$ Indicates statistical difference when treatment group(s) are compared to control

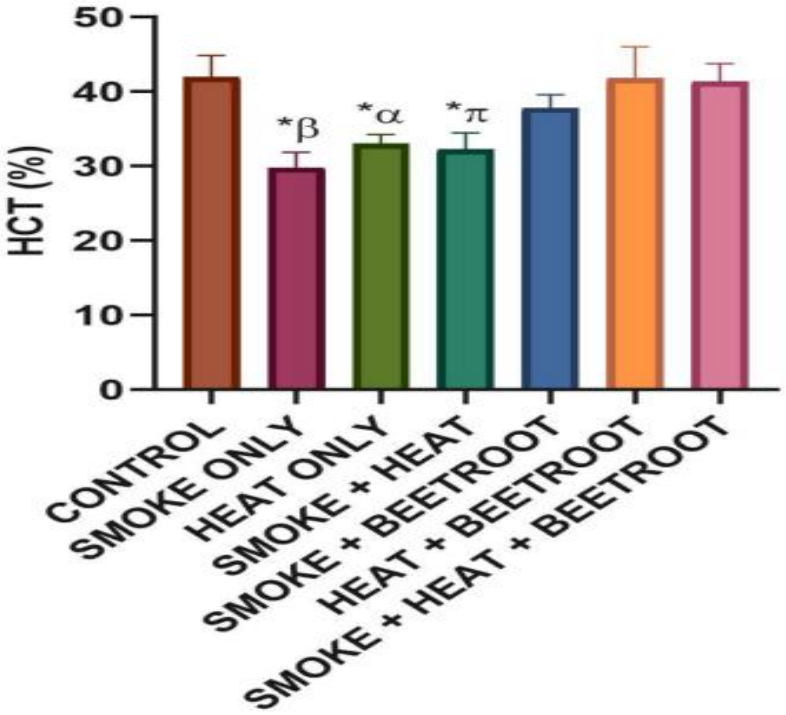

Figure 4.7: Chart showing the effect of smoke, heat and beetroot on HCT of wistar rats after 28 days.

There was a statistically significant decrease in HCT in the smoke only, heat only and smoke + heat groups of wistar rats compared to control ($p<0.05$, respectively). But no statistically significant difference in the other treatment groups compared to control ($p<0.05$, respectively). There was a statistically significant decrease in the smoke only group compared to the smoke + beetroot ($p<0.05$). There was also a statistically significant decrease in the heat only group compared to the heat + beetroot group ($p<0.05$) and a statistically significant decrease in the smoke + heat group compared to the smoke + heat + beetroot group ($p<0.05$).

*$P<0.05$ Indicates statistical difference when treatment group(s) are compared to control

β$p<0.05$ Indicates statistical difference when smoke only group is compared to smoke + beetroot group

α$p<0.05$ Indicates statistical difference when heat only group is compared to heat + beetroot group

π$p<0.05$ Indicates statistical difference when smoke + heat group is compared to smoke + heat + beetroot group.

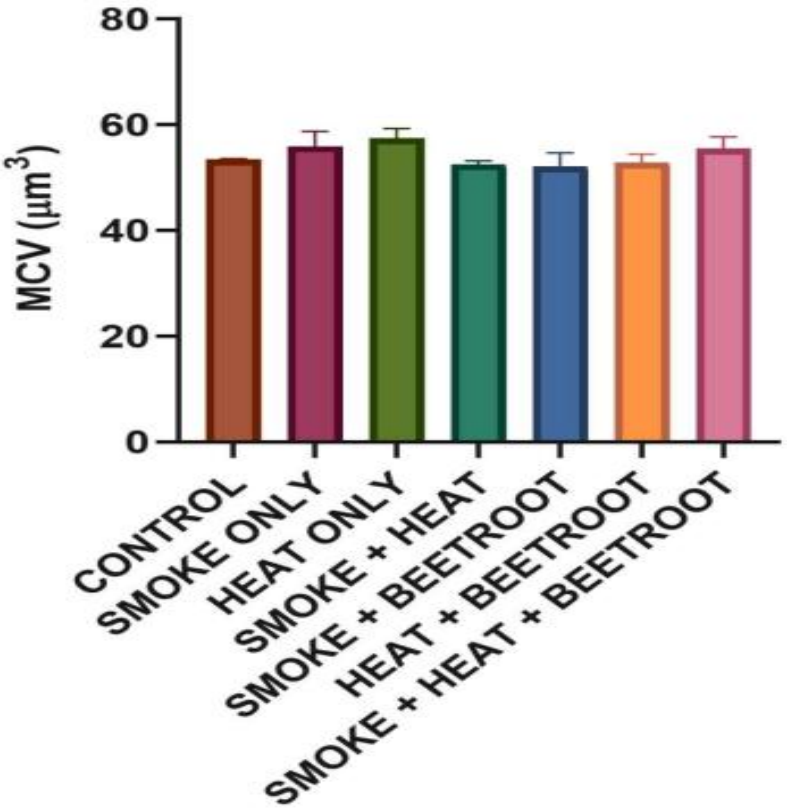

Figure 4.8: Chart showing the effect of smoke, heat and beetroot on MCV of wistar rats after 28 days.

The result shows no statistically significant difference in MCV in Wistar rats across all treatment groups compared with control ($p>0.05$, respectively).

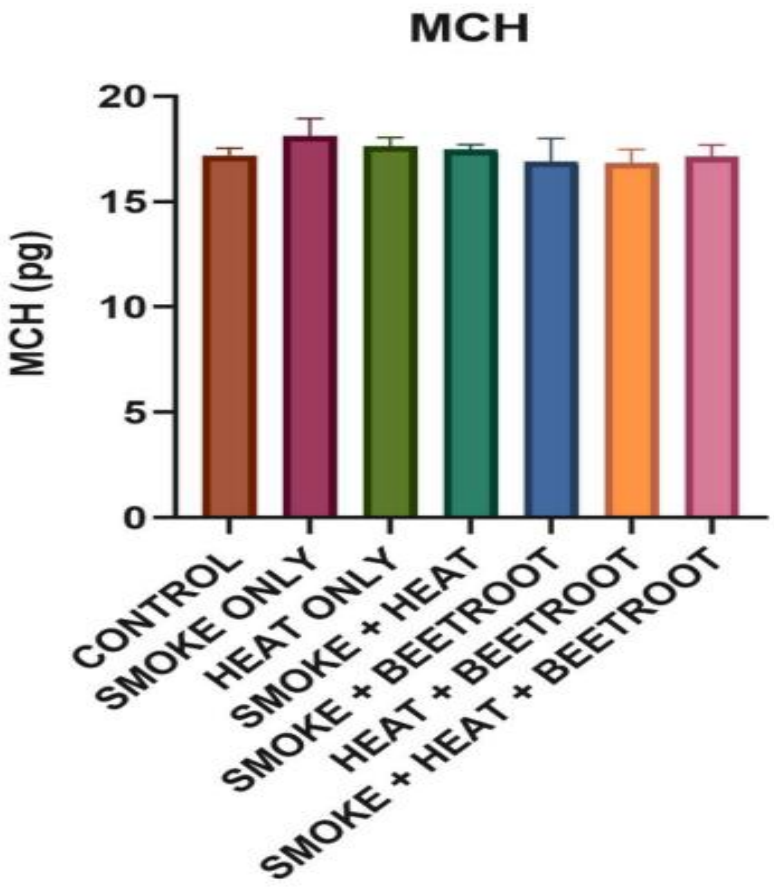

Figure 4.9: Chart showing the effect of smoke, heat and beetroot on MCH of wistar rats after 28 days.

The result shows no statistically significant difference in MCH in Wistar rats across all treatment groups compared with control ($p>0.05$, respectively).

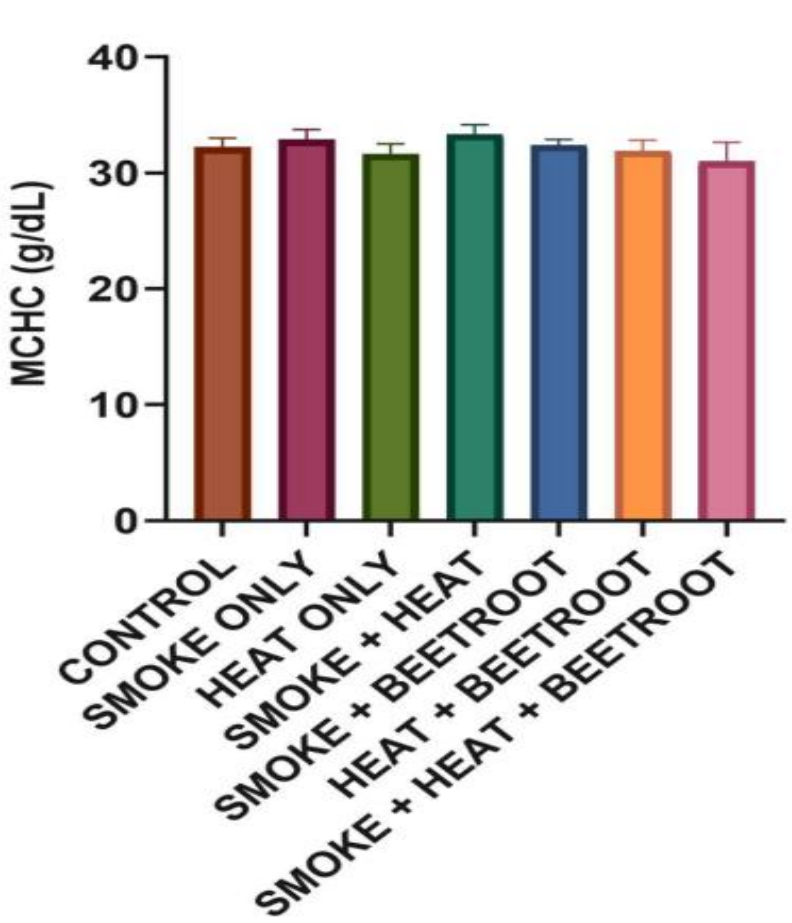

Figure 4.10: Chart showing the effect of smoke, heat and beetroot on MCHC of wistar rats after 28 days.

The result shows no statistically significant difference in MCHC in Wistar rats across all treatment groups compared with control ($p>0.05$, respectively).

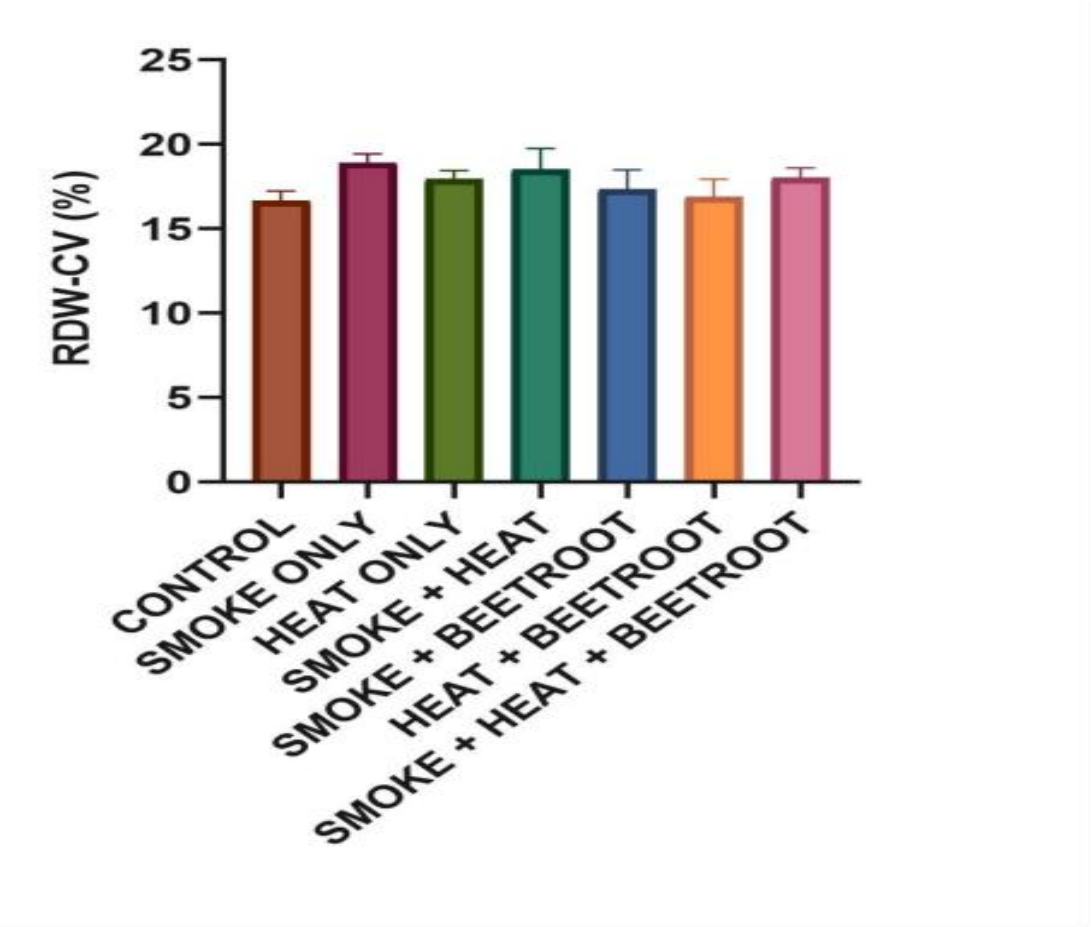

Figure 4.11: Chart showing the effect of smoke, heat and beetroot on RDW-CV of wistar rats after 28 days.

The result shows no statistically significant difference in RDW-CV in Wistar rats across all treatment groups compared with control ($p>0.05$, respectively).

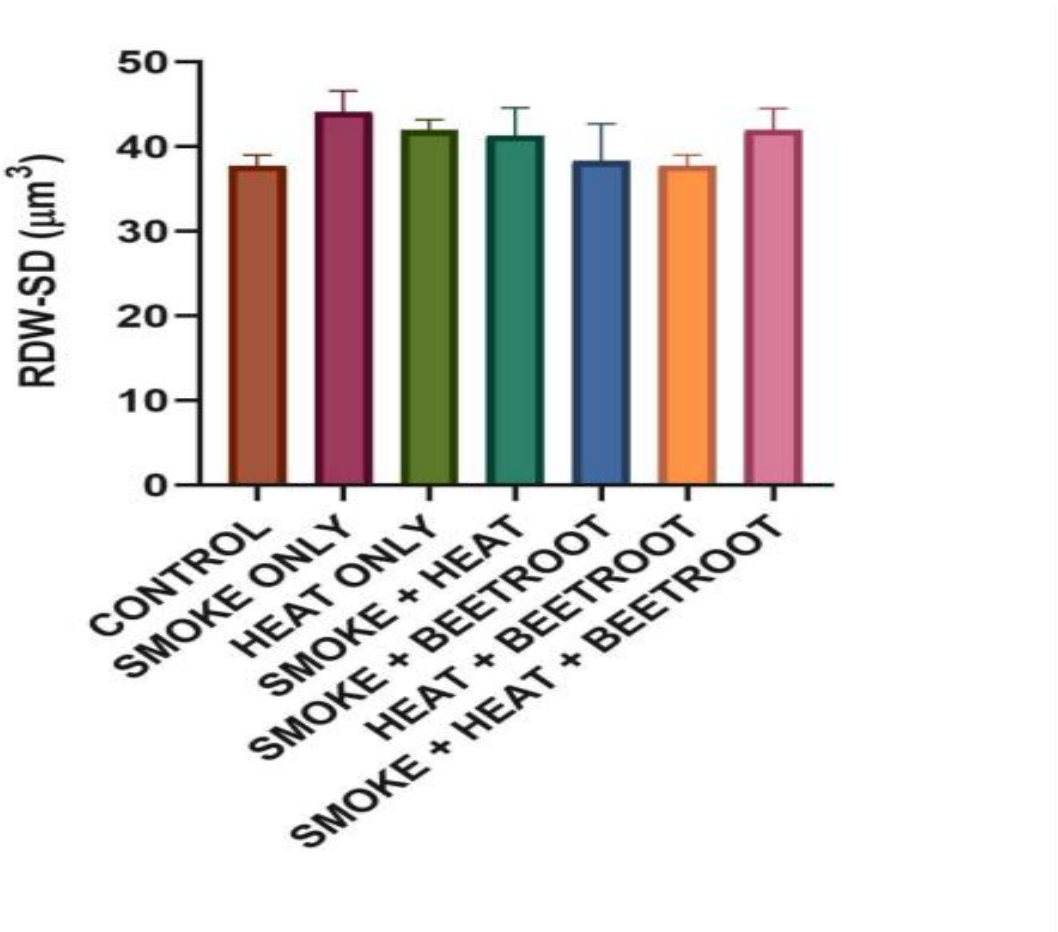

Figure 4.12: Chart showing the effect of smoke, heat and beetroot on RDW-SD of wistar rats after 28 days.

The result shows no statistically significant difference in RDW-SD in Wistar rats across all treatment groups compared with control ($p>0.05$, respectively).

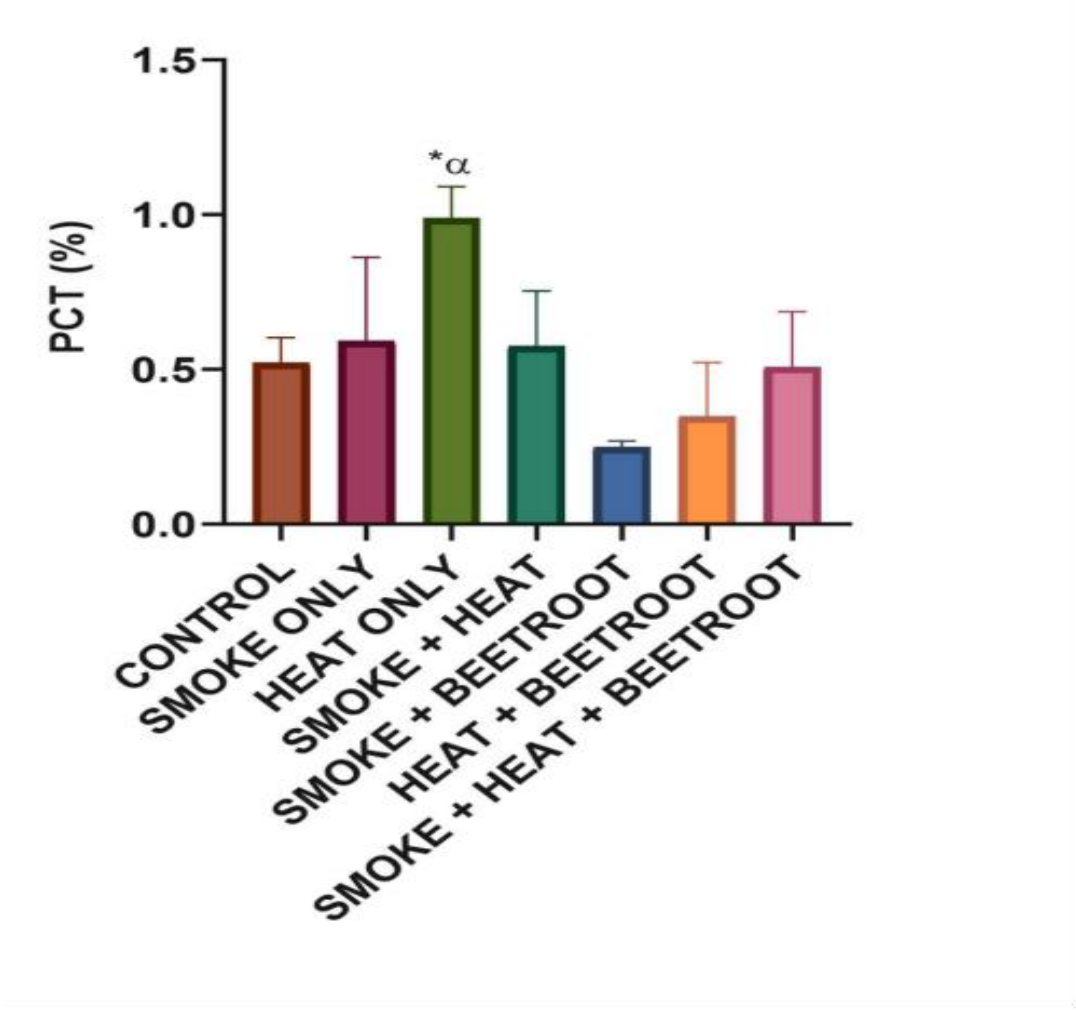

Figure 4.13: Chart showing the effect of smoke, heat and beetroot on PCT of wistar rats after 28 days.

There was a statistically significant increase in PCT in the heat only group of wistar rats compared to control ($p<0.05$), but no statistically significant difference in the other treatment groups compared to control ($p<0.05$, respectively). There was a statistically significant increase in the heat only group compared to the heat + beetroot ($p<0.05$).

*$P<0.05$ Indicates statistical difference when treatment group(s) are compared to control

α$p<0.05$ indicates statistical difference when heat only group is compared to heat + beetroot treated group.

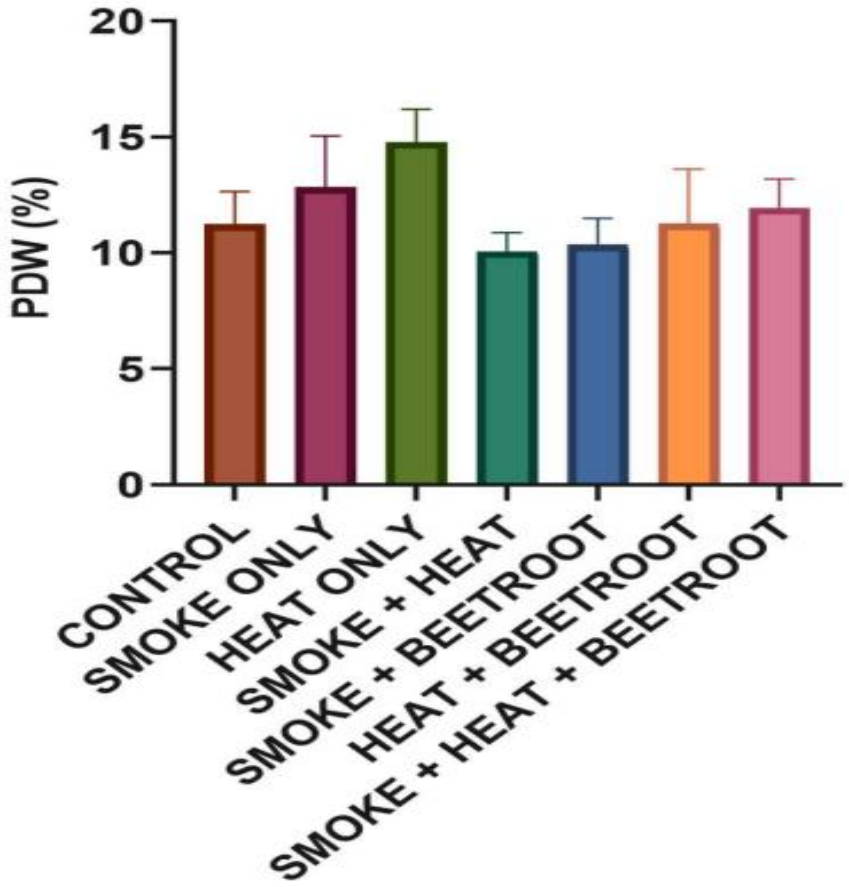

Figure 4.14: Chart showing the effect of smoke, heat and beetroot on PDW of wistar rats after 28 days.

The result shows no statistically significant difference in PDW in Wistar rats across all treatment groups compared with control ($p>0.05$, respectively).

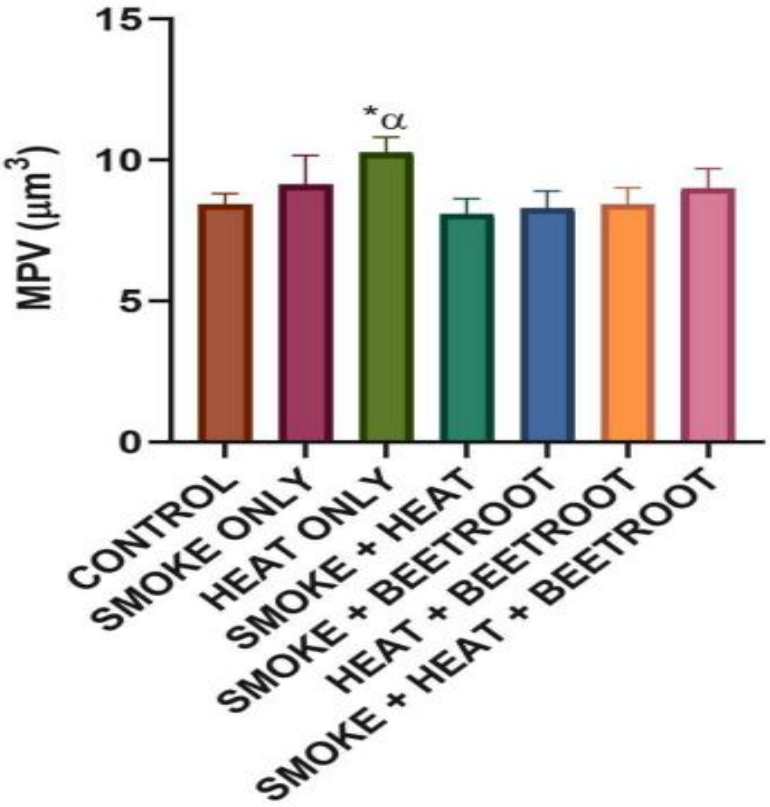

Figure 4.15: Chart showing the effect of smoke, heat and beetroot on MPV of wistar rats after 28 days.

There was a statistically significant increase in the heat only group when compared to control ($p<0.05$), but no statistically significant difference in the other treatment groups when compared to control ($p<0.05$).

*$P<0.05$ Indicates statistical difference when treatment group(s) are compared to control

α$p<0.05$ Indicates statistical difference when heat only group is compared to heat + beetroot treated group.

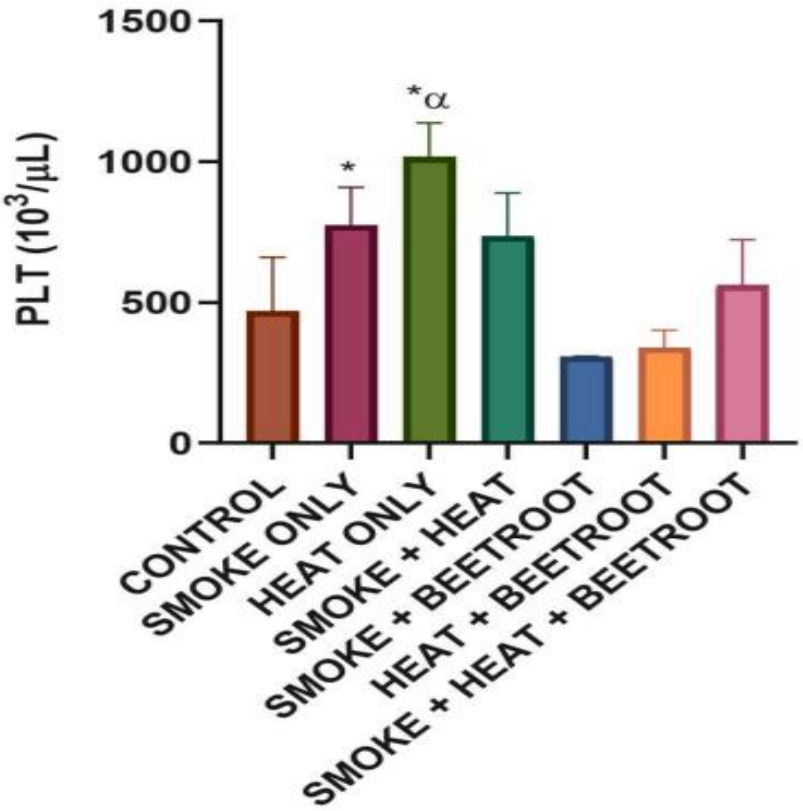

Figure 4.16: Chart showing the effect of smoke, heat and beetroot on PLT of wistar rats after 28 days.

There was a statistically significant increase in PLT in the smoke only and heat only group when compared to control ($p<0.05$), but no statistically significant difference in the other treatment groups when compared to control ($p<0.05$). There was also a statistically significant increase in the heat only group when compared to heat + beetroot treatment group ($p<0.05$).

*$P<0.05$ Indicates statistical difference when treatment group(s) are compared to control

α$p<0.05$ Indicates statistical difference when heat only group is compared to heat + beetroot treated group.

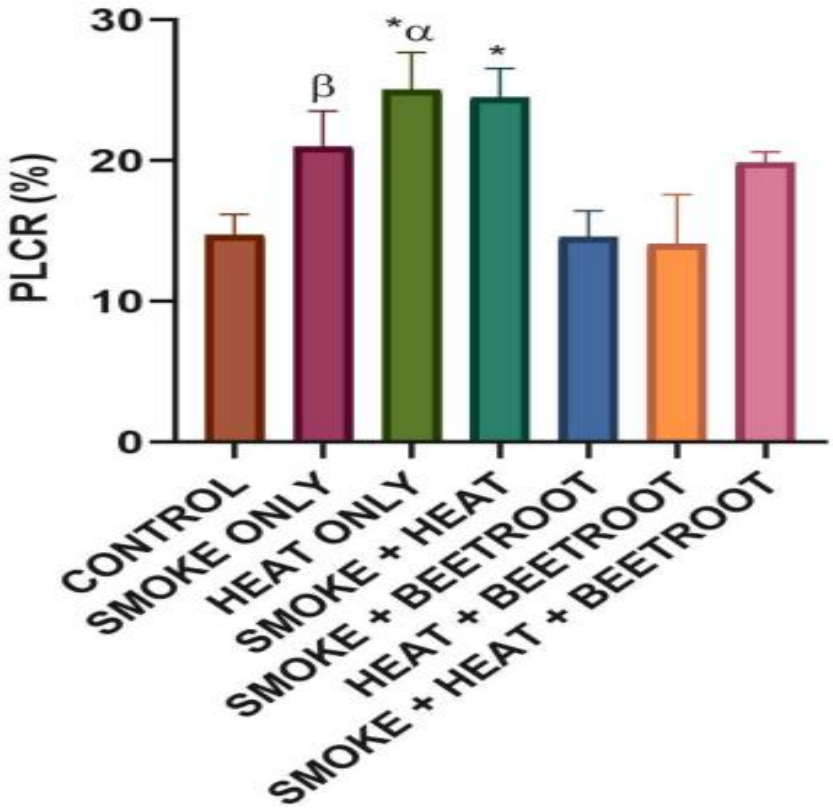

Figure 4.17: Chart showing the effect of smoke, heat and beetroot on PLCR of wistar rats after 28 days.

There was a statistically significant increase in PLCR in the heat only group and smoke + heat group when compared to control ($p<0.05$), but no statistically significant difference in the other treatment groups when compared to control ($p<0.05$). There was also a statistically significant increase in the heat only group when compared to heat + beetroot treatment group ($p<0.05$). There was a statistically significant increase in the smoke only group when compared to smoke + beetroot group ($p<0.05$), and the heat only compared to the heat + beetroot group ($p<0.05$).

*$P<0.05$ Indicates statistical difference when treatment group(s) are compared to control

α$p<0.05$ Indicates statistical difference when heat only group is compared to heat + beetroot treated group

β$p<0.05$ Indicates statistical difference when smoke only group is compared to smoke + beetroot group.

4.2 HISTOLOGY OF THE LUNGS (After 28 Days of Exposure)

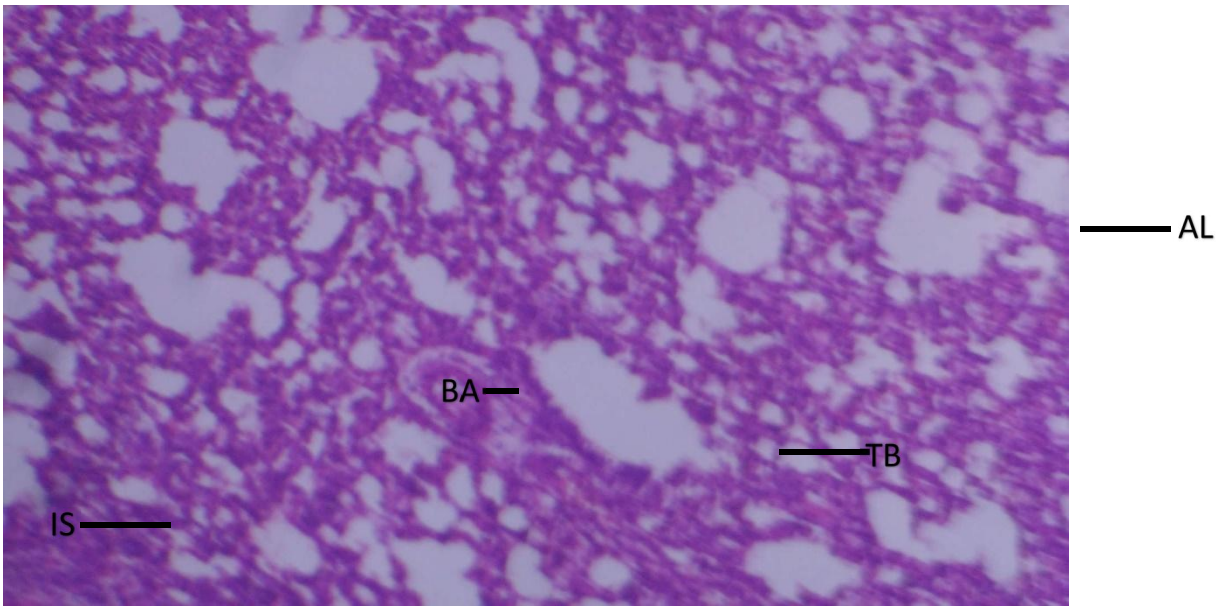

Plate 4.1: Rat lungs control showing: alveoli (AL), interstitial space (IS), bronchial artery (BA) and terminal bronchiole (TB): H&E 100X.

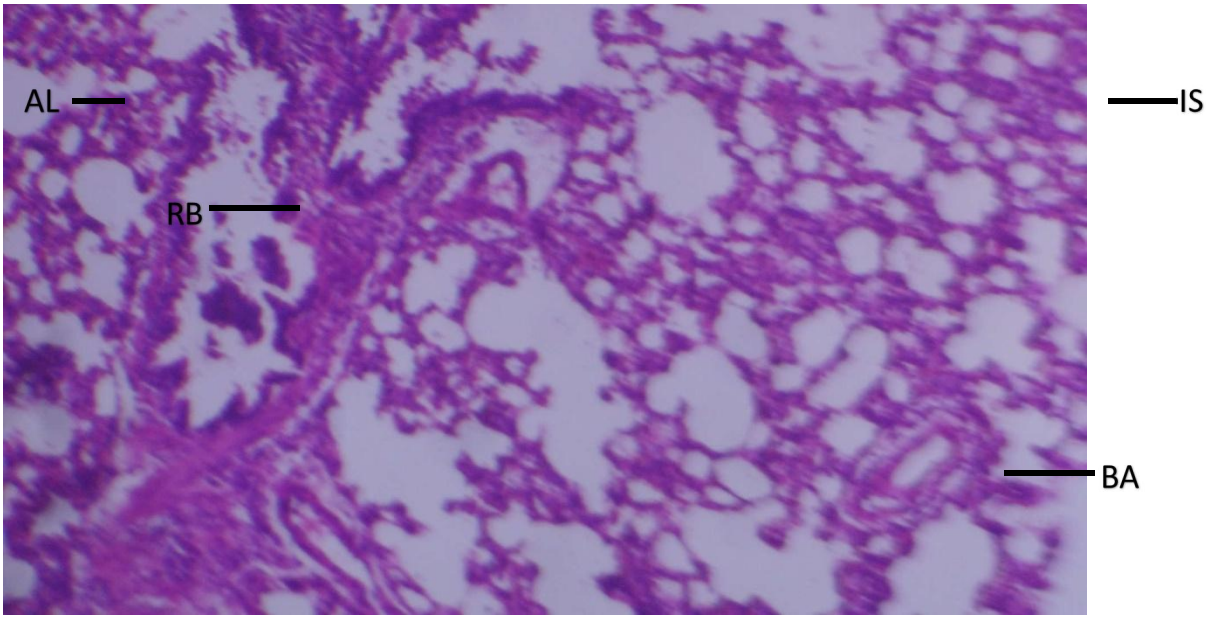

Plate 4.2: Rat lungs control showing: alveoli (AL), interstitial space (IS), bronchial artery (BA) and respiratory bronchiole (TB): H&E 100X.

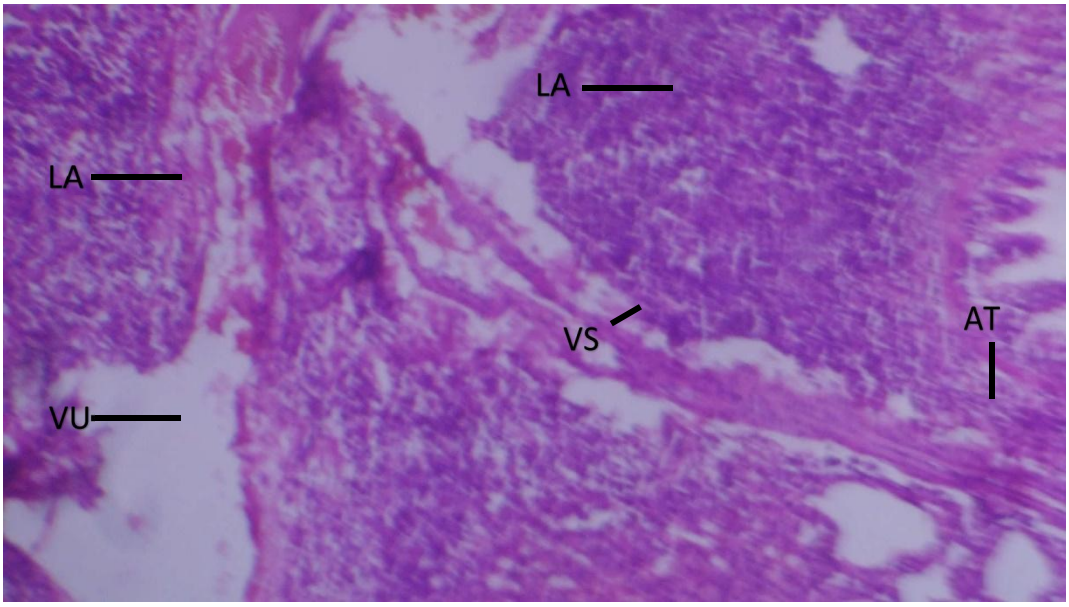

Plate 4.3: Rat lungs exposed to smoke only showing: severe bronchiolo-alveolar lymphoid tissue activation (LA), vascular ulceration (VU) and stenosis (VS), atelectasis (AT): H&E 100X.

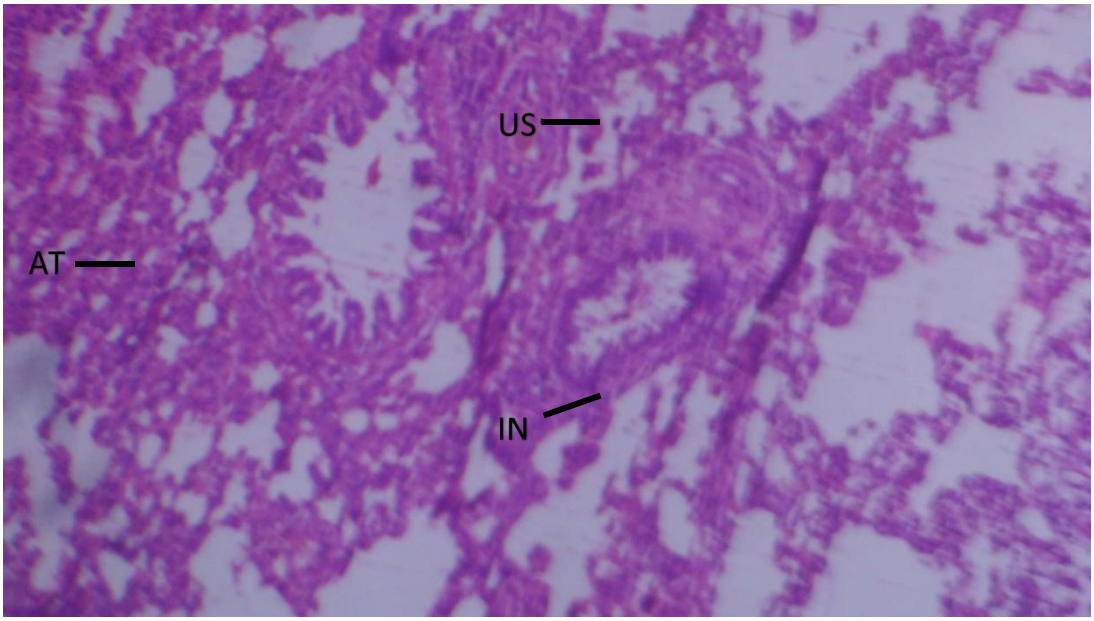

Plate 4.4: Rat lungs exposed to smoke only showing: interstitial infiltrates of inflammatory cells (IN), vascular ulceration and stenosis (US), atelectasis (AT): H&E 100X.

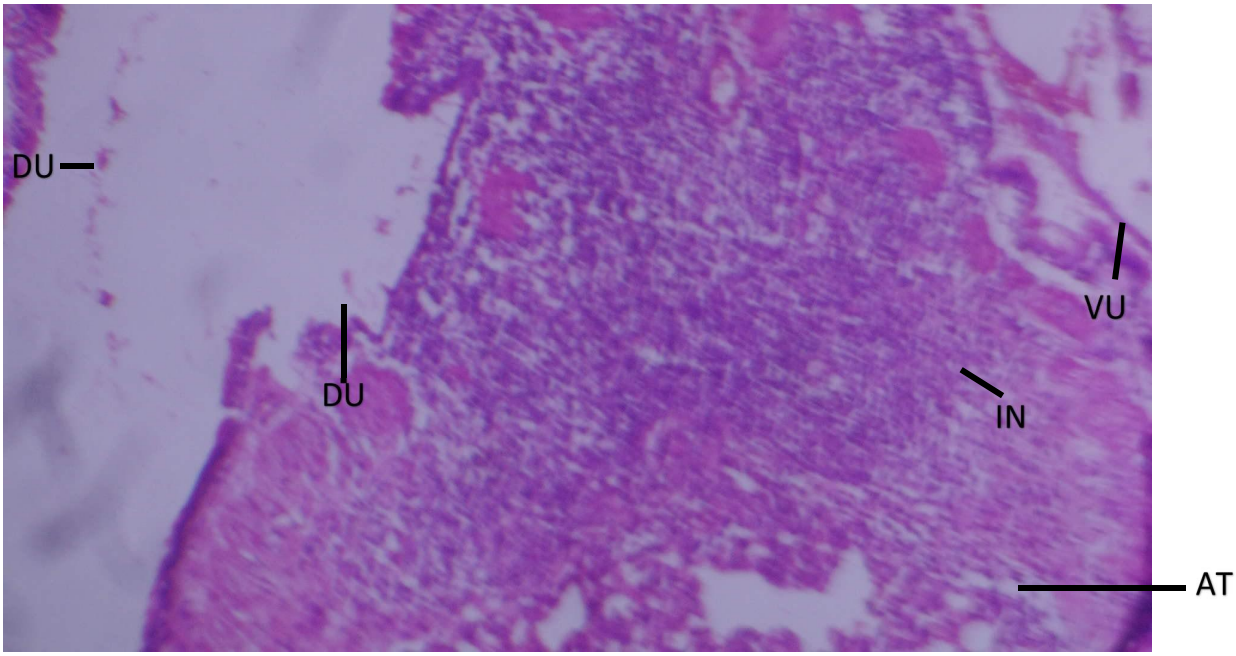

Plate 4.4: Rat lungs exposed to heat only showing: severe bronchiolar dilation and ulceration (DU), atelectasis (AT), vascular ulceration (VU) and severe inflammation (IN): H&E 100X.

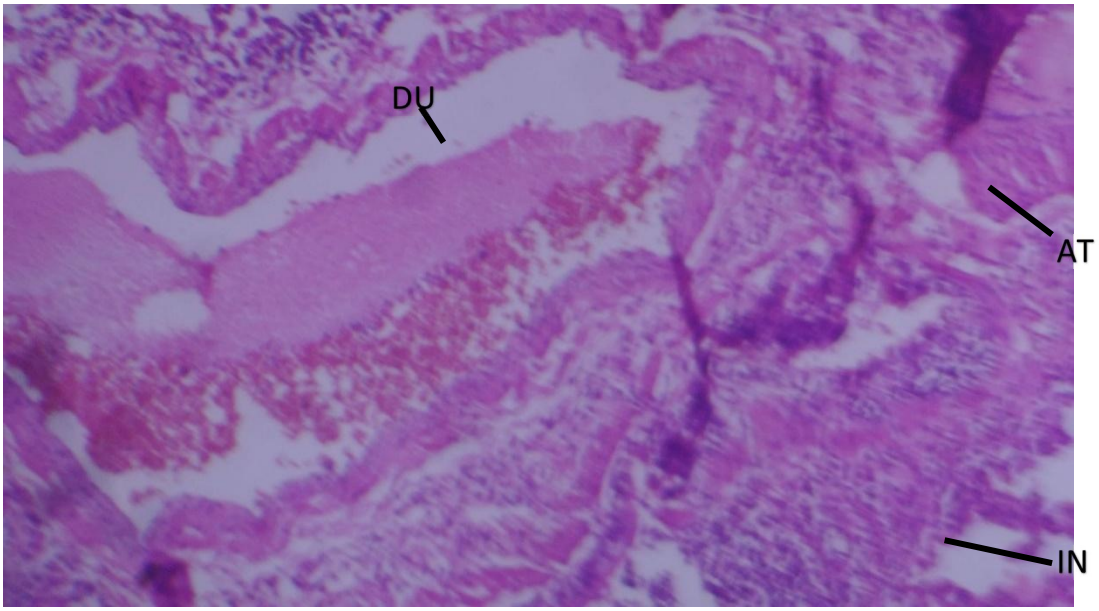

Plate 4.5: Rat lungs exposed to heat only showing: severe vascular dilatation and ulceration (DU), atelectasis (AT) and severe inflammation (IN): H&E 100X.

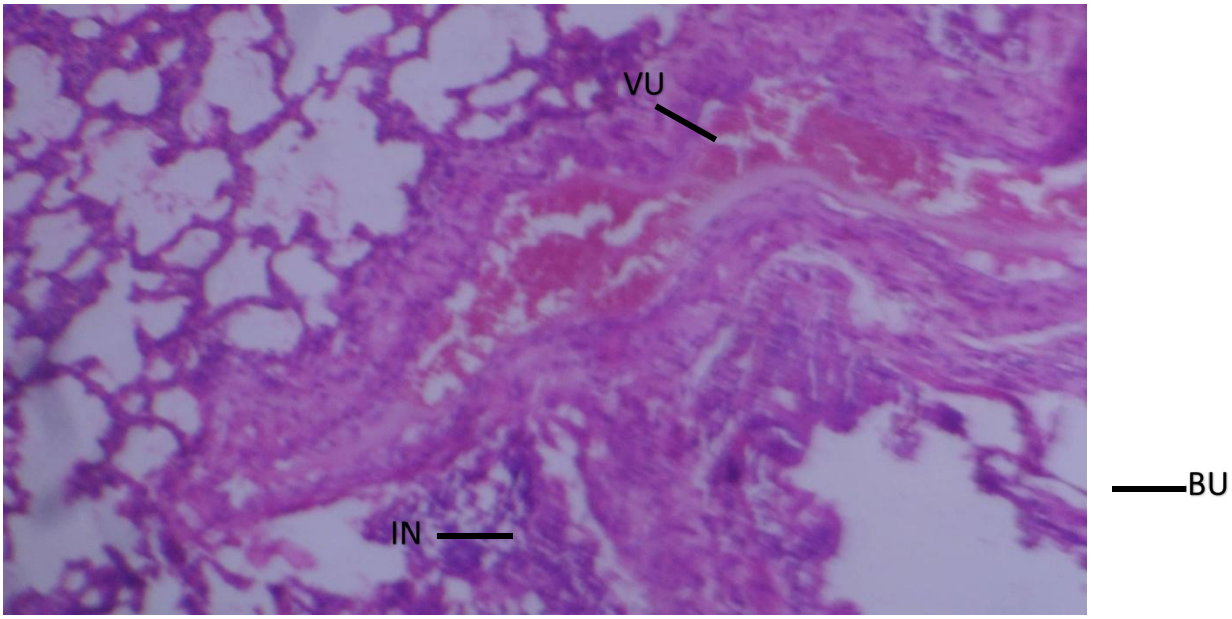

Plate 4.6: Rat lungs exposed to heat and smoke showing: severe vasodilatation and ulceration (DU), bronchiolar ulceration (BU) and interstitial inflammation (IN): H&E 100X

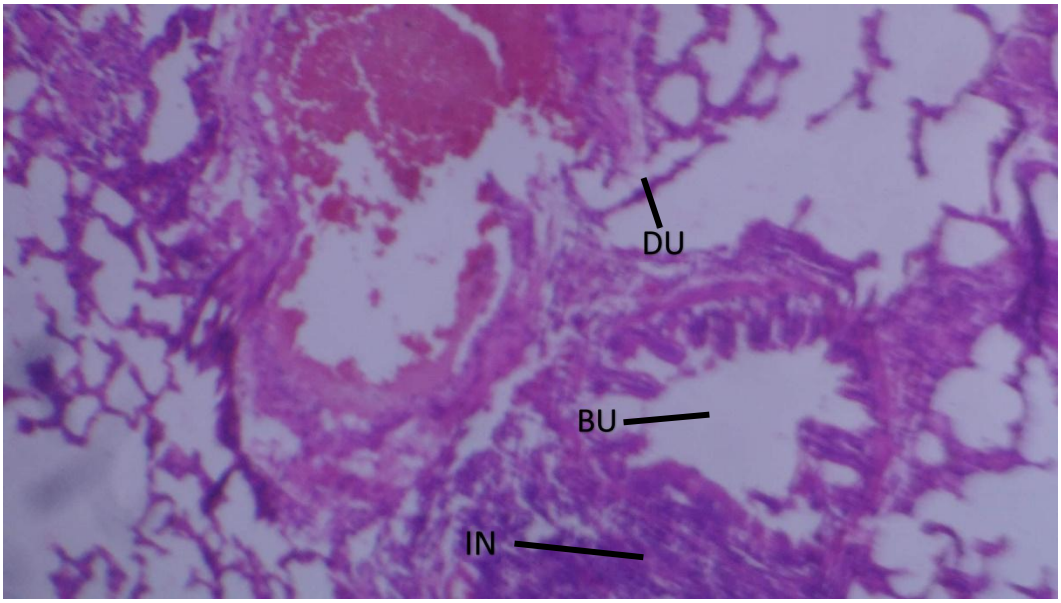

Plate 4.7: Rat lungs exposed to heat and smoke showing: severe vasodilatation and ulceration (DU), bronchiolar ulceration (BU) and interstitial inflammation (IN): H&E 100X.

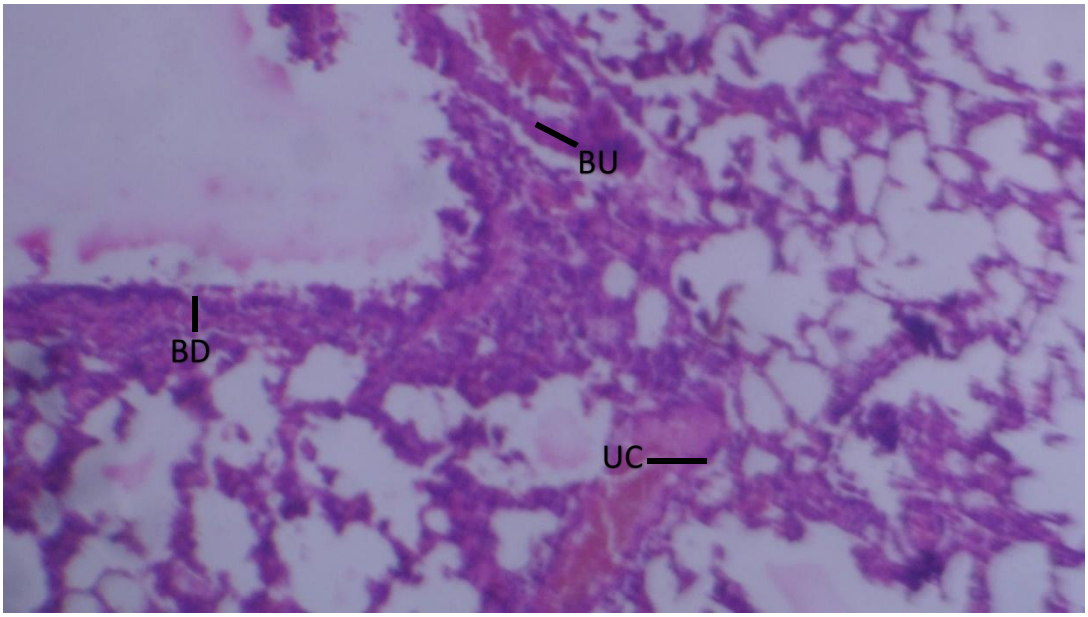

Plate 4.8: Rat lungs exposed to heat after treatment with beetroot extract showing: bronchiolar dilation (BD), brochiolar ulceration (BU), vascular ulceration and congestion (UC): H&E 100X.

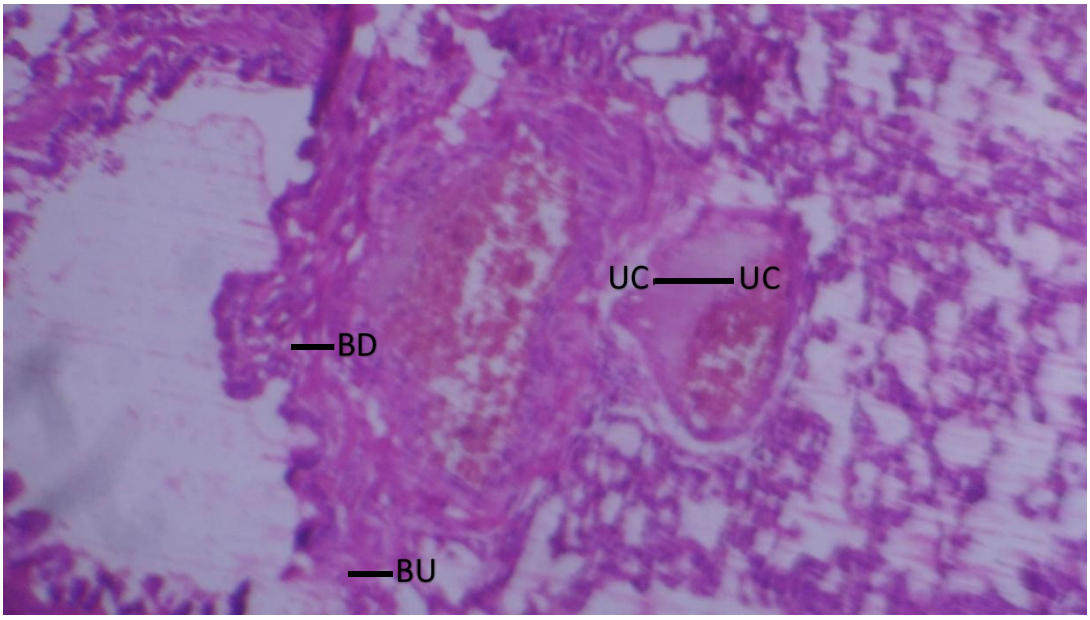

Plate 4.9. Rat lungs exposed to heat after treatment with beetroot extract showing: bronchiolar dilation (BD), brochiolar ulceration (BU), vascular ulceration and congestion (UC): H&E 100X.

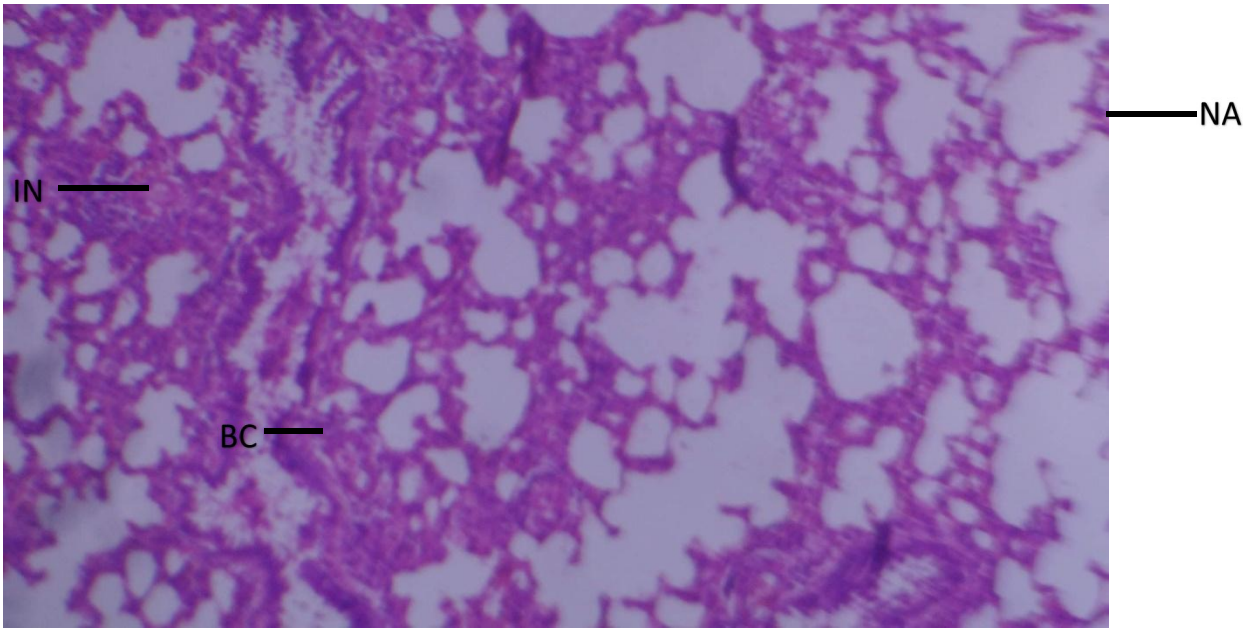

Plate 4.10: Rat lungs exposed to smoke after treatment with extract showing: normal alveoli (NA), bronchiolar constriction (BC) and interstitial inflammation (IN): H&E 100X.

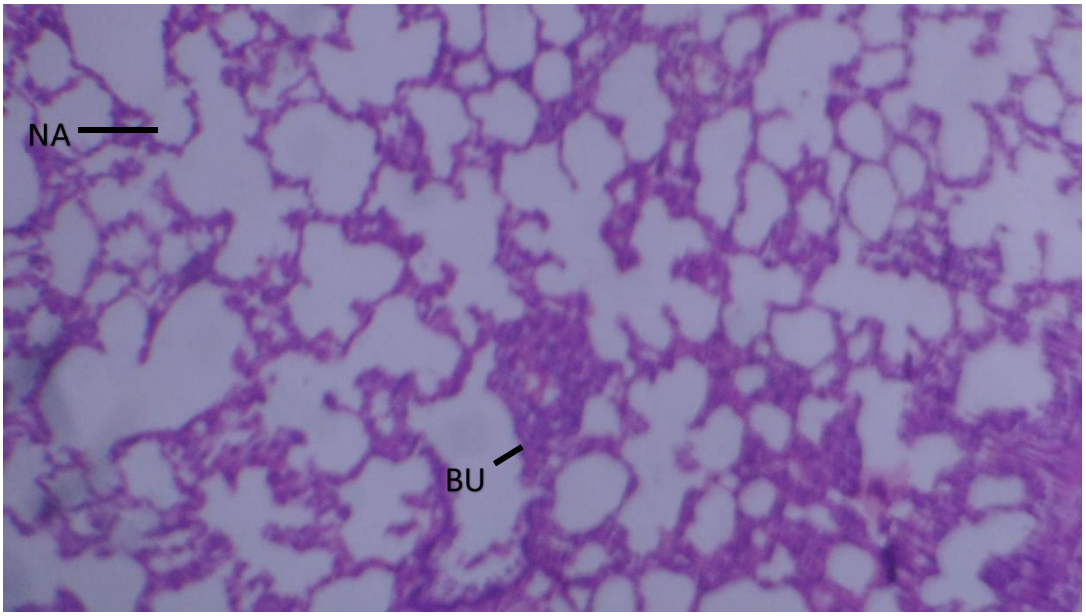

Plate 4.11: Rat lungs exposed to heat and smoke after treatment with extract showing: normal alveoli (NA) and focal bronchiolar ulceration (BU): H&E 100X.

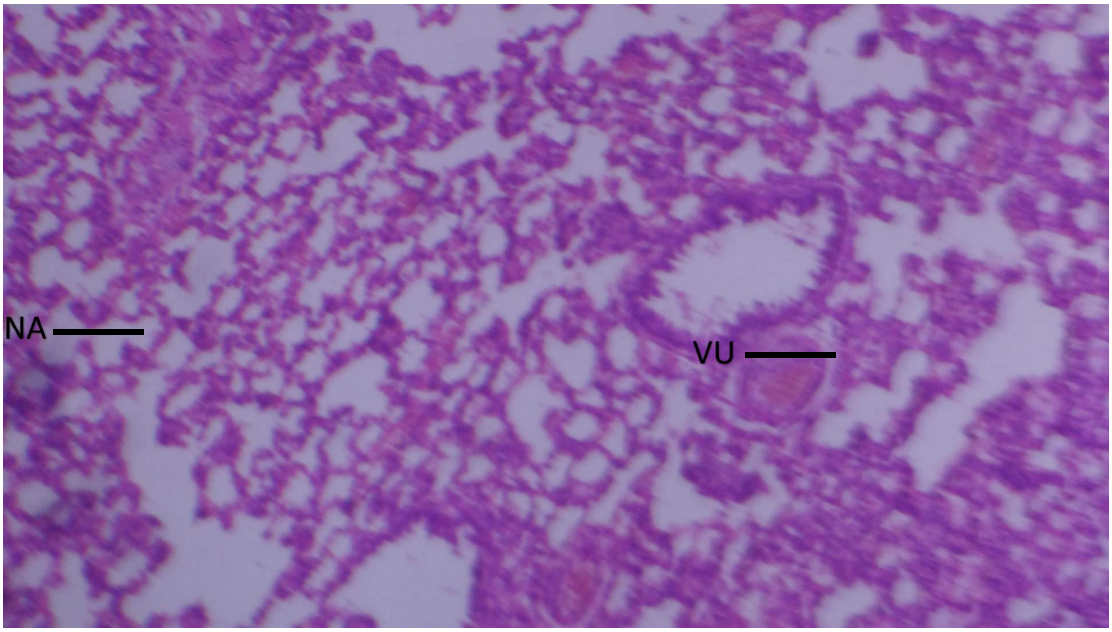

Plate 4.12: Rat lungs exposed to heat and smoke after treatment with extract showing: normal alveoli (NA) and vascular ulceration (VU): H&E 100X.

CHAPTER FIVE

DISCUSSION

The purpose of this study was to evaluate the protective effects of beetroot (*Beta vulgaris* L.) extract on hematological parameters in Wistar rats exposed to both heat and biomass smoke, two environmental stressors. Exposure to environmental heat and smoke is known to cause hematological abnormalities, inflammation, and systemic oxidative stress, all of which impair circulatory and pulmonary health (Po *et al*., 2011; Kurmi *et al.,* 2012; Mukherjee and Agrawal, 2017). The argument was based on the known oxidative and inflammatory pathology caused by these exposures, which beetroot's rich profile of bioactive compounds, such as betalains, polyphenols, and inorganic nitrates, provides a powerful natural defense against. While also highlighting the substantial restorative potential of beetroot, the results show a complex interaction between these stressors and the hematopoietic system.

The observed significant decrease in total white blood cell (T. WBC) and granulocyte counts (GAN) in the exposure groups (at $p<0.05$) suggests a state of stress-induced leukopenia. This result significant because leukopenia is a change from the chronic inflammatory response, which is typically characterized by leukocytosis, or an increase in the number of white blood cells. (Ghio *et al.,* 2012). This could be a sign of early immunosuppression or, more likely, a large-scale influx of these cells from the circulation, followed by their spreading and migration into injured tissues, like the lungs, which would reduce their blood count. This supports the findings of Umeh and Bruno

(2023) and offers a hematological relationship to the inflammatory cell infiltration in lung tissues noted by Kanter *et al*. (2004), who reported similar heat-induced leukopenia. Leukopenia implies a weakened immune system, which makes a person more vulnerable to infections. Beetroot extract's capacity to dramatically halt this decline highlights its immunomodulatory potential, most likely as a result of its anti-inflammatory properties, which lessen the chemotactic signals that push leukocytes out of the vasculature (Chavda *et al*., 2024).

The significant restoration of T. WBC and GRAN counts in the beetroot-treated groups (smoke + beetroot and heat + beetroot) underscores the extract's immunomodulatory potential. This protective effect can be attributed to the potent anti-inflammatory properties of beetroot's bioactive compounds, particularly betalains, which are known to inhibit key pro-inflammatory pathways like NF-κB, thereby reducing the expression of cytokines and adhesion molecules that promote leukocyte migration (Clifford *et al.,* 2015; Krajka-Kuźniak *et al.,* 2017).

Also, the study showed that the exposed groups had anemia, as shown by the significant decreases in hematocrit (HCT), hemoglobin (HGB) concentration, and red blood cell (RBC) count in the smoke-only and combined exposure groups (at $p<0.05$). This hematological disorder is caused by a variety of factors, such as inflammatory suppression of erythropoietin production, systemic toxin-induced bone marrow suppression, and oxidative damage that results in hemolysis. Biomass smoke contains carbon monoxide, which binds irreversibly to hemoglobin to form carboxyhemoglobin,

further reducing the oxygen-carrying capacity and functional lifespan of red blood cells (Kurmi *et al.,* 2012). The extract's effectiveness in maintaining erythrocyte integrity and function is shown by the evident rise in these parameters in the beetroot-treated groups, particularly the smoke + heat + beetroot group. Biologically, this is supported by the rich composition of beetroot: its dietary nitrates increase nitric oxide (NO) bioavailability, which improves microvascular blood flow and oxygen delivery; its antioxidants (betalains, vitamin C) shield red blood cells from oxidative lysis (Ninfali and Angelino, 2013). The effectiveness of beetroot extract in reversing this decrease is supported by the study by Adji *et al*. (2022).

A more complex situation was obtained from the examination of platelet indices. In the group exposed to heat and smoke, both plateletcrit (PCT) and platelet count (PLT) increased, suggesting a stress-induced hematological response marked by platelet activation and thrombocytosis. Due to these alterations, environmental stressors like heat and biomass smoke trigger the production of inflammatory cytokines, which in turn lead to release of platelets and production of megakaryocytes. In accordance with findings from research that showed increased platelet activity and aggregation under hyperthermic or oxidative conditions, the elevated platelet indices represent the body's attempt to combat tissue damage and oxidative stress (Gao *et al.,* 2019; Niu *et al.,* 2021). Beetroot-treated groups, however, showed no significant changes in PCT or PLT compared to controls, suggesting a protective effect. The antioxidant and anti-inflammatory properties of beetroot helped stabilize platelet levels, counteracting the pro-thrombotic effects of

heat and smoke exposure and maintaining normal hematological balance. A thermally induced change in platelet production and activation is indicated by the heat-only group's significant increase in Mean Platelet Volume (MPV) and Platelet Larger Cell Ratio (PLCR) ($p<0.05$), even though many other parameters stayed the same. Hemoconcentration and endothelial activation brought on by heat stress can result in the bone marrow releasing larger, more reactive platelets, which may be a pro-thrombotic risk factor (Umeh and Bruno, 2023). This shift may be decreased by beetroot consumption, as indicated by the normalization of these indices in the heat + beetroot group. The nitrate-NO pathway, which lowers the stimulus for platelet activation by promoting vasodilation and decreasing endothelial dysfunction, is most likely the mechanism underlying this effect (Clifford *et al.,* 2015).

The heat-only and smoke-only groups had significantly higher monocyte (MID) counts, which is consistent with these stressors' known ability to cause systemic inflammation. Monocytes are drawn to areas of damage and are important mediators of inflammation. Beetroot extract's strong anti-inflammatory qualities are further supported by its capacity to dramatically lower MID counts in the corresponding treatment groups, most likely by inhibiting the release of pro-inflammatory cytokines (Kujawska *et al.,* 2009).

It's also important to highlight the parameters, like the lymphocyte (LYM) count and red cell indices (MCV, MCH, and MCHC), that were not significantly different between groups. A cause other than nutritional deficiencies, such as the direct oxidative and toxic effects of the exposures, may be indicated by the stability of MCV, MCH, and MCHC,

which indicates that the induced anemia was normocytic and normochromic. Further research into particular lymphocyte groups is necessary because the lack of a discernible change in the lymphocyte count, in spite of changes in other leukocyte lines, suggests that the stressors have varying effects on different immune cell populations.

According to the histological analysis of lung tissues, male Wistar rats suffered significant pulmonary damage as a result of exposure to biomass smoke and intense heat. Intense peribronchiolar and interstitial inflammation, vascular ulceration, and alveolar collapse were observed in the smoke-only group, whereas bronchiolar ulceration, vascular congestion, and edema were observed in the heat-only group. These results are in line with earlier research showing that oxidative stress, inflammatory cell infiltration, and lung epithelial damage are caused by particulate matter and heat stress exposure (Torres-Duque *et al.,* 2021; Tamagawa *et al*., 2008). The complex mixture of carbon monoxide, nitrogen oxides, and polycyclic aromatic hydrocarbons found in biomass smoke causes tissue damage and vascular congestion by inducing the release of cytokines like TNF-α and IL-6 from neutrophils and macrophages (Forchhammer *et al*., 2012; Migliaccio and Mauderly, 2010). Likewise, heat stress causes endothelial disruption and alveolar damage by increasing vascular permeability and initiating inflammatory cascades (Liu *et al.,* 2019). More significant changes were seen in the combined exposure group, indicating a synergistic interaction between oxidative and inflammatory pathways brought on by heat and smoke co-exposure. On the other hand, groups that received beetroot extract beforehand demonstrated a notable reduction in histopathological lesions,

including reduced inflammatory cell infiltration, vascular ulceration, and preservation of alveolar architecture. This protective effect is consistent with well-established reports that extracts from *Beta vulgaris*, which are rich in phenolic antioxidants and betalains, stabilize cell membranes, and inhibit inflammatory responses (Clifford *et al.,* 2015). Beetroot supplementation lowers tissue oxidative stress and enhances histological results in models of pulmonary and hepatic injury, according to earlier in vivo research (Clifford *et al.,* 2015; Moreno-Ley *et al.,* 2021). Thus, the present histological results support the potential of beetroot extract as a natural antioxidant and protective agent against environmental lung insults by indicating that it reduces oxidative and inflammatory damage in Wistar rats that is caused by heat and smoke.

CONCLUSION

Exposure to heat and biomass smoke caused significant hematological and histological alterations in Wistar rats, including immune disruption, impaired oxygen transport, inflammation, vasculopathy, and alveolar damage. These effects reflect systemic toxicity and pulmonary injury resulting from oxidative and inflammatory stress. However, treatment with beetroot extract notably reduced these adverse changes, preserving normal blood parameters and lung architecture. Overall, beetroot extract demonstrated strong protective and cytoprotective effects against combined heat and smoke-induced damage.

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