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Research Article

HISTOLOGICAL EFFECTS OF CHRONIC CONSUMPTION OF ACTION BITTERS ON THE LUNGS, LIVER AND KIDNEYS OF ADULT MALE WISTAR RATS

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Ehebha Ehizokhale Santos
Department of Human Anatomy and Cell Biology, Delta State University, Abraka, Nigeria

Ahama Endurance Efe
Department of Human Anatomy and Cell Biology, Delta State University, Abraka, Nigeria

Glorious Praise Chinenye
Department of Human Anatomy and Cell Biology, Delta State University, Abraka, Nigeria

Obohwemu Oberhiri Kennedy
Department of Health, Wellbeing & Social Care, Global Banking School, Oxford Brookes University, Birmingham, United Kingdom; and PENKUP Research Institute, Birmingham, United Kingdom

Omakpokpose Gloria Oyovwikemo
Department of Human Anatomy and Cell Biology, Delta State University, Abraka, Nigeria

Amromare Peace Ochuko
Department of Human Anatomy and Cell Biology, Delta State University, Abraka, Nigeria

Kaine Omashim
Department of Human Anatomy and Cell Biology, Delta State University, Abraka, Nigeria

Corresponding Author: Obohwemu Kennedy Oberhiri, PhD

ABSTRACT

Introduction: Action bitters, commonly used in traditional medicine, have an unclear safety profile and potential effects on lung, liver, and kidney tissues. This experimental study investigated the histological

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effects of action bitters on the lungs, liver, and kidney tissues of adult male Wistar rats. Understanding these effects is crucial, as it can provide insights into the safety and potential risks associated with the use of action bitters in traditional medicine.

Methodology: A total of 18 adult male rats were used in this study. The rats were divided into three groups, each consisting of six rats (n = 6). Group A served as the normal control group, receiving no action bitters. Group B was administered 10% of the lethal dose 50 (LD50) of action bitters, equivalent to 0.66 ml/kg. Group C received 5% of the LD50, equivalent to 0.33 ml/kg. The administration of action bitters continued for 28 days. After this period, lung and kidney tissues were harvested and examined using hematoxylin and eosin staining.

Results: The results of the study showed no significant histological changes or inflammation in the lung and kidney tissues of all groups. This indicates that, under the conditions of this experiment, the administration of action bitters did not cause observable damage or alterations in the histology of these organs. However, it is important to note that these findings are specific to the conditions and dosages used in this study, and further research is needed to confirm these results across different conditions and dosages.

Conclusion: The study suggests that action bitters may have a limited impact on the histology and function of the lungs, liver, and kidneys. These findings contribute to our understanding of the safety profile of action bitters, indicating that, at least at the tested doses, they do not cause significant histological damage to these organs. However, the study also highlights the need for further research to fully define the effects of action bitters on lung, liver, and kidney tissues.

KEYWORDS

Histological Effects, Chronic Consumption, Action Bitters, Lungs, Liver, Kidneys, Wistar Rats.

INTRODUCTION

Action Bitters are a type of botanical-based beverage that originated in 18th-century Europe. They are typically made by infusing a combination of roots, herbs, barks, and fruits in a

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neutral spirit or glycerin, resulting in a concentrated, flavourful extract. These bitters contain a mixture of bioactive compounds, including alkaloids, glycosides, terpenes, and phenolic acids. Bitters are used in traditional medicine for their digestive, anti-inflammatory, and antimicrobial properties (Baydoun et al., 2015). The characteristics of bitters include their distinctive, often bitter flavour profiles, which can range from sweet and herbal to dry and medicinal. Due to their potency, bitters are usually consumed in small amounts, either as a shot or mixed with other beverages.

There are several types of bitters, including digestive bitters, which contain ingredients like gentian, angelica, and sarsaparilla. These bitters aid digestion and relieve symptoms of indigestion and bloating (Gruenwald et al., 2010). Aperitif bitters are used to stimulate appetite before meals and often contain ingredients like orange peel, rhubarb, and cassia. Cocktail bitters, a key ingredient in many classic cocktails, come in various flavours. Bitters contain a mixture of bioactive compounds, including alkaloids, glycosides, terpenes, and phenolic acids. Alkaloids are a major constituent of bitters and are responsible for their bitter taste (Newman & Cragg, 2012). Glycosides have been shown to have anti-inflammatory and antimicrobial properties (Kumar et al., 2013). Terpenes are a class of compounds found in bitters with antimicrobial and anti-inflammatory properties (Sahoo et al., 2016). Phenolic acids, a type of polyphenol found in bitters, have antioxidant and anti-inflammatory properties (Zhang et al., 2015).

The benefits of bitters include aiding digestion by stimulating digestive enzymes, relieving indigestion, and reducing inflammation. Certain bitters have been shown to exhibit antimicrobial activity against pathogens. Some popular brands of bitters include Alomo Bitters (Ghana), made with ginger, cloves, and wormwood, often used as a digestif or in traditional medicine. Action Bitters (Nigeria), made with a blend of ginger, garlic, and bitter leaf, have a bold, spicy flavor and are often consumed as an energizing tonic. Angostura Bitters (Trinidad and Tobago), made with gentian, orange peel, and cinnamon, are commonly used in cocktails like the Old Fashioned. Campari Bitters (Italy), made with a blend of rhubarb, orange peel, and chinotto, are often consumed as an aperitif or used in cocktails.

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Action Bitters, a brand of cocktail bitters, is an infusion of bitter substances from various herbs, roots, and botanicals, traditionally used to aid digestion and detoxification (Carissa, 2019; Kim et al., 2019). Despite its widespread use, little is known about its safety and potential histological effects on lung, liver, and kidney tissues (Sun et al., 2018). Action Bitters has been widely used in traditional medicine for its purported health benefits, including digestive and anti-inflammatory properties (Adewole et al., 2016; Nwodo et al., 2017). However, concerns have been raised regarding its potential toxicity and adverse effects on various organs, including the testes and lungs (Olaleye et al., 2013; Oyagbemi et al., 2015; Ehebha et al., 2024). Some studies have found that Action Bitters has potential benefits for lung health, such as anti-inflammatory properties that could help alleviate inflammation in the lungs (Esimone et al., 2011). The herbal remedy has also been found to have antioxidant properties, which could help protect the lungs from oxidative stress and damage (Oyedemi et al., 2012). Additionally, some studies suggest that Action Bitters may have expectorant properties, which could help relieve respiratory symptoms such as coughing and congestion (Igoli et al., 2005).

Bitters have been claimed to help heal piles/hemorrhoids and improve sexual function, enhance blood circulation, purify blood by the kidneys, regulate blood pressure through arterial dilatation, and prevent the formation of kidney stones. They are also said to cleanse the colon of impurities and possess anti-tumor properties (Oforibika, 2020). Bitters are also reported to have anti-inflammatory (Anayasor and Ogunbiyi, 2017), antibiotic, and antifungal properties (Mohammed, 2020; Qing et al., 2017). They ensure good digestion of fats and oils and proper functioning of the excretory system, reduce accumulated fat, triglycerides, and cholesterol levels, thereby conferring hypolipidemic properties (Helen et al., 2021). Bitters are said to reduce excess body fat, promote healthy weight loss, act as body detoxifiers, and enhance liver function (Hoffmann, 2002; McDonald, 2014). They act on the pancreas, helping to normalize blood sugar levels and promote the production and release of pancreatic enzymes (Anionye and Onyeneke, 2016).

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Action bitters are generally claimed to be effective in curing allergic, metabolic, and immunological conditions where the diagnosis points to a fault in the digestive process. They improve immunity, help in anaemia, wound healing, and blood clotting by increasing the population of red blood cells, white blood cells, and platelets in tissues. Bitters help with inflammatory conditions of the gastrointestinal tract, such as colitis, Crohn's disease, and nonspecific inflammation. They strengthen the tone of tissues throughout the digestive tract and aid in the healing of damaged mucous membranes.

Action Bitters are said to regenerate and heal the mucosal lining of the gastrointestinal tract, especially in cases of duodenal and gastric ulcers, helping resolve conditions ranging from gastroesophageal reflux to leaky gut syndrome. They are claimed to help heal piles/hemorrhoids, improve sexual function, enhance blood circulation, purify blood by the kidneys, regulate blood pressure through arterial dilatation, and prevent the formation of kidney stones. Bitters are also said to possess anti-tumour properties, especially protecting against colorectal cancers. They have anti-inflammatory, antibiotic, and antifungal properties (Anionye and Onyeneke, 2016).

Made from various chemical compounds taken from herbs and roots, Action Bitters is an herbal alcoholic beverage. Many consider these alcoholic bitters as a multipurpose medicine and use them without considering their effects on the liver or other organs. Many people use herbal remedies for treating a wide range of diseases due to the claims of their efficacy by manufacturers (Helen et al., 2021). However, there is little insight into the mode of action and possible toxic effects of these popular herbal formulations on organs such as the liver and kidneys.

Action Bitters, a polyherbal blend commonly consumed in Nigeria, is believed to possess numerous health benefits, including preventing kidney and bladder infections, lowering blood pressure, and aiding digestion (Ogbonnia et al., 2010). Despite its widespread use, there is limited scientific evidence on its potential toxicity and harmful effects on organs like the kidney (Akande et al., 2010). Herbal remedies, such as Action Bitters, have gained popularity worldwide due to their perceived health benefits (Kumar et al., 2017).

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The lungs, vital organs responsible for gas exchange and respiratory function (Hall, 2015; West, 2016), are susceptible to damage from chronic exposure to harmful substances. This study seeks to address the knowledge gap regarding the histological effects of long-term Action Bitters consumption on lung tissue. The significance of this study lies in its potential to inform public health policy and ensure the safe usage of Action Bitters (Smith et al., 2020).

Action Bitters, an herbal formulation, has been used for centuries to promote digestion and overall well-being. However, its safety profile and potential histological effects on lung, liver, and kidney tissues remain unknown, despite its widespread use as an herbal remedy. Previous studies have demonstrated the potential toxicity of herbal preparations on lung and kidney tissue, leading to conditions such as pulmonary fibrosis, inflammation, and oxidative stress (Oyagbemi et al., 2015; Adewole et al., 2016).

The Wistar rat, a commonly used animal model, has been shown to exhibit similar lung, liver, and kidney histology to humans, making it an ideal choice for studying lung and kidney toxicity (Crapo et al., 2017). This knowledge gap necessitates an investigation into the histological effects of Action Bitters on the lungs of adult male Wistar rats to determine its potential impact on pulmonary health. This study aims to investigate the histological effects on lung, liver, and kidney tissues resulting from chronic Action Bitters consumption in adult male Wistar rats. The study's objectives include evaluating histological changes in lung, liver, and kidney tissue, assessing fibrosis and inflammation, and establishing a causal link between Action Bitters consumption and lung histology, as well as any alterations in liver and kidney tissues.

METHODOLOGY

Materials and Methods

This study employed an experimental design to investigate the histological effects of chronic consumption of Action Bitters on the lungs and kidney tissues of adult male Wistar rats. Eighteen male adult rats were randomly selected using simple random sampling and divided into three groups (A, B, and C), with each group consisting of six rats. Group A (control) received standard feed and water. Group B received 10% of the lethal dose 50 (LD50) of Action Bitters (0.66 ml/kg).

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Group C received 5% of the LD50 of Action Bitters (0.33 ml/kg). The materials and equipment used included a digital microscope, dissecting boards, microscopic slides, tissue processors, microtome, embedding machine, staining racks, measuring cylinders, syringes, feeds (grower mash), oral cannula, and weighing scale.

Animal Housing and Care

Eighteen male adult Wistar rats were obtained from Delta State University's Animal House in Abraka, Nigeria. The inclusion criteria for the study included adult male Wistar rats with no pre-existing respiratory conditions, normal lung function, normal liver function, normal kidney function, and normal histology. Exclusion criteria included female rats, rats with pre-existing medical conditions, and those exposed to substances affecting lung, liver, and kidney histology. The Wistar rats were housed in plastic cages with regulated environmental conditions, including controlled temperature and humidity, and a 12-hour light/dark cycle. They were allowed ad libitum access to water and feed throughout the study period.

Animal Sacrifice and Sample Collection

After 28 days of treatment, the Wistar rats were left to fast overnight to ensure an empty gastrointestinal tract. The rats were then euthanized humanely using an overdose of anesthesia. Following euthanasia, the lungs, liver, and kidney tissues were carefully harvested. The tissues were immediately fixed in 10% formal saline to preserve their structure and prevent autolysis. The fixed tissues were then processed for histological examination.

Histological Processing

The fixed tissues underwent a series of steps for histological processing. Initially, the tissues were dehydrated using a graded series of ethanol solutions to remove water. This was followed by clearing the tissues in xylene to remove the ethanol and prepare the tissues for embedding. The tissues were then embedded in paraffin wax using an embedding machine, which provided support for sectioning. Thin sections of the tissues, approximately 5 micrometers thick, were cut using a microtome. These sections were mounted on microscopic slides and stained using hematoxylin and eosin (H&E) staining. H&E staining is a widely used technique in histology

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that provides contrast to the tissue sections, highlighting the cellular and structural details.

Instruments and Data Collection

A light microscope was used to examine the stained histological sections. The microscope was equipped with various magnification lenses to allow detailed observation of the tissue architecture. A pilot study was conducted to ensure the validity of the instruments and the staining process. Reliability was ensured through repeated examinations of the tissue sections by multiple observers to confirm consistency in the observations.

Ethical Considerations

Ethical approval for the study was obtained from the Delta State University Department of Human Anatomy and Cell Biology (RBC/FBMC/DELSU/24/423). The study adhered to ethical guidelines for the care and use of laboratory animals, ensuring that all procedures were conducted humanely and with minimal distress to the animals. The principles of the 3Rs (Replacement, Reduction, and Refinement) were followed to minimize the number of animals used and to enhance their welfare.

Data Analysis

The histological data collected from the tissue sections were analyzed qualitatively. Observations focused on identifying any histological changes, such as inflammation, fibrosis, or cellular damage, in the lungs, liver, and kidney tissues. Comparisons were made between the control group and the treatment groups to assess the impact of Action Bitters on tissue morphology. The findings were documented with photomicrographs taken using a digital camera attached to the light microscope.

Results

Lung Histology

In the control group (Group A), lung sections showed normal architecture characterized by multiple alveoli separated by loose connective tissue stroma. The alveoli were lined by lymphocytes, and the alveolar ducts, respiratory bronchioles, and terminal bronchioles were clearly visible. The bronchioles had columnar epithelium-lined walls with smooth muscle, and the vasculature included both arterial and venous origins. The presence of blood vessels and

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lymphoid aggregates further indicated healthy lung tissue.

In Treatment Group B (0.66 ml/kg Action Bitters), lung sections revealed alveoli of varying sizes bordered by Type I and Type II lymphocytes. The intervening loose connective tissue stroma contained arteries, veins, and lymphocytes. The respiratory and terminal bronchioles were prominent, with epithelium-lined walls and smooth muscle. The normal bronchi and the overall lung architecture were maintained, indicating no significant histological changes compared to the control group.

In Treatment Group C (0.33 ml/kg Action Bitters), lung sections showed multiple alveoli separated by loose connective tissue stroma, similar to the control group. The bronchioles, blood vessels, and lymphoid aggregates were present, and the alveolar and bronchiolar architecture appeared normal. The vasculature included both arterial and venous origins, and no significant histological changes were observed compared to the control group.

Overall, all three groups (control and treatment) exhibited normal lung tissue morphology. The absence of significant histological changes in the treatment groups compared to the control group suggests that the administration of Action Bitters at the tested doses did not adversely affect the lung tissue.

Liver Histology

In Group A (control), liver sections appeared entirely normal. The central veins were surrounded by hepatic plates, and the sinusoids intervening between the cords of hepatocytes were lined by healthy endothelial cells. Perisinusoidal spaces were present between the endothelium and hepatocytes, indicating normal liver architecture and function.

In Group B, liver sections showed a well-defined central vein, with multiple plates of hepatocytes separated by vascular spaces or sinusoids. The sinusoids were lined with healthy endothelial cells, and there was no evidence of liver damage, scarring, or disease. The overall liver architecture was maintained, indicating that the administration of 0.66 ml/kg Action Bitters did not cause significant histological changes.

In Group C, liver sections revealed central veins surrounded by hepatic plates, with sinusoids separating the cords of hepatocytes. The

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sinusoids were lined with healthy endothelial cells, and perisinusoidal spaces were present between the endothelium and hepatocytes. The liver architecture appeared normal, and no significant histological changes were observed compared to the control group.

All three groups showed normal liver histology, suggesting that the administration of Action Bitters at the tested doses did not adversely affect the liver tissue.

Kidney Histology

In Group A (control), kidney sections revealed a cortex and glomerulus surrounded by a layer of connective tissue. There were many glomeruli of varying sizes divided by tubules. The renal corpuscle consisted of a Bowman's capsule, Bowman's space, and glomerulus. The capsule was bordered with flattened epithelium in the glomerular pole, while the glomerulus was lined by podocytes. The tubules and glomerular bodies were separated by a loose connective tissue stroma. The tubules were lined with cuboidal or flattened epithelium. Juxtaglomerular apparatuses were also prevalent. The medulla contained various tubular structures lined with low cuboidal to columnar epithelial cells. Blood vessels were also seen in the medullary area of renal tissues. These characteristics are compatible with those of a normal kidney.

In Group B, kidney sections appeared normal with clear areas of cortex and medulla. The cortex had many glomeruli, Bowman's capsules, and tubules surrounded by connective tissue. The tubules had cuboidal or flattened cells, and juxtaglomerular apparatuses were present. The medulla had tubular structures with cuboidal to columnar cells along the blood vessels. The overall kidney architecture was maintained, indicating that the administration of 0.66 ml/kg Action Bitters did not cause significant histological changes.

In Group C, kidney sections appeared perfectly healthy with a clear distinction between the outer cortex and inner medulla. The cortex was packed with tiny filtering units called glomeruli surrounded by delicate tubules and connective tissue. The tubules were lined with specialized cells, and there were also juxtaglomerular apparatuses that helped regulate blood pressure. The medulla contained tubular structures with a variety of cell types, along with blood vessels that supplied the kidney with oxygen and nutrients. The overall kidney architecture appeared normal,

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and no significant histological changes were observed compared to the control group.

All three groups showed normal kidney histology, suggesting that the administration of Action Bitters at the tested doses did not adversely affect the kidney tissue.

Detailed Observations

In the control group (Group A), the lung sections showed a well-organized structure with multiple alveoli separated by loose connective tissue stroma. The alveoli were lined by lymphocytes, and the alveolar ducts, respiratory bronchioles, and terminal bronchioles were clearly visible. The bronchioles had columnar epithelium-lined walls with smooth muscle, and the vasculature included both arterial and venous origins. The presence of blood vessels and lymphoid aggregates further indicated healthy lung tissue.

In Treatment Group B (0.66 ml/kg Action Bitters), lung sections revealed alveoli of varying sizes bordered by Type I and Type II lymphocytes. The intervening loose connective tissue stroma contained arteries, veins, and lymphocytes. The respiratory and terminal bronchioles were prominent, with epithelium-lined walls and smooth muscle. The normal bronchi and the overall lung architecture were maintained, indicating no significant histological changes compared to the control group.

In Treatment Group C (0.33 ml/kg Action Bitters), lung sections showed multiple alveoli separated by loose connective tissue stroma, similar to the control group. The bronchioles, blood vessels, and lymphoid aggregates were present, and the alveolar and bronchiolar architecture appeared normal. The vasculature included both arterial and venous origins, and no significant histological changes were observed compared to the control group.

Overall, all three groups (control and treatment) exhibited normal lung tissue morphology. The absence of significant histological changes in the treatment groups compared to the control group suggests that the administration of Action Bitters at the tested doses did not adversely affect the lung tissue.

In Group A (control), liver sections appeared entirely normal. The central veins were surrounded by hepatic plates, and the sinusoids intervening between the cords of hepatocytes were lined by healthy endothelial cells.

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Perisinusoidal spaces were present between the endothelium and hepatocytes, indicating normal liver architecture and function.

In Group B, liver sections showed a well-defined central vein, with multiple plates of hepatocytes separated by vascular spaces or sinusoids. The sinusoids were lined with healthy endothelial cells, and there was no evidence of liver damage, scarring, or disease. The overall liver architecture was maintained, indicating that the administration of 0.66 ml/kg Action Bitters did not cause significant histological changes.

In Group C, liver sections revealed central veins surrounded by hepatic plates, with sinusoids separating the cords of hepatocytes. The sinusoids were lined with healthy endothelial cells, and perisinusoidal spaces were present between the endothelium and hepatocytes. The liver architecture appeared normal, and no significant histological changes were observed compared to the control group.

All three groups showed normal liver histology, suggesting that the administration of Action Bitters at the tested doses did not adversely affect the liver tissue.

In Group A (control), kidney sections revealed a cortex and glomerulus surrounded by a layer of connective tissue. There were many glomeruli of varying sizes divided by tubules. The renal corpuscle consisted of a Bowman's capsule, Bowman's space, and glomerulus. The capsule was bordered with flattened epithelium in the glomerular pole, while the glomerulus was lined by podocytes. The tubules and glomerular bodies were separated by a loose connective tissue stroma. The tubules were lined with cuboidal or flattened epithelium. Juxtaglomerular apparatuses were also prevalent. The medulla contained various tubular structures lined with low cuboidal to columnar epithelial cells. Blood vessels were also seen in the medullary area of renal tissues. These characteristics are compatible with those of a normal kidney.

In Group B, kidney sections appeared normal with clear areas of cortex and medulla. The cortex had many glomeruli, Bowman's capsules, and tubules surrounded by connective tissue. The tubules had cuboidal or flattened cells, and juxtaglomerular apparatuses were present. The medulla had tubular structures with cuboidal to columnar cells along the blood vessels. The overall kidney architecture was maintained, indicating that the

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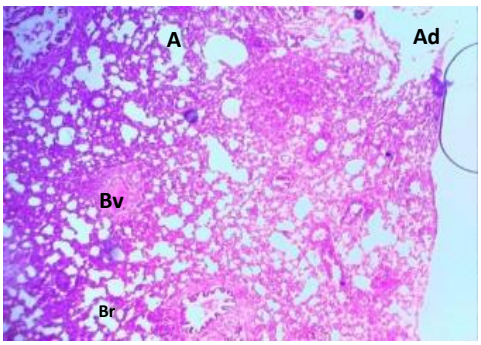
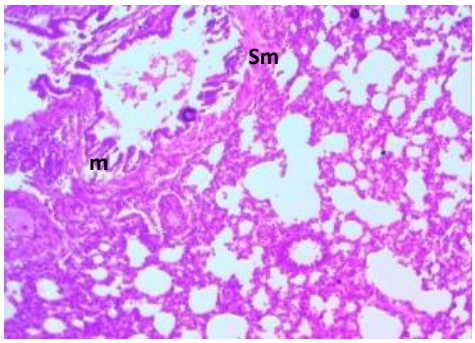
administration of 0.66 ml/kg Action Bitters did not cause significant histological changes.

In Group C, kidney sections appeared perfectly healthy with a clear distinction between the outer cortex and inner medulla. The cortex was packed with tiny filtering units called glomeruli surrounded by delicate tubules and connective tissue. The tubules were lined with specialized cells, and there were also juxtaglomerular apparatuses that helped regulate blood pressure. The medulla contained tubular structures with a variety of cell types, along with blood vessels that supplied the kidney with oxygen and nutrients. The overall kidney architecture appeared normal, and no significant histological changes were observed compared to the control group.

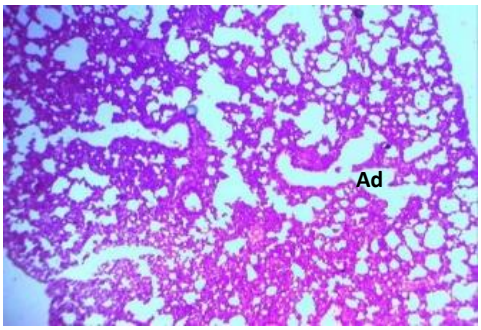
All three groups showed normal kidney histology, suggesting that the administration of Action Bitters at the tested doses did not adversely affect the kidney tissue.

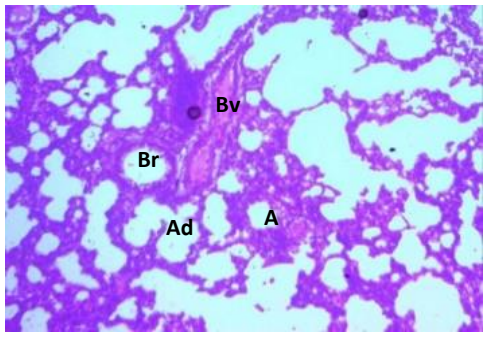




Lungs (Group A) Ax100 **BX400**

Keys: Br: bronchioles, A: Alveoli, B: Blood vessels, Ad: Alveolar duct, As: Alveolar sac, M: Mucosa, Sm: Smooth muscle.





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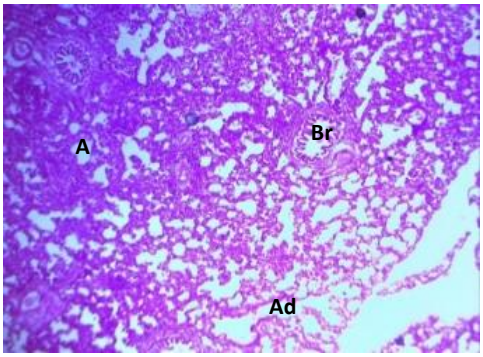
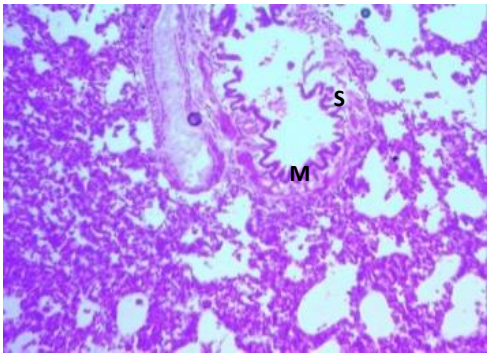
Lungs (Group B) AX100 **BX400**

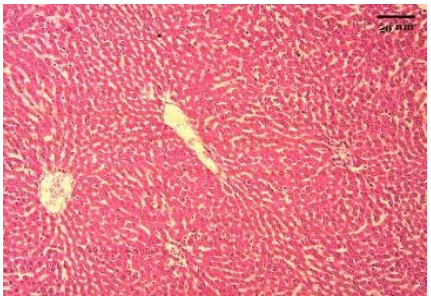
Keys: Br: Bronchioles, A: Alveoli, Bv: Blood vessel, Ad: Alveolar duct.

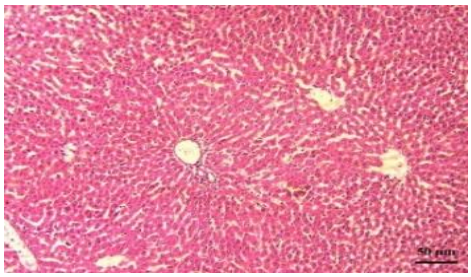




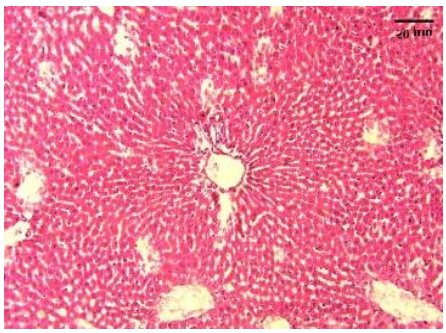
Lungs (Group C) AX100 **BX400**

Key: Br: Bronchioles, A: Alveoli, Ad: Alveolar duct, Sm: Smooth muscle, M: Mucosa.





Liver (Group A) AX100 **BX100**



Liver (Group) BX100 **BX100**
Keys: Bpv; Central vein

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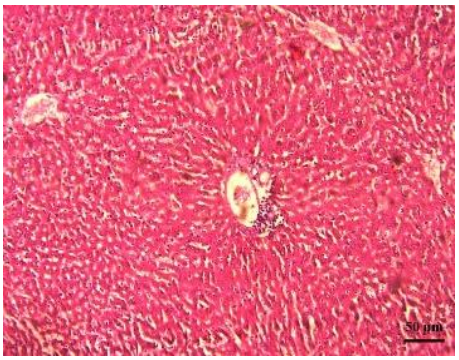
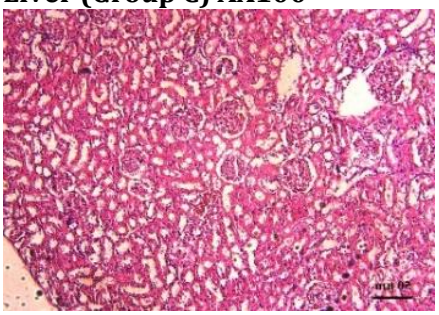
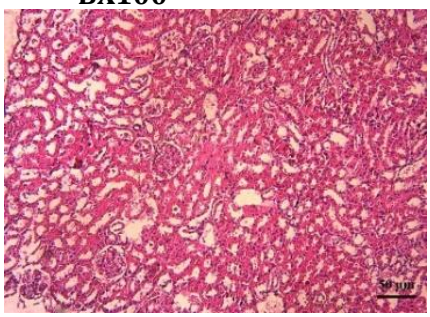


Liver (Group C) AX100

BX100



Kidney (Group A) AX100



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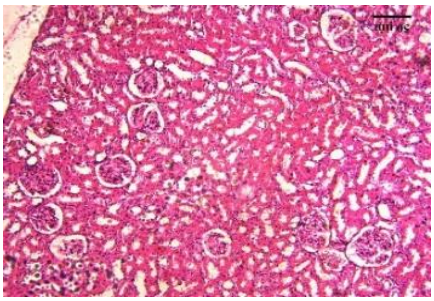
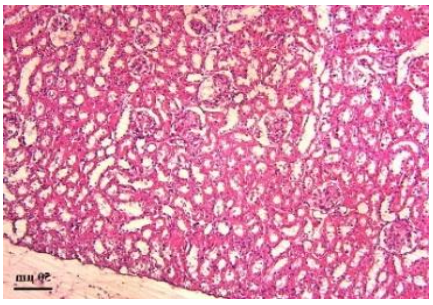


Kidney (Group B) AX100



BX100

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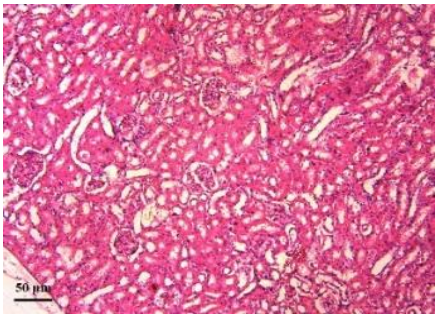
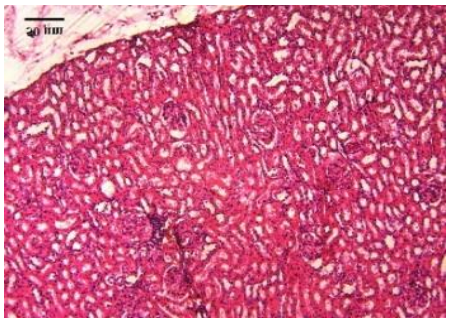

Kidney (Group C) AX100 **BX100**

DISCUSSION

The results of this study offer valuable insights into the histological effects of Action Bitters on lung, liver, and kidney tissues. The absence of significant histological changes in these tissues of adult Wistar rats exposed to Action Bitters suggests that this herbal remedy may be safe for use and may not have a profound impact on lung, liver, and kidney histology. This finding is particularly important given the widespread use of Action Bitters in traditional medicine.

The normal cellular morphology and lack of inflammation or necrosis in the lung, liver, and kidney tissues of treated rats indicate that Action Bitters may not possess inflammatory or carcinogenic properties. This is consistent with previous studies that have reported the anti-inflammatory and antioxidant activities of certain herbal remedies (Aladejana, 2023; Ayustaningwarno et al., 2024. For instance, the anti-inflammatory properties of herbal compounds can help mitigate cellular damage and oxidative stress, which are common pathways leading to tissue inflammation and carcinogenesis. The antioxidant properties, on the other hand, can neutralize free radicals, thereby protecting cells from oxidative damage (Chen et al., 2024).

However, it is essential to note that this study had a limited scope and duration. The findings may not be generalizable to humans or other animal species, and the long-term effects of Action Bitters on lung, liver, and kidney tissues remain unknown. The short duration of the study (28 days) may not be sufficient to observe chronic

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effects or delayed toxicity that could manifest over a longer period. Therefore, while the initial findings are promising, they should be interpreted with caution.

Further research is necessary to confirm these findings and investigate the potential hazards associated with the use of Action Bitters. Long-term studies involving different dosages and extended exposure periods are needed to fully understand the safety profile of Action Bitters. Additionally, studies involving other animal models and eventually human trials would be crucial to determine the relevance of these findings to human health (Hong et al., 2024).

The exact mechanisms by which Action Bitters may exert its effects on lung, liver, and kidney tissues are unclear. Understanding these mechanisms is vital for elucidating how Action Bitters interacts with cellular and molecular pathways. Future studies should aim to elucidate the biochemical and molecular pathways involved in the interaction between Action Bitters and lung, liver, and kidney tissues. This could involve investigating the role of specific bioactive compounds present in Action Bitters and their interactions with cellular receptors and signalling pathways (Aladejana, 2023; Ehebha et al., 2024).

Moreover, the potential therapeutic benefits of Action Bitters, such as its anti-inflammatory and antioxidant properties, should be explored in more detail. Identifying the active components responsible for these effects could lead to the development of targeted therapies for conditions involving inflammation and oxidative stress. For example, isolating and characterizing these compounds could provide insights into their potential use in treating respiratory conditions, liver diseases, and kidney disorders (Ayustaningwarno et al., 2024; Chen et al., 2024).

The study's findings also highlight the importance of considering the dosage and formulation of herbal remedies. While Action Bitters did not show significant histological changes at the tested doses, it is possible that higher doses or different formulations could have different effects. Therefore, it is crucial to establish safe dosage ranges and standardized formulations to ensure the safe use of herbal remedies like Action Bitters.

In summary, the study provides preliminary evidence that Action Bitters may not have significant adverse effects on lung, liver, and

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kidney tissues in adult Wistar rats. However, the limited scope and duration of the study necessitate further research to confirm these findings and explore the long-term safety and potential therapeutic benefits of Action Bitters. Understanding the mechanisms of action and identifying the active components will be key to fully elucidating the effects of this widely used herbal remedy.

LIMITATIONS

This study faced several limitations that may have impacted the results and their generalizability. Firstly, financial constraints limited the scope of the research. Adequate funding is crucial for conducting comprehensive studies, including the procurement of advanced equipment, reagents, and other necessary resources. The lack of sufficient financial support restricted the ability to explore more extensive and varied experimental conditions.

Secondly, the duration of the study was relatively short. The 28-day period may not have been sufficient to observe the long-term effects of Action Bitters on lung, liver, and kidney tissues. Chronic exposure studies over several months or even years are often necessary to fully understand the potential cumulative effects and delayed toxicity of substances. Therefore, the findings of this study should be interpreted with caution, as they may not reflect the long-term impact of Action Bitters.

Additionally, the study was limited to routine hematoxylin and eosin (H&E) staining for histological examination. While H&E staining is a standard and widely used technique for assessing tissue morphology, it may not provide detailed insights into specific cellular and molecular changes. Advanced staining techniques, such as Periodic Acid-Schiff (PAS) and Masson's Trichrome, could offer more comprehensive information on tissue structure, fibrosis, and other pathological changes. The reliance on H&E staining alone may have limited the ability to detect subtle histological alterations.

RECOMMENDATIONS

Based on the findings and limitations of this study, several recommendations can be made. Firstly, it is advisable to consume Action Bitters in moderation. While the study suggests that Action Bitters may not have significant adverse effects on lung, liver, and kidney tissues at the tested

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doses, moderation is key to minimizing any potential risks.

Further research is essential to confirm the findings of this study and to investigate the long-term effects of Action Bitters. Extended studies with longer durations and varied dosages should be conducted to provide a more comprehensive understanding of its safety profile. Additionally, research should focus on elucidating the mechanisms underlying the observed histological changes. Understanding the biochemical and molecular pathways involved can help identify potential therapeutic targets and improve the safety of herbal remedies.

The utilization of various staining techniques, such as PAS and Masson's Trichrome, is recommended for enhanced tissue analysis. These techniques can provide more detailed information on tissue structure, fibrosis, and other pathological changes, offering a deeper understanding of the effects of Action Bitters on organ tissues.

Finally, financial support for students and researchers is crucial to facilitate comprehensive experimental studies. Adequate funding can enable the acquisition of advanced equipment, reagents, and other resources necessary for conducting high-quality research. Supporting young researchers can also foster innovation and contribute to the advancement of scientific knowledge in the field of herbal medicine.

Conclusion

The present study provides preliminary evidence that Action Bitters may be safe for use and may not have a significant impact on lung, liver, and kidney histology. However, the shorter duration of the study and other limitations suggest that these findings should be interpreted with caution. Further research is necessary to fully understand the effects of Action Bitters on lung, liver, and kidney tissues and to define its safety profile. Extended studies with longer durations, varied dosages, and advanced staining techniques are essential to provide a more comprehensive evaluation of the potential hazards and therapeutic benefits of Action Bitters. Financial support for researchers is also crucial to facilitate these comprehensive studies and to advance our understanding of herbal remedies.

CONFLICTS OF INTEREST

The authors report no conflicts of interest.

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