THE USA JOURNALS
THE AMERICAN JOURNAL OF INTERDISCIPLINARY INNOVATIONS AND RESEARCH (ISSN- 2642-7478)
VOLUME 06 ISSUE11

PUBLISHED DATE: - 18-11-2024
DOI: - https://doi.org/10.37547/tajiir/Volume06Issue11-11 **PAGE NO.: - 194-203**

RESEARCH ARTICLE **Open Access**

HISTOLOGICAL EVALUATION OF THE EFFECTS OF ACTION BITTERS ON THE TESTES OF ADULT MALE WISTAR RATS

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Abstract

Action Bitters, a popular herbal concoction, has been traditionally used to treat various health conditions, including those related to the male reproductive system. Despite its widespread use, there is a dearth of scientific evidence on its effects on the testes. This study aimed to investigate the histological changes in the testes of adult male Wistar rats following administration of Action Bitters.

Methods: Fifteen adult male Wistar rats were randomly divided into three groups (n = 5). Group A served as the normal control, while groups B and C received 10% (0.66ml/kg) and 5% (0.33ml/kg) lethal dose 50 (Ld50) of Action Bitters, respectively. The Ld50 values were calculated based on the median lethal dose of the herbal remedy. The rats were administered the herbal remedy for a period of one month, after which they were sacrificed, and their testes collected for histological examination.

Results: Microscopic examination of the testicular tissue samples revealed no evidence of abnormal cellular changes or tissue damage. The tissue samples appeared normal, with no signs of inflammation, necrosis, or apoptosis.

Discussion: The findings of this study suggest that Action Bitters does not induce histological changes in the testes of adult male Wistar rats. These results provide new insights into the potential mechanism of action of the herbal medication on testicular function. The study highlights the importance of considering both histological and biochemical assessments when evaluating the effects of herbal remedies on reproductive health.

Conclusion: This study contributes to the growing body of knowledge on the effects of herbal remedies on the male reproductive system. The findings suggest that Action Bitters may be a safe and effective herbal remedy for treating reproductive health issues, and further research is warranted to explore its potential benefits and mechanisms of action.

Keywords Action Bitters, testes, histology, male reproductive system, herbal remedy.

INTRODUCTION

Action Bitters, a rich wine-colored spirit with a slightly bitter, mouth-watering aroma, has been a popular herbal remedy globally for its purported health benefits (Ojo et al., 2020). The ingredients of Action Bitters may vary depending on the specific brand and formulation (Garcia et al., 2018), but its traditional use in folk medicine has been well-documented (Schoenberg et al., 2020). Despite its widespread use, the potential adverse effects of excessive consumption on male reproductive health remain understudied (Wang et al., 2020). This research aims to investigate the changes in the testis of male Wistar rats following chronic consumption of Action Bitters at the cellular level (Livingston, 2023).

The use of bitter substances, including Action Bitters, in traditional medicine dates back centuries, with bioactive compounds being the primary drivers of their therapeutic effects (Schoenberg et al., 2020). Action Bitters, containing herbs like Symphonia Globulifera, Garcinia Kola, Tetrapleura Tetraptera, Lannea Welwitschi, demineralized water, ethyl alcohol, color: E150, and brandy flavoring, are consumed for various health benefits (Ogechi & Ibioku, 2019). Some of these herbs, such as fennel and ginger, have been traditionally used to increase libido and improve sexual performance (Molina, 2012), while others, like dandelion root and burdock root, may help increase testosterone levels (Molina, 2012), which may improve sexual

function in men.

However, chronic consumption of Action Bitters can lead to adverse health effects. Previous studies have shown that certain herbal medicines can harm male reproductive health, affecting hormonal levels, sperm production, and testicular function (Adedosu et al., 2018). The testes, or testicles, are a crucial part of the male reproductive system, responsible for producing and storing sperm until they are mature enough to ejaculate (Sadler-Riggleman et al., 2018). They also produce testosterone, which is responsible for libido, fertility, and muscle and bone development (Tim., 2018).

The scrotum, a sac of skin that contains the testicles, hangs outside the body in the front of the pelvic region near the upper thighs (Sadler-Riggleman et al., 2018). The testes are a pair of organs located in the scrotum, and their proper functioning is essential for male fertility and overall health. Despite the potential benefits of Action Bitters, there is a need to investigate its effects on male reproductive health, particularly the testes.

Action Bitters has been used for centuries in traditional medicine to treat various health conditions, including digestive issues, liver problems, and sexual dysfunction (Ogechi & Ibioku, 2019). The exact mechanism of action of Action Bitters is not fully understood, but it is believed to work by stimulating the digestive system, improving liver function, and increasing testosterone levels (Molina, 2012). However, the long-term effects of chronic consumption of Action Bitters on male reproductive health are unknown.

Previous studies have shown that certain herbal remedies can affect male reproductive health, including reducing sperm count and motility, and altering hormone levels (Adedosu et al., 2018). For example, studies have found that chronic consumption of a herbal remedy containing ginseng and licorice root reduced sperm count and motility in male rats (Wang et al., 2018). Another study found that a herbal remedy containing saw palmetto and stinging nettle root reduced prostate size and improved urinary symptoms in men with benign prostatic hyperplasia (BPH) (Molina, 2012).

The testes are a complex organ that is sensitive to changes in the body's hormonal balance and environmental factors (Sadler-Riggleman et al., 2018). Chronic consumption of Action Bitters may affect the testes by altering hormone levels, reducing sperm production, and damaging testicular tissue (Adedosu et al., 2018). However, the exact mechanisms by which Action Bitters affects the testes are unknown and require further study.

This study aims to investigate the histological effects of chronic Action Bitters consumption on the testis of male Wistar rats. The study will examine the changes in testicular tissue, including the presence of any abnormal cellular changes or tissue damage, and the effects of Action Bitters on sperm production and hormone levels. The study will also investigate the potential mechanisms by which Action Bitters affects the testes, including the role of bioactive compounds and hormonal changes.

The findings of this study will provide valuable insights into the potential health risks associated with excessive consumption of Action Bitters and will contribute to the growing body of knowledge on the effects of herbal remedies on male reproductive health. The study will also provide a foundation for future research on the effects of Action Bitters on male reproductive health and will inform the development of evidence-based guidelines for the use of Action Bitters in traditional medicine.

METHODS

This study employed a quasi-experimental research design to evaluate the histological effects of chronic administration of Action Bitters on the testes of adult male Wistar rats. While it does not involve a true control group with random assignment, it does compare the effects of different doses of Action Bitters on the testicular tissue of rats to a control group.

MATERIALS AND EQUIPMENT

The study utilized various materials and equipment, including:

- Digital microscopes (Olympus CX41, Olympus Corporation, Tokyo, Japan) for histological examination
- Dissection plates (Fisher Scientific, Pittsburgh, PA, USA) for tissue dissection
- Slides (Fisher Scientific, Pittsburgh, PA, USA) for histological staining
- Pipettes (Fisher Scientific, Pittsburgh, PA, USA) for tissue processing
- Centrifuges (Eppendorf Centrifuge 5415R, Eppendorf AG, Hamburg, Germany) for tissue processing
- Tissue cassettes (Fisher Scientific, Pittsburgh, PA, USA) for tissue embedding
- Embedding machines (Leica EM UC6, Leica Microsystems, Wetzlar, Germany) for tissue embedding
- Hematoxylin and eosin (H&E) staining kit (Sigma-Aldrich, St. Louis, MO, USA) for histological staining
- Xylene (Fisher Scientific, Pittsburgh, PA, USA) for tissue processing
- Ethanol (Fisher Scientific, Pittsburgh, PA, USA) for tissue processing
- Distilled water (Fisher Scientific, Pittsburgh, PA, USA) for tissue processing

ANIMAL SELECTION, GROUPING AND CARE

Fifteen male adult Wistar rats (Rattus norvegicus) were randomly selected and divided into three groups (A, B, and C) of five rats each. Group A served as the control, while Groups B and C received 0.66 ml/kg (10% LD50) and 0.33 ml/kg (5% LD50) of Action Bitters, respectively. Rats were housed in well-ventilated plastic cages with partitions, maintained under controlled environmental conditions (temperature: 25 ± 5°C, relative humidity: 50 ± 5°C, and 12-h light/dark cycle), and fed standard farm puree with free access to water. Action Bitters were administered orally using an oral cannula without dilution for 28 days.

The rats were monitored daily for any signs of illness or distress, and their body weights were recorded weekly. The rats were euthanized at the end of the 28-day period using a humane method (CO_2 asphyxiation), and their testes were collected for histological analysis.

TISSUE COLLECTION AND HISTOLOGICAL ANALYSIS

After 28 days, rats were euthanized, and testes were collected, fixed in 10% buffered formalin, and processed for histological analysis using H&E staining. The testes were then embedded in paraffin wax, sectioned into 5-µm thick slices, and mounted on glass slides. The slides were then stained with H&E using a standard protocol.

The stained sections were examined under a light microscope at various magnifications to assess histological changes, including changes in seminiferous tubules, interstitial tissue, spermatogenesis, and Leydig cells. The histological changes were evaluated using a standardized

scoring system, which included the following criteria:

- Seminiferous tubules: normal, slightly dilated, or severely dilated
- Interstitial tissue: normal, slightly inflamed, or severely inflamed
- Spermatogenesis: normal, slightly impaired, or severely impaired
- Leydig cells: normal, slightly atrophic, or severely atrophic

The histological changes were evaluated by a blinded observer, and the results were recorded and photographed for documentation and further analysis.

DATA COLLECTION

Histological data were systematically recorded and photographed for documentation and further analysis. The data were analyzed using descriptive statistics, including means and standard deviations, and were presented in tables and figures.

ETHICAL CONSIDERATIONS

The study was approved by the Institutional Animal Ethics Committee (IAEC) of the Faculty of Basic Medicine, Delta State University, Nigeria. The study was conducted in accordance with the principles of animal welfare and the guidelines for the care and use of laboratory animals.

The study was conducted in accordance with the guidelines for the care and use of laboratory animals, and the animals were handled and cared for in accordance with the principles of animal welfare. The study was designed to minimize animal suffering and to ensure that the animals were treated with respect and dignity. The study was conducted in a humane and ethical manner, and the results were analysed and presented in a responsible and transparent manner.

RESULTS

The histological examination of testicular tissue from the three groups (A, B, and C) was performed using a Primo Star ZEISS light microscope. Photomicrographs of the tissue sections are presented below.

The testicular tissue of Group A exhibited normal histo-architecture, characterized by seminiferous tubules of varying sizes surrounded by round to oval structures with centrally placed nuclei (Figure 1). The tubules were lined by germ cells, including Spermatozoa, Primary Spermatocytes, Spermatids, and Basal Spermatogonia, with Sertoli cells present within the tubular lumen. The germ cells were arranged in a specific pattern, with Spermatozoa and Primary Spermatocytes present in the basal compartment, and Spermatids and Basal Spermatogonia present in the apical (adluminal) compartment. The Sertoli cells were observed to be in close contact with the germ cells and were responsible for providing support and nutrition to the developing spermatozoa.

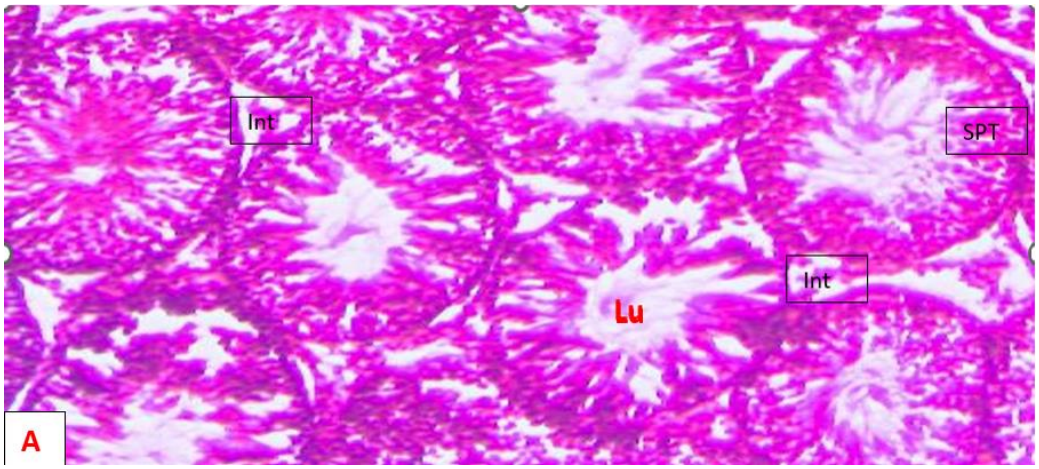

Figure 1: Histology of Wistar rat's testes in Group A (H & E stain x 100)

Key: Int (Interstitium); Lu (Lumen); SPT (Spermatocyte – Preleptotene)

The testicular tissue of Group B showed no significant differences from the control group (Group A) (Figure 2). Seminiferous tubules of varying sizes were observed, surrounded by round to oval structures with centrally placed nuclei, and lined by germ cells and Sertoli cells. The germ cells were arranged in a similar pattern to that observed in Group A, with Spermatozoa and Primary Spermatocytes present in the basal compartment, and Spermatids and Basal Spermatogonia present in the adluminal compartment. The Sertoli cells were also observed to be in close contact with the germ cells and were responsible for providing support and nutrition to the developing spermatozoa.

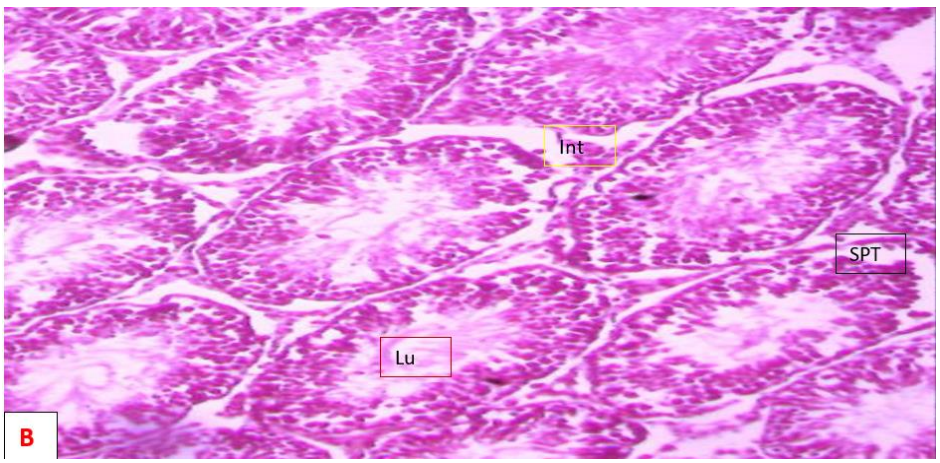

Figure 2: Histology of Wistar rat's testes in Group B (H & E stain x 100)

Key: Int (Interstitium); Lu (Lumen); SPT (Spermatocyte – Preleptotene)

Similarly, the testicular tissue of Group C exhibited no significant differences from the control group (Group A) and Group B (Figure 3). Normal seminiferous tubules and germ cell morphology were observed, with Sertoli cells present within the tubular lumen. The germ cells were arranged in a similar

pattern to that observed in Groups A and B, with Spermatozoa and Primary Spermatocytes present in the basal compartment, and Spermatids and Basal Spermatogonia present in the adluminal compartment. The Sertoli cells were also observed to be in close contact with the germ cells and were responsible for providing support and nutrition to the developing spermatozoa.

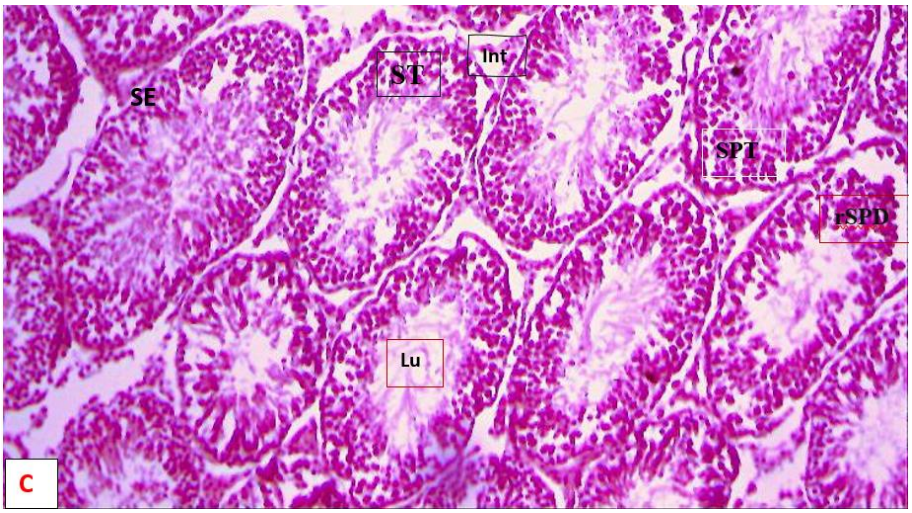

Figure 3: Histology of Wistar rat's testes in Group C (H & E stain x 100)

Key: ST (Seminiferous Tubule); Int (Interstitium); SE (Seminiferous Epithelium); Lu (Lumen); rSPD (Round Spermatid); SPT (Spermatocyte – Preleptotene)

The results of this study demonstrate that the administration of Action Bitters to adult male Wistar rats for 28 days did not cause any significant changes in the histological architecture of the testicular tissue. The seminiferous tubules and germ cells appeared normal, with no signs of damage or degeneration. These findings suggest that Action Bitters may not have any adverse effects on the testicular tissue of adult male Wistar rats.

DISCUSSION

The current study investigated the histological effects of Action Bitters on the testes of male Wistar rats. The results showed that the treated groups exhibited normal histological features, with seminiferous tubules of varying diameters, Leydig cells, and germ cells, including basal spermatogonia, spermatids, primary spermatocytes, and spermatozoa. Sertoli cells were also visible in the tubular lumen, similar to the control group. These findings align with previous studies by Yakubu et al. (2015) and Oremosu et al. (2017), which reported no histopathological changes in rats treated with Action Bitters.

However, they contradict the results of Adedosu et al. (2018) and Oyeyipo et al. (2019), which found significant histopathological changes and oxidative stress in rats administered Action Bitters. The discrepancy between these studies may be attributed to differences in the experimental design, including the dose and duration of Action Bitters administration, as well as the species and strain of rats used.

The absence of abnormal cellular changes or tissue

damage in the current study suggests that Action Bitters may not have adverse effects on testicular histology at the doses and duration used. However, it is essential to note that normal histological results do not entirely rule out the possibility of disease, as some conditions may not be detectable by this method. For example, some studies have reported that Action Bitters can cause oxidative stress and DNA damage in cells, which may not be detectable by histological examination (Oyeyipo et al., 2019).

Furthermore, the current study did not investigate the effects of Action Bitters on sperm quality or fertility, which are important endpoints in the evaluation of the reproductive toxicity of a substance. Therefore, future studies should investigate the effects of Action Bitters on sperm quality and fertility to provide a more comprehensive understanding of its reproductive toxicity.

In general, the results of this study suggest that Action Bitters may not have adverse effects on testicular histology at the doses and duration used. However, further studies are needed to investigate the effects of Action Bitters on sperm quality and fertility, as well as to determine the optimal dose and duration of administration for its use as a herbal remedy. Future research should aim to examine the impact of Action Bitters on sperm count, motility, and morphology, as well as its potential effects on fertility rates and reproductive outcomes. This will involve conducting randomized controlled trials and observational studies to gather more robust evidence on the effects of Action Bitters on male reproductive health.

IMPLICATIONS

The findings of this study provide preliminary evidence suggesting that moderate consumption of Action Bitters may not have a detrimental effect on male reproductive health, which is a crucial consideration given the widespread use of traditional remedies, particularly in developing countries where access to modern healthcare may be limited. This is a significant finding, as it suggests that Action Bitters may be a safe and effective option for men seeking to improve their overall health and well-being, without compromising their reproductive health. However, it is crucial to interpret these results cautiously, considering the limitations of the study.

LIMITATIONS

Several limitations of this study should be acknowledged. Firstly, the sample size was relatively small, which may have limited the statistical power to detect significant effects. Secondly, the duration of the study was relatively short, and further research is needed to assess the long-term effects or interactions with other substances. Thirdly, the study focused on a specific dosage range, and it is unclear whether higher or lower doses may have different effects. Further research with larger, more diverse populations and longer follow-up periods is necessary to confirm these findings and fully understand the implications of Action Bitters consumption on male reproductive health.

FUTURE DIRECTIONS

To further elucidate the impact of Action Bitters on male reproductive health, several avenues for future research are suggested:

1. Long-term Studies: Conducting long-term studies to assess the cumulative effects of Action Bitters consumption over extended periods.
2. Dose-Response Studies: Investigating the effects of different dosages of Action Bitters on various reproductive parameters.
3. Mechanistic Studies: Exploring the

underlying mechanisms of action of Action Bitters on the male reproductive system, including potential molecular, biochemical, and genetic effects.

4. Clinical Trials: Conducting randomized controlled trials to rigorously evaluate the efficacy and safety of Action Bitters in improving male reproductive health.
5. Multicenter Studies: Collaborating with multiple research institutions to increase sample size and enhance the generalizability of findings.
6. Exploration of Other Traditional Remedies: Investigating the impact of other traditional remedies, particularly those used for male reproductive health, on reproductive parameters.

CONCLUSION

Action Bitters is a popular herbal remedy that has been used for centuries in traditional medicine to treat various health conditions. However, the potential adverse effects of excessive consumption on male reproductive health remain understudied. This study investigated the histological effects of chronic Action Bitters consumption on the testis of male Wistar rats and provides valuable insights into the potential health risks associated with excessive consumption. The study also contributed to the growing body of knowledge on the effects of herbal remedies on male reproductive health and informs the development of evidence-based guidelines for the use of Action Bitters in traditional medicine.

While this study provides valuable insights into the potential effects of Action Bitters on male reproductive health, further research is necessary to establish a definitive conclusion. Future studies should address the limitations of this study and delve deeper into the mechanisms of action, long-term effects, and optimal dosage of Action Bitters. By conducting rigorous scientific investigations, we can gain a better understanding of the potential benefits and risks associated with the use of traditional remedies and make informed decisions about their incorporation into modern healthcare practices.

Availability of Data and Materials

The authors declare consent for all available data present in this study.

FUNDING

This research did not receive any grant from funding agencies in the public, commercial, or not-for-profit sectors.

Authors' Contributions

The entire study procedure was conducted with the involvement of all writers.

Competing Interests

The authors declare no conflicts of interest.

ACKNOWLEDGEMENTS

The authors would like to acknowledge the management and technical staff of PENKUP Research Institute, Birmingham, United Kingdom for their excellent assistance and for providing medical writing and editorial support in accordance with Good Publication Practice (GPP3) guidelines.

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