ORIGINAL ARTICLE

ABELMOSCHUS ESCULENTUS ADVERSE EFFECT ON THE SPLENIC CYTO-ARCHITECTURAL AND MORPHOLOGICAL INTEGRITY OF MALE WISTAR RATS

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ABSTRACT

Background:

Abelmoschus esculentus herbaceous hairy annual plant of the mallow family is widely consumed globally. High consumption of this plant may compromise the immune system, if it affects the functions of the spleen, whereas, the spleen is the main filter for blood-borne pathogens and antigens, as well as a key organ for iron metabolism and erythrocyte homeostasis.

Objective:

Our study was aimed at determining the possible toxicological effects of *Abelmoscus esculentus*on the spleen of adult Wistar rats.

Methods:

Twenty-one (21) adult Wistar rats weighing about 150-170g were used for this study and were divided into three (3) groups of 7 animals each (n=7). Groups (I-III) received; normal rat feed and water, a low dose of *Abelmoschus esculentus* 1.0mg/kg BW and a high dose of *Abelmoschus esculentus* 3.0mg/kg BW respectively. All extracts were given daily by the oral gavage method for fourteen (14) days. Twenty-four hours after the last administration, the animals were sacrificed by cervical dislocation. The spleen was harvested, weighed, and fixed in 10% buffer formalin, sectioned, and processed for microscopic studies.

Results:

Following the administration of *Abelmoschus esculentus*, there was a significant increase in the test group. Also, histological observation showed *Abelmoschus esculentus* could result in various grades of degenerative changes, while prolonged intake at high concentrations may cause adverse effects on the cells of the spleen which may alter the functionality of the spleen.

Conclusion: Hence, herbal remedies may help improve organ health, but can cause serious side effects if taken inappropriately.

Keywords: Spleen, *Abelmoschus esculentus, Toxicology, Wistar Rats*

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INTRODUCTION

Medicinal plants are involved in several types of herbal healing (herbology or herbal medicine) (1). The use of plants for medicinal purposes has gained popularity and the study includes the fruit, seed, stem bark, flower, leaf, stigma, or a root, as well as non-woody plants, trees, and shrubs (1). Plants synthesize hundreds of chemical compounds for functions including defense against insects, fungi, diseases, and herbivorous mammals. Numerous phytochemicals with potential or established biological activity have been identified. However, since a single plant contains many phytochemicals, the use of a whole plant as medicine may not be effective (2). In many African countries, traditional medicine is still the major source of healing and most of the drugs and cures come from natural resources (2). Globally, it has been reported that every year, the export value of fifty thousand to seventy thousand plants with medicinal properties was estimated to be 2.2 billion dollars in amount in 2012 and, subsequently in 2017, the potential global market for botanical extracts and medicines was estimated at several hundred billion dollars (2).

Vegetables with green leaves provide a high content of microelements, carotene, and ascorbic acids, which play significant roles in body metabolism and reduction in many known diseases (3). Alternative medicine is still recognized and preferred primary health care system in many African communities with above 60% of the world population and around 80% in developing countries depending directly on medicinal plants for their medical purposes (4).

Abelmoschusesculentus is an important vegetable, widely distributed around the world, this plant is commonly known as ladies finger, okra (4). The fibers were this lady's finger help to stabilize blood glucose levels; by regulating the rate at which sugar is absorbed from the intestinal tract. Studies have reported that polysaccharides found in okra possess so many antioxidants activities hepato- (5-8). Furthermore, it has been established that the richest part of the ladies' finger plant is the dried seeds (9). The ladies finger has many constituents, like oil which is consumable, and the residual meal after oil extraction is a rich source of protein, also, the bast fiber found on the stem of the plant has industrial uses (9), mucilage produced by the okra plant can be used for the removal of turbidity from wastewater because of its properties (10), having a composition similar to a thick polysaccharide film, okra mucilage serves as a biodegradable food packaging (9). Abelmoschusesculentus (Okra) is said to have many health benefits, however, its adverse effect on immune integrity has not been evaluated.

The spleen is the largest lymphoid organ of the body and plays a key role in body fluid balance and metabolism (11). It is located in the upper left quadrant of the abdomen, which is left to the stomach (11). The spleen has varying sizes and shapes between different races and plays important supportive roles in the body. The spleen is a soft organ that generally looks purple and it is made up of two different types of tissues. These tissues include red pulp tissue that filters the blood and gets rid of old, damaged red cells and the white pulp tissue which contains the immune cells (i.e. T cells and B cells) (11). The spleen generally has several functions which include also serving filter of blood cells. Old red blood cells recycle, while the platelet, which are fragments of megakaryocytes and the leucocyte are stored inside them (11). The spleen is specific in its fight against certain bacteria. Damage to the spleen, may lead to blood disorders such as idiopathic thrombocytopenic purpura (ITP), thalassemia, hemolytic anemia, sickle cell anemia), and cancer such as lymphoma, Hodgkin disease, and leukemia (11).

MATERIAL AND METHODS

Sample Collection and Extract Preparation

Fresh pods of okra (Abelmoschusesculentus) were gotten from the local market in Okuku, Yala Local Government Area of Cross River State, Nigeria. After collection, the pods were washed to clean up debris, sliced, and dried at room temperature at 270C. After which they were blended and kept in an air-tight container before extraction.

The okra powder was dispensed in 10,000 m/s of distilled water in a plastic container (distilled container).

Then the mixture was vigorously stirred intermittently with a stick and allowed to stand for 24 hours before it was filtered with a cloth sieve. The filtrate was evaporated at 500C with a water bath to obtain the crude solid extract for one month and the extract obtained was stored in a refrigerator.

Experimental Design

Twenty-one (21) adult male Wistar rats were used for this study. The animals were distributed into three (3) groups, seven (7) animals for each group (3n=7). The animals were housed in a plastic cage under a controlled light schedule (12 hours of light & 12 hours of the dark cycle) and were fed with standards growers' food and water and weighed prior to the experiments. Experimental protocols complied with the guideline for animal research, as stated in the NIH Guidelines for the Care and Use of Laboratory Animals (12).

The three (3) groups of animals include control, low dose, and high dose (group A-C) according to their weight respectively. Group A-C animals received water and food only, Low dose of aqueous extract of Abelmoschus esculentus at 1.0ml/kg BW, and a High dose of aqueous extract of Abelmoschus esculentus at 3.0ml/kg BW. At the end of the two (2) weeks period of administration, of the aqueous extract of Abelmoschus esculentus, animals in all groups were sacrificed a day after the end of the administration by cervical dislocation. The spleen of animals from each group was excised and washed with 5% sucrose solution.

Histological Studies

The tissues were preserved and labeled in bottles containing 10% buffered formalin. They were allowed to stand for 72 hours to achieve good tissue penetration and effective fixation. After these, they were placed in ascending grades of ethanol for dehydration. First, they were treated with two changes of 70% ethanol each lasting for one (1) hour followed by 95% ethanol and then absolute alcohol for the same duration. Following dehydration, the tissues were cleared in three changes of xylene each lasting for thirty (30) minutes. Impregnation in molten paraffin wax at 580C was carried out overnight and the following morning, the tissues were embedded in wax to form blocks. These tissue blocks were trimmed and sectioned using a rotary microtome. The sectioning was floated in warm water (280C) and taken up on albuminized glass slides. They were air-dried and stained using Haematoxylin and Eosin.

Tissue processing

Tissue blocks were sectioned at 4µ with a rotary microtome and dewaxed in xylene for two (2) minutes per two changes. The xylene was cleared in 95% alcohol for one (1) minute per two (2) changes and 70% alcohol for another minute. The sections were then hydrated in running tap water until sections turned blue. They were thereafter counterstained with 1% alcohol eosin for one (1) minute, followed by rapid dehydration through ascending grades of alcohol, after which they have cleared in xylene again and mounted with DPX mutant. The stained sections were viewed under a light microscope.

Morphological Studies

All the animals were weighed using sensitive weighing balance before and after the administration of Parkiabiglobosa to ascertain the morphological observation.

Statistical Analysis

Statistical analysis was done using Statistical Package for Social Sciences (SPSS) Version 16. Also, a one-way analysis of variance, followed by Bonferroni's Multiple Data Comparison test was used. Results of descriptive statistics of the experimental data were presented as the mean and standard error of the mean (Mean ± SEM). While paired sample T-test was considered statistically significant at $P<0.05$.

RESULTS

Histological Observations

The histological result of the study shows that the control animals revealed a normal architecture with red pulp and white pulp, with no pathological observation (fig.1).

Administration of Abelmoschusesculentus at a low dose of 1.0mg/kg BW shows increased cellularity of

interfollicular tissue in the red pulp area with a prognosis of metastatic and primary vascular loss (circled area) (fig.1) Photomicrograph of spleen of albino Wistar rats in the high dose group given 3.0mg/kg BW of Abelmoschusesculentus shows interfollicular tissue in the red pulp area, with a prognosis of metastatic and primary vascular loss (circled area) (fig. 1).

Morphological Observations

Morphological observation from the study shows an observable significant ($p<0.05$) increase in the final mean body weight when compared with the initial body weight observable in control vs low dose, control vs high dose, and low dose vs control but not observable in low dose vs high dose and high dose vs control dose. The final body weight of the control animals (133.9 ± 7.058) was significantly ($p<0.05$) higher than its initial body weight (111.4 ± 8.162). However, the mean final bodyweight of the low dose group (142.3 ± 4.716) and high dose group (145.7 ± 4.786) were significantly ($p<0.05$) higher than their initial body weights (128.0 ± 6.856) and (130.3 ± 6.157) respectively.

DISCUSSION

Plants synthesize hundreds of chemical compounds for functions including defense against foreign intruders. Numerous phytochemicals with potential biological activity have been identified in these plants. However, since a single plant contains many phytochemicals and using a whole plant for medicine is not advisable (2).

Histological studies

The histological findings of the control group show no pathology while low dose and high dose revealed degeneration with increased cellularity of inter-follicular tissue in the red pulp area. The red pulp area is the site of the spleen that plays a primary function in the filtration of the blood of antigens, microorganism, and defective or worn-out red blood cells, and also act as a reservoir for monocytes. Which are found in clusters in the red pulp cords that can be rapidly mobilized to leave the spleen and assist in tackling ongoing infections. However, in the case of infection or damage to the spleen in form of focal hyperplasia of the red pulp has been reported in aging Fischer rats (14). The lesion is typically a solitary, well-demarcated nodule protruding from the spleen. Also, focal White Pulp is seen as a well-demarcated, focal expansile lesion that occasionally occurs in the F344 rat as a spontaneous lesion (14).

Morphological Observation

The morphological observation from this study shows an observable significant increase ($p<0.05$), in the final body weight when compared with the initial body weight observable in control vs low dose, high dose but not observable in low dose vs high dose, and high dose vs control. The final body weight of the animal was significantly higher than its initial body weight. However, the mean final bodyweight of the low dose group and high dose group were significantly higher than their initial body weight at ($p< 0.05$) respectively.

Hence, an increase in the final body weight could be as a result of the nutritional value of hibiscus esculentus and its high fibers which help to stabilize blood sugar by regulating the rate at which sugar is reabsorbed from the intestinal tract, and its polysaccharide possesses, which hepato-protective, antidiabetic, anticancer, anti-inflammatory, laxative, antihyperlipidemic, antifungal and analgesic activities (5,8). The mucilage produced by the okra plant can be used for the remover of turbidity in wastewater, by virtue of its flocculent properties (3) having a composition similar to a thick polysaccharide film. Okra mucilage is under development as a biodegradable food packing (9). Interestingly, spleen enlargement which is the cause of increased body weight is commonly seen in tumor cell transplantable models (15).

Conclusion

The histological and morphological observation suggests that aqueous extract of Abelmoschus esculentus showed various serious degenerative changes and prolonged intake in high concentration may cause adverse effects on the cells of the spleen which may alter the functionality of the spleen. Therefore, herbal is known to improve organ health, although can cause serious side effects if taken inappropriately.

References

1. Mahta A. Introduction and importance of medicinal plants and herbs. National Health Portal India; 2016.[Internet][[cited 2019 Jul 24]].
2. Ahn K. The worldwide trend of using botanical drugs and strategies for developing global drugs. BMB Rep. 2017 Mar;50(3):111–6.
3. Olaiya C, Adebisi J. Phyto-evaluation of the nutritional values often green leafy vegetables in South-Western Nigeria. Internet J Nutr Wellness. 2010;9(2):1–5.
4. Susan O, Tonny A, Maryann A, Peter A, Daniel B, Dorris K, et al. Medicinal plants for Treatment of Prevalent Diseases. Pharmacognosy-Medicinal Plants; 2019. pp. 1–19.
5. Hu L, Yu W, Li Y, Prasad N, Tang Z. Antioxidant activity of extract and its major constituents from okra seed on rat hepatocytes injured by carbon tetrachloride. BioMed Res Int. 2014; Special Issue:1–9.
6. Sabitha V, Ramachandran S, Naveen KR, Panneerselvam K. Antidiabetic and antihyperlipidemic potential of Abelmoschus esculentus (L.) Moench. in streptozotocin-induced diabetic rats. J Pharm Bioallied Sci. 2011 Jul;3(3):397–402.
7. Olorunnipa T, Igbokwe C, Lawal T, Adeniyi B, Mahady G. Anti-helicobacterpylori activity of *Abelmoschus esculentus L.moench* (okra): an invitro study. Afr J Pure App Chem. 2013;7(9):330–6.
8. 8. Saha D, Jain B, Jain VK. Phytochemical evaluation and characterization of hypoglycemic activity of various extract of Abelmoschus esculentus Linn.Fruit. Int. J. Pharm Sci 2011; 183–5.
9. De W, Tairou F, Van A. Reduction in neural tube defect safer folic fortificationin Canada. N Engl J Med. 2007;357(2):135–42.
10. Konstantinos A, Dimitrose K, Evan D. Flocculation behavior of mallow and okra mucilage in treating waste water. Desalination. 2009;249(2):786–91.
11. Chaudhry SR, Luskin V, Panuganti KK. Anatomy, Abdomen and Pelvis, Spleen. Stat Pearls. Treasure Island (FL): Stat Pearls Publishing; 2021.
12. Guide for the care and use of laboratory animals, 2011. 8th ed. Retrieved from http://www.google.com.ng/?gfe_rd=cr&ei=KWkSVvy7MtPH8gehuLvQDg#q=nih+guidelines+animal+care. [Accessed: October 05, 2015].
13. Panadda K, Walairorn T, Noppaku P, Maitree S, Piyanete C. Antioxidative activities and phenolic content of extracts from Okra (*Abelmoschus esculentus L*. Res J Biol Sci. 2010;5(4):310–3.
14. Suttie AW. Histopathology of the spleen. Toxicol Pathol. 2006;34(5):466–503.
15. Gabrilovich DI, Ostrand-Rosenberg S, Bronte V. Coordinated regulation of myeloid cells by tumours. Nat Rev Immunol. 2012 Mar;12(4):253–68.

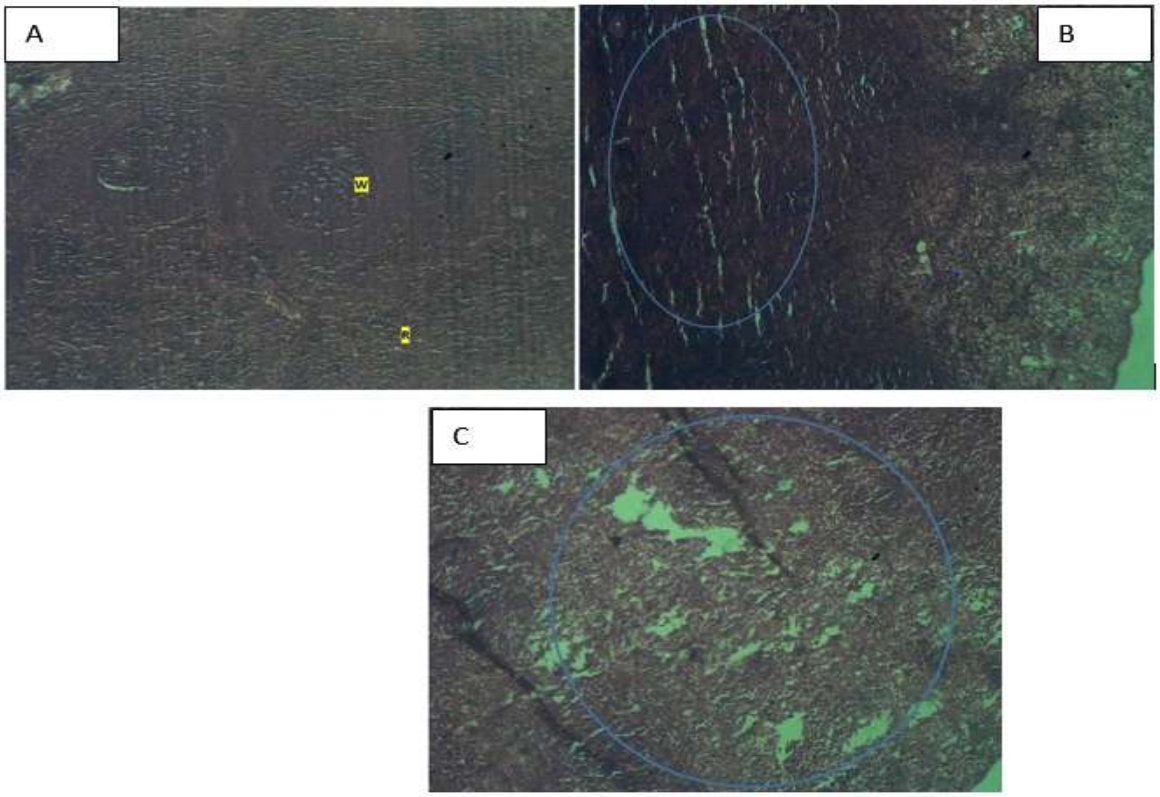

Figure 1: photomicrographs of the spleen A. Control of spleen showing normal architecture with the red pulp and the white pulp. B. Low dose of spleen showing an increased cellularity of interfollicular tissue in the red pulp area (circled area). C. High dose of spleen showing interfollicular tissue in the red pulp area (circled area). H & E X 40

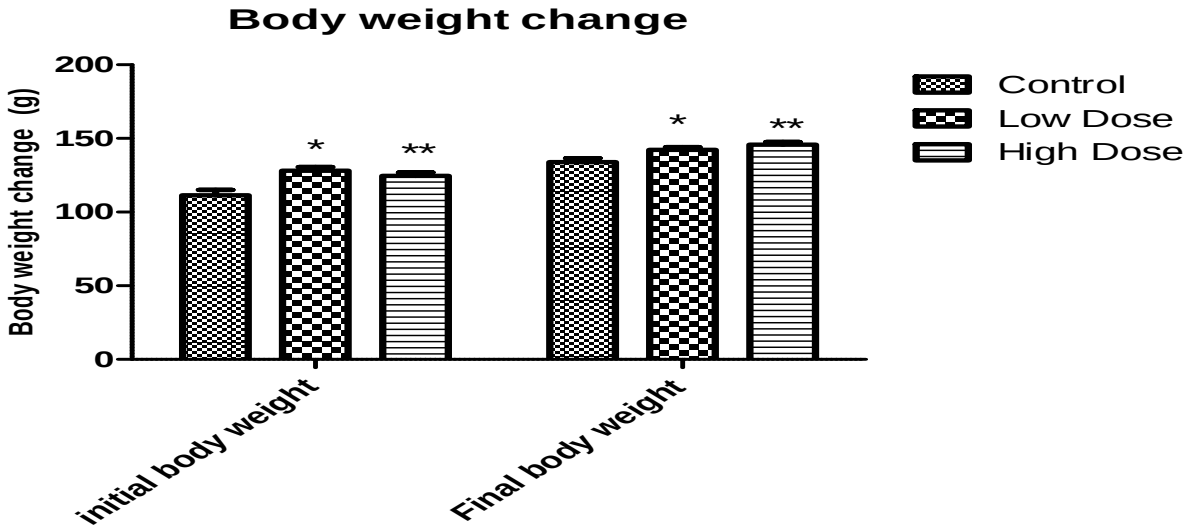

Figure 2: showing effect of daily administration of
Values are expressed in Mean +SEM, n=7, p<0.05
**=P<0.05 vs control