

**The Effect of Aqueous Solution of *Hibiscus sabdariffa* (Zobo Drink)
on Albino Wistar Rats Ovarian Tissue**

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Abstract

Hibiscus sabdariffa is a flowering plant that grows in tropical regions. It is used in the preparation of a non-alcoholic beverage called Zobo drink. This current study investigated the ovarian histological changes and hormonal level outcome that occur after administration of Hibiscus sabdariffa aqueous solution (Zobo drink) on albino Wistar rat. Twenty (20) female Wistar rats were purchased and divided into five (5) groups, group A, group B, group C, group D and group E with each group having four (4) Wistar rats. Group A, the control group was unexposed to any form of foreign substance. Group B were given 0.5ml of Zobo drink once daily (morning). Group C were given 0.5ml of Zobo drink twice daily (morning and afternoon). Group D were given 0.5ml of Zobo drink three times daily (morning, afternoon and evening). Group E were given the Zobo drink concurrently all through the day and water only at night. In the end, the rats were sacrificed and the ovaries were removed and fixed in 10% formalin saline solution. The ovaries were stained with hematoxylin and eosin. Their blood was collected through the femoral

artery for hormonal level assay. The histological result of group A, B, C and D showed normal histological structures. Group E showed hemorrhage in the corpus luteum of the ovaries. The result of the hormonal analysis showed an increase in the level of progesterone, follicle stimulating hormone and luteinizing hormone and decrease in the level of estrogen all through the five groups. This lower estrogen level could impede menstruation. Therefore, Zobo drink can damage the ovary if taken in excess quantity and cause fluctuation in hormonal level of the ovary.

Keywords: Hibiscus sabdariffa; Zobo drink; Antioxidants; Ovarian histology

INTRODUCTION

Hibiscus sabdariffa, also known as roselle, is a plant species originating from tropical regions. with well-documented medicinal properties that has been traditionally used in many cultures around the world. This plant is typically produced as an annual crop in more temperate climates, where it can grow up to two or three meters in height.^[2] There are two main varieties of *Hibiscus sabdariffa*. The first, *Hibiscus sabdariffa* var. *altissima* Wester and the second *Hibiscus sabdariffa* L. ^{[4],[6]} It is grown in some part of the world is a home garden crop.^[3] In Nigeria, it is used to produce a locally made non-alcoholic beverage known as Zobo. The term "Zobo" is a Hausa word which is one of the primary languages spoken in Nigeria. The Yoruba refer to it as "isápá.". while the Igbo people refer to it as "ojō" or "òkwór'À-ozo". Zobo drink is made from the dried calyxes of the *Hibiscus sabdariffa*.^[5] Throughout the world, it is referred to by a number of names including hibiscus tea, sorrel tea, sour tea, agua de Jamaica, bissap, karkade, and Sudanese tea. It is mostly served during occasions as a refreshing drink. Roselle calyx extract is a rich source of antioxidants, particularly anthocyanins, and has been associated with antitumor properties and inhibitory effects on the growth of several cancer cells.^[1]

MATERIALS AND METHODS

Twenty neonatal albino Wistar rats were purchased from national Veterinary Research Institution, Vom, Plateau State and were used for the experiment. Plastic cages with iron wire netting, saw dust, animal feed, latex laboratory gloves, weighing balance, dissecting set and board, measuring cylinder, sample bottles, cotton wool, glass slides and slide rack, cover slips, light microscope and microtome. The animals were grouped into five (5)

groups. Group A (control) were not given Zobo drink, Group B (Morning) were given 0.5ml of Zobo drink once daily in the morning. Group C (Morning and afternoon) were given 0.5ml of Zobo drink twice daily in the morning and afternoon. Group D (Morning, afternoon and evening) were given 0.5ml of Zobo drink three times a day. Group E (Concurrently) were given Zobo drink from morning to evening and were given water in the evening.

Animal Sacrifice and Tissue Collection

At the end of the experimental administration, the following meticulous procedures were followed to ensure accurate data collection and meaningful insights:

Sacrifice Method: Chloroform Inhalation

The animals were humanely sacrificed using the chloroform inhalation method.

This approach ensures a quick and painless transition for the animals.

The use of a closed chamber minimizes stress and discomfort.

Organ Dissection and Fixation:

Following an abdominal incision, the required organs were carefully dissected out.

These organs were then fixed in 10% formal saline fluid to preserve the tissues and allow for subsequent histological studies.

Ovarian Handling:

The two ovaries were also dissected and suspended in 0.9% normal saline.

Proper handling ensures the integrity of ovarian tissue for further analysis.

Blood Collection and Serum Extraction:

The heart was exposed, and a cardiac puncture was performed using a needle and syringe.

Blood was collected into properly labeled sample bottles for each group.

After centrifugation at 3000 revolutions for 15 minutes, serum was carefully extracted using a micro-pipette.

This serum was then used for hormonal assay which provided critical insights into the study.

RESULTS

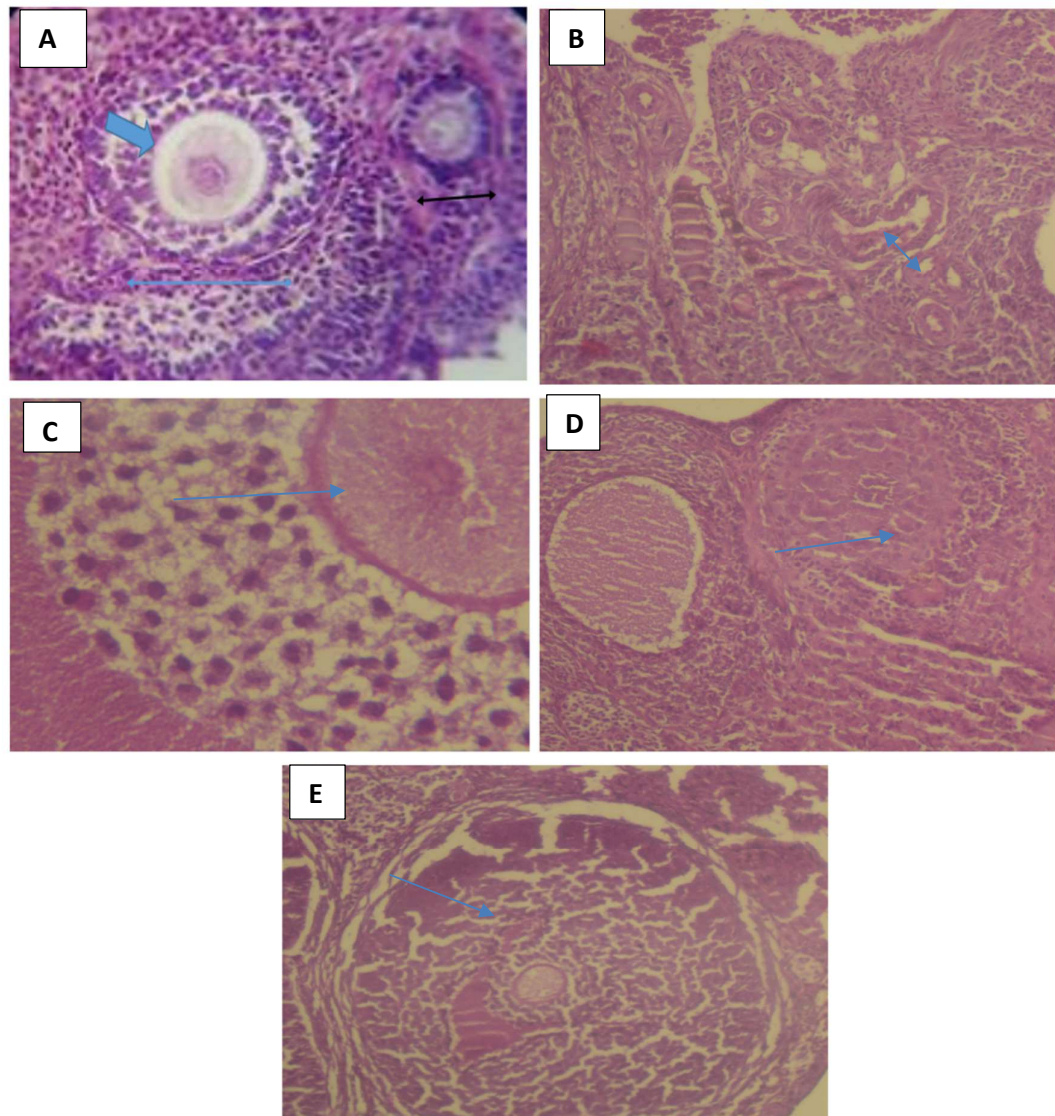


Figure 1: Photomicrograph (A) shows a transverse section of the histology of the ovary of the control group showing ovarian follicles (blue and black line), maturing oocyte cell (blue arrow) and intact stroma. Photomicrograph (B) shows the transverse section of the histology of the ovary of the group given Zobo drink once daily (morning) with the histology showing ovarian follicles at different stages (blue arrow) and intact stroma. Photomicrograph (C) shows the transverse section of the histology of the ovary of the group given Zobo drink twice daily (morning and afternoon) with the histology showing a fully matured corpus luteum (blue arrow). Photomicrograph (D) shows the transverse section of the histology of the ovary of the group given Zobo drink three times a day

(morning, afternoon and evening) with the histology showing a normal ovarian follicle (blue arrow). Photomicrograph (**E**) shows the transverse section of the histology of the ovary of the group given Zobo drink concurrently with the histology showing blood in the corpus luteum (blue arrow).

Hormonal analysis

Luteinizing hormone: Luteinizing hormone level showed a progressive increment throughout all the groups with Group E having the highest level of increase.

Follicle stimulating hormone: follicle stimulating hormone level showed a gradual increment from Group B to Group D.

Estrogen: estrogen level showed a gradual decrease from Group B to Group D.

Progesterone: progesterone level showed a gradual increment from Group A to group D.

DISCUSSION

The experimental results revealed intriguing insights into the impact of Zobo drink on ovarian tissue. The key findings were;

1. Histological changes: Groups B to D (administered 0.5 ml of Zobo drink) showed no significant histological changes in the ovaries. However, Group E exhibited hemorrhage in the corpus luteum, suggesting that frequent Zobo drink consumption may lead to this specific alteration.

2. Hormonal Assay Results: Across all groups, there was an increase in progesterone, follicle-stimulating hormone (FSH), and luteinizing hormone (LH) levels. Conversely, estrogen levels decreased.

CONCLUSION

The regular Zobo drink intake can impact ovarian histology and hormonal balance. The observed changes may disrupt the normal menstrual cycle in females. As we continue to explore the effects of herbal medicinal beverages, understanding their implications on reproductive health remains crucial.

Limitation: Specific research on Zobo drink itself is limited, therefore we can draw insights from related studies on herbal extracts and their impact on reproductive health.

Recommendations : Some documented plausible mechanisms of Zobo drink worthy of note;

☑ Phytochemical composition: Zobo drink contains various phytochemicals, including flavonoids, polyphenols, and anthocyanins. These compounds may interact with cellular receptors, enzymes, and signaling pathways in the ovaries.

☑ Antioxidant Properties: Zobo drink's antioxidant activity could protect ovarian tissue from oxidative stress. This oxidative stress contributes to tissue damage and inflammation, affecting overall ovarian health.

☑ Anti-Inflammatory Effects: Some components in Zobo may exhibit anti-inflammatory properties. It is known that chronic inflammation can disrupt ovarian function and tissue integrity.

☑ Hormonal Modulation: Zobo drink might influence hormone levels, including estrogen, progesterone, and gonadotropins (FSH and LH). These hormonal changes could impact ovarian follicle development and ovulation.

☑ Vascular Effects: Zobo's bioactive compounds may affect blood vessels within the ovaries. And it well recognized that proper blood flow is crucial for nutrient supply and waste removal in ovarian tissue.

☑ Metabolic Pathways: Zobo drink could alter metabolic pathways related to glucose regulation and lipid metabolism. Dysregulated metabolism may indirectly affect ovarian health.

☑ Immunomodulation: Components in Zobo may modulate the immune response within the ovaries and immune balance is essential for tissue homeostasis.

☑ Direct Cellular Effects: Zobo's compounds may directly interact with ovarian cells, affecting cell proliferation, apoptosis, and differentiation.

☑ Microbiome Influence: Gut microbiota play a role in metabolizing dietary compounds. Zobo drink consumption might indirectly impact ovarian health via gut-microbiome interactions.

☑ Individual Variability: Responses to Zobo drink can vary based on individual genetics, health status, and lifestyle factors. In summary, while we don't have specific data on Zobo drink's effects on ovarian tissue, these potential mechanisms provide a framework for further investigation. Future research should explore Zobo's impact on ovarian histology,

hormone levels, and overall reproductive health. Remember that individual responses may differ, and caution is essential when interpreting herbal effects.

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