

GORDON DRY GIN (MORINGA CITRUS BLEND) INDUCE ADENOMATOUS HYPERPLASIA IN FEMALE SPRAGUE DAWLEY RATS

ABSTRACT

Objectives; This study aim to investigate the histopathological effects of binge consumed Gordon's dry gin moringa citrus blend on the uterus of adult female wistar rats.

Materials; Fifty outbred sprague dawley rats (~~fifty females~~) weighing between $120\text{g}\pm 2.6\text{-}250\text{g}\pm 3.5$ were divided into four groups. Group A, B and C is the test groups while Group D is control group. Rats in group A received 0.3ml of Gordon's dry gin moringa citrus blend, morning and evening orally vusing orogastric tube. Animals in group B received 0.3ml of 43% ethanol, morning and evening orally via orogastric route using orogastric tube. Group C were administered with 0.3ml of 200mg/kg Moringa extract, morning and evening orally via orogastric route using orogastric tube. Five (5) animals each from group A, B and C was sacrificed on the 7th, 14th and 21st day of administration. Group D served as control and was sacrificed at the end of the study of which the organ of interest, uterus was harvested, processed and stained with haematoxylin and eosin protocol for histological studies. **Result;** Results of the study revealed histological alterations in the uterus in the treatment groups. Such alterations include adenomatous hyperplasia and cystic hyperplasia.

Conclusion; Binge consumption of Gordon's dry gin moringa citrus blend from this study induces cystic and adenomatous hyperplasia which may lead to infertility and endometrial cancer.

Comment [L1]: Remove the extra space

Comment [L2]: "Wistar" should start from capital letter

Comment [L3]: Replace by "Fifty outbred female Sprague Dawley rats"

Comment [L4]: Replace by " $120\pm 2.6\text{ g-}250\pm 3.5\text{g}$ ". This was already mentioned by the reviewer in previous comments, but the authors have chosen to ignore.

Comment [L5]: The authors cite three groups but use the singular "is".

Comment [L6]: Rewrite as follows: "Fifty female rats (weighing range: $120\pm 2.6\text{-}250\pm 3.5\text{ g}$) were divided into four groups (A, B, C & D). Group A, B, and C (15 animals each) were orally administered with Gordon's dry gin moringa citrus blend, 43% ethanol, and 200mg/kg moringa extract, respectively, while Group D (5 animals) did not receive any treatment and was used as negative control. In groups A, B, and C, five animals were sacrificed on the 7th, 14th and 21st day of administration, whereas animals of group D were sacrificed on day 21. The uterus of each rat was harvested, processed and stained with haematoxylin and eosin solution for histological analyses."

Comment [L7]: Replace by "Results of the study revealed histological alterations in the uterus of treated animals."

Comment [L8]: Replace by "The oral administration of gordon's dry gin moringa citrus blend and moringa extract at 200 mg/kg induced cystic and adenomatous hyperplasia in rat uterus, inferring that moringa might yield antifertility activity."

Keywords: Uterus, Adenomatous, Hyperplasia, Binge, Infertility, Cancer, Alcohol, Rats.

INTRODUCTION

The adverse effects of alcohol is common to both male and female, however, evidence suggests that many of these effects pose a greater risk to women's physical health at lower consumption levels than men¹. Consumption of alcoholic drinks by women is common worldwide but its use in pregnancy has become a major public health problem². In USA, alcohol use by pregnant women with rates up to 16.2% have been reported annually while that of non-pregnant women is as high as 56.3%³. The rates of alcohol use by African women are on the increase ranging from 1% to 30% for current use drinkers and 4% to 41% for heavy drinkers⁴.

A "binge" is a pattern of drinking alcohol that brings blood alcohol concentration to 0.08 gram percent or above. For adults this pattern corresponds to consuming 5 or more drinks (male), or 4 or more drinks (female), in about 2 hours. Binge drinking is clearly dangerous for the drinker and for society⁵. This definition achieved three main goals. First, it quantified the definition of a binge by anchoring it to an indicator of the dose ingested. Second, it recognize that circumstances like gender other than simply the "number of drinks" could influence blood alcohol concentration. Thirdly, it established a de facto threshold for intoxication (80 mg %), implying that observable behavioral intoxication is a

Comment [L9]: Refer to this site "<http://www.journaliammr.com/index.php/JAMMR/about/submissions#authorGuidelines>" to prepare the manuscript. The numbers referring the references in the text should be in parentheses [].

Comment [L10]: Same remark as the previous.

Comment [L11]: Replace by "1 to 20%"

Comment [L12]: Replace by "4 to 41%"

Comment [L13]: What do the authors mean by gram percent? Is this the unit of concentration?

Comment [L14]: There should be a comma here.

Comment [L15]: Reduce the space.

Comment [L16]: There is a comma missing here.

Comment [L17]: Is this the concentration of alcohol provided according to reference [5]? This reference [5] talk about 80 mg/dL or 0.08 g/dL. How come the authors highlighted as 80 mg%? Please be consistent.

defining characteristic of binge drinking. By stating the approximate real-life number of standard drinks, it provided researchers and treatment professionals a route to approximately quantify this type of drinking. This has come to be termed “the 5/4 rule”⁵. This definition was subsequently adopted by the Centers for Disease Control and Prevention of the USA. This study is of great importance because the effect of binge drinking of alcohol to the individual and to society is clear. There are immediate risks such as injury, driving accidents, unwanted pregnancy, infertility and death. Therefore this study will help create an awareness especially for females on the effects of consuming alcoholic beverages. It will also help policy makers to formulate policies on alcohol consumption. The aim of this study is to investigate the histopathologic effects of binge consumption of Gordon’s dry gin moringa citrus blend on the uterus of adult female Wistar rats.

Comment [L18]: Instead of providing this explanation, the authors would have highlighted some previous in vitro or preclinical (in vivo) studies related to binge drinking.

Comment [L19]: This was already mentioned in previous reviewer’s comments but the authors decided to ignore. What is the motivation for the authors to study the histopathological effects of Moringa. Are there reports showing that this type of study help to conclude about the activity on fertility, pregnancy etc... Why the authors have chosen to carry out their study on uterus? And not any other organ of the reproductive system? The authors should highlight some previous in vitro or preclinical (in vivo) studies related to the effects of binge drinking.

Comment [L20]: Omit ‘an’

Comment [L21]: Replace by “women”

MATERIALS AND METHODS

Location of Study

This study was carried out in the Department of Medical Laboratory Science, Faculty of Basic Medical Sciences, College of Health Sciences, Niger Delta University, Wilberforce Island, Amassoma, Bayelsa state of Nigeria.

Procurement of Guinness Gordon’s Moringa Citrus Blend

The Gordon’s Dry Gin Moringa Citrus blend is produced by GUINNESS NIGERIA PLC, OBA AKRAN AVENUE, IKEJA, LAGOS, NIGERIA with NAFDAC Reg No.: 08-3821 and batch number L7287ZI002 and was bought at Yenagoa and transported to the Niger Delta University, Bayelsa where it was stored in a refrigerator at $4 \pm 2^{\circ}\text{C}$. Also fresh moringa leaves were harvested and air dried at room temperature for 3 days. The dried leaves was meshed in a mortar in the laboratory and ethanolic extraction using the soxhlet extractor was done to collect the active substance

Comment [L22]: There is a comma missing here.

Comment [L23]: Replace by “were”

Comment [L24]: Omit “in the laboratory”

Comment [L25]: What concentration of ethanol? 95%? 70%?

Comment [L26]: Replace by “was carried out”

Comment [L27]: Replace by “to afford the ethanol extract”

Animal Housing

Fifty outbred sprague dawley rats (fifty females) weighing between 120g±2.6-250g±3.5 were used for this study. These animals were obtained from the Animal House of the Pharmacology Department of Niger Delta University, of Bayelsa, Nigeria. They were housed under standard condition of temperature (27 ± 2°C) with twelve hours light/dark periodicity. These animals were housed in clean gauzed cages in groups and fed on standard feed pellet (Guinea feed® Nigeria Plc) and clean water *ad libitum* throughout the duration of the study. Acclimatization was for two weeks. Animals were handled in this study according to institutions guidelines for experiments involving the use of animals.

Experimental Design

The animals were weighed and divided into four groups. The duration of this study was for thirty five (35) days, the animals were allowed to acclimatize for fourteen (14) days. Administration of Gordon's Dry Gin Moringa Citrus Blend was commenced immediately after acclimatization in a binge manner for twenty one (21) days. Groups A, B & C served as the test groups while Group D served as control group. Rats in group A received 0.3ml of Gordon's Dry Gin Moringa Citrus Blend, morning and evening orally via orogastric route using orogastric tube. Animals in group B received 0.3ml of 43% ethanol, morning and evening orally via orogastric route using orogastric tube. Group C were administered with 0.3ml of 200mg/kg Moringa extract, morning and evening orally via orogastric route using orogastric tube. Five (5) animals each from group A, B and C was sacrificed on the 7th, 14th and 21st day of administration. Group D served as controls and was sacrificed at the end of the study.

Comment [L28]: Replace by "Sprague Dawley"

Comment [L29]: This repetition is not needed and this was already mentioned in previous comments by the reviewer. Do mention the age of the rats instead.

Comment [L30]: Should be written as follows: 120±2.6 g - 250±3.5 g. This was already mentioned in the previous comments sent to the authors.

Comment [L31]: Why is animal house starting from cap lock letters?

Comment [L32]:

Comment [L33]: There is no word or sentence approximate. As already mentioned in previous comments, there is a word missing in this sentence. "The acclimatization was carried out during two weeks" OR "The acclimatization was retained for two weeks" OR something similar.

Comment [L34]: Replace by "institutional" or something similar.

Comment [L35]: Replace by "twice a day"

Comment [L36]: Omit "via orogastric route" to avoid repetition

Comment [L37]: Omit "via orogastric route" to avoid repetition.

Comment [L38]: Same remark as previous

Comment [L39]: Five animals "were"

Comment [L40]: Replace by "control" because it is one group that serve as control.

Table 1: Experiment Layout

	GROUP A (GORDON DRY GIN)	GROUP B (43% ALCOHOL) POSITIVE CONTROL	GROUP C (MORINGA)	GROUP D (CONTROL)NEG ATIVE CONTROL
DAY 7	5	5	5	5
DAY 14	5	5	5	
DAY 21	5	5	5	

Histological processing

The (uterus) was cut in slabs of about 0.5µm thick and fixed in 10% formal saline. The organ processed according to the paraffin wax embedding method using an Automatic Tissue Processor. Sections of 5µm thick were obtained using the Rotary Microtome (Heitz 150 Rotary Microtome, Cambridge model). The sections were stained routinely using Haematoxylin and eosin staining technique¹⁶.Statiscal analysis was done using the graph pad software and the results presented as graphs and bars.

Microscopy and Photomicrography

The sections were examined using Olympus binocular microscope with in-built lighting system. The sections were then photomicrographed using a digital microscope camera (Samsung Model SS850) attached to an Olympus trinocular microscope.

Comment [L41]: Replace by "The uterus of each rat' OR something similar.

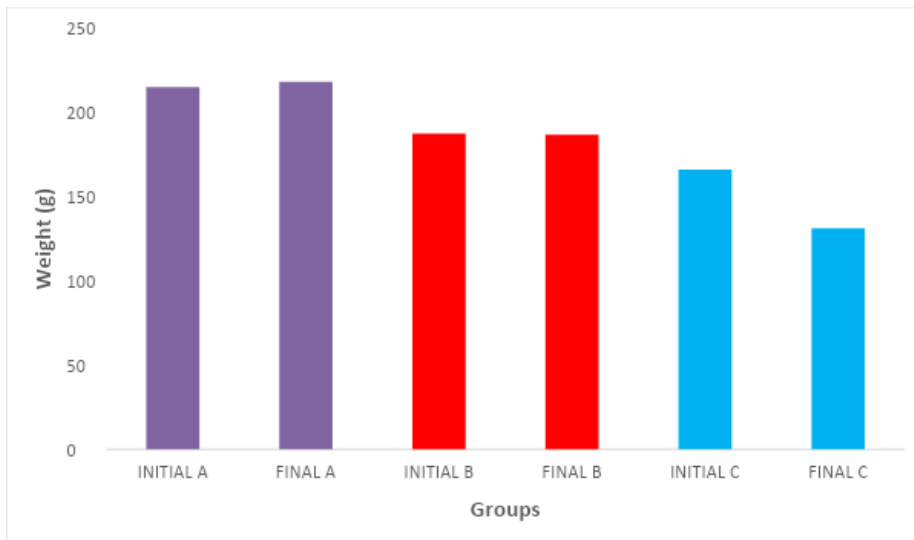
Comment [L42]: "was"

Comment [L43]: Reduce the space

Results

Weight of animals

Figure I, II and III are bar chart showing the comparison of initial and final weights of animals after 7, 14 and 21 days of treatment with Gordon dry gin moringa citrus blend, 43% ethanol and moringa.



Comment [L44]: Did the authors perform the statistical analyses? This is found nowhere in this manuscript. This was already mentioned in comments to the authors. The significance of the results should be mentioned on the bars of figures I, II, and III. Generally, number of stars (*, **, ***) or letters (a, b, c ...) are used to show the significance of the results after performing statistical analyses.

Figure I: Comparison of Initial and Final weight after 7 days of treatment

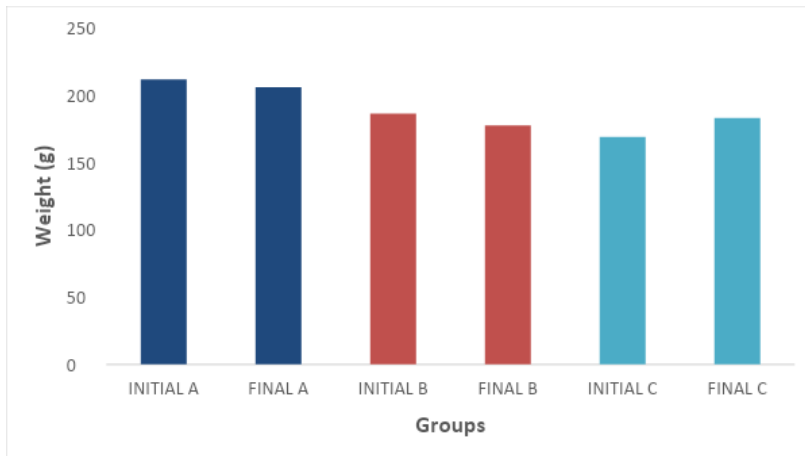


Figure II: Comparison of Initial and Final weight after 14 days of treatment

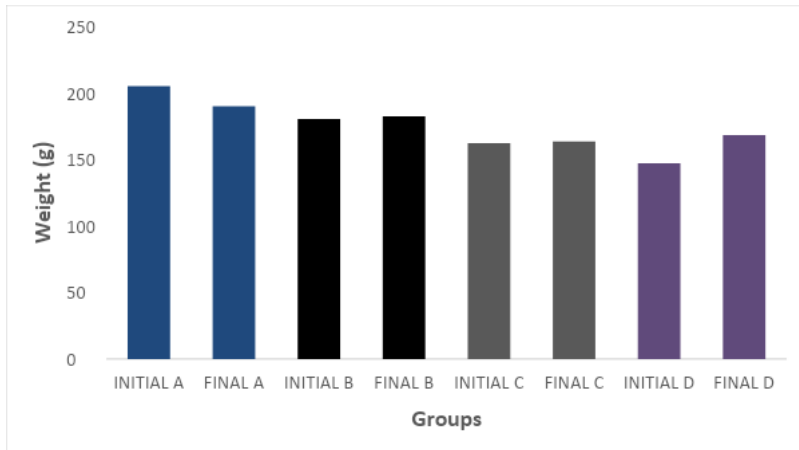


Figure III: Comparison of Initial and Final weights after 21 days of treatment

Histological slides

The histological photomicrograph of the uterus of the adult dawley sprague rat,

Plate 1 shows the morphology of the uterus after treatment with Gordon's dry gin moringa citrus blend, 43% Alcohol, and 200mg/kg Moringa for seven (7) days and exposed to normal feeds. The endometrial glands show cystic hyperplasia in the slide labelled moringa, citrus blend and alcohol, compared with normal morphology of the uterus in the slide labelled normal.

PLATE 2, shows the endometrial glands which show cystic hyperplasia in the slide labelled Moringa and adenomatous hyperplasia in the slide labelled alcohol and citrus blend after administration for 14 days while the slide labelled normal shows normal histology of the uterus.

Plate 3, shows the morphology of the uterus after treatment with Gordon's dry gin moringa citrus blend, 43% Alcohol, and 200mg/kg Moringa for 21 days and

Comment [L45]: Query regarding statistical analyses was already raised in previous comments by the reviewer. The significance of the results should be mentioned on the bar graphs. Generally this is mentioned by using stars "****" or letters "a, b, c etc..". Results from figures/graphs cannot be published in this form.

Comment [L46]: As already mentioned in previous comments and ignored by the authors, this statement is unclear. Explain.

Comment [L47]: It was also suggested to the authors to replace plates 1, 2, 3, etc.... by figures 4, 5, and 6 etc..... But the authors have chosen to ignore.

Comment [L48]: Why is this starting from cap lock letter?

Comment [L49]: Same remark as the previous

exposed to normal feeds. The slide labeled Citrus Blend shows adenomatous hyperplasia while the slide labelled alcohol shows hyperplasia of the endometrial glands and the slide labelled moringa shows cystic hyperplasia. The slide labelled normal shows the normal histo-architecture of the uterus.

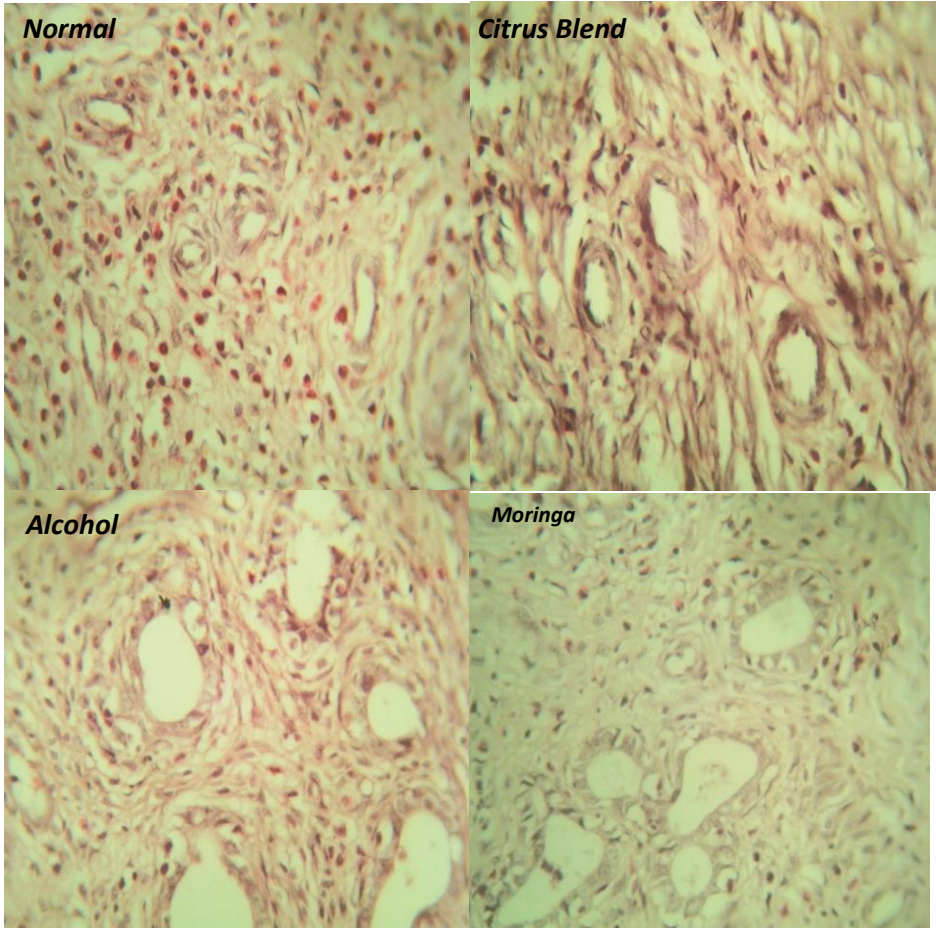
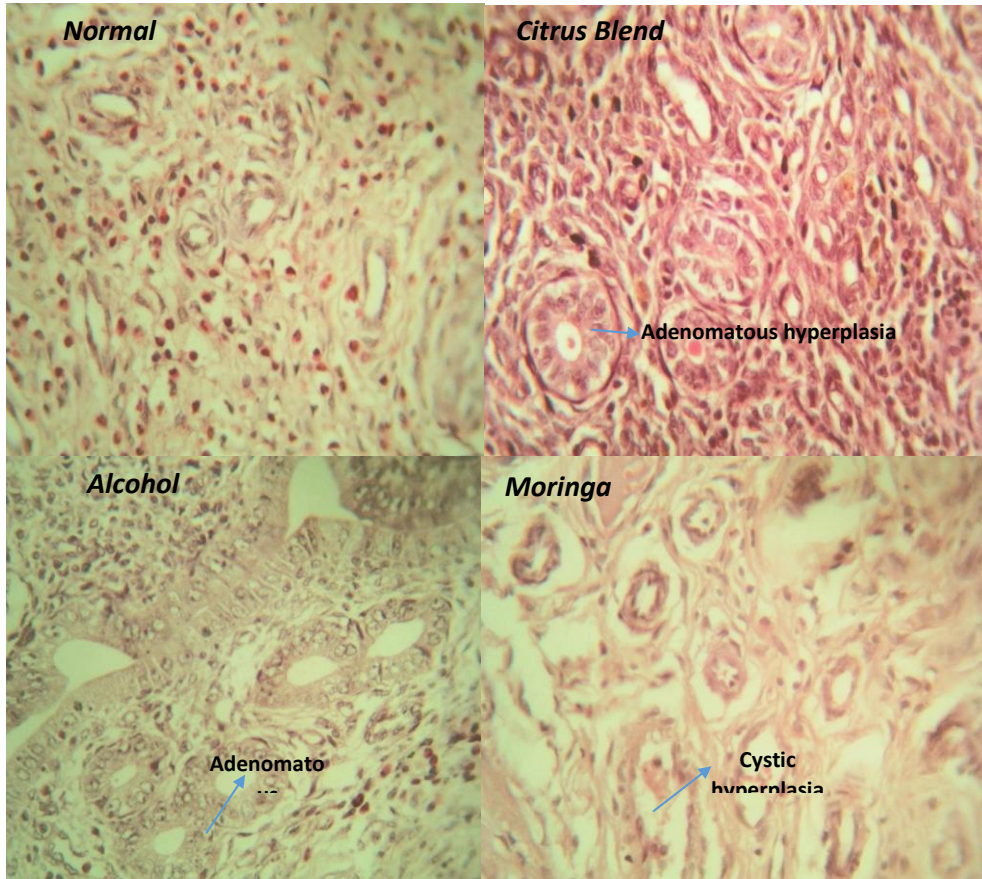


PLATE i: shows the morphology of the uterus after the various treatments for 7 days. Slide labelled **Normal** shows normal morphology of the uterus. The slide labelled **Citrus Blend, Alcohol and Moringa** shows the endometrial glands depicting cystic hyperglycemia compared with normal for the groups given moringa citrus blend, alcohol and moringa extracts. X400

Comment [L50]: Replace by figure...



Comment [L51]: The writing is not visible.

*PLATE ii: Shows the Morphology of the uterus after treatment for 14days. The slide labelled **Normal** shows the normal morphology of the uterus. The slide labelled **citrus blend** and **alcohol** shows Adenomatous hyperplasia of the endometrial glands. The slide labelled **moringa** shows cystic hyperplasia compared with normal. X400*

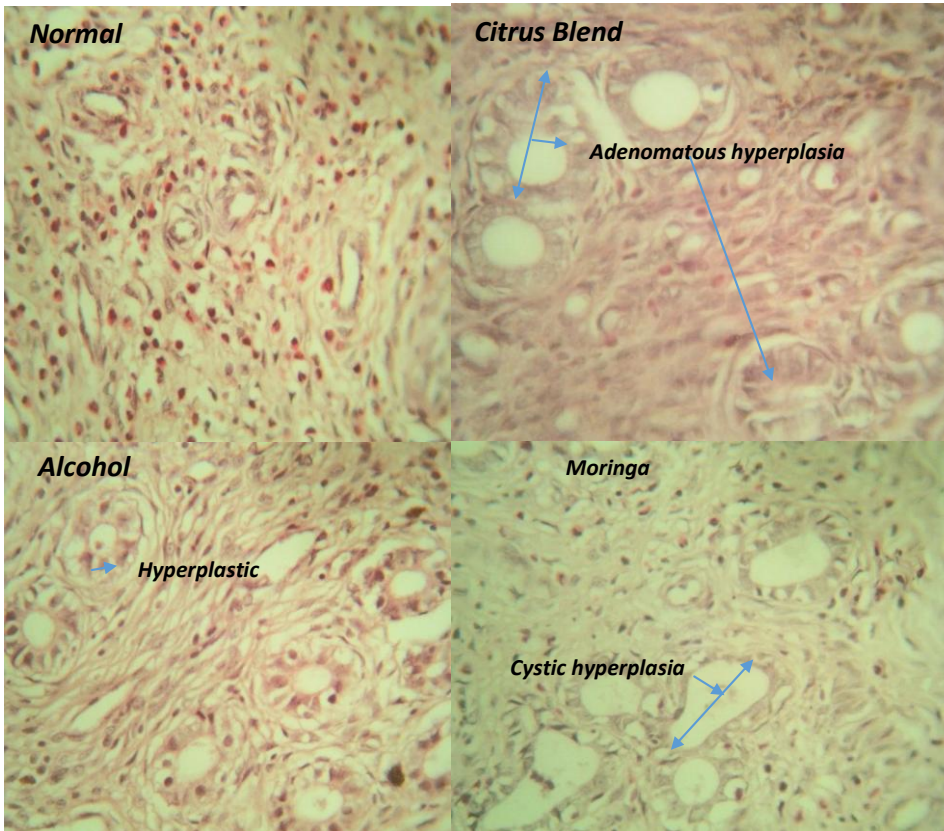


PLATE iii Morphology Als. The slide labelled **Citrus Blend** shows Adenomatous hyperplasia of the endometrial glands. The slide labelled **alcohol** shows hyperplasia of the endometrial glands. The slide labelled **moringa** shows cystic hyperplasia compared with normal.

Comment [L52]: What is "Als"?

Discussion

The oral administration of Gordon dry gin (moringa citrus)blend to dawley sprague rats for seven(7),fourteen(14) and twenty one(21) days respectively led to the changes seen in the plates i-iv. The slides labelled Citrus Blend, Alcohol and Moringa shows endometrial glands that show cystic hyperplasia. This finding correlates with studies done by (6)(7) in a study where it states that there is a direct

Comment [L53]: Replace by "Sprague Dawley"

Comment [L54]: Replace by "figures"

Comment [L55]: Replace by "reported"

Comment [L56]: This is very confusing. How can the authors state: "This finding with studies done by (6) (7)....." This is not meaningful. Re-write.

effect of consumption of alcohol on estrogen levels which increases it leading to mitotic proliferation of endometrial cells with endometrial growth.

Plate ii shows the morphology of the uterus after administration of Gordon's dry gin moringa citrus blend, 43% alcohol, and 200mg/kg moringa for 14 days. The slide labelled Citrus Blend shows adenomatous hyperplasia. Adenomatous hyperplasia is also referred to as endometrial hyperplasia which is a condition of excessive proliferation of the cells of the endometrium, or inner lining of the uterus¹³. It is characterized by an increase in the number of endometrial glands that results in a greater than normal gland-stroma ratio⁸. Endometrial hyperplasia results from high levels of estrogens, combined with insufficient levels of progesterone hormone which counteracts estrogen's proliferative effects on this tissue. If ovulation does not occur, progesterone is not made and the lining of the endometrium is not shed. The endometrium may continue to grow in response to estrogen. The cells that make up the lining may crowd together and become abnormal¹³. Studies such as (9) showed that acute alcohol exposure in female rats disrupt female cycling. Possible mechanisms underlying alcohol's disruption of the female cycle in the rat model may be due to temporary elevation of estradiol¹⁰. This reaffirms the findings in this study, as the slide labelled citrus blend shows the effect of treatment with Gordon's dry gin moringa citrus blend on the uterus of **dawley sprague** rats. Adenomatous hyperplasia is an indication that there is higher concentration of the hormone estrogen and subsequently reduced concentration of progesterone to provoke such changes in the uterus. This may result in infertility as the female may be infertile until cessation of alcohol consumption as a result of the interplay of the hormones on the hypothalamus caused by the consumption of alcohol. This conforms to a study by (11) in which he states that alcoholic women are known to have menstrual disorders such as irregular cycles to complete cessation of menses, absence of ovulation and infertility. The slide labelled

Comment [L57]: Review the references.

Comment [L58]: This is meaningless. The authors can state as follows: "LaPaglia et al. [9] showed that....." OR "LaPaglia and coworkers [9] showed that....." OR something similar.

Comment [L59]: Replace by "Sprague Dawley"

Comment [L60]:

Comment [L61]: Replace by "This conforms with the study of Mello et al. [11] who reported the adverse effects (menstrual disorders such as irregular cycles to complete cessation of menses, absence of ovulation and infertility) of alcohol consumption in women."

moringa showed cystic hyperplasia which also indicates endometrial hyperplasia of which (17) stated that *Moringa oleifera* can cause biochemical and physiologic alterations in the female reproductive organs of cyclic rats. And thus, inclusion of *Moringa oleifera* in the Gordon's dry gin moringa citrus blend will definitely produce similar alterations in the uterus.

Comment [L62]: Replace by "Shukla *et al.* [11] stated....."

Plate iii shows the histo-architecture of the uterus after administration of Gordon's dry gin moringa citrus blend, 43% Alcohol, and 200mg/kg Moringa for 21 days. The slide labelled citrus blend and alcohol also shows hyperplasia but on a grander scale as a result of continuous exposure of the uterus to the deleterious effects of alcohol and moringa which is combined in the Gordon's dry gin moringa citrus blend. The slide labelled moringa also showed cystic hyperplasia which is a strong indication that continuous consumption of moringa over a period of time has effects on the uterus via direct interaction on the estrous cycle as confirmed by a previously reported study [12]. ~~study which explains that there is disruption of the estrous cycle due to the effect of moringa on the ovary which disrupts ovarian functions and estrous cycle via ovarian and extra ovarian hormones¹².~~

Comment [L63]: Replace by figure....

Conclusion

The result of this study shows that binge consumption of Gordon's dry gin moringa citrus blend has histopathological effects on the uterus of sprague dawley rats. Alcohol and moringa has been shown to illicit histopathological effects on the uterus of dawley sprague rats and a combination of these two substances in the Gordon's dry gin moringa citrus blend has been proven to cause such cystic hyperplastic changes in the endometrium at day 7, 14 and 21 .

Comment [L64]: This is "Sprague Dawley" and not "dawley sprague"

The consumption of Gordon's dry gin moringa citrus blend in a binge manner over a period of time to induced cystic and adenomatous hyperplasia which could lead to irregular menstruation, infertility as well as endometrial cancer.

Comment [L65]: Omit "to"

Conflict of interest

The author(s) have no conflict of interest to declare.

Ethical approval

The experimental protocol was approved by the Animal Ethics Committee of the College of health Sciences, Niger Delta University Wilberforce Island, Nigeria.

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