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Gordon Dry Gin (Moringa Citrus Blend) Induce Adenomatous Hyperplasia in Female Sprague Dawley Rats

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Authors' contributions

This work was carried out in collaboration among all authors. Author EUE designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors TOA and EFE managed the analyses of the study. Author EFE managed the literature searches. All authors read and approved the final manuscript.

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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 dawley Sprague rats.
Materials: Fifty female rats (weighing range; 120±2.6 g - 250±3.5 g) were divided into four groups (A, B and C & D). Group A, B, C (15 animals each) were orally administered with Gordon's dry gin moringa citrus blend, 43% ethanol, and 200 mg/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 analysis.
Results: Results of the study revealed histological alterations in the uterus of treated animals. Such alterations include adenomatous hyperplasia and cystic hyperplasia.

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Conclusion: The oral administration of gordon's dry gin moringa citrus and moringa extract at 200 mg/kg induced cystic and adenomatous hyperplasia in rat uterus, inferring that both gordon dry gin and moringa might yield antifertility activity.

Keywords: Uterus; adenomatous; hyperplasia; binge; infertility; alcohol; rats.

1. 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 me [1] Consumption of alcoholic drinks by women is common worldwide but its use in pregnancy has become a major public health problem [2]. 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 20% for current use drinkers and 4 to 41% for heavy drinkers [4]. A "binge" is a pattern of drinking alcohol that brings blood alcohol concentration to 0.08 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 society [5]. The facto threshold for intoxication (80 mg/dL), implying that observable behavioral intoxication is a defining characteristic of binge drinking [5], also repeated episodes of binge drinking displayed lipid peroxidation in the liver and brain also harmful histological effect were observed in the liver associated to steatosis and loss of parenchymal architecture in female rat [6]. The ethanol extract of both the leaf and seed of moringa oleifera has shown abortificient effect in that they caused a decrease in the number of litters from animals treated with the extract. The ethanol extract also causes atretic follicle and tissue engorgement follicle in a dose dependent manner in the ovaries as a sign of high levels of degenerating pre-ovulatory follicle and an absence of the steroid hormones. The abortificient effect was also observed in the uterus where it causes endometrial polyps and shrinkage of the uterine gland as well as making the endometrial milieu to become unfavourable for the implantation of the fertilized ovum [7]. Therefore this study will help create awareness especially for women 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.

2. MATERIALS AND METHODS

2.1 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.

2.2 Procurement of Guinness Gordon's Moringa Citrus Blend

The Gordon's Dry Gin Moringa Citrus blend is produced by GUINESS 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 ± 2ºC. Also, fresh moringa leaves were harvested and air dried at room temperature for 3 days. The dried leaves were meshed in a mortar and 95% ethanolic extraction using the soxhlet extractor was carried out to afford the ethanol extract.

2.3 Animal Housing

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

2.4 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 0.3 ml of Gordon's Dry Gin Moringa Citrus Blend, twice a day orally via orogastric route using orogastric tube. Animals in group B received 0.3 ml of 43% ethanol, morning and evening orally using orogastric tube. Group C were administered with 0.3 ml of 200 mg/kg Moringa extract, morning and evening orally via using orogastric tube. Five (5) animals each from group A,B and C were sacrificed on the 7^{th},14^{th} and 21^{st} day of administration. Group D served as control and was sacrificed at the end of the study.

2.5 Histological Processing

The uterus each rat was cut in slabs of about 0.5µm thick and fixed in 10% formal saline. The organ was 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 technique [8]. Statiscal analysis was done using the graph pad software and the results presented as graphs and bars.

2.6 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.

3. RESULTS

3.1 Photomicrograph Slides

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

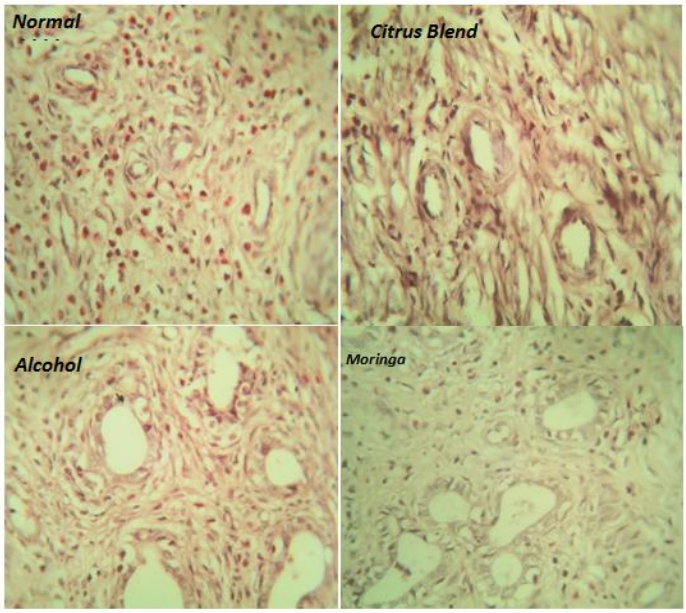

Fig. 1. Shows the morphology of the uterus after the various treatments for 7 days. Slide labelled Normal shows normal morphology of the uterus. The slide labelled Citrus Blend, Alcohol and Moringa shows the endometrial glands depicting cystic hyperglycemia compared with normal for the groups given moringa citrus blend, alcohol and moringa extracts. X400

Table 1. Experiment layout

	Group A (Gordon Dry Gin)	Group b (43% alcohol) Positive control	Group c (moringa)	Group d (control)negative control
DAY 7	5	5	5	5
DAY 14	5	5	5	
DAY 21	5	5	5	

Fig. 2 shows the endometrial glands which shows 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.

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

4. DISCUSSION

The oral administration of Gordon dry gin (moringa citrus) blend to Sprague Dawley rats for seven (7), fourteen (14) and twenty one (21) days respectively led to the changes seen in the Figs. 1-3. The slides labelled Citrus Blend, Alcohol and Moringa shows endometrial glands that show cystic hyperplasia. This finding correlates with studies reported by Akhmedkhanov et al. [9] and Ljungkvist et al. [10] where they separately affirm that there is a direct 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 200 mg/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 uterus [11].

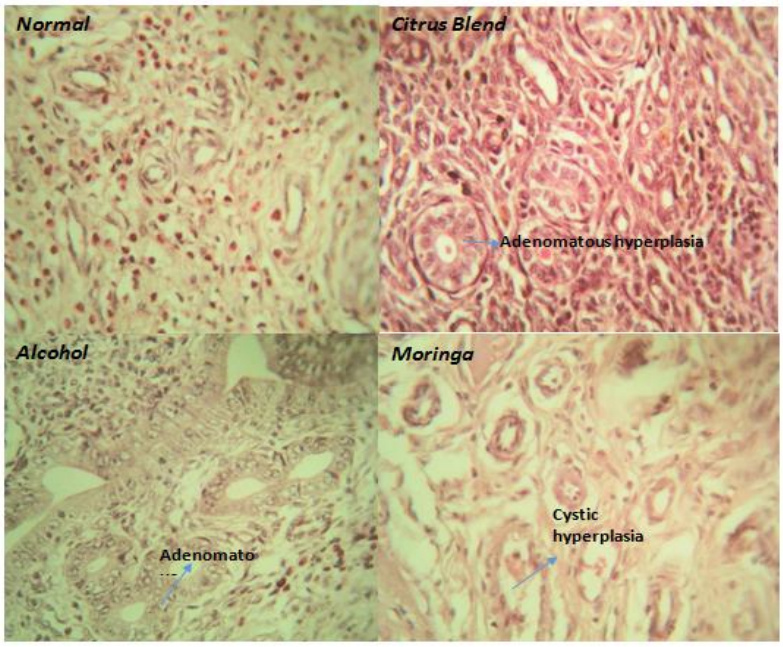

Fig. 2. Shows the morphology of the uterus after treatment for 14 days. The slide labelled normal shows the normal morphology of the uterus. The slide labelled citrus blend and alcohol shows Adenomatous hyperplasia of the endometrial glands. The slide labelled moringa shows cystic hyperplasia compared with normal. X400

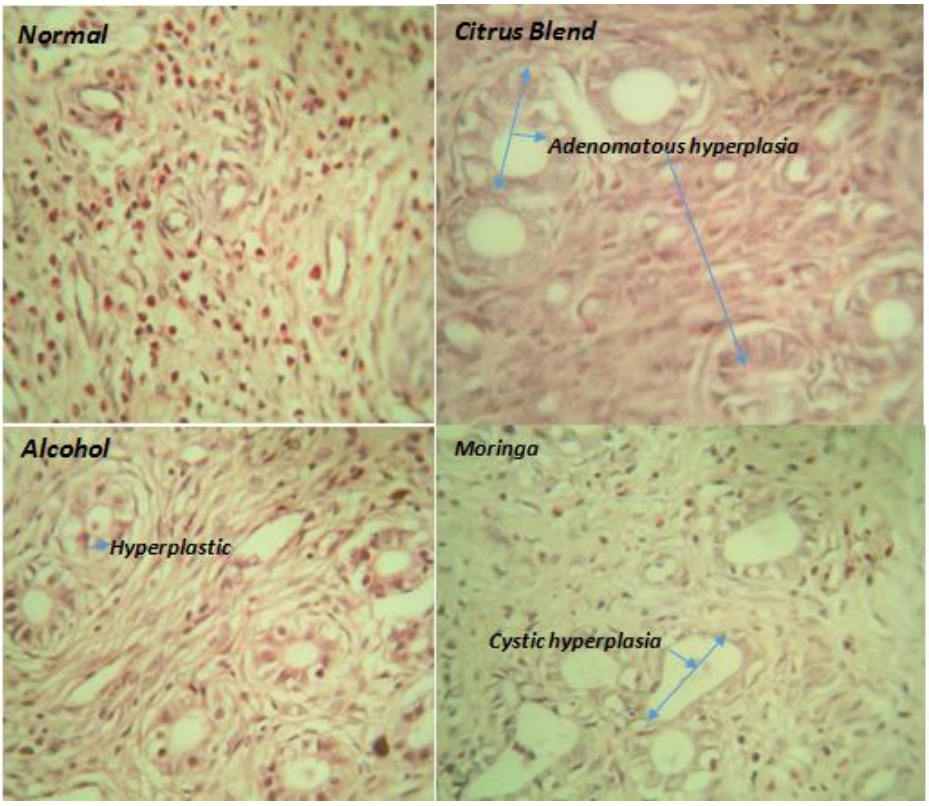

Fig. 3.The slide labelled Citrus Blend shows Adenomatous hyperplasia of the endometrial glands. The slide labelled alcohol shows hyperplasia of the endometrial glands. The slide labelled moringa shows cystic hyperplasia compared with normal

It is characterized by an increase in the number of endometrial glands that results in a greater than normal gland-stroma ratio [12]. 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 abnormal [11] LaPaglia et al. [13] 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 estradiol [14]. 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 Sprague Dawley 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 with the study of Mello et al. [15] who reported the adverse effects (menstrual disorders such as irregular cycles to complete cessation of menses, absence of ovulation and fertility) of alcohol consumption in women. The slide labelled moringa showed cystic hyperplasia which also indicates endometrial hyperplasia of which Shukla et al. [16] 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.

Fig. 1 shows the histo-architecture of the uterus after administration of Gordon's dry gin moringa

citrus blend, 43% Alcohol, and 200 mg/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 [17].

5. 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 Sprague Dawley 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.

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

CONSENT

It is not applicable.

ETHICAL APPROVAL

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

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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