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

Comment [u1]: Which was used "Sprague Dawley or Wistar 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.

Comment [u2]:

Materials;Fifty outbred sprague dawley rats (fifty females) weighing between 120g±2.6-250g±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.

Comment [u3]: Average ± standard deviation not range

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.

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

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 men[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 30% 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 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 society[5] . 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 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.

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 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 $4 \pm 2^{o}C$. 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

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^{o}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.

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	

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 technique[16].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.

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.

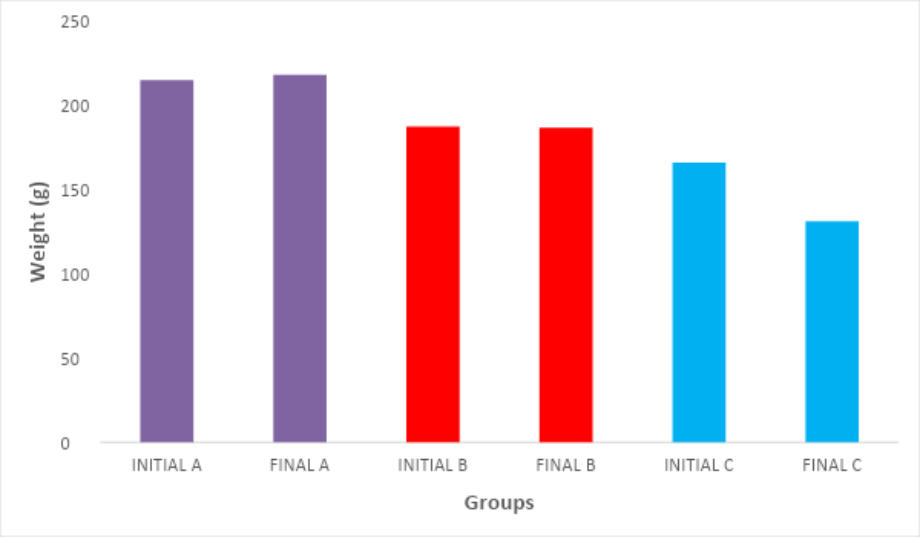

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Figure I: Comparison of Initial and Final weight after 7 days of treatment

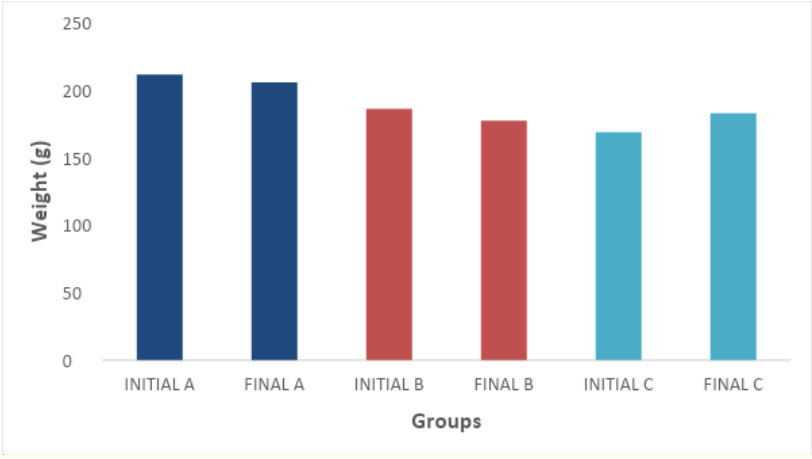

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Figure II: Comparison of Initial and Final weight after 14 days of treatment

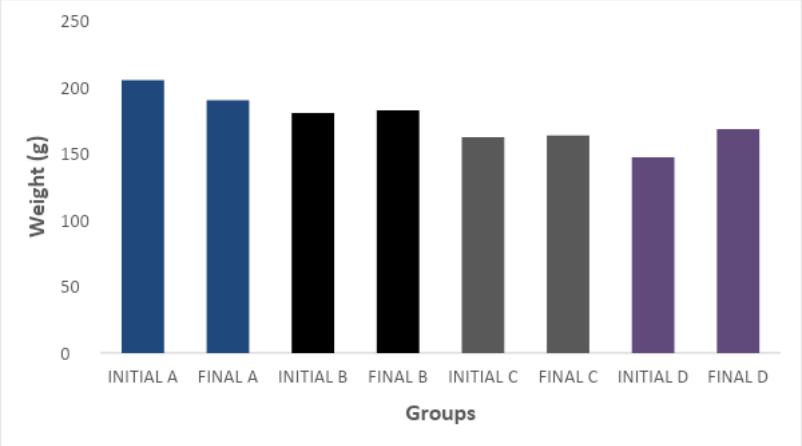

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Figure III: Comparison of Initial and Final weights after 21 days of treatment

Histological slides

The histological photomicrograph of the uterus of the adultdawley 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 shows 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 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.

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 21days 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.

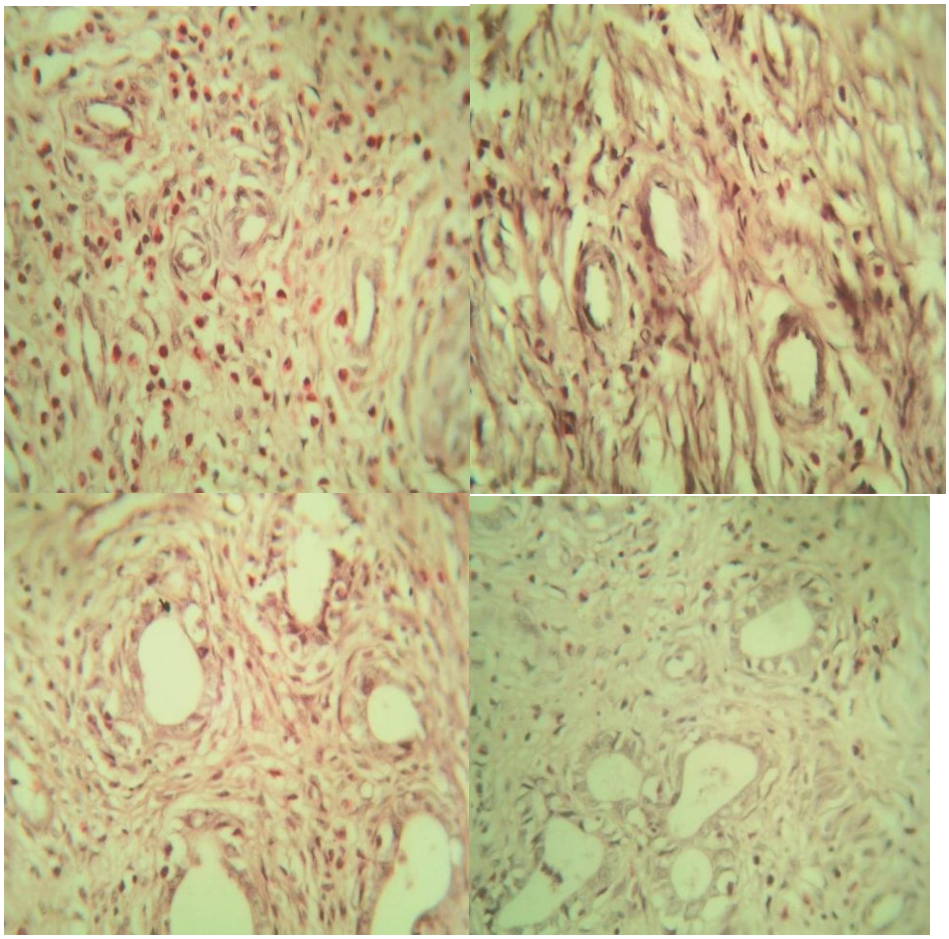

PLATE i: shows the morphology of the uterus after the various treatments for 7 days. Slide labelled ***Normal*** *shows normal morphology of the uterus. The slide labelled* ***Citrus Blend***, **Alcohol** *and* **Moringa** *shows the endometrial glands depicting cystic hyperglycemia compared with normal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

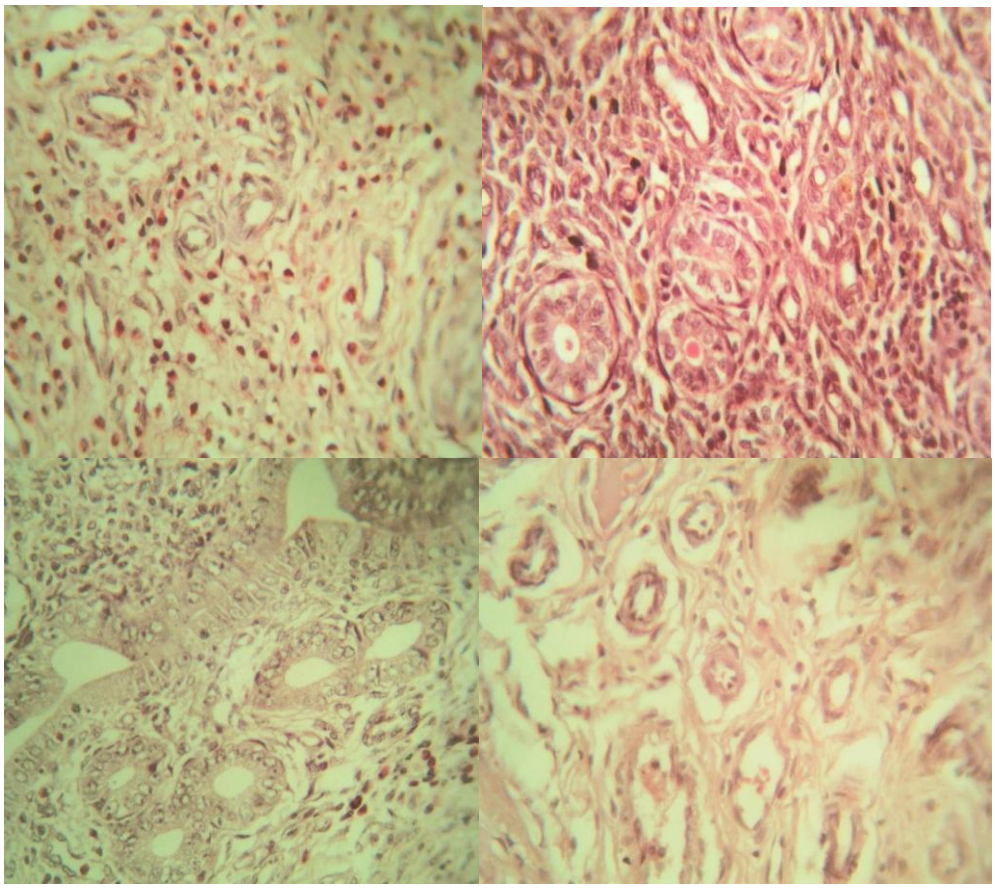

PLATE ii: Shows the Morphology of the uterus after treatment for 14days. The slide labelled ***Normal*** *shows the normal morphology of the uterus. The slide labelled c****itrus blend*** *and a****lcohol*** *shows Adenomatous hyperplasia of the endometrial glands. The slide labelled m****oringa*** *shows cystic hyperplasia compared with normal. X400*

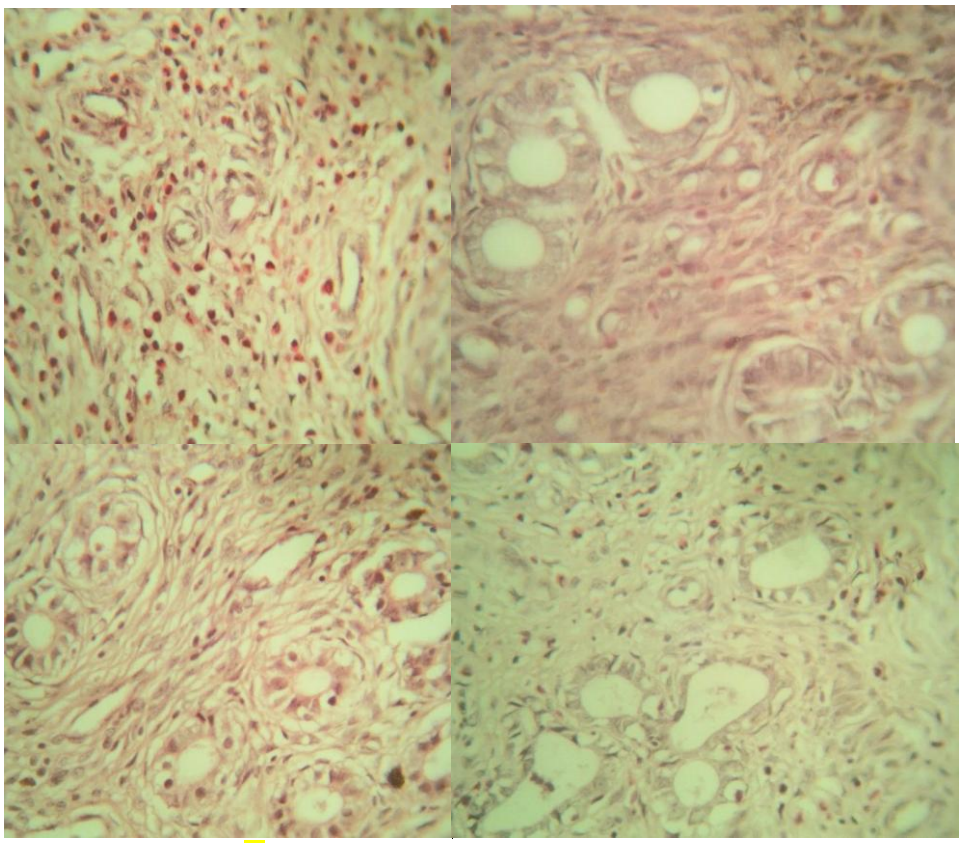

PLATE iii *Morphology Als. The slide labelled* ***Citrus Blend*** *shows Adenomatous hyperplasia of the endometrial glands. The slide labelled a****lcohol*** *shows hyperplasia of the endometrial glands. The slide labelled m****oringa*** *shows cystic hyperplasia compared with normal.*

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

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 uterus[13] . It is characterized by an increase in the number of endometrial glands that results in a greater than normal gland-stroma ratio[8]. 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[13].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 estradiol[10]. 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

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.

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 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 hormones[12] .

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 .

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.

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.

REFERENCES

1.Eggert, J., Theobald, H. and Engfeld t, P. Effects of alcohol consumption on female fertility during an 18-year period. Fertility and Sterility.2004*;81*: 379 – 383.

2.Charya G., Bhuvaneswar, M.D., Grance Change, M.D., Theodore A. Sterm, M.D. Alcohol use in pregnancy: Prevalence and Impact. Journal of Clinical Pschiatry. 2007; *9*(6) 455-459.

3.Dafina kanny,Yong liu,Robert D,Brewer. Binge drinking, United state supplement. National center for chronic disease, Prevention and health promotion CDC.2011; 60(1) 101-104

4.Julia Martinez, Kenneth, S., Philip wood. Drinking consequences and subsequent Drinking in college students over 4yrs.Psychol Addict Behav. 28(4):1240-1245.

5. NIAAA Council Approves Definition of Binge Drinking. (2004). NIAAA Newsletter 2004.Winter Vol. 3. Bethesda, MD: DHHS-NIH; Ref Type: Newspaper.

6. Akhmedkhanov, A., Zeleniuch-Jacquotte, A., Toniolo, P. Role of exogenous and endogenous hormones in endometrial cancer: review of the evidence and research perspectives. Ann N Y Acad Sci. 2001*; 943:296-315*

7. Ljungkvist, I. Attachment reaction of rat uterine luminal epithelium. The effect of estradiol estrone and estriol on the morphology of the luminal epithelium of spayed virgin rats. Acta of the Society of Medicine Uppsala. 1971;*76*: 139- 157.

8. Medscape. May 13, 2016. Drugs and Diseases. Obstetrics and Gynecology. Retrieved: March 10. 2018. https://emedicine.medscape.com/article/269919-overview.

9. LaPagalia,N., Steiner,J., Kirsteins,L., Emanuele,M.A. and Emanuele,N.V. The impact of acute ethanol on reproductive hormone synthesis, processing, and secretion in female rats at proestrous. *Alcoholism: Clinical and Experimental Research.*1997;*21*(9): 1567 – 1572.

10. Emanuele, N.V., Lapaglia, N., Steiner, J., Kirsteins, L., Emanuele, M.A. Effect of chronic ethanol exposure on female rat reproductive cyclicity and hormone secretion. *Alcoholism: Clinical and Experimental Research.2001*; *25***:** 1025 – 1029.

11. Mello, N.K., Mendelson, J.H., and Teoh, S.K. (1993): Overview of the

effects of alcohol on the neuroendocrine function in women. In: Zakhari, S., ed. Alcohol and the Endocrine System. National Institute on Alcohol Abuse and Alcoholism Research Monograph No. 23. NIH Pub. No. 93–3533. Bethesda, MD: National Institutes of Health. pp. 139 – 169.
12. Lerner, L.J. The biology of non-steroidal antifertilityIn Contraception, chemical control of fertility. Edited by Lednicer D, Marcel Derker Inc, New York. 1996; 161.
13. Emanuele, M.A. and Emanuele, N.V. Alcohol's effects on male reproduction. Alcohol health and research world. 1998; *3*(2): 195 – 201.
14.Emanuele, N.V, and Emanuele, M.A. The endocrine system: Alcohol alters critical hormonal balance. Alcohol Health and Research World. 1997; *21*(1): 53 – 64.
15. Alfonso, M., Duran, R. and Marco, J. Ethanol–induced alterations in gonadotrophins secretion during the estrous cycle of rats. Alcohol and Alcoholism.1993: 667 – 674
16.Ochei,J.and A,Kolhatar .Medical Laboratory Science.Theory and Practice .Tata Mcgraw-Hill Publishing Company Limited;New Delhi.2nd Edition pp 231-234.2000
17.Shivendra shukla,Annayya Aroor,Ricardo Restrepo,Kusum Kharbanda,Jamal Ibadah.Invivo Acute on Chronic Ethanol Effect in the liver;A mouse model exhibiting exacerbated injury,altered metabolic and epigenetic responses.Biomolecules,2015;5(4):3280 DOI:10.3390/biom5043280