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SEMINIFEROUS HYPERTROPHY IN MALE WISTAR RAT ADMINISTERED WITH GORDON DRY GIN (MORINGA CITRUS BLEND)

Eric Emmanuel Uchenna*[1] and Harrison Oju Uket[1]

Department of Medical Laboratory Science, Niger Delta University, Wilberforce Island, Yenagoa. Bayelsa State.

***Corresponding Author: Dr. Eric Emmanuel Uchenna**
Department of Medical Laboratory Science, Niger Delta University, Wilberforce Island, Yenagoa. Bayelsa State.

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ABSTRACT
The consumption of herbal products has in recent times increased worldwide especially among the people of Africa. Gordon dry gin moringa citrus blend is an alcoholic drink composed of mainly moringa, alcohol and citrus. This study seeks to ascertain the histological effect of Gordon dry gin on the liver and testes of adult rats. Thirty five (35) adult male rats weighing 120±20g-180±30g were used for the study and were allowed to climatize for two weeksand administered with the various treatment for three weeks. They are divided into five groups designated group A,B,C,D. Group A(control)received water and feed. Group B; group B1 and B2 received high and low doses of Gordon dry gin (moringa citrus blend) respectively. Group C; C1 and C2 received high and low doses of 43% of alcohol respectively. Group D; D1 and D2 received high and low doses of 200mg/kg of moringa leaf extract respectively. At the end of 21 days the animals were sacrificed, the liver and the testes harvested, fixed in 10% formal saline and the tissue processed in parrafin wax. The photomicrograph of the liver showed no significant change at the dose administered in all the groups. The photomicrograph of the testes showed enlargement in the size of the seminiferous tubule measuring 2.6cm in group B and 2.8cm in group C.Conclusion the dose of moringa citrus blend used in this work did not produce any significant effect on the liver but hypertrophic effect was seen in the testes.

KEYWORD: Gordon dry gin, testes, liver, moringa, seminiferous tubules.

INTRODUCTION
The consumption of herbal products has in recent times increased worldwide especially among the people of Africa, this increase is attributed to the notion that they are safe and effective for medicinal purposes, recreational purposes, and as food.[1] Gordon dry gin moringa citrus blend is an alcoholic drink composed of mainly moringa, alcohol and citrus Both chronic and acute consumption of alcohol has been reported to cause fertility disturbances such as low sperm count and motility, reduced serum/plasma testosterone level, testicular atrophy and irregularity in the diameter of the seminiferous tubules in men and laboratory animals.[2,3,4] Alcohol has been shown to impact spermatogenesis on multiple levels. Alcohol suppresses the hypothalamic–pituitary–testis (HPT) axis in mice as well as humans and has direct toxic effects on Leydig and Sertoli cells[5], thus affecting spermatogenesis at both the level of the pituitary and the testes.

Alcohol causes numerous atrophies in the testes and damaged spermatogenic cells. *M.oleifera* and vitamin C however exhibited protective and reversibility effects. *M.oleifera* ameliorates alcohol induced testicular toxicities with its antioxidant properties comparable to vitamin C. The alcoholic extract from the leaf have aphrodisiac function[6,7] and can improve the testicular function and sexual performance in stressed rats.[15] Earlier studies have also demonstrated that *Moringa oleifera* leaf alcoholic extract can attenuate the chromium-induced testicular[7] and cyclophosphamide-induced urinary bladder toxicity.[16] Also, administration of alcoholic moringa extract ameliorates the CP-induced toxic effects on the pre-pubertal testes and improves the functional characteristics of spermatozoa after attaining the puberty.[7,8] Ethanol has been reported to be among the most widely abused drug which can suppress reproductive function and sexual behaviour in laboratory animals and humans. Alcohol abuse has been considered as one of the problems associated with poor semen production and sperm quality.[9,10] Both chronic and acute consumption of alcohol has been reported to cause fertility disturbances such as low sperm count and motility, reduced serum/plasma testosterone level, testicular atrophy and irregularity in the diameter of the seminiferous tubules in men and laboratory animals.[11,12,13,14] In addition, Martinez et al.[13] reported histological abnormalities in testicular tissue of alcoholic animals. These include intense intercellular spaces, irregular diameter of seminiferous tubules and high

amount of necrotic cells in the lumen compared with controls. Epididymal sperm motility also decreased in ethanol-treated rats.

Liver is an organ in the upper abdomen that aids in digestion and removes waste products and worn-out cells from the blood. It is a vital organ present in vertebrate and some other animals, which has a wide range of functions including detoxification and protein synthesis. The liver is our greatest chemical factory, it builds complex molecules from simple substances absorbed from the digestive tract, it neutralizes toxins, it manufactures bile which aids fat digestion and removes toxins through the bowels.[17] Buraimoh *et al*[18] reports that the ethanolic extract of *Moringa Oleifera* leaf has an appreciable ability to prevent damage to the liver. Numerous scientific reports have shown elevation of a variety of antioxidant enzymes and organ biomarkers as a result of treatment with *M. oleifera* or with phytochemicals isolated from *M.oleifera*.[19,20] In another research *Moringa Oleifera* administration after alcohol challenge in Wistar rat showed improved liver histology, function and oxidative stress.[21]

Objective of Study

1. To determine the histological effect of Gordon dry gin moringa citrus blend on the liver of male wistar rats
2. To determine the histological effect of Gordon dry gin moringa citrus blend on the testes.

MATERIALS AND METHOD

3.1 Substance of Study

Several bottles of "Gordon dry gin moringa citrus blend" where purchased from a Guinness product dealer in Yenagoa Bayelsa State, Nigeria with NAFDAC registration number: 08-3821 and Batch Number: L7287ZI002. These details were checked to ensure the authenticity of the products purchased.

3.2 Experimental Animals

Thirty five adult male Wister rats weighing 120 ± 20g - 180 ±30g were procured from the animal house of the Department of Pharmacology, College of Health Sciences Niger Delta University, Amassoma Bayelsa state and moved to the animal house of the department of Medical Laboratory science Niger Delta University Amassoma, Bayelsa State, Nigeria, where they were housed under standard condition of temperature (27 ± 2^{o}c) with twelve hours light/dark periodicity in plastic cages. The rats were allowed to acclimatize for two weeks and were fed ad libitum during this period, with water and grower mesh feed. Animals where handles throughout the period of study according to institutions guidelines for experiment involving the use of animals.

3.3 Experimental Design The animals were weighed and assigned into four major groups after the period of acclimatization: a control group "A" and three test groups (B, C and D) subdivided into B1 and B2, C1 and C2, and D1 and D2 respectively.

3.5 Substance Administration

All the animal groups were fed with feed (growers mash) plus water given ad libitum. However, as group A (control) received water and feed(grower mesh) only, test groups B: B1 received 0.4ml (High dose) of Gordon dry gin Moringa citrus blend while sub-group B2 received 0.2ml (Low dose) of Gordon dry gin Moringa citrus blend. Group C subgroup C1 received 0.4ml (high dose) of 43% alcohol (Ethanol) and C2 subgroup received 0.2ml (low dose) of 43% alcohol. Group D1 and D2 received 0.4ml and 0.2ml of alcoholic *Moringa oleifera* leaf extract respectively. The route of administration was oral with the aid of orogastric tube. The substance administered/time duration and the groups and doses are bellow tabulated.

3.6 Study Duration

This study lasted for five weeks (two weeks for acclimatization and three weeks for substance administration. During the five weeks study period, the animals were fed and observed for various behaviors.

3.7 Sample Collection

At the end of three weeks of administration, the rats were sacrificed by administering chloroform as anesthesia. The rats were then dissected to harvest the liver and the testes which were then fixed immediately in 10% formalin.

3.8 Statistical analysis

Analysis of variance (ANOVA) was used for all statistical calculations. Differences were considered probably significant at a P-value of <0.05 and significant at a value of <0.01.

RESULT AND DISCUSSION

Histological studies

The histological photomicrograph of the liver of Adult male wistar rat.

Plate 4.1shows the slide labeled Normal; which represent animals in the control group. The slide labeled Citrus represent animals given 0.4ml of Gordon dry gin citrus blend. The slide labeled Alcohol represent animals given 0.4ml of 43% alcohol (ethanol) and the slid labeled Moringa represent animals given 0.4ml of ethanolic extract of *Moringa oleifera* leaf.

Plate 4.2 shows the slide labeled Normal; which Lrepresent animals in the control group. The slide labeled Citrus represent animals given 0.2ml of Gordon dry gin citrus blend. The slide labeled Alcohol represent animals given 0.2ml of 43% alcohol (ethanol) and the slid labeled Moringa represent animals given 0.2ml of ethanolic extract of *Moringa oleifera* leave.

The histological photomicrograph of the testes of Adult male wistar rat.

Plate 4.3 shows the slide labeled Normal; which represent animals in the control group. The slide labeled Citrus represent animals given 0.4ml of Gordon dry gin citrus blend. The slide labeled Alcohol represent animals given 0.4ml of 43% alcohol (ethanol) and the slid labeled Moringa represent animals given 0.4ml of ethanolic extract of *Moringa oleifera* leaf.

Plate 4.4 shows the slide labeled Normal; which represent animals in the control group. The slide labeled Citrus represent animals given 0.2ml of Gordon dry gin citrus blend. The slide labeled Alcohol represent animals given 0.2ml of 43% alcohol (ethanol) and the slid labeled Moringa represent animals given 0.2ml of ethanolic extract of *Moringa oleifera* leaf.

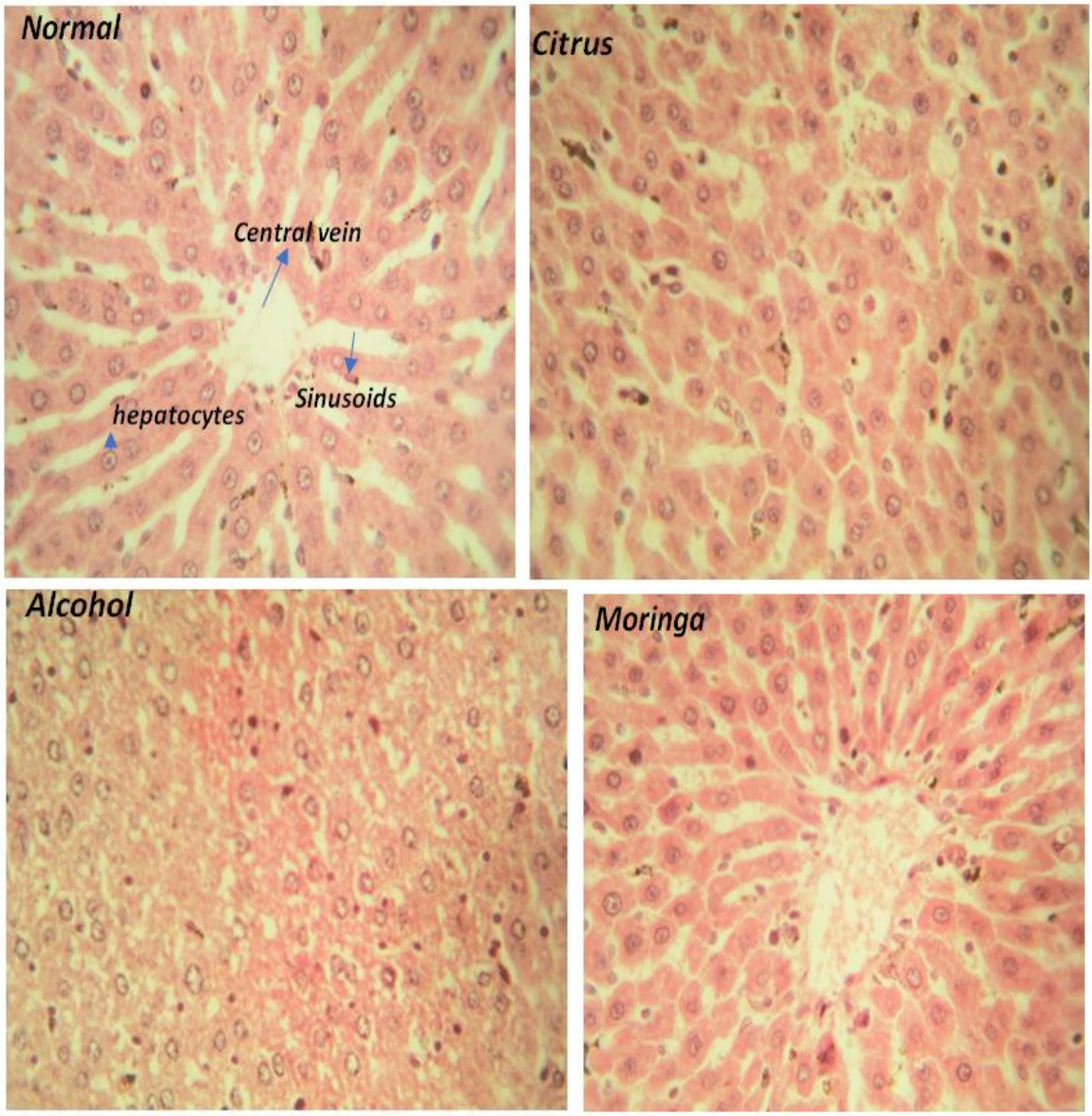

Plate 4.1: Liver morphology of rats given low dose of alcohol and extracts for 21 days compared with normal. Citrus and group shows prominent hepatocytes while the alcohol group showed no changes compared with control. The substances administered are not hepatotoxic at the concentration, dose, and duration of study compared with the control group.

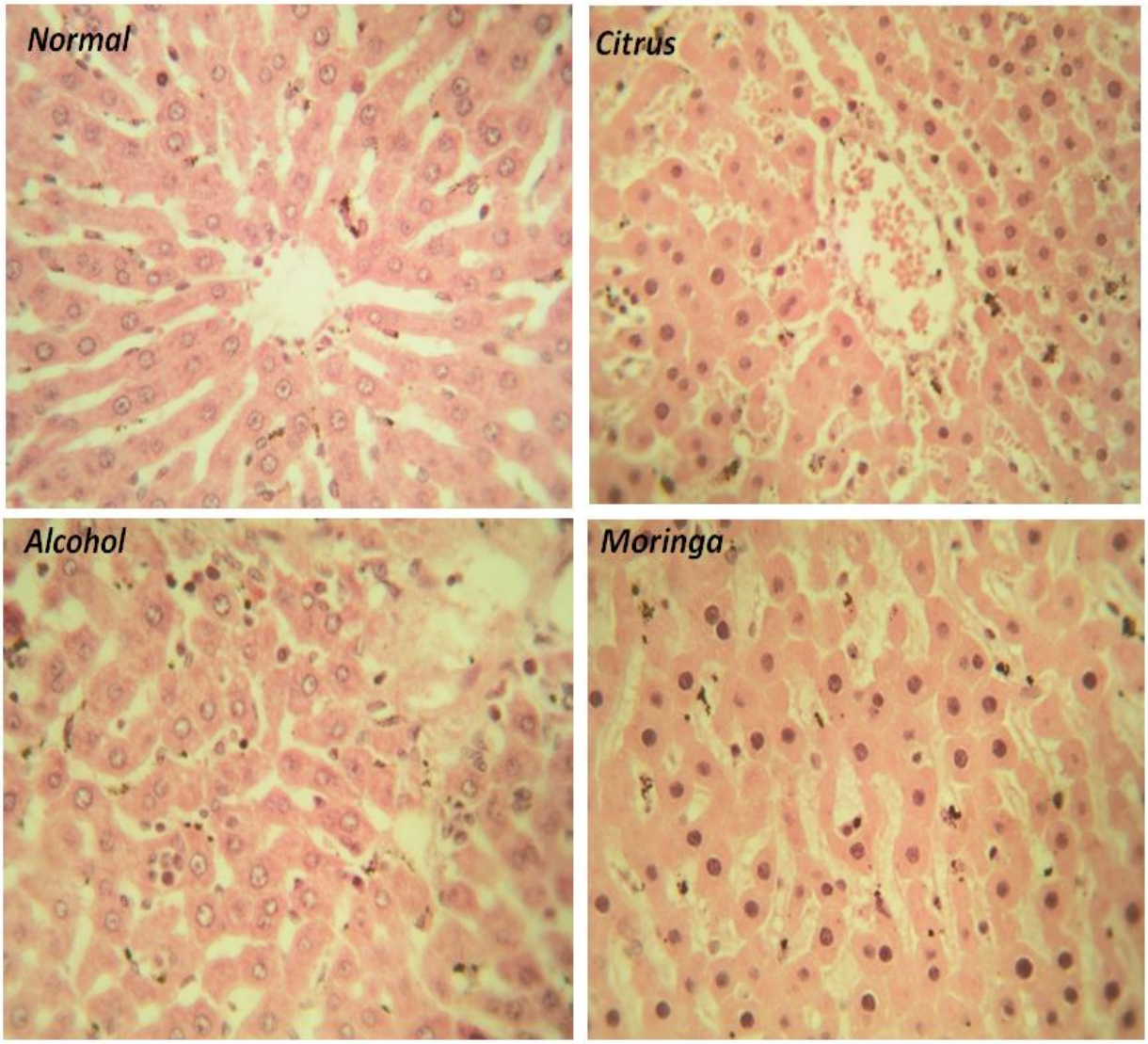

Plate 4.2: Liver morphology of rats given high dose of alcohol and extracts for 21 days compared with normal. Citrus and group shows prominent hepatocytes while the alcohol group showed no changes compared with control. The substances administered are not hepatotoxic at the concentration, dose, and duration of study compared with the control group.

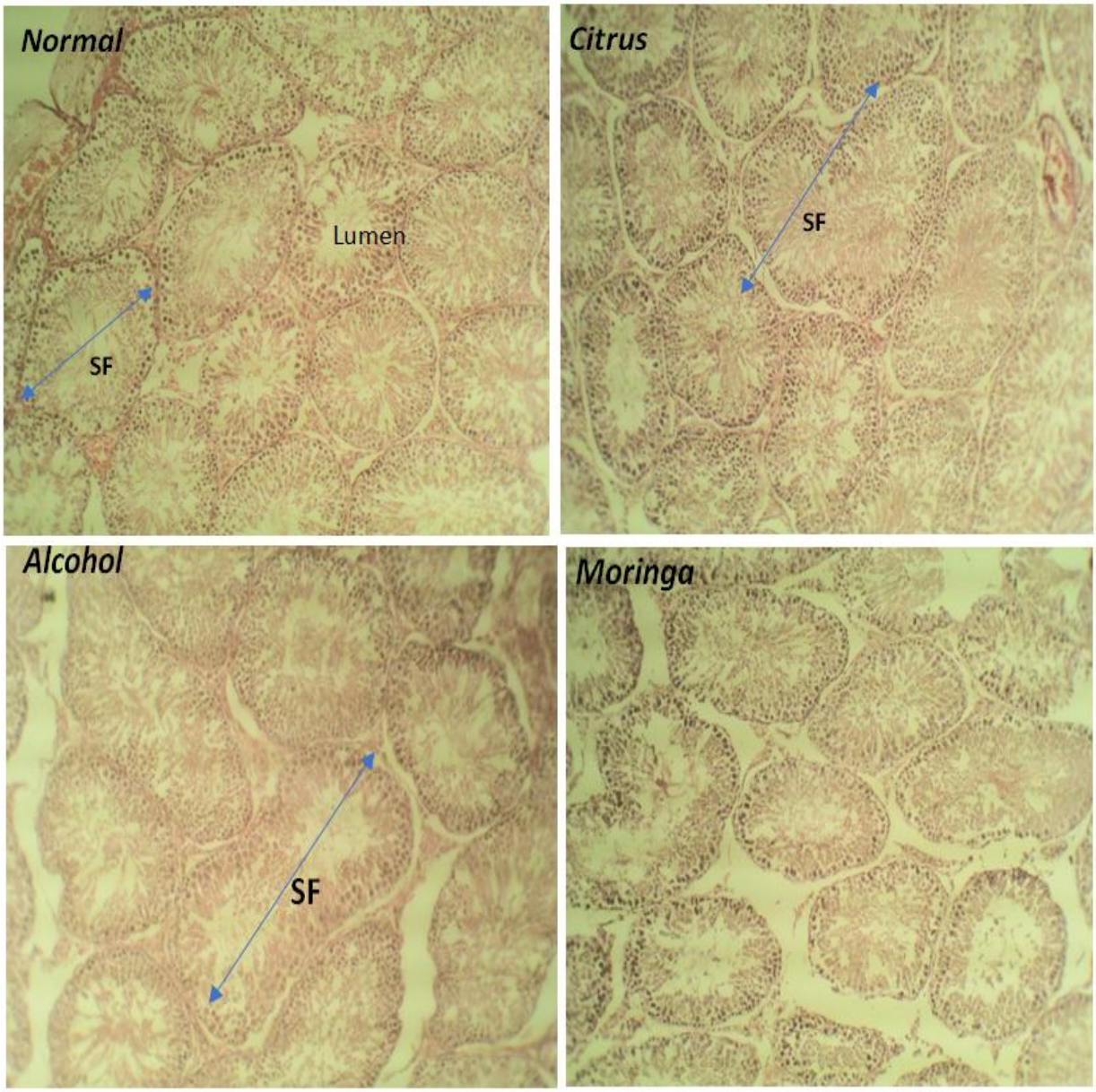

Plate 4.3: Testicular morphology of rats given low dose of alcohol and extracts for 21 days compared with normal.

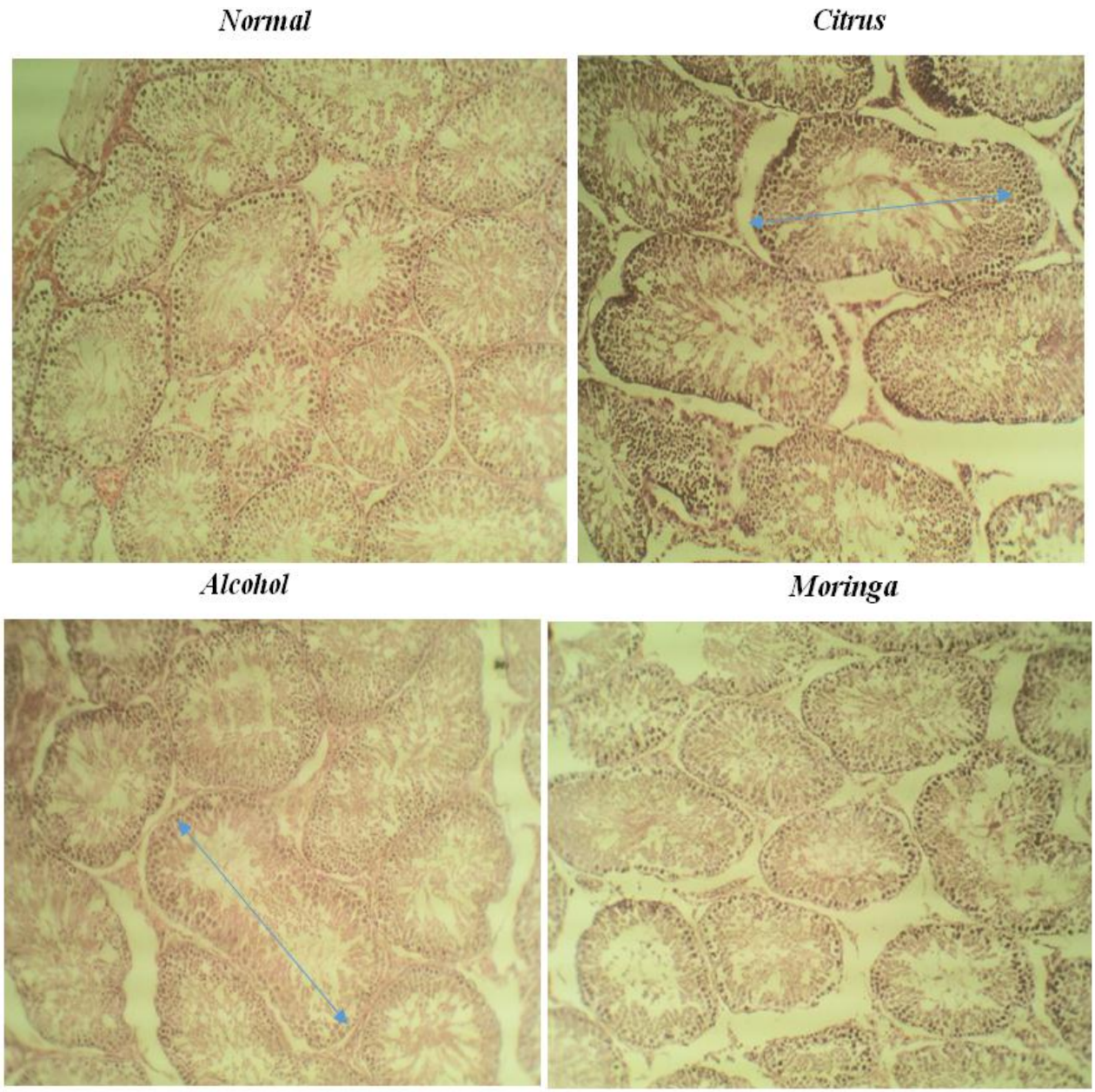

Plate 4:4: Testicular morphology of rats given high dose of alcohol and extracts for 21 days compared with normal. No toxic effect.

The plates labeled 4.1-4.2 represent the liver of animals used in the above study. The slide labeled normal represent animals in the control group which were administered with normal feed, the slide labeled citrus represent animals in the group administered with high dose and low dose Gordon dry gin moringa citrus blend respectively, the slide labeled Alcohol represent animals administered with high and low dose of 43% alcohol respectively and the slide Moringa represent animals administered with high and low dose respectively of 200mg/kg ethanolic extract of *moringa oleifera* leaf.

The plates labeled 4.3-4.4 represent the testes of animals used in the above study. The slide labeled normal represent animals in the control group which were administered with normal Feed, the slide labeled citrus represent animals in the group administered with high and low dose of Gordon dry gin moringa citrus blend respectively, the slide labeled Alcohol represent animals administered with high and low dose of 43% ethyl alcohol respectively and the slide Moringa represent animals administered with high and low dose of 200mg/kg ethanolic extract of *Moringa Oleifera* leaf respectively.

The plates 4.1-4.2 shows normal liver histology in slide label citrus as compared to slide label normal (control) and plate 4.3-4.4 also show normal histology of the testes in slide labeled Citrus as compared to normal (control).

Chronic and acute consumption of alcoholic beverages have been reported to inhibit testosterone production

(Mendelson *et al.*, 1977). Prior to this time Van Thiel and colleagues (1974) reported that impotence occurred more frequently among patient with greater liver damage than those without liver damage which is also demonstrated in this present study as there was no significant histomorphological effect on the liver and testes. The effect of alcohol and moringa on the liver and testes has been reported to be dose dependent (Talabi *et al*, 2011, Maneesh *et al*, 2006, Martinez *et al*, 2009 and Dasumu *et al*, 2010). Histological abnormalities in testicular tissue of animals administered alcohol was reported by Martinez *et al* in 2009 to include intense spaces, irregular diameter of seminiferous tubules and high amount of necrotic cells in lumen.

In this study plate 4.3 shows enlargement in the size of seminiferous tubules with animals administered with only alcohol having a diameter of 2.8cm and animals admininstered with moringa citrus blend having a diameter of 2.6cm as compared with animals in the normal control group with a diameter of 1.7cm. Similarly plate 4.4 show enlargement in the size of seminiferous tubules with animals administered with only alcohol having a diameter of 3.1cm and animals admininstered with moringa citrus blend having a diameter of 3.4cm as compared with animals in the normal control group with a diameter of 1.7cm.

In 2005 and 2012, Maneesh *et al* and Awodele respectively reported that orally administered moringa leaf extract at their estimated sub-lethal dosages were relatively safe for the testes and liver (body organs). Alcohol has also been said to cause testicular atrophy and damage spermatogenic cells which moringa and vitamine C have been seen to exhibit protective and reversible effect. Buraimoh *et al* (2011) also reported that ethanol and moringa leaf has an appreciable ability to prevent damage to the liver which effect was seen in this study. (null effect).

Table 4.1 shows the comparative weight difference of all animals use in the study showing that across the groups there were no statistically significant weight differences within the duration of study.

It is clear that at certain concentrations i:e >130g per week as reported by Curtis and colleagues (1997), >60g per day by Mills and Meacham (2007), >90g per day Vicari and colleagues (2002), and >165g per day reported by Ser\mondade and colleagues (2010) of alcohol and moringa produce effects that alters the histomorphological architecture of body organs of which the liver and testes are inclusive such effects that included liver cirrhosis, fatty liver disease, liver hepatitis (for the liver) and testicular atrophy, azoospermia (for the testes) which in comparison with the administered dosage of 0.4ml per day in this study proves that at such administered dose they do not produce pathological change to the histomorphological architecture of the liver at the administered dose, however hypertrophic changes are visible in the testes. Gordon dry gin moringa citrus blend could not produce significant effect on the histomorphological architecture of the liver but produce hypertrophic change s in the seminiferous tubules when administered at high doses.

5.2 CONCLUSION

In conclusion, the effect of Gordon dry gin moringa citrus blend on the liver and testes of adult male wistar rats is dose dependent irrespective of the duration of administration and at the dose used for this work there was no significant effect on the histomorphological architecture of the liver but hypertrophic changes are seen in the testes.

5.3 RECOMENDATION

Based on the result from this study, I recommend that further studies should be carried out at higher doses to determine if the hypertrophic effect on the testes are reversible and how long does take to revert if reversible.

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