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Original Research Article

A Comparative Study of the Cytohistomorphological and Biochemical Effect of Alcoholic Beverages (Brandy, Beer, Sour Wine, Dry Gin) on the Testes of Adult Male Wistar Rats

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ABSTRACT

This study was designed to evaluate the cytohistomorphological and biochemical effect of alcoholic beverages on the prostate gland of adult male Wistar rats. Sixty-five (65) rats weighing between 180-230g were used for this experiment. They were randomly divided into 13 groups of five (5) animals each. Group 1 was the normal control. Group 2-13 were the experiment groups. Group 2, 3 and 4 were treated with 1.23mg/kg, 2.45mg/kg and 3.68mg/kg bodyweight of brandy respectively. Group 5, 6 and 7 were treated with 17.32mg/kg, 34.64mg/kg and 51.96mg/kg body weight of beer respectively. Group 8, 9 and 10 were treated with 12.25mg/kg, 24.96mg/kg and 36.74mg/kg bodyweight of soured wine respectively. Group 11, 12 and 13 were treated with 1.73mg/kg, 3.46mg/kg and 5.20mg/kg bodyweight of dry gin respectively. Administration was done daily for 28 days and orally using orogastric tube. On the 29th day, the animals were sacrificed using chloroform inhalation anaesthesia. The blood samples were aspirated via cardiac puncture and centrifuged for biochemical analysis, and testicular tissues were harvested, fixed in 10% buffered formalin, processed, and stained with haematoxylin and eosin. Body weight showed significant ($p<0.05$) increase in brandy administered groups compared to control. For testicular weight, there was an insignificant increase in all the treated groups except the group administered with 3.69mg/kg which showed insignificant decrease compared to control. Results for TT showed a general significant ($p<0.001$) decrease in all administered groups compared to control. FSH showed significant ($p<0.05;0.01;0.001$) decrease in group administered 3.69mg/kg of brandy, all administered groups of soured wine and group administered 5.20mg/kg of dry gin compared to control. Histology showed narrowed and elongated lumen of seminiferous tubule, hypertrophied sertoli cells, destroyed interstitial cells of leydig, distorted seminiferous tubules with degenerating spermatogenic cells in the administered groups compared to control. In conclusion, alcohol beverages pose adverse effects on the testes.

KEYWORDS: Alcoholic Beverages, Testes, Histology, Testosterone, FSH.

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INTRODUCTION

Alcoholic beverages are fermented liquor that contains ethyl alcohol or ethanol as an intoxicating agent; they are produced by sugar containing liquids by fermentation [1].

Alcoholic consumption becomes a problem when the individual engages in chronic drinking pattern that puts him/her health at risk of developing adverse health event [2].

Metabolism is anywhere from 10-30mg/dL/hour and depend upon a multitude of factors, including vital organs size [3].

The level of intoxication and peak is affected by age, gender, weight, presence of food in the

stomach, number of drinks per hour, percentage of alcohol in drink and presence of other drugs that interact with the alcohol [3]. The most consumed alcoholic beverages are brandy, beer, sour wine, dry gin etc. [4]. Brandy is a liquor produced by distilling wine. It generally contains 35%-6% alcohol by volume and its typically consumed as an after-dinner digestif [5].

Beer is one of the oldest and most widely consumed alcohol and is produced by brewing and fermentation of starches mainly derived from cereal, grains mostly from malted barley, though wheat, maize, oats and rice are also used [6]. Brewing beer involves microbial activity at every stage, from raw material production and malting to stability in the package. Most of these activities are desirable but others represent threats to the quality of the final product and must be controlled actively through careful management [7]. Wine contains about 6-21% alcohol by volume [8] and contain substances that have a significant effect on the cardiovascular diseases and some chronic diseases [9]. Dry gin is a distilled alcoholic drink that derives its predominant flavor from juniper berries [10]. Gin today is produced in different ways from a wide range of herbal ingredients giving rise to a number of distinct styles and brands. It contains an alcoholic volume of 35%-60% [11].

The Testes are the male gonads and are primarily the male reproductive organs [12].

They fulfill two functions, the production of gametes (sperm) and the secretion of hormones particularly the male hormone, testosterone [13].

A Testosterone test measure the blood test level of the male sex hormone and follicle stimulating hormone is a hormone produced by gonadotropin cells in the anterior pituitary gland [14].

However, with the recent issues associated with alcoholic beverages, this research is aimed at evaluating the possible effect of alcoholic beverages on the histomorphology of the testes of adult male Wistar rats based on its toxicity considering the duration, quantity or chronic drinking pattern.

MATERIALS AND METHODS

Materials: The materials that was used in this experiment include the following: Experimental rats (60 male wistar rats), well ventilated wooden cages, feed, feeder, wood shavings, tissue, 2ml and 5ml syringes, cannula, beaker, sample bottles, cotton wool, dissecting board, dissecting blade, light microscope, wooden block, rotary microtome, weighing balance, forceps, hand gloves, masking tape, markers, embedding mold, electric water bath, detergent, 10% buffered formalin, chloroform, normal saline, alcohol (Absolute, 95%, 70%), xylene, DPX mount, haematoxylin and eosin. Alcoholic beverages included: brandy, soured wine, dry gin and beer.

Ethical Consideration: Consent and approval was given for the use of the animal house by the Ethics Committee, Faculty of Pharmacy, University of Uyo. Approval letter is attached to the manuscript. Experimental procedures involving the animals and their care was conducted in accordance with the Guide and Care for the Use of Laboratory Animals in biomedical research [15].

Animal Care and Protocol: Sixty-five (65) wistar rats weighing 180g-230g were used for the study. They were obtained from animal house, Faculty of Pharmacology and were acclimatized for two weeks. They were housed in wooden cages under standard housing conditions (Ventilated room with 12/12hour light/dark cycle at 24 ± 2°C). The rats will be fed with standard rat chow and water given *ad-libitum*.

Drug Preparation and Administration: There were four (4) different alcoholic beverages used for this research. The beverages were obtained at a wine store in Uyo city of Akwa Ibom State, Nigeria. The alcoholic beverages include: Brandy (Red Label), Sour Wine (Lambrusco), Dry Gin (Seaman) and Beer (Heineken). The alcoholic beverages were administered orally through an orogastric tube.

Determination of the Median Lethal Dose (LD_{50}): In determining the LD_{50} of the different alcoholic beverages, the Lorke's method was used. Sixty (60) mice weighing between 15g-25g were collected and grouped into four (4) groups according to the number of

alcoholic beverages used. Each group consisted of 3 mice which were well labelled. All animals were observed for restlessness, increased heartbeat, excitation of tissues and death within 24 hours. The LD50 was calculated as the geometric means of the maximum dosage producing 0% mortality (A) and the minimum dosage producing 100% mortality or the dosage in which half of the animals show signs of toxicity and die.

$LD_{50}=\sqrt{AB}$ [16].

Table 1: Experimental Design.

Groups	Regimen	Duration
1-Control	No treatment	28 days
2	Brandy (1.23mg/kg) (low dose)	28 days
3	Brandy (2.4mg/kg) (middle dose)	28 days
4	Brandy (3.68mg/kg) (high dose)	28 days
5	Beer (17.32mg/kg) (low dose)	28 days
6	Beer (34.63mg/kg)(middle dose)	28 days
7	Beer (51.96mg/kg) (high dose)	28 days
8	Soured Wine (12.25mg/kg) (low dose)	28 days
9	Soured Wine (24.29mg/kg)(middle dose)	28 days
10	Soured Wine (36.74mg/kg) (high dose)	28 days
11	Dry Gin (1.73mg/kg)(low dose)	28 days
12	Dry Gin (3.46mg/kg) (middle dose)	28 days
13	Dry Gin (5.20mgs/kg) (high dose)	28 days

Termination of Experiment: On 24 hours after stoppage of administration, the animals were sacrificed by inhalation of chloroform intraperitoneally on day 29. The blood sample was obtained using cardiac puncture for biochemical analysis. Shortly after, the organs (Testes) were harvested, rinsed in Normal Saline and fixed immediately for tissue processing and staining for histomorphological analysis

Morphometric Analysis: The weight of the kidney was assessed with the aid of a weighing balance.

Histopathology studies: The liver was excised and immediately transferred into 10% neutral buffered formalin and processed for light microscopic study, using an automatic tissue processor machine (Shandon 2000, Leica, Frankfurt, Germany). Tissues were dehydrated in various grades of alcohol then cleared in two changes of xylene, infiltrated in two changes of wax bath and finally embedded in paraffin wax. Five microns thick paraffin sections were obtained, which were finally stained using the Hematoxylin and Eosin staining procedure and the sections mounted with DPX and examined microscopically by means of ×10 objective lenses [17].

Biochemical Analysis: Venous blood samples were collected at days 0, 10 and 30 and used for biochemical analysis. The parameters measured included

Determination of Total Testosterone: To determine Total Testosterone, a desired number of coated wells in the holder. 10ìl of standards, specimens and controls was dispensed into appropriate wells. 100ìl of Testosterone-HRP Conjugate Reagent was also dispensed into each wells. 50ìl of anti-Testosterone reagent was dispensed to each well, thoroughly mixed for 30 seconds (it is very important to mix them completely), incubated at 370C for 90 minutes. The incubated mixture was removed by fickling plate contents in a whole container, after which was rinsed and the microtiter was flicked for 5 time with deionized or distilled water (not tap water). The wells were striked sharply onto absorbent paper to remove all residual water droplets. 100ìl of TMB Reagent was dispensed into each well and gently mixed for 5 seconds, incubated at room temperature for 20 minutes. The reaction was stopped by adding 100ìl of Stop Solution to each well, gently mixed for 30 seconds (It is important to make sure that all the blue color changes to yellow completely). Absorbance was read at 450nm with a micromtiter well reader within 15 minutes [14].

Determination of Follicle Stimulating Hormone: The Follicle Stimulating Hormone determination is based on the principle of solid phase enzyme-linked immunosorbent assay (ELISA). The assay system utilizes mouse monoclonial anti-a-LH for solid phase (microtiter wells) immobilization and a mouse monoclonal anti-B-LH antibody-enzyme (horseradish peroxides) conjugate solution. The test sample is allowed to react simultaneously with the antibodies, resulting in the LH molecules being sandwiched between the olid phase and enzyme-linked antibodies. After 45 minutes incubation at room temperature, the wells are washed with water to remove unbound-labeled antibodies. A solution of TMB Reagent is added and incubated for 20minutes, resulting in the development of a blue color. The

color development is stopped with the addition of stop solution and the color is changed to yellow and measured spectrophotometrically at 450nm. The concentration of LH is directly proportional to the color intensity of the test sample [18].

Statistical Analysis: All data are expressed as mean ± SD. Data was statistically analysed using one-way analysis of variance (ANOVA) using the Graph Pad Insta3 software package. The Bonferri's test was applied for the detection of significance between different groups with respect to control. The P value of <0.05 was deemed to be statistically significant.

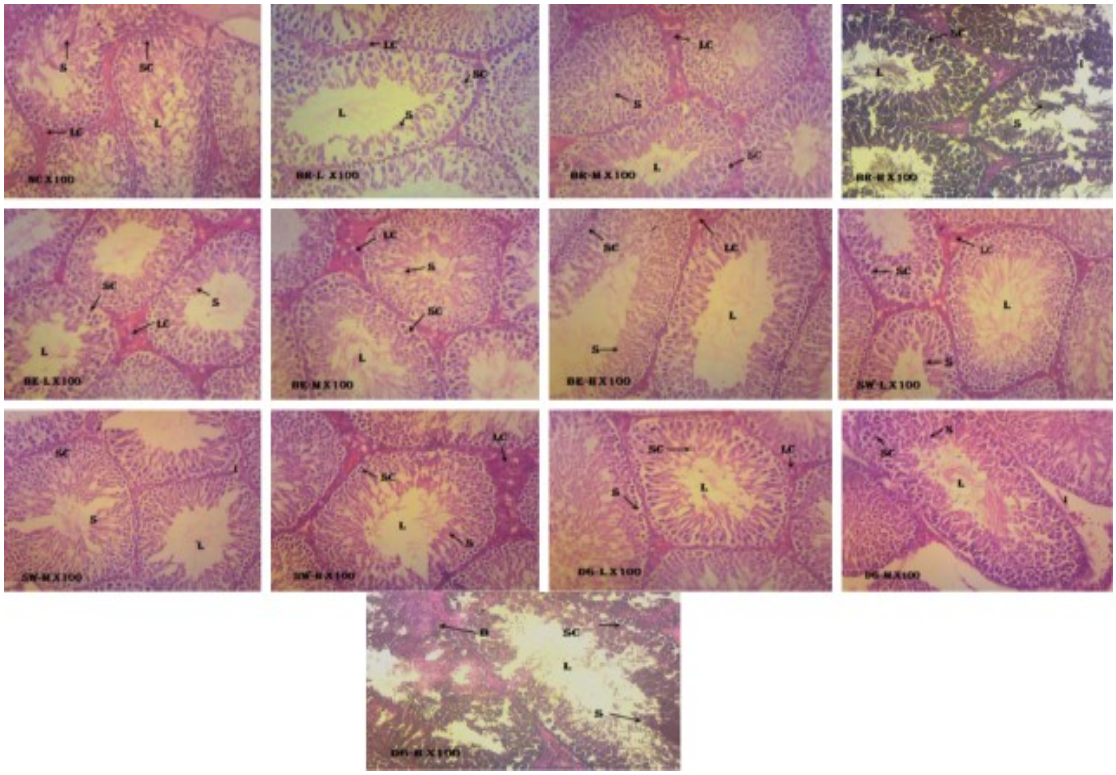

Fig. 1: Photomicrographs of the testis treated with 5.20 mg/kg body weight of dry gin showing s= seminiferous tubules distorted with degenerating spermatogenic cells. l= lumen of seminiferous tubule dilated. sc= sertoli cells. b= blood deposits on seminiferous tubules. using haematoxylin and eosin (h&e) stain at magnification x100. interference: severely affected

RESULTS

Body Weight: The body weight of the rats was taken before the administration commenced, every seven (70 days and at the last day after administration. Student paired T-test was used as a statistical tool for the analysis of the bodyweight before and after the administration. There was a general marked difference in body weight of all the groups but groups administered 1.23mg/kg, 2.46mg/kg and 3.69mg/kg bodyweight of brandy showed significant increase in the final body weightcompared to the initial weight at p<0.05 respectively. Only group 13 administered with 5.20mg/kg bodyweight of dry gin showed a slight insignificants decrease in final bodyweight when compared to initial bodyweight (Table 2).

Organ Weight: For testis, there was an insignificant increase in all the treated groups except the group administered with 3.69mg/kg which showed insignificant decrease compared to control (Table 3).

Hormonal Assay: Results for testosterone showed a general significant decrease in all treated groups of brandy, beer, soured wine and dry gin compared to control at $p < 0.001$ respectively. For follicle stimulating hormone (FSH), we observed a significant decrease in group administered 3.69mg/kg bodyweight of brandy compared to control at $p< 0.001$. There was also a significant decrease in all treated groups of soured wine compared to control at $p < 0.05$ and $p < 0.01$ respectively. In addition, group administered 5.20mg/kg bodyweight of dry gin also showed significant decrease compared to control at $p < 0.05$ (Table 4).

Table 2: Showing body weight difference.

Groups	Initial Body Weight (g)	Final Body Weight (g)	Weight Difference (g)
1. Normal Control	150.2±3.54	161.6±4.18	11.4±0.64
2. Brandy(1.23mg/kg)	129.4±3.61	154.6±6.11*	25.2±2.50
3. Brandy(2.46mg/kg)	147.8±11.50	171.4±17.44*	23.8±5.94
4. Brandy(3.69mg/kg)	142.6±9.08	163.6±12.31*	21.0±3.23
5. Beer(17.32mg/kg)	164.6±8.88	183.6±9.90	19.0±1.02
6. Beer(34.64mg/kg)	154.4±12.52	171.4±15.39	17.0±2.87
7. Beer(51.96mg/kg)	183.6±9.99	181.3±15.01	2.3±5.02
8. Soured Wine(12.25mg/kg)	179.0±11.03	188.4±9.15	9.4±1.88
9. Soured Wine (24.49mg/kg)	190.6±10.73	207.5±11.55	16.9±0.82
10.Soured Wine(36.74mg/kg)	168.8±9.89	183.0±8.51	14.2±1.48
11. Dry Gin (1.73mg/kg)	181.6±13.53	1832.2±12.24	0.6±1.29
12. Dry Gin (3.46mg/kg)	187.8±13.66	185.4±10.75	2.4±2.91
13. Dry Gin (5.20mg/kg)	185.8±4.66	182.2±8.50	3.6±3.84

Values are expressed in Mean±SEM

*indicates significance from initial body weight at $p < 0.05$

Table 3: Showing results for testis weight.

Groups	Testes (g)
1. Normal Control	0.26±0.05
2. Brandy(1.23mg/kg)	0.36±0.01
3. Brandy(2.46mg/kg)	0.31±0.05
4. Brandy(3.69mg/kg)	0.21±0.03
5. Beer(17.32mg/kg)	0.30±0.02
6. Beer(34.64mg/kg)	0.27±0.04
7. Beer(51.96mg/kg)	0.31±0.04
8. Soured Wine(12.25mg/kg)	0.34±0.06
9. Soured Wine (24.49mg/kg)	0.38±0.04
10.Soured Wine(36.74mg/kg)	0.30±0.01
11. Dry Gin (1.73mg/kg)	0.38±0.03
12. Dry Gin (3.46mg/kg)	0.39±0.07
13. Dry Gin (5.20mg/kg)	0.37±0.04

Values are expressed in Mean±SEM

Table 4: Showing results for hormonal assay.

Groups	Testosterone (ng/ml)	FSH (m/u/ml)
1. Normal Control	3.13±0.26	1.50±0.33
2. Brandy(1.23mg/kg)	1.83±0.24***^{a}	0.85±0.18
3. Brandy(2.46mg/kg)	1.12±0.11***^{a}	0.82±0.12
4. Brandy(3.69mg/kg)	0.55±0.06***^{a}	0.46±0.06***^{a}
5. Beer(17.32mg/kg)	0.99±0.16***^{a}	1.08±0.11
6. Beer(34.64mg/kg)	0.94±0.18***^{a}	1.05±0.12
7. Beer(51.96mg/kg)	0.57±0.04***^{a}	0.88±0.15
8. Soured Wine(12.25mg/kg)	0.78±0.06***^{a}	0.66±0.09*^{a}
9. Soured Wine (24.49mg/kg)	0.76±0.10***^{a}	0.69±0.15*^{a}
10.Soured Wine(36.74mg/kg)	0.57±0.10***^{a}	0.62±0.12**^{a}
11. Dry Gin (1.73mg/kg)	0.78±0.19***^{a}	0.90±0.1
12. Dry Gin (3.46mg/kg)	0.91±0.11***^{a}	0.99±0.14
13. Dry Gin (5.20mg/kg)	0.70±0.04***^{a}	0.72±0.12*^{a}

Values are expressed in Mean±SEM

*a, **a, ***a indicates significance from control at $p < 0.05$, $p < 0.01$ and $p < 0.001$ respectively.

DISCUSSION

Alcohol beverages are common and a norm in our society especially during events and celebrations [19]. They can be consumed singly or in combination; sometimes individuals take brandy and beer, dry gin and soured wine etc, intermittently depending upon their desires. Both chronic and acute consumption of alcohol has been reported to cause infertility disturbances such as low sperm count and mortility, reduced serum/plasma testosterone level, testicular atrophy and irregularity in the diameter of the seminiferous tubules in men and laboratory animals [20]. In this study, there was a general marked difference in body weight of all the groups but groups administered 1.23, 2.46 and 3.69mg/kg bodyweight of brandy showed significant increase in the final body weight compared to the initial weight respectively. Only group administered with 5.20mg/kg bodyweight of dry gin showed a slight insignificant decrease in final bodyweight when compared to initial bodyweight. This is obvious because brandy has a high alcoholic content and is been reported to increase weight of affected organs thereby increasing the weight of the body [3]. In the present study, for testis weight, there was an insignificant increase in all the treated groups except the group administered with 3.69mg/kg which showed insignificant decrease compared to control.

Forquer *et al*. [21] have also reported significant reductions in androgen levels following ethanol intoxication in males. Undoubtedly, ethanol consumption produces a significant decrease in the percentage of motility concentration [22] and normal morphology in human and animal spermatozoa [22]. In agreement, results from our study for testosterone showed a general significant decrease in all treated groups of brandy, beer, soured wine and dry gin compared to control at $p < 0.001$ respectively. Report from Ren *et al*. [24] showed that alcohol does not only affect LH and FSH synthesis but also impedes their secretion.

Muthusami and Chinnaswamy [25] reported that alcohol has a direct toxic effect on the testis which leads to decreased seminiferous tubular functions. For follicle stimulating hormone (FSH), we observed a significant decrease in group administered 3.69mg/kg bodyweight of brandy compared to control at $p< 0.001$. There was also a significant decrease in all treated groups of soured wine compared to control respectively. In addition, group administered 5.20mg/kg bodyweight of dry gin also showed significant decrease compared to control.

A study conducted by Martinez *et al*. [26] reported histological abnormalities in testicular tissue of alcoholic animals which included; intense inter cellular spaces, irregular diameter of seminiferous tubules and high amount of necrotic cells in the lumen compared with controls and epididymal sperm mortality also decreased in ethanol treated rats. One effect of ethanol consumption on the testis is probably a change in the structure of the mitochondria [27].

In agreement to the above, the histological findings showed that rats testes administered with 2.46 mg/kg body weight of Brandy showed narrowed lumen of seminiferous tubule, and 3.69 mg/kg body weight of Brandy showed seminiferous tubules with spermatogenic cells partially destroyed, sertoli cells hypertrophied and interstitial cells of Leydig destroyed. Rats administered with 51.96 mg/kg body weight of Beer showed distorted seminiferous tubules and lumen of seminiferous tubule elongated. Rats administered with 36.74 mg/kg body weight of Soured showed seminiferous tubules with spermatogenic cells distorted and atrophy of the sertoli cells. Rats administered with 3.46 mg/kg body weight of Dry Gin showed seminiferous tubules with spermatogenic cells distorted, sertoli cells hypertrophied and interstitial cells of Leydig destroyed; and 5.20mg/kg body weight of Dry Gin showed seminiferous tubules distorted with degenerating spermatogenic cells, lumen of seminiferous tubule dilated and blood deposits on seminiferous tubules.

CONCLUSION

The result of this study showed that alcoholic beverages has an adverse effect on the testes

of albino rats. The organ weight was insignificantly affected and the biochemical analysis were significantly higher compared to the control group. The histomorphological alterations and degenerations showed that alcohol can be harmful to the testes when consumed excessively.

Conflicts of Interests: None

There was no competing interest among the authors in this article.

Author Contributions

Dr Idorenyin Umoh made substantial contributions in conception and design of the work, have been involved in drafting the manuscript, revised critically for important intellectual content, and have given final approval of the manuscript version to be published.

Mr Samuel Umanah helped in the analysis and interpretation of data, drafting and revising the manuscript critically for important content.

Miss Abasiekeme Ephraim contributed in the conception and design of the work and acquisition of data.

Miss Patience Udoh contributed in the conception and design of the work and acquisition of data.

Miss Precious Azodo contributed in the conception and design of the work and acquisition of data.

Miss Emilia Eshiet contributed in the conception and design of the work and acquisition of data.

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