Biochemical And Histological Effect Of Alcoholic Beverages On The Kidney Of Adult Wistar Rats

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RUNNING TITLE: Comparative Effect of Alcoholic Beverages

Abstract*—This study was designed to evaluate the histomorphological and biochemical effect of different alcoholic beverages; brandy, beer, soured wine and dry gin, on the kidney of adult Wistar rats. Sixty-five (65) rats weighing between 180-230 g were used for this research. They were randomly divided into 13 groups of five (5) rats each. Group 1 was the control group. Group 2-13 were the experimental groups. Group 2, 3 and 4 were administered with 1.23 mg/kg, 2.45 mg/kg and 3.68 mg/kg of brandy respectively. Group 5, 6 and 7 received 17.32 mg/kg, 34.64 mg/kg and 51.96 mg/kg of beer respectively. Group 8, 9 and 10 received 12.25 mg/kg, 24.96 mg/kg and 36.74 mg/kg of soured wine respectively. Group 11, 12 and 13 were administered with 1.73mg/kg, 3.46 mg/kg and 5.20 mg/kg of dry gin respectively. Administration was orally using orogastric tube daily for 28 days. On the 29th day, the animals were sacrificed under chloroform inhalation anaesthesia. The blood samples were obtained via cardiac puncture for biochemical analysis and kidney tissues were harvested, fixed in 10% buffered formalin, processed, and stained with haematoxylin and eosin for histological studies. Histologically, results showed cytoarchitectural alterations with atrophied and haemorrhagic glomerulus, dilated bowman's space, and collecting ducts in the groups of rats administered with high doses of the alcoholic beverages compared to control. Urea was significantly ($p<0.05$) higher in high doses of brandy, soured wine and dry gin compared to control, respectively, chloride was significantly ($p<0.05$) lower in high dose of dry gin compared to control, no significant difference in K, Na+, creatinine and HC03, respectively. This study showed that alcoholic beverages may be detrimental to the kidney and its functions especially in high doses with longer and consistent duration. Keywords: Alcoholic beverages, Kidney, Histology, Kidney enzymes

Introduction

According to the World Health Organization report in 2016, about 43 % of the world's population that are over 15 years of age, have been reported to drinking of alcohol in the past 12 months (21). According to Chaiyasong *et al*, (5), the drinking rate of men and women was 75.3 % and 45.7 %, respectively (17). An alcoholic drink or beverage is a drink that contains ethanol, a type of alcohol produced by fermentation of grains, fruits, or other sources of sugar (9). Alcohol consumption has various effects on health but however, previous studies have reported that light to moderate alcohol consumption has some beneficial effects such as reduction in the risk of cardiovascular disease and type II diabetes (2, 9). Alcohol consumption has known to be associated with health problems including liver disease, pancreatitis, neurologic complications, and certain cancers (2).

The kidney's function is to filter blood as it removes wastes, control the body's fluid balance, and keep the right levels of electrolytes (12). This common knowledge may lead to the assumption that alcohol consumption may also cause kidney damage and consequently contribute to a decline in kidney function. Animal studies have shown that alcohol may cause kidney injury by inducing oxidative stress and inflammation (10). However, a study by Yuan *et al,* (23) found that alcohol preserved renal function by reducing tubular damage and inflammation in mice with renal ischemic/reperfusion injury (23). Another study also suggested an inverse association between alcohol consumption and the risk of renal impairment or chronic kidney disease (CKD) (12).

There are several potential mechanisms to explain the inverse association between alcohol consumption and decline in kidney function. First of all, polyphenolic compounds in alcoholic beverages exhibit antioxidant and anti-inflammatory properties, both of which may have renal protective effects (22). Moreover, even polyphenol-free alcoholic beverages have been found to exert anti-inflammatory or antioxidant effects (8), even though they were less effective than those with high amounts of polyphenol (7). Kidney function, assessed by measurement of the glomerular filtration rate declined by about 8 mL/min/1.73 m2 per decade after age 40 years (6). The decline in kidney function may be accelerated due to various factors such as hypertension, diabetes, primary renal disorders, and some medications causing kidney injury. It is an observed problem that patients with impaired kidney function also consume alcohol (6). Previous studies have shown that about

20– 36 % of patients with CKD consume alcohol either occasionally or daily, and 10 % of patients even drink heavily (4). Notwithstanding, the association between alcohol consumption and kidney function has received relatively less attention and studies have been inconclusive.

A study reported that alcohol consumption was associated with the development or progression of CKD (11). In another study, however, alcohol consumption was not associated with kidney function; rather, it was inversely associated with the risk of chronic kidney disease (12). Higher total alcohol intake, more frequent alcohol consumption, and binge drinking were associated with a lesser decline in the estimated glomerular filtration rate (eGFR). This association was more pronounced among non-smokers, participants aged below 60 years, and those with albuminuria; however, it was completely attenuated among women (3). Notwithstanding, the association between alcohol consumption and kidney function has received relatively less attention and studies have been inconclusive. This research was however, designed to investigate the influence of different alcohol beverage consumption on kidney histology and function, and the progression of any renal damage using adult Wistar rats.

Materials and Method

Materials

The materials that was used in this experiment include the following: Experimental rats, well ventilated wooden cages, feed, feeder, wood shavings, tissue, 2 ml and 5 ml 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 mountant, haematoxylin and eosin. Alcoholic beverages included: brandy, soured 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 (14).

Animal Care and Protocol

Sixty-five (65) Wistar rats weighing 180 - 230 g 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 Soured Wine store in Uyo City of Akwa Ibom State, Nigeria. The alcoholic beverages include: Brandy (Red Label), Sour Soured Wine (Lambrusco), Dry Gin (Seaman) and Beer (Heineken). The alcoholic beverages were administered orally through an orogastric tube.

Determination of the Median Lethal Dose (LD50)

In determining the LD_{50} of the different alcoholic beverages, the Lorke's method was used. Sixty (60) mice weighing between 15 g -25 g 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 LD_{50} 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.

$LD50=\sqrt{AB}$ (13).

Experimental Design

Groups	Regimen	Duration
1-Control	Distilled Water	28 days
2 Experimental	Brandy LD (1.23mg/kg)	28 days
3 Experimental	Brandy MD (2.4mg/kg)	28 days
4 Experimental	Brandy HD (3.68mg/kg)	28 days
5 Experimental	Beer LD (17.32mg/kg)	28 days
6 Experimental	Beer MD (34.63mg/kg)	28 days
7 Experimental	Beer HD (51.96mg/kg)	28 days
8 Experimental	Soured Wine LD (12.25mg/kg)	28 days
9 Experimental	Soured Wine MD (24.29mg/kg)	28 days
10 Experimental	Soured Wine HD (36.74mg/kg)	28 days
11 Experimental	Dry Gin LD (1.73mg/kg)	28 days
12 Experimental	Dry Gin MD (3.46mg/kg)	28 days
13 Experimental	Dry Gin HD (5.20mgs/kg)	28 days

Legend: LD= Low dose, MD= Middle dose, HD= High dose

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 organ (kidney) 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 (1).

Biochemical Analysis

Determination of Creatinine

Normal creatinine clearance for healthy women is 88-128 ml/min and 97-137ml/min in males. A Jaffe rate reaction in which creatinine reacts with picrates in an alkaline solution to form a red-creatinine picrate complex was used. The intensity of the colour is proportional to the optical density which was measured at 520 nm (15).

Determination of Urea

Urea reacted with diacetyl in hot acid solution at nearly 100 $^{\circ}$C, which was released from diacetyl monoxime by an oxidative condensation reaction to give a coloured product. The absorbance colour developed was measured at 480 nm. The intensity of the colour developed was proportional to the concentration of urea present in the sample (15).

Determination of Electrolytes

The different biochemical methods for determination of sodium ions, potassium ions and chloride ions using standard biochemical procedures and principles were employed. Sodium was precipitated as the triple salt, sodium magnesium uranyl aceate, with the excess uranium being reacted with ferrocyanide to produce chromophore whose absorbance varied indirectly with the concentration of sodium in the test sample (19). The amount of potassium was determined by using sodium tetraphenylboron, a specifically prepared mixture to produce a colloidal suspension. Chloride was estimated using the method described by Skeggs and Hochestrasser (19).

Results

Body Weight

There was a general marked difference in body weight of all the groups but groups administered 1.23, 2.46 and 3.69 mg/kg bodyweight of brandy showed significant ($p<0.05$) increase in the final body weight compared to the initial weight, respectively. Only group administered with 5.20 mg/kg bodyweight of dry gin showed a slight insignificant decrease in final bodyweight when compared to initial bodyweight (Table 1).

Table 1: Showing body weight difference

Groups	Initial Body Weight (g)	Final Body Weight (g)	Weight Difference (g)
Normal Control	150.2±3.54	161.6±4.18	11.4±0.64
Brandy LD (1.23mg/kg)	129.4±3.61	154.6±6.11	25.2±2.50*
Brandy MD (2.4mg/kg)	147.8±11.50	171.4±17.44	23.8±5.94*
Brandy HD (3.68mg/kg)	142.6±9.08	163.6±12.31	21.0±3.23*
Beer LD (17.32mg/kg)	164.6±8.88	183.6±9.90	19.0±1.02
Beer MD (34.63mg/kg)	154.4±12.52	171.4±15.39	17.0±2.87
Beer HD (51.96mg/kg)	183.6±9.99	181.3±15.01	2.3±5.02
Soured Wine LD (12.25mg/kg)	179.0±11.03	188.4±9.15	9.4±1.88
Soured Wine MD (24.29mg/kg)	190.6±10.73	207.5±11.55	16.9±0.82
Soured Wine HD (36.74mg/kg)	168.8±9.89	183.0±8.51	14.2±1.48
Dry Gin LD (1.73mg/kg)	181.6±13.53	183.2±12.24	0.6±1.29
Dry Gin MD (3.46mg/kg)	187.8±13.66	185.4±10.75	2.4±2.91
Dry Gin HD (5.20mgs/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

4.1.2:Organ Weight

Result of the kidney revealed that there was an insignificant increase in all treated groups except group treated with 1.23 mg/kg bodyweight of brandy, compared to control. Groups treated with 24.49 mg/kg bodyweight of soured wine and 3.46 mg/kg bodyweight of dry gin were significantly higher compared to group administered 1.23 mg/kg bodyweight of brandy at p<0.05. Dr gin high dose was significantly higher compared to control and group administered 1.23 mg/kg bodyweight of brandy at p<0.05, respectively (Table 2).

Table 2: Showing results of kidney weight

Groups	Kidney (g)
Normal Control	1.05±0.05
Brandy LD (1.23mg/kg)	0.93±0.01
Brandy MD (2.4mg/kg)	1.19±0.09
Brandy HD (3.68mg/kg)	1.12±0.03
Beer LD (17.32mg/kg)	1.22±0.04
Beer MD (34.63mg/kg)	1.21±0.10
Beer HD (51.96mg/kg)	1.25±0.05
Soured Wine LD (12.25mg/kg)	1.11±0.06
Soured Wine MD (24.29mg/kg)	1.29±0.06*^{b}
Soured Wine HD (36.74mg/kg)	1.17±0.05
Dry Gin LD (1.73mg/kg)	1.24±0.10
Dry Gin MD (3.46mg/kg)	1.30±0.08*^{b}
Dry Gin HD (5.20mg/kg)	1.34±0.08*^{a,b}

Values are expressed in Mean ± SEM

*^{a} indicates significance from control at p<0.05

*^{b} indicates significance from 1.23 mg/kg bodyweight of brandy at p<0.05

4.1.3Biochemical Analysis

Potassium (K), as well as sodium (Na^{+}) showed insignificant decrease in all treated groups compared to control. Chlorine also showed insignificant decrease in most treated groups compared to control but group administered with 5.20 mg/kg bodyweight of dry gin showed significant decrease compared to control at p<0.05 (Table 3).

Results of urea showed significant increase in groups administered with 3.69mg/kg bodyweight of brandy and 5.20 mg/kg of dry gin compared to control at p<0.05 respectively. Group treated with 36.74 mg/kg bodyweight of soured wine also showed significant decrease compared to control at p<0.01. Creatinine showed slight insignificant decrease in all treated groups compared to control and HCO_3 showed no marked difference in all the treated groups compared to control (Table 3).

Table 3: Showing results of kidney function tests

Groups	K (mmol/l)	Na+ (mmol/l)	Cl- (mmol/l)	Urea (mmol/l)	Creatinine (umol/l)	HCO3 (mmol/l)
Normal Control	5.15±0.45	171.3±5.22	41.75±1.49	9.15±0.93	153.8±14.74	**26.75±2.29**
Brandy LD (1.23mg/kg)	5.78±0.19	168.5±6.46	31.00±1.83	9.10±0.63	142.3±4.54	**25.75±1.93**
Brandy MD (2.4mg/kg)	5.70±0.92	170.5±15.97	33.75±2.18	9.18±0.28	147.3±10.08	**27.25±1.79**
Brandy HD (3.68mg/kg)	4.88±0.72	168.0±9.54	36.75±1.25	10.43±0.84*a	154.8±13.48	**28.25±1.75**
Beer LD (17.32mg/kg)	5.25±0.72	170.0±18.25	38.50±1.56	12.00±0.48	144.8±9.26	**25.50±2.06**
Beer MD (34.63mg/kg)	5.15±0.83	168.3±10.32	34.00±0.91	9.53±0.35	147.3±5.01	**26.25±1.80**
Beer HD (51.96mg/kg)	4.96±0.38	165.8±8.54	31.25±2.93	10.35±0.38	151.5±11.69	**27.00±2.74**
Soured Wine LD (12.25mg/kg)	4.60±0.27	165.3±8.23	38.25±1.65	9.83±0.57	136.3±7.76	**28.00±1.08**
Soured Wine MD (24.29mg/kg)	5.93±0.23	164.3±16.80	40.00±1.58	10.68±0.43	143.3±9.73	**31.50±0.65**
Soured Wine HD (36.74mg/kg)	5.00±0.33	160.3±9.18	39.50±3.60	12.73±0.31**a	151.5±8.93	**27.00±0.58**
Dry Gin LD (1.73mg/kg)	4.75±0.63	164.5±9.37	31.75±1.93	9.28±1.47	144.3±23.56	**25.00±1.47**
Dry Gin MD (3.46mg/kg)	4.78±0.27	161.5±1.26	30.75±1.75	10.10±0.56	155.3±10.17	**30.25±3.38**
Dry Gin HD (5.20mgs/kg)	5.10±0.52	164.8±5.02	31.25±1.70*a	12.40±0.51*a	157.8±5.39	**28.00±1.68**

Values are expressed in Mean±SEM

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

Histological Findings

Photomicrography of the kidney of normal control showed normal glomerulus, bowman's space and collecting ducts. Photomicrography of rats' kidney administered with 1.23 mg/kg body weight of Brandy showed haemorrhage in the glomerulus, normal bowman's space and collecting duct. Photomicrography of rats' kidney administered with 2.46 mg/kg body weight of Brandy showed atrophied glomerulus, normal bowman's space and haemorrhagic collecting ducts. Photomicrography of rats' kidney administered with 3.69 mg/kg body weight of Brandy showed haemorrhagic glomerulus, normal bowman's space and dilated collecting duct (Fig. 1).

Photomicrography of rats' kidney administered with 17.32 mg/kg body weight of Beer showed splitted glomerulus, and normal bowman's space and convulated ducts. Photomicrography of rats' kidney administered with 36.64 mg/kg body weight of Beer showed distorted glomerulus, unaffected bowman's space, and dilated collecting ducts. Rats' kidney administered with 51.96 mg/kg body weight of Beer showed distorted glomerulus, vacuolated bowman's space, and dilated collecting ducts, with haemorrhage (Fig. 1).

Photomicrography of rats' kidney administered with 12.25 mg/kg body weight of Soured Wine showed distorted glomerulus, normal bowman's space and collecting ducts. Photomicrography of rats' kidney administered with 24.49 mg/kg body weight of Soured Wine showed distorted glomerulus, vacuolated bowman's space and normal convulated ducts. Rats' kidney administered with 36.74 mg/kg body weight of Soured Wine showed haemorrhagic and distorted glomerulus, vacuolated bowman's space, and dilated collecting ducts (Fig. 1).

Photomicrography of rats' kidney administered with 1.73 mg/kg body weight of Dry Gin showed haemorrhagic glomerulus, mild shrinkage in bowman's space and collecting ducts. Photomicrography of rats' kidney administered with 3.46 mg/kg and 5.20 mg/kg body weight of Dry Gin showed haemorrhagic glomerulus, shrinked bowman's space, and dilated collecting ducts (Fig. 1).

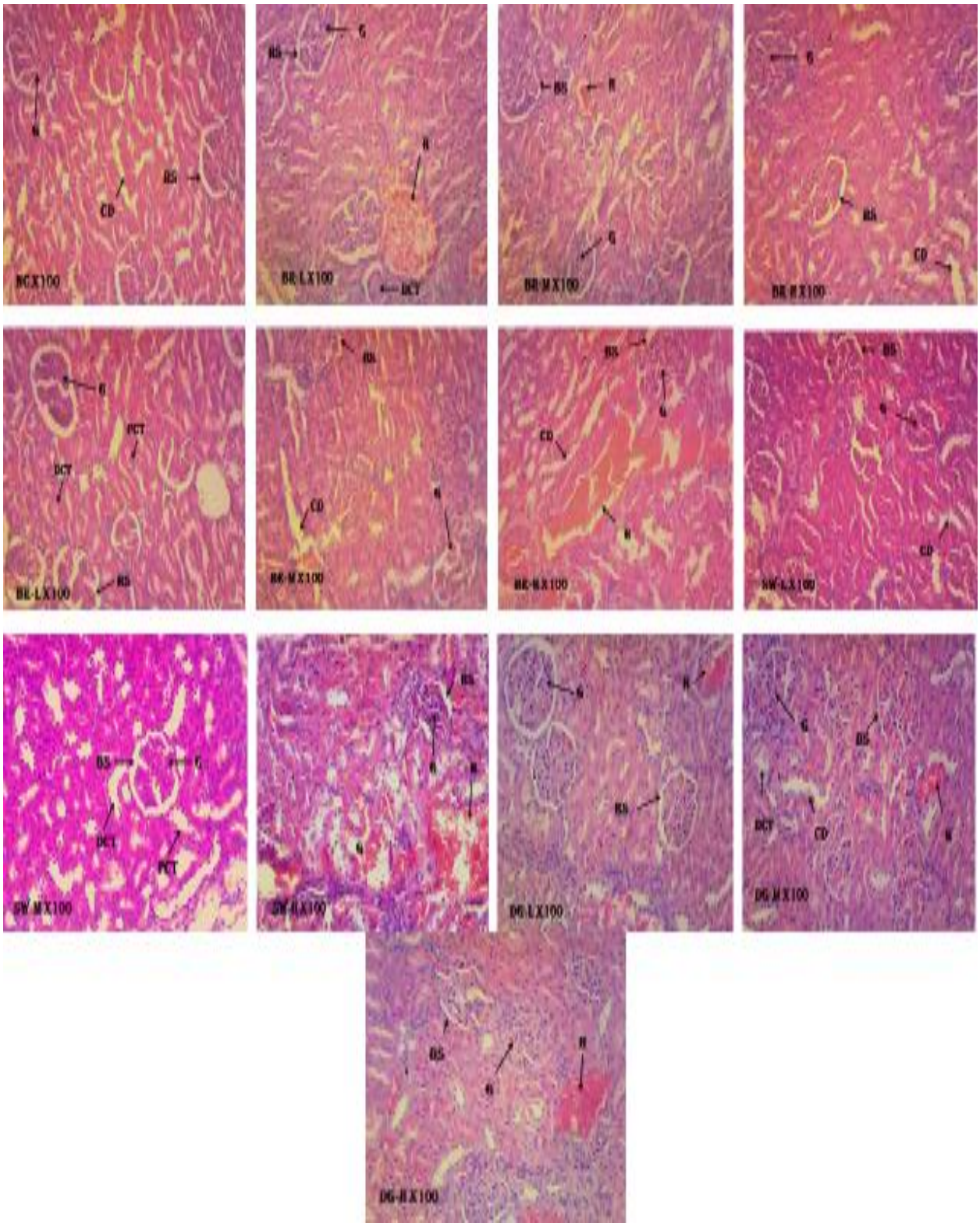

Fig. 1: Photomicrographs of the Kidney administered with alcoholic beverages showing G= Haemorrhagic Glomerulus, BS= Bowman's Space shrinked, CD= Collecting Duct dilated, H= Haemorrhage. Stained with H&E at x100 magnification

Discussion

Over the years, alcohol has been known to be most common social drug to be consumed worldwide, with an average annual consumption of 6.2 L of pure alcohol per capita or 13.5 g of pure alcohol per day (20). Alcohol consumption in the population is influenced by different aspects, including the volume of alcohol consumed, the drinking pattern, and the age and gender of the drinker (5, 10, 20). Alcohol intake is a widespread activity globally and alcohol energy can be a contributing factor to weight gain.

In the present study, rats treated with brandy had increased body weight compared to the control and other alcoholic beverage treated groups. A study

conducted by Joo *et al,* (11) showed that the energy content in 1gram of alcohol is 29 KJ and can lead to weight gain. According to Koning *et al,* (12), light to moderate alcohol intake, especially wine intake, may be more likely to protect against weight gain, whereas consumption of spirits has been positively associated with weight gain. Rats treated with moderate doses of soured wine and dry gin were significantly higher in kidney weight compared to low dose of brandy. However, only the high dose of dry gin caused increase in rats' kidney compared to the control. Concomitantly, Reynolds *et al*, (16) compared kidney structure and function in alcohol-fed and control rats. The alcohol-fed group experienced kidney swelling and significantly reduced kidney function; in addition, under microscopic examination, the kidneys of alcohol-fed rats were found to have cells enlarged with increased amounts of protein, fat and water, compared with those of control animal.

Potassium (K), as well as sodium (Na^+) showed insignificant decrease in all treated groups compared to control. Chlorine also showed insignificant decrease in most treated groups compared to control but group administered with 5.20 mg/kg bodyweight of dry gin showed significant decrease compared to control. According to Sloan *et al,* (18), alcohol consumption has historically been found to reduce the amount of potassium excreted by the kidney. According to Yuan *et al,* (23), alcohol consumption has major effects on the absorption, elimination and serum concentrations of many physiologically important electrolytes such as sodium, potassium, calcium and chlorine. Results of urea showed significant increase in groups administered with 3.69 mg/kg bodyweight of brandy and 5.20 mg/kg of dry gin compared to control. According to a study carried out by Harris *et al,* (10), it indicates that alcohol down regulates urea synthesis, possibly via a redox effect. Group treated with 36.74 mg/kg bodyweight of soured wine also showed significant decrease compared to control at. Creatinine showed slight insignificant increase in all treated groups compared to control and $HC0_3$ showed no marked difference in all the treated groups compared to control.

Histologically, kidney of control rats showed normal glomerulus, bowman's space and collecting ducts. Rats' kidney administered with low dose of Brandy showed haemorrhage in the glomerulus, normal bowman's space and collecting duct. Brandy intermediate dose showed atrophied glomerulus, normal bowman's space and haemorrhagic collecting ducts, while high dose of Brandy showed haemorrhagic glomerulus, normal bowman's space and dilated collecting duct. Low dose of Beer showed splitted glomerulus, and normal bowman's space and convulated ducts, Intermediate dose of Beer showed distorted glomerulus, unaffected bowman's space, and dilated collecting ducts. However, high dose of Beer showed distorted glomerulus, vacuolated bowman's space, and dilated collecting ducts, with haemorrhage.

Rats' kidney administered low dose of Soured Wine showed distorted glomerulus, normal bowman's space and collecting ducts, intermediate dose showed distorted glomerulus, vacuolated bowman's space and normal convulated ducts, and high dose showed haemorrhagic and distorted glomerulus, vacuolated bowman's space, and dilated collecting ducts. Low dose of Dry Gin showed haemorrhagic glomerulus, mild shrinkage in bowman's space and collecting ducts. Intermediate and high doses of Dry Gin showed haemorrhagic glomerulus, shrinked bowman's space, and dilated collecting ducts. In previous investigation, several striking alterations after chronic alcohol administration was observed and the basement membrane of the glomerulus became abnormally thickened which was characterized by cell proliferation (3), and this supports the present study.

Conclusion

This study showed that alcoholic beverages may be detrimental to the kidney and its functions especially in high doses

Recommendation

A shorter duration of study should be considered to investigate if perhaps there may not be detrimental effects, as compared to longer duration. Also, special staining methods like immunohistochemical staining be employed to ascertain toxicity of these alcoholic beverages.

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