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Comparative Histopathological and Biochemical Study Of The Effect Of Alcoholic Beverages On The Liver Of Adult Wistar Rats

Case Study

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Abstract

This study was designed to evaluate the histopathological and biochemical effect of different alcoholic beverages; brandy, beer, soured wine and dry gin, on the liver of adult 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. Group 2-13 were used as the experiment group. 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 liver 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, and no significant difference in liver weight. Histology showed haemorrhagic central and portal vein; atrophied, dilated and vacuolated sinusoids, dilated bile ducts and general distortion in cyto-architecture of administered groups compared to control. AST, total and conjugated bilirubin showed significant ($p<0.01$, 0.001) decrease in high dose of brandy, beer and soured wine compared to control respectively. ALT showed significant ($p<0.01$) decrease in high dose of beer compared to group C and Total protein and Albumin showed no significant difference. In conclusion, brandy and dry gin caused more detrimental effect to the liver than the other alcoholic beverages.

Keywords: Alcoholic Beverages; Liver; Histology; Liver Function.

Introduction

Alcohol (ethanol or ethyl alcohol) is the ingredient found in beer, wine and spirits which causes drunkenness [22]. At low doses, alcohol can act as a stimulant, inducing feelings of euphoria and talkativeness, but drinking too much alcohol at one session can lead to drowsiness, respiratory depression, coma or even death [12]. As well as its acute and potentially lethal sedative effect at high doses, alcohol has effects on every organ in the body, and these effects depend on the blood alcohol concentration (BAC) over time [30]. Most of the metabolism, or breaking down of alcohol from a toxic substance to water and carbondioxide is performed by the liver, with the rest excreted through the lungs (allowing alcohol breath tests), through the kidneys (into urine) and in sweat [30]. Chronic heavy alcohol use can damage the liver, causing alcoholic liver disease. This occurs across a spectrum from fatty liver, to acute alcoholic hepatitis, to cirrhosis [3].

Beer is a fermented carbonated alcoholic beverage produced from malted barley using yeast as biological catalyst and hops (Humulus Lupulus) as spice which gives bitterness to the beer [4]. The specific mechanisms through which beer and its minor components may affect the liver are not fully understood and poorly elucidated. Experimental studies in humans showed that there are only limited data from human studies investigating the effect of beer drinking on liver enzymes [24]. Wine is made by fermenting the juices of grapes or other fruits such as apples (cider), cherries, berries, or plums [1]. Brandy is strong alcoholic spirit made from fruit juice or distilled wine. Brandy is derived from wine, yet it is aged in oak barrels, which increases the alcohol content and also

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gives it a unique colour [18]. Brandy can affect normal functioning of the liver [5].

Gin is a colourless spirit obtained by distilling an aqueous mixture of alcohol together with aromatic plant materials, generally juniper berries (juniperuscommunis L.), to which water and alcohol and at times fruit juices, extracts, and/or essential oils of fruits may be added [14]. Gin has a lethal effect on the liver cells and duration of this alcohol consumption is a major determinant of the degree of alcoholic liver disease [15]. This study was carried out in order to estimate or see the damaging effect of different alcohol beverages on the histology and function of the liver.

Materials and Method

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 mountant, haematoxylin and eosin. Alcoholic beverages included: brandy, soured wine, dry gin and beer.

Ethical Approval

The experimental procedure was approved by the Akwa Ibom State Ministry of Health Ethical Committee, Uyo, Nigeria. The protocol was in accordance to National Guidelines for care and use of laboratory animals (National Research Council, 2011).

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 12/12 hour 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 (LD50)

In determining the LD50 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.

$LD50=\sqrt{AB}$(Lorke, 1983).

Experimental Design

Table 1.

Termination of Experiment

On 24 hours after stoppage of administration, the animals were sacrificed by inhalation of chloroform intraperitoneally on day 29. The liver tissues were harvested for histological studies and blood aspirated for biochemical analysis.

Morphometric Analysis

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

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

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 [2].

Biochemical Analysis

Venous blood samples were collected at days 0, 10 and 30 and used for biochemical analysis. The parameters measured included aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), total protein, albumin and bilirubin (total and conjugated). AST, ALT, ALP, total protein and albumin were analyzed using commercial diagnostic kits from Randox laboratory, United Kingdom. The kits employed the procedure of [21] for the analysis of AST, ALT and ALP, while total protein was estimated using the procedure of [28]. Albumin and bilirubin (total and conjugated)was estimated using the method of Grant (1987).

Statistical Analysis

The results were analysed using (SPSS) version 22. The data were expressed using descriptive statistics and Analysis of Variance (ANOVA). Multiple comparisons for the groups were done using Post Hoc Turkey to test for the level of significance between means. A $p < 0.05$ was considered to be statistically significant. Values are expressed in Means ±Standard Deviation (M±SD). Superscript c: indicates significant difference ($p < 0.05$).

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 body weight before and after the administration. There was a general marked difference in bodyweight 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 weight compared to the initial weight at $p<0.05$ respectively. Only group 13 administered with 5.20mg/kg bodyweight of dry gin showed a slight insignificant decrease in final bodyweight when compared to initial bodyweight (Table 4.1).

Organ Weight

Results of the liver weight showed no marked difference in all treated groups compared to control (Table 4.2).

Biochemical Analysis

Result of AST showed significant decrease in group administered 3.69mg/kg bodyweight of brandy compared to control at $p < 0.001$. Groups administered 51.96mg/kg bodyweight of beer and 36.74mg/kg bodyweight of soured wine were also significantly lower compared to control at $p < 0.01$ respectively. ALT showed significant decrease in groups administered with 51.96mg/kg bodyweight of beer and 5.20mg/kg bodyweight of dry gin compared to group administered with 3.69mg/kg bodyweight of brandy at $p < 0.01$ respectively (Table 4.3). ALP showed a general insignificant decrease in all treated groups compared to control except group administered with 1.23mg/kg bodyweight of brandy. There was a significant decrease in groups administered 3.69mg/kg bodyweight of brandy, 34.64 and 51.96mg/kg bodyweight of beer, all treated groups of soured wine and 5.20mg/kg bodyweight of dry gin compared to group administered 1.23mg/kg bodyweight of brandy at $p < 0.05$, $p < 0.01$ and $p < 0.001$ respectively (Table 4.3).

Total protein and Albumin showed no marked difference in the treated groups compared to control. Total bilirubin showed significant decrease in groups administered 3.69mg/kg bodyweight of brandy, 51.96mg/kg bodyweight of beer and 36.74mg/kg bodyweight of soured wine compared to control at $p < 0.01$ and $p < 0.001$ respectively. Conjugated bilirubin also showed signifi-

Table 4.1: 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
1.23mg/kg body weight of Brandy	129.4±3.61	154.6±6.11*	25.2±2.50
2.46mg/kg body weight of Brandy	147.8±11.50	171.4±17.44*	23.8±5.94
3.69mg/kg body weight of Brandy	142.6±9.08	163.6±12.31*	21.0±3.23
17.32mg/kg body weight of Beer	164.6±8.88	183.6±9.90	19.0±1.02
34.64mg/kg body weight of Beer	154.4±12.52	171.4±15.39	17.0±2.87
51.96mg/kg body weight of Beer	183.6±9.99	181.3±15.01	2.3±5.02
12.25mg/kg body weight of Soured Wine	179.0±11.03	188.4±9.15	9.4±1.88
24.49mg/kg body weight of Soured Wine	190.6±10.73	207.5±11.55	16.9±0.82
36.74mg/kg body weight of Soured Wine	168.8±9.89	183.0±8.51	14.2±1.48
1.73mg/kg body weight of Dry Gin	181.6±13.53	1832.2±12.24	0.6±1.29
3.46mg/kg body weight of Dry Gin	187.8±13.66	185.4±10.75	2.4±2.91
5.20mg/kg body weight of Dry Gin	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 4.2: Showing results of the liver weight.

Groups	Liver (g)
Normal Control	4.48±0.56
1.23mg/kg body weight of Brandy	5.74±0.78
2.46mg/kg body weight of Brandy	5.04±0.44
3.69mg/kg body weight of Brandy	5.38±0.35
17.32mg/kg body weight of Beer	5.31±0.56
34.64mg/kg body weight of Beer	6.10±0.26
51.96mg/kg body weight of Beer	5.55±0.19
12.25mg/kg body weight of Soured Wine	6.26±0.20
24.49mg/kg body weight of Soured Wine	5.55±0.24
36.74mg/kg body weight of Soured Wine	5.51±0.21
1.73mg/kg body weight of Dry Gin	4.57±1.25
3.46mg/kg body weight of Dry Gin	5.37±0.31
5.20mg/kg body weight of Dry Gin	4.86±0.33

Values are expressed in Mean±SEM

Table 4.3: Showing results of Liver function tests (AST, ALT and ALP).

Groups	AST	ALT	ALP
Normal Control	56.6±4.43	23.5±1.85	21.0±2.38
1.23mg/kg body weight of Brandy	62.6±4.18	24.5±2.53	27.3±4.59
2.46mg/kg body weight of Brandy	59.6±2.36	26.3±1.75	25.0±1.87
3.69mg/kg body weight of Brandy	31.0±3.29***a	20.8±0.85	14.25±0.85**b
17.32mg/kg body weight of Beer	57.8±3.51	23.8±1.30	18.0±1.47
34.64mg/kg body weight of Beer	56.6±2.98	21.3±0.48	16.3±1.03*b
51.96mg/kg body weight of Beer	32.4±4.01**a	16.5±0.96**c	12.3±0.63***b
12.25mg/kg body weight of Soured Wine	56.2±5.25	19.0±1.87	15.8±1.32*b
24.49mg/kg body weight of Soured Wine	49.4±2.54	18.3±1.65	16.5±1.56*b
36.74mg/kg body weight of Soured Wine	32.6±2.02**a	18.0±1.58	12.5±0.65***b
1.73mg/kg body weight of Dry Gin	54.4±5.50	18.5±2.06	18.5±2.40
3.46mg/kg body weight of Dry Gin	53.4±2.42	18.8±0.85	17.3±1.84
5.20mg/kg body weight of Dry Gin	42.8±4.15	16.5±1.56**c	15.3±1.60**b

Values are expressed in Mean±SEM
*, **, *** indicates significance at $p < 0.05$ and $p < 0.01$ respectively.
a, b and c represent significance from control, 1.23mg/kg and 2.46mg/kg bodyweight of brandy

cant decrease in groups administered 3.69mg/kg bodyweight of brandy, 51.96mg/kg bodyweight of beer and 36.74mg/kg bodyweight of soured wine compared to control at $p < 0.01$ and $p < 0.001$ respectively (Table 4.4).

Discussion

The liver is a critical organ in the human body and a hub for numerous physiological processes [29] which include macronutrient metabolism, blood volume regulation, immune system support, endocrine control for growth signalling pathways, lipid and cholesterol homeostasis, and the breakdown of xenobiotic compounds, including many current drugs [29]. Thus, the liver is a prime target for damage because it processes everything that enters the mouth and is swallowed [27]. Liver disease is the major cause of death every year; Approximately 29 million people suffer from chronic liver condition [7]. The most common causes of liver disease worldwide are alcohol, non-alcoholic steatohepatitis associated with obesity and metabolic syndrome, chronic hepatitis A [7-9].

Our findings showed that there was a general marked difference in body weight of all the administered groups but however rats administered with 1.23 mg/kg, 2.46 mg/kg and 2.69 mg/kg body weight of brandy showed significant increase in body weightrespectively. Only rats administered with 5.20 mg/kg body weight of dry gin showed a slight insignificant decrease in final body weight when compared to initial body weight. Alcohol is not only an addictive substance; it is a high- caloric beverage that interferes with metabolic function [8] and when consumed to intoxicating levels, it can affect an individual's weight status [10]. Results also

Table 4.4: Showing results of Liver function tests (T.P, Albumin, T.B, C.B).

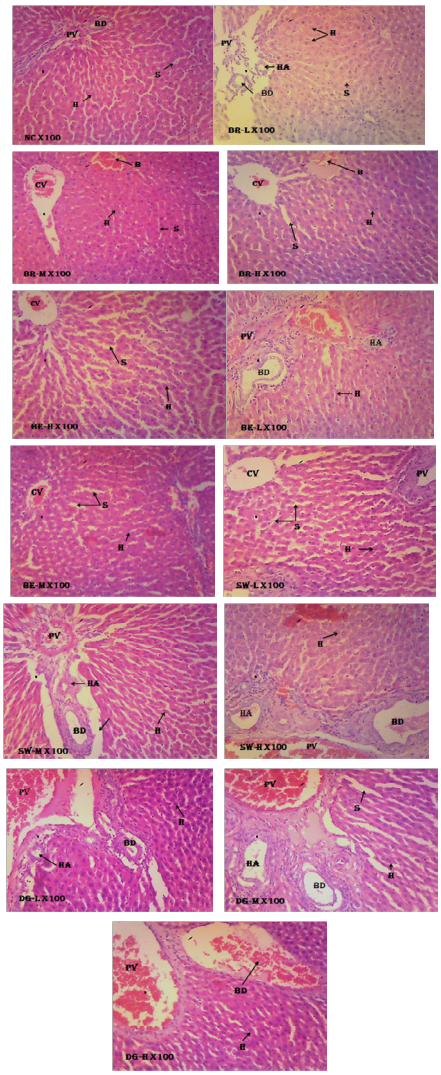
Groups	Total Protein	Albumin	Total Bilirubin	Conjugated Bilirubin
Normal Control	62.3±4.33	38.0±3.24	10.98±0.69	6.75±0.83
1.23mg/kg body weight of Brandy	65.5±2.53	40.0±2.35	10.50±0.19	7.50±0.41
2.46mg/kg body weight of Brandy	59.5±4.41	35.8±4.89	11.83±0.28	7.60±0.40
3.69mg/kg body weight of Brandy	75.0±6.06	37.3±3.90	7.70±0.43**a	4.03±0.20***a
17.32mg/kg body weight of Beer	58.5±4.33	36.5±1.85	10.63±0.30	7.83±0.61
34.64mg/kg body weight of Beer	70.3±23.50	40.3±2.02	10.50±0.21	6.53±0.58
51.96mg/kg body weight of Beer	58.5±4.81	32.3±4.05	7.88±0.66**a	4.68±0.27**a
12.25mg/kg body weight of Soured Wine	70.5±2.53	39.8±1.03	9.88±0.54	5.70±0.79
24.49mg/kg body weight of Soured Wine	67.3±5.98	34.3±2.66	9.90±0.50	5.95±0.64
36.74mg/kg body weight of Soured Wine	67.5±8.99	35.3±4.33	6.93±0.42***a	4.00±0.39***a
1.73mg/kg body weight of Dry Gin	68.0±5.72	40.5±4.79	10.91±0.74	6.75±0.73
3.46mg/kg body weight of Dry Gin	69.8±7.66	36.5±4.74	10.98±0.23	7.88±0.14
5.20mg/kg body weight of Dry Gin	75.8±4.11	34.8±5.81	10.28±0.38	6.35±0.25

Values are expressed in Mean±SEM

**a, ***a indicates significance from initial body weight at $p < 0.01$ and $p < 0.001$ respectively

Figure 4.1-4.13: Photomicrographs of the Liver showing PV= Haemorrhagic and dilated Portal Vein, CA= Haemorrhagic Central Vein, HA= Hepatic Artery, Bd= Dilated Bile duct, S= Sinusoids, H= Hepatocytes. Stained with H&E at x100 magnification.

Legend: NC- Normal Control, BR-L= Brandy Low, BR-M= Brandy Middle, BR-H= Brandy High, BE-L= Beer Low, BE-M= Beer Middle, BE-H= Beer High, SW-L= Soured Wine Low, SW-M= Soured Wine Middle, SW-H= Soured Wine High, DG-L= Dry Gin Low, DG-M= Dry Gin Middle, DG-H= Dry Gin High.



showed no marked difference in liver weight of the rats in all the administered groups compared to control.

The most common health-related consequences include alcoholic liver disease (ALD), which is a condition associated with various morphological changes in the liver, ranging from steatosis to advanced steatosis accompanied by inflammation, fibrosis, and / or cirrhosis [20, 13]. Histological findings showed normal histological features in the liver of normal control and rats administered with 1.23mg/kg body weight of Brandy; central vein with haemorrhage, blood deposits and atrophied sinusoids in animals administered with 2.46mg/kg body weight of Brandy; haemorrhagic central vein, blood deposits, Vacuolated sinusoids, and general distortions in cyto architecture in 3.69 mg/kg body weight of Brandy administered rats.

Rats administered with 17.32 mg/kg body weight of Beer showed portal vein with haemorrhage; 34.64 mg/kg body weight of Beer showed central vein with haemorrhage, blood deposits and vacuolatedsinusoids; 51.96 mg/kg body weight of Beer showed central Vein with blood deposits, dilated sinusoids, and general distortions. Animals administered with 12.25 mg/kg body weight of Soured Wine showed normal central and portal vein, and dilated sinusoids; 24.49 mg/kg body weight of Soured Wine showed normal portal Vein, hepatic artery, bile duct, vacoulated portal triad area and normal hepatocytes; 36.74 mg/kg body weight of Soured Wine showed haemorrhagic portal vein, dilated bile duct and blood droplets.Animals administered with 1.73 mg/kg body weight of Dry Gin showed haemorrhagic portal vein, but normal hepatic artery, bile duct and hepatocytes; 3.46 mg/kg body weight of Dry Gin showed haemorrhagic portal vein, and dilated Sinusoids; 5.20 mg/kg body weight of Dry Gin showed haemorrhagic and dilated portal vein, and dilated Sinusoids.

In the present study, the result of aspartate aminotransferase (AST) showed significant decrease in groups administered 3.69mg/kg bodyweight of brandy, 51.96mg/kg bodyweight of beer and 36.74mg/kg bodyweight of soured wine compared to control respectively. Alanine aminotransferase (ALT) showed significant decrease in groups administered with 51.96mg/kg bodyweight of beer and 5.20mg/kg bodyweight of dry gin compared to group administered with 3.69mg/kg bodyweight of brandy respectively. Experimental studies in humans showed that there are only limited data from human studies investigating the effect of beer drinking on liver enzymes [24]. Two independent cross-over trials indicated that up to 4 beers/day do not affect liver enzymes significantly. No evidence of an increase in gamma-glutamyltransferase (GGT), aspartate animotranasferase (AST) and alanine aminotransferase (ALT) after 3 weeks of daily intake of 4 beers was found in 11 men [24]. However, in a small cross-over trial of 10 men consuming 4 beers/day and of a post-menopausal women consuming 3 beers/day for a period of three weeks, found a slight increase of GGT and ALT but only in women [23]. Alkaline Phosphatase (ALP) showed a general insignificant decrease in all treated groups compared to control except group administered 1.23mg/kg bodyweight of brandy. There was a significant decrease in groups administered 3.69mg/kg bodyweight of brandy, 34.64mg/kg bodyweight of beer and 51.96mg/kg bodyweight of beer, all treated groups of soured wine and 5.20mg/kg of dry gin compared to administered 1.23mg/kg bodyweight of brandy 1 respectively. Total protein and Albumin showed no marked difference in the treated groups compared to control.

Total bilirubin and conjugated bilirubin showed significant decrease in groups administered 3.69mg/kg bodyweight of brandy, 51.96mg/kg bodyweight of beer and 36.74mg/kg bodyweight of soured wine compared to control respectively. One of the polyphenols of wine is resveratrol [6], and study have showed that oral administration of 20mg of resveratrol daily for six weeks can significantly prevent the loss of liver weight and inhibit serum alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, and bilirubin levels [16].

Conclusion

From this research, it has been observed that Alcoholic beverages has the ability to cause histomorphological and biochemical alterations. The biochemical parameters were altered.

Recommendation

Alcoholic beverages is proven to be of high toxicity and should not be consumed for prolonged periods of time. It is pertinent for more research to be conducted to ascertain its toxic effect using special staining techniques and immunohistological methods.

References

[1]. Akubor, P. I., Obio, S. O., Nwadomere, K. A. and Obiomah, E..The Technology of Wine Making. Plant Foods Hum. Nutr.2013; 58(3):6-13.

[2]. Anwioro OG. Histochemistry and tissue pathology; Principlesand Techniques.3rdEdition. Society for Cellular Pathology Scientist of Nigeria.2014: 97-98.

[3]. Baan, R., Straif, K., Bouvard, V. and Ghissassi, F..Carcinogenicity of Alcoholic beverages. Lancet Oncol.2017; 8(4): 292-293.

[4]. Belitz HD, Grosch W, Schieberle P. Alcoholic beverages. Food Chem. 2009;353 (17468):892-937.

[5]. Bertrand, A. Brandy and Cognac-Armagnac and their Manufacture.Ecyclopedia of Food Science and Nutrition.2nd Edition.Edited by Caballero, B., Turgo, I. C. and Finglas, P.M. Academic Press, Oxford.2013: 584-601.

[6]. Bishayee A, Politis T, Darvesh AS. Resveratrol in the chemoprevention and treatment of hepatocellular carcinoma. Cancer Treat. Rev. 2010 Feb 1;36(1):43-53.

[7]. Blachier M, Leleu H, Peck-Radosavljevic M, Valla DC, Roudot-Thoraval F. The burden of liver disease in Europe: a review of available epidemiological data. J. Hepatol. 2013 Mar 1;58(3):593-608.

[8]. Casbon TS, Curtin JJ, Lang AR, Patrick CJ. Deleterious effects of alcohol intoxication: diminished cognitive control and its behavioral consequences. J Abnorm Psychol. 2003 Aug;112(3):476-87.Pubmed PMID: 12943026.

[9]. Diew, A. M. Effects of Alcohol on Liver Regeneration.Alcohol Health and research World. 2016;17(4): 279-283.

[10]. Field M, Mogg K, Bradley BP. Alcohol increases cognitive biases for smoking cues in smokers. Psychopharmacology. 2005 Jun;180:63-72.

[11]. Grant GH. Amino Acids and Proteins. In: Fundamentals ofClinical Chemistry, Tietz NW, 3rd edition, Philadephia, USA: WB Saunders Company. 1987: 328-329.

[12]. Griswold, M. G., Tapp, A. G., Abrar, M. M., and Aghayan, S. A. A Systematic Analysis for the Global Burden of Disease Study.The Lancet.2018;392(10152): 1015-1035.

[13]. Hagström H, Hemmingsson T, Discacciati A, Andreasson A. Alcohol consumption in late adolescence is associated with an increased risk of severe liver disease later in life. J. Hepatol. 2018 Mar 1;68(3):505-10.

[14]. Hernandez, E. G. Gin Composition and Analysis. Encyclopedia of Food Sciences and Nutrition, 2nd Edition.2003:2894-2898.

[15]. Idonije, O. B. and Okojie, F. O. Liver Enzymes Activities in Nigerian Local Gin Consumers. Cent. Eur. J. Biol.2012; 1(4) : 131-133.

[16]. Lee ES, Shin MO, Yoon S, Moon JO. Resveratrol inhibits dimethylnitrosamine-induced hepatic fibrosis in rats. Arch. Pharmacal Res. 2010 Jun;33:925-32.

[17]. Lorke D. A new approach to practical acute toxicity testing. Arch. Toxicol. 1983 Dec;54:275-87.

[18]. Masami, O., Guymon, J. F., and Crowell, E. A. Changes in Some Volatile Constituents of Brandy During Aging. Am. J. Enol. Vitic.2016; 28:152-158.

[19]. National Research Council .Guide to the care and use of laboratory animals, 8th edition. The National Academics Press: Washington, DC. 2011:240.
[20]. Osna NA, Donohue Jr TM, Kharbanda KK. Alcoholic liver disease: pathogenesis and current management. Alcohol research: current reviews. 2017;38(2):147-161.
[21]. Reitman S, Frankel S. A colorimetric method for determination ofserum glutamate oxaloacetate and glutamic pyruvate transaminase. Am J Clin Pathol.1957; 28:56-58.
[22]. Roehrs, T. and Roth, T. Sleep, Sleepiness, and Alcohol use. Alcohol Research and Health. 2011;25(2): 101-109.
[23]. Sierksma A, van der Gaag MS, Kluft C, Hendriks HF. Moderate alcohol consumption reduces plasma C-reactive protein and fibrinogen levels; a randomized, diet-controlled intervention study. Eur J Clin Nutr. 2002 Nov;56(11):1130-6.Pubmed PMID: 12428180.
[24]. Steele, C. M., and Josephes, R. A. Alcoholic Myopia Dangerous Effects. Am Psychol.2009; 45(8): 921-933.
[25]. Subramaniam S, Hedayathullah Khan HB, Elumalai N, Sudha Lakshmi SY. Hepatoprotective effect of ethanolic extract of whole plant of Andrographis paniculata against CCl 4-induced hepatotoxicity in rats. Comparative Clinical Pathology. 2015 Sep;24(5):1245-51.
[26]. Suwa, Y., Kohda, H. and Yomo, H. Influence of Moderate Drinking in Purine and Carbohydrate Metabolism.Alcoholism. 2016;26(1):20-25.
[27]. Tanimizu, N. and Mitaka, T. Morphogenesis of Liver Epithelial Cells: A Review. J. Hepatol. 2017; 46(10): 964-976.
[28]. Tietz NW. Clinical guide to laboratory tests.3rd Edition.Philadephia USA: WB Saunders Company.1995 :518-519.
[29]. Trefts, E., Gannon, M. and Wasserman, D. H. The Liver.Current Biology.2017; 27(21): 1147-1151.
[30]. Zakhari, S. Alcoholism: An Overview-How Alcohol is metabolized by the body. Alcohol Res. Health.2016; 29(4): 245-254.