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ORIGINAL PAPER

Assessment of hepatorenal biochemical indices in male Sprague Dawley rats preceding concurrent oral administration of Ghana alcoholic bitters and natural cocoa powder

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ABSTRACT

Introduction and aim. There has been a surge in the consumption of Ghanaian alcoholic bitters. Ghanaian alcoholic bitters are formulated from a maceration of not less than three plant parts making the organic composition very complex. There appear to be no studies on the effect of Ghanaian alcoholic bitters on the hepatorenal biochemistry indices. The study aimed to assess the effects of alcoholic bitters and cocoa powder on the liver and kidney serum biochemistry.

Material and methods. Twenty-four healthy male Sprague Dawley rats, age 11–12 weeks, weighing 150–250 g were used. The rats were randomly assigned to four groups (n=6). At the end of the experimentation, a blood sample was taken by cardiac puncture and centrifuged to obtain the serum for biochemical assays and analysis.

Results. The liver enzymes showed no significant difference between the treatment and control groups. There were higher mean values for total bilirubin and direct bilirubin for alcoholic bitters and natural cocoa powder groups respectively than the control group and the co-administration of alcoholic bitters and natural cocoa powder group.

Conclusion. The study concludes that alcoholic bitters consumption might cause injury to the liver and kidney resulting in anomaly of the hepatorenal indices from rat blood serum biochemistry.

Keywords. biochemistry, Ghanaian alcoholic bitters, hepatorenal indices, natural cocoa powder, Sprague Dawley rat

Introduction

Ghanaian alcoholic bitters are alcohol-based preparations obtained by macerating fresh or dried plant parts such as the bark, root, leaves and/or seeds in alcohol of 18% to 45% concentration.[1-3] The Ghanaian alcoholic bitters are formulated from a combination of not less than three plant parts making the organic composition of these drinks very complex. Most of the alcoholic bitters used plant parts from *Xylopia aethiopica*, *Anthocleista nobilis* and *Khaya senegalensis* for the concoction preparation.

Alcoholic bitters are widely consumed all over Ghana and neibouring countries including, Côte d'Ivoire, Togo and Nigeria.[4] This high consumption is linked to the claimed health benefits consumers intend to derive from its intake. Excess use of alcoholic bitters negatively

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affects human health, and is a serious worldwide problem.[5] The reasons for drinking alcoholic beverages vary, and they include being part of a standard diet, medical objectives, relaxing effects, euphoric effects, recreational purposes, artistic inspiration, alleged aphrodisiac benefits, and happiness.[6] Alcoholic bitters, however, play a direct role in the production of reactive oxygen and nitrogen species, creating an environment prone to oxidative stress.[5] Among the most severe health problems caused by alcohol, is its adverse effect on the liver and kidney. The damage to the liver and kidney is mostly detected when there are anomalies of hepatorenal indices from liver and kidney function tests. These hepatorenal indices include aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP) and gamma-glutamyl transpeptidase (GGT), total protein, albumin, creatinine, urea and bilirubin.[7] For instance, alcoholic herbal bitters was found to cause a combined elevation in urea and creatinine and bilirubin suggesting a moderate to severe form of kidney and liver damage in rats.[7] There have been no studies conducted on the effect of Ghanaian alcoholic bitters on the hepatorenal indices. This study's findings would serve as the baseline for diagnosing hepatorenal disorders using these biochemical indices. This would be easier, more affordable and less riskier than performing biopsies for liver and kidney tissues which is invasive.

Furthermore, there is no intervention that could counteract the oxidative stress on the liver and kidney tissues preceding Ghanaian alcoholic bitters consumption. Therefore, utilizing natural cocoa powder known to be a rich source of flavonoid, theobromine,[8] and several important natural antioxidants could provide protection for the body against free radicals.[9]

Aim

The aim of this study is to determine if simultaneous consumption of Ghanaian alcoholic bitters with natural cocoa powder has a protective effect on the liver and kidneys. The aim was accomplished by assessing liver and kidney damage through changes in the hepatorenal biochemical indices using Sprague Dawley rats.

Material and methods

Study design

This experimental study used Sprague Dawley rats as a model for evaluation of Ghanaian alcoholic bitters consumption and its effect on the hepatorenal biochemical indices. This experimental research design using animals was used to increase human knowledge and significantly offer solutions to questions in biological and biomedical sciences.[10] The authors followed the criteria for the alcohol model because the Ghanaian herbal alcoholic bitters are a mixture of at least three plant parts (concoction) in alcohol with a bitter, sour or bittersweet flavor.[11]

Alcoholic bitters

A popularly used alcoholic bitters in Ghana made with *Xylopia aethiopica, Anthocleista nobilis* and *Khaya senegalensis* in 42% (v/v) alcohol was selected for this study.

Natural cocoa powder

Natural cocoa powder (NCP) was purchased from the manufacturer. The content of the natural cocoa powder as stated in the manusfacturer's information sheet is shown in Table 1.

Table 1. Composition of Natural coacoa powder according to manufacturer's specification

Component	Amount (g/100 g)
Total dietary fiber	34.3
Total carbohydrate	15.5
Protein	24.0
Moisture	6.0
Free fatty acid	1.2
Theobromine	1.2
Total ash	5.7
Acid insoluble ash	0.1

A solution of the NCP (20% (w/v)) was freshly prepared daily with warm water. The procedure for the preparation of the natural cocoa solution (NCS) was as follows:

- 1 g of the NCP was measured using a chemical weighing scale,
- 5 mL of warm water was added to the 1 g NCP to make a natural cocoa solution (NCS),
- The 20% (w/v) NCS solution was given to the rats through oral administration.

Animals

Twenty-four healthy Sprague Dawley rats of 11–12 weeks of age and weighing 150–250 g were acquired from the Center for Scientific Research into Plant Medicine, Mampong, Ghana. They were housed in the laboratory animal house of the Department of Anatomy, University of Health and Allied Sciences, Ho Teaching Hospital, for two weeks to achieve acclimatization before the commencement of the experiment. They were kept in cages at a laboratory temperature condition of 24–27°C and a 12-hour light-dark cycle. The animals were fed on grower mash and had free access to water ad libitum throughout the study. All the animals were treated according to the National Institute of Health Guidelines for the care and use of laboratory animals (NIH, Department of Health, and Human Services Publication no. 85-23, revised 1985). The study was approved by the Committee on Human Research, Publication, and Ethics of the School of Medicine and Dentistry, Kwame Nkrumah University of Science and Technology, with reference number: CHRPE/RC/205/17. Only male rats were used for the study because female rats are more

susceptible to alcoholic hepatotoxicity and are more likely to die early.[12]

Experimental design

The 24 mature male albino rats were randomly assigned to four groups (n=6) as follows:

1. Group A: This was the control group given 1.5 mL of physiological saline through oral administration every day for 63 days
2. Group B or the alcoholic bitters (ALC_B) group received a daily dose of 1000 mg/kg of 42% (v/v) alcoholic bitters
3. Group C or the NCP group – the third experimental rats were given 1000 mg/kg body weight of a 20% (w/v) NCS
4. Group D: Alcoholic Bitters + NCP (ALC_B + NCP) – the group D rats were given Alcoholic Bitters + NCS at a dosage of 1000 mg/kg of 42% (v/v) of alcoholic bitters and 1000 mg/kg of 20% (w/v) of NCS daily.

All treatments were administered between 9:00–11:00 am by oral administration daily for 63 days following a recommendation by Srikanth et al. and Siervo et al.[13-14] The body weight of each rat was determined using an animal weighing balance on the first day to serve as a baseline and repeated weekly throughout the entire period of the treatment.

Determination of parameters for biochemical assays

Twenty-four hours after the last treatment, the rats were put under light thiopental sodium anaesthesia and blood samples were taken by cardiac puncture. The blood samples were collected into plain tubes for liver and renal biochemical assays. The blood samples in the plain tubes were centrifuged at 4,000 rpm for 15 minutes and the supernatant (serum) was separated and stored at a temperature of –4°C until it was used for assaying. The serum biochemistry was performed with the VITROS® 5600 Integrated System (Ortho Clinical Diagnostics, Raritan, New Jersey, USA). Parameters that were determined for the liver function test were aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), gamma-glutamyl transpeptidase (GGT), total bilirubin (T-BIL), direct bilirubin (DBIL), indirect bilirubin (I-BIL), total-protein (T-PROT), albumin and globulin. The renal function test parameters were serum sodium (Na^+), serum potassium (K^+), chloride (Cl^-), blood urea nitrogen (BUN) and serum creatinine.

Data analysis

Statistical analyses were carried out using the IBM Statistical Package for Social Sciences (SPSS) software (SPSS 24.0 version, Inc., Chicago, IL, USA) and GraphPad Prism 8.4.2 (GraphPad Software, Inc., San Diego, CA, USA). The values were expressed in means±standard error of the mean (SEM). Normal distribution was tested with the one-sample Kolmogorov-Smirnov test and Shapiro-Wilk normality test. Mean differences among the control and treatment groups were tested with the analysis of variance test (ANOVA), followed by Tukey's Multiple Comparison (TMC) Test. The level of statistical significance was determined at $p < 0.05$ (95% confidence interval).

Results

Descriptive statistics of the weight of organs of the Sprague Dawley rats

Table 2 presents the descriptive statistics from a one-way analysis of variance (ANOVA) of the weight of the liver and the kidneys of the rats post-treatment. The weight of the liver in the control group was 6.54±0.58 g. This was higher than observed for alcoholic bitters and in the NCP groups (6.27±0.50 and 6.19±0.47 g) respectively, but lower than in the ALC+NCP (6.55±0.32 g) group. However, the differences were not statistically significant (p=0.9255). The mean weight of the right and left kidneys in the control group were 0.60±0.05 g and 0.66±0.87g espectively. There was no statistically significant variations of means of the liver and kidney indices among the groups (p>0.05).

Table 2. Weights of organs of Sprague Dawley rats treated with alcoholic bitters and NCP*

Organs	Control	ALC_B	NCP	ALC_B+NCP	p
Liver (g)	6.54±0.58	6.27±0.50	6.19 ±0.47	6.55 ±0.32	0.926
Right kidney (g)	0.60±0.05	0.63 ±0.03	0.76 ±0.09	0.67 ±0.03	0.476
Left kidney (g)	0.66±0.87	0.65 ±0.05	0.70 ±0.09	0.64 ±0.01	0.733

* Data recorded in mean±standard error of the mean, ALC_B – alcoholic bitters, NCP – natural cocoa powder, ALC_B/NCP – alcoholic bitters + NCP, statistically significant difference (p<0.05)

Descriptive statistics and ANOVA of liver serum biochemical indices of Sprague Dawley rats

Table 3 shows the mean values and one-way analysis of variance (ANOVA) of the liver function test results of the Sprague Dawley rats fed with alcoholic bitters and natural cocoa powder. The four liver enzymes; AST, ALT, ALP and GGT, demonstrated no statistically significant differences between the groups. There was, however, a numerically higher mean value of GGT (53.80±27.74 U/L) of ALC_B than the other treatment groups as well as the control group. Also, the mean values of ALT (121.50±40.89 U/L) and ALP (339.00±64.31 U/L) of the control group were numerically higher than in the treatment groups. The AST/ALT ratio was not statistically different between the groups (p>0.05). Regarding the bilirubin parameters, the total and direct bilirubin

showed no significant difference between all the groups. There were, however, higher mean values for total bilirubin (43.81±24.2 µmol/L) and direct bilirubin (21.84±0.2 µmol/L) for the ALC_B than the control group and the ALC_B + NCP group. The indirect bilirubin mean values for the ALC_B and NCP were significantly higher (p=0.0021) than the control group. On the protein indices, total protein, albumin, and Alb/Glob ratio indicated no significant variations between the groups. The globulin level (38.33±1.71 g/L) of the ALC_B group was significantly higher (p=0.0124) than the NCP, ALC_B + NCP and the control groups.

Table 3. Liver serum biochemical indices of Sprague Dawley rats fed with alcoholic bitters and NCP*

Parameters	Control	ALC_B	NCP	ALC_B+NCP	p
AST (U/L)	147.40±9.03	325.20±46.51	300.30±85.05	286.80±70.82	0.152
ALT (U/L)	121.50±40.89	96.40±19.31	103.67±45.24	43.33±15.38	0.437
GGT (U/L)	10.25±0.25	53.80±27.74	11.00±0.30	11.67±1.67	0.333
ALP (U/L)	339.00±64.31	251.80±61.37	215.00±95.28	215.00±52.37	0.571
AST/ALT ratio	3.14±1.69	2.80±0.65	2.73±0.82	2.55±0.19	0.996
T/Bilirubin (µmol/L)	13.00±5.37	43.81±24.2	8.99±2.59	5.48±3.32	0.357
D/Bilirubin (µmol/L)	13.00±5.39	21.84±0.2	7.78±0.51	3.41±2.23	0.271
I/Bilirubin (µmol/L)	0.39±0.39	5.82±0.69	4.23±0.45	2.25±2.24	**0.002**[ab]
T/protein (g/L)	66.26±3.94	77.33±5.46	78.67±4.81	76.50±3.94	0.095
Albumin (g/L)	35.00±1.91	58.80±21.68	40.67±4.06	34.33±2.60	0.592
Globulin (g/L)	31.28±2.2	38.33±1.71	37.77±1.18	37.23±2.31	**0.012**[ade]
Alb/Glob ratio	1.15±0.05	1.00±0.06	1.00±0.03	1.05±0.02	0.199

***** Data recorded in mean±standard error of the mean, ALC_B – slcoholic bitters, NCP – natural cocoa powder, ALC_B+NCP – alcoholic bitters + NCP, a – control versus ALC_B, b – control versus NCP, c – control versus ALC_B + NCP, AST – aspartate aminotransferase, ALT – alanine aminotransferase, GGT – gamma glutamyl transpeptidase, ALP – alkaline phosphatase, Alb/Glob – albumin/globulin ratio, statistically significant difference (p<0.05)

Descriptive statistics and ANOVA of kidney serum biochemical parameters of Sprague Dawley rats

Table 4 shows the renal biochemical parameters of the Sprague Dawley rats that received the alcoholic bitters and the natural cocoa powder for the period of the treatment. The one-way analysis of variance (ANOVA) of the renal parameters showed no statistically significant variations among the groups. There was no significant difference in the means of urea nitrogen between the treatment groups and the control group. However, the mean creatinine level (50.37±4.11 mmol/L) of the co-administered group was found to be statistically significantly higher than the rats that received alcoholic bitters only ((ALC_B) and control group).

Table 4. Renal serum biochemical Indices of Sprague Dawley rats fed with alcoholic bitters and NCP*

Parameters	Control	ALC_B	NCP	ALC_B+NCP	p
Sodium (mmol/L)	139.75±0.85	137.6±2.42	136±0.1	137.67±1.2	0.58
Potassium (mmol/L)	7.50±0.81	8.36±1.63	8.83±0.96	5.70±0.81	0.451
Chloride (mmol/L)	101.75±0.75	101.40±2.8	105±0.04	100.67±1.2	0.7
Urea nitrogen (mmol/L)	7.40±1.38	5.70±0.74	8.30±0.66	5.33±0.18	0.18
Creatinine (mmol /L)	33.36±2.89	37.06±3.26	39.73±4.6	50.37±4.11	0.006[ce]

***** Data recorded in mean±standard error of the mean, ALC_B – slcoholic bitters, NCP – natural cocoa powder, ALC_B+NCP – alcoholic bitters + NCP, statistically significant difference (p<0.05)

Discussion

The biochemical profile including the liver function test and kidney function test were assessed in the current study. The present study revealed no stastitically significant difference in the values of the liver enzymes (AST, ALT, GGT and ALP) of the rats fed with alcoholic bitters, natural cocoa powder and the co-administered groups. The findings of the present study disagree with a previous study by Johnson et al. who observed a significant increase in AST, ALT, GGT and ALP in rats that received alcoholic bitters.[15] Another study by Adias et al. reported a dramatic rise of AST and GGT among chronic alcohol drinking participants in Nigeria compared with their nondrinking counterparts.[16] Similarly, a previous report in Ghana among 60 total abstainers, 56 social drinkers and 100 alcoholics, indicated that GGT and AST were sufficiently sensitive to detect chronic alcoholics and that the serum GGT and AST showed a progressive increase with increasing alcohol intake.[17] The enzymes AST and ALT were also found to increase in a study by Shair et al.[18] The present study, though not statistically significant, it showed the AST and GGT values were numerically higher in the alcoholic bitters group than the control, NCP only and co-administered groups. Typically, elevated ALT and AST levels reflect the extent of hepatocellular injury while elevated serum ALP and GGT levels indicate the extent of impaired bile flow or cholestasis.[19] In particular, GGT is well known as a marker of alcohol consumption and correlates to alcohol consumption.[20] For instance, GGT was reported to be the most significant associations and ALT level the weakest relationship in alcohol consumption.[21] This could be the reason for the increased level of GGT in the present study. And while AST and ALP did not increase after alcoholic bitters treatment, these enzymes may not be sufficiently sensitive to detect minimal acute hepatotoxicity.[22] Gamma-glutamyl transpeptidase is present in the cell membranes of the tissues of certain organs, including the liver, kidney, spleen, pancreas and heart. Chronic alcohol use causes those cells to become inflamed and necrotic causing an increase in the amount of GGT to leak out.[23] The finding of the high

GGT of this study could therefore be a demonstration of hepatocellular injury and/or other organs in the rats that received the alcoholic bitters treatment. The differences in finding of the present study and the previous studies could be due to the pattern of drinking and type of drinks consumed. Aside from this, alcoholic liver disease has a multi-factorial aetiology, including environmental factors and genetics and might not be exactly same between animal and human studies.[24] Stranges et al. reported the possibility of intermittent heavy use of alcohol to have a greater influence on GGT blood levels than a regular one.[21] In this study, alcoholic bitters were regularly administered to rats and of the same type and this might be the reason for the lack of a significant increase in GGT because according to Bellentani et al., drinking alcohol outside mealtimes and drinking multiple different alcoholic beverages increase the risk of developing alcohol induced liver damage.[25] The postulation is that, heavy drinkers tend to have a raised mean cell volume, high GGT, but only a slightly raised serum alkaline phosphatase.[26] The mean GGT for the rats in the alcoholic bitters group in the present study was numerically higher than the control group, natural cocoa powder and co-administered natural cocoa powder and alcoholic bitters groups as well as previous studies using similar rats.[27,28] This indicates that cocoa might offer a protective effect on the liver of the rats which were fed with the cocoa and alcohol bitters. It is also confirmed in natural cocoa powder treated rats not having significant increase of the liver enzymes and this corroborates previous similar study, where consumption of cocoa extract alone showed no significant difference compared to control group for the liver enzymes.

Furthermore, this study found that the total bilirubin (direct and indirect) was significantly higher in the rats that received alcoholic bitters than the control and the other treatment groups. This supports previous studies by O'Malley et al. and Tanaka et al. where increased mean serum total bilirubin following alcohol consumption were observed.[29,30] The high total bilirubin level might be due to alcohol being a competitive inhibitor of bilirubin conjugation. Consistent with this possibility, both indirect (unconjugated) bilirubin and direct (conjugated) bilirubin of rats fed with the alcoholic bitters are significantly higher than those in the natural cocoa powder and co-administered natural cocoa powder and alcoholic bitters groups. Thus, while hepatic clearance of ethanol is primarily catalyzed by alcohol dehydrogenase, the microsomal ethanol-oxidizing system and aldehyde dehydrogenase, a minor, but forensically significant clearance pathway, involves ethanol conjugation with glucuronic acid.[29,31] The pathway for glucuronidation of ethanol involves UDP-glucuronosyltransferase 1A1 (UGT1A1), which is also primarily responsible for glucuronidation of bilirubin.[29] Ethyl glucuronide (EtG) is a direct phase-II metabolite of ethanol formed through the UGT1A1 catalyzed conjugation of ethanol with glucuronic acid.[32] Thus, forensic scientists have been using the advantageous properties of EtG in studying drunk driving cases, covert alcohol use among psychiatric inpatients and multiple other situations in which alcohol consumption was thought to play a role.[31] The high bilirubin observed in the present study among rats fed with alcoholic bitters might be due to slight hemolysis. For instance, Padmini and Sundari reported that erythrocytes from alcoholics have significantly decreased resistance to hemolysis in comparison to non-alcoholics.[33] However, the rats that received the co-administration of alcoholic bitters and natural cocoa powder has low bilirubin values. This indicates that cocoa plays a role in the excretion of bilirubin.

The kidney is the site of accumulation of chemicals; hence urea and creatinine are sensitive and reliable biochemical indices for evaluation of renal function.[34] In the present study, the means of urea nitrogen and creatinine of the group treated with alcoholic bitters alone were higher than the natural cocoa powder only, co-administered and control groups. This finding corroborates several previous study reports where higher than normal values of the parameters were recorded in rats.[29,35,36] The elevated serum creatinine is an indication of injury of the kidney due to the alcoholic bitters intake and hence the bitters can be said to have a reno-toxic effect on the kidneys of the rats. Thus, urea is formed in the liver, and is excreted by the kidney and elevation of it indicate kidney injury, with resultant reduced glomerular filtration.[37.]

The rat that received co-administered alcoholic bitters and natural cocoa powder showed no elevation of urea nitrogen, creatinine as well as electrolytes. This implies that the natural cocoa powder preserved the renal integrity and did not affect their capacity to excrete these ions. A previous study reported the capability of cocoa to prevent or reduce the complications in chronic kidney disease.[38]

Study limitations

The authors wish to acknowledge some limitations of the study which could be considered in future similar studies. The concentration of the Ghanaian alcoholic bitters, and the natural cocoa powder were not given in difference doses to difference groups. That could determine the effective dosage of the natural cocoa powder. Furthermore, the antioxidants and methylxathine quantities were not determined in the natural cocoa powder that was used, though only theobromine was stated in the manufacturer's information sheet. The study was also limited in the fact that the constituents of the Ghanaian alcoholic bitters were not determined.

Conclusion

This study found no significant difference of the liver enzymes between control and treatment groups. There was, however, numerically high values of AST and GGT in Sprague Dawley rats treated with Ghanaian alcoholic bitters indicating injury to the liver. The Ghanaian alcoholic bitters also appeared to have a reno-toxic effect as there were elevated serum creatinine in Ghanaian alcoholic bitters fed rats. The natural cocoa powder could offer a protective effect on the liver and kidney of the rats which were fed with both alcoholic bitters and cocoa powder together. Further studies on different doses of alcoholic bitters and natural cocoa powder are recommended.

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Declarations

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Author contributions

Conceptualization, R.S.M. and C.S.A.; Methodology, R.S.M.; Software, R.S.M.; Validation, R.S.M., S.B. and M.L.P.; Formal Analysis, M.L.P.; Investigation, R.S.M.; Resources, C.S.A.; Data Curation, M.B.K.; Writing – Original Draft Preparation, R.S.M.; Writing – Review & Editing, S.B.; Visualization, M.L.P; Supervision, C.S.A; Project Administration, R.S.M.; Funding Acquisition, R.S.M.

Conflicts of interest

The authors declared no conflict of interest

Data availability

The datasets used during the current study are available from the first author on reasonable request.

Ethics approval

Ethical approval was obtained from the Committee on Human Research, Publication, and Ethics of the School of Medicine and Dentistry, Science Kwame Nkrumah University of Science and Technology, with reference number: CHRPE/RC/205/17.

References

1. Kouassi GK, Kouakou LK, Oussou JBN, Malan FD, Yapo PA. Acute toxicity and effect of subacute administration of "4 heures du matin" bitters on anthropometric and hematological parameters in Wistar rats. *GSC Biological and Pharmaceutical Sciences*. 2022;19(02):121-130. doi: 10.5281/zenodo.6627500
2. Kyeremeh K, Agbemafo FW, Appiah-Opong R. Quantitative analysis of chemical contaminants in Ghanaian herbal alcoholic bitters. *International Journal of Chemistry and Applications*. 2013;5(2):153-167.
3. Vandebroek I, Balick MJ, Ososki AL, et al. The importance of botellas and other plant mixtures in Dominican traditional medicine. *J Ethnopharmacol*. 2010;128(1):20-41. doi: 10.1016/j.jep.2009.12.013
4. Kouassi KG, Malan DF, Kouakou KL, Diop AL, Litta AL. Trade and consumption of bitters, alcoholic macerates, in the district of Abidjan (southern Côte d'Ivoire). *Journal of Medicinal Plants*. 2023;11(1):113-125.
5. Baydilli N, Can Akınsal E, Doğanyiğit Z, Ekmekçioğlu O, Silici S. Protective role of poplar propolis against alcohol-induced biochemical and histological changes in liver and testes tissues of rats. *J Clin Pract Res*. 2020;42(2):132-138. doi: 10.14744/etd.2020.83097
6. Odey MO, Ofonime ME, Ogar I, et al. Biochemical and Haematological Responses to Chronic Consumption of some Herbalized Nigerian Alcoholic Drinks. *Nigerian Journal of Biochemistry and Molecular Biology*. 2022;37(3):181-187.
7. Adeyemi MM, Afolabi OM, Balogun N. Hepatorenal function of Wistar rats treated with Alamo and Jekomo, an alcoholic herbal bitters in Nigeria. *Journal of Phytomedicine and Therapeutics*. 2022;21(2):992-1002. doi: 10.4314/jopat.v21i2.18
8. Aidoo E, Addai FK, Ahenkorah J, Hottor BA, Bugyei KA, Gyan B. Natural cocoa ingestion reduced liver damage in mice infected with Plasmodium berghei (NK65). *Res Rep Trop Med*. 2012;3:107-116. doi: 10.2147/RRTM.S33149
9. Khwaldeh A, Shraideh Z, Badran DH, et al. Ameliorative Effect of Turmeric and Cocoa Extract against Acute Second hand Exposure of Tobacco Smoking on Hepatocytes and Enterocytes in Albino Rats: Ultra structural Study. *Biomedical and Pharmacology Journal*. 2021;14(1):199-206. doi: 10.13005/bpj/2114
10. Andersen ML, Winter LMF. Animal models in biological and biomedical research - experimental and ethical concerns. *Anais Da Academia Brasileira De Ciencias*. 2019;91(1):e20170238. doi: 10.1590/0001-3765201720170238
11. Kayode OT, Kayode AAA, Nwonuma CO. Alcoholic bitters modulates sex hormones and some biochemical parameters of testicular function in male Wistar rats. *F1000Research*. 2018;7:1838. doi: 10.12688/f1000research.16648.1
12. Sokpor G, Addai FK, Gyasi RK, Bugyei KA, Ahenkorah J, Hottor BA. Voluntary Ingestion of Natural Cocoa Extenuated Hepatic Damage in Rats with Experimentally Induced Chronic Alcoholic Toxicity. *Functional Foods in Health and Disease*. 2012;2(5):166. doi: 10.31989/ffhd.v2i5.91

13. Srikanth V, Malini T, Govindarajulu P, Balasubramanian K. Effects of ethanol ingestion on epididymal glycosidases and fertility in the rat. *Int J Androl*. 1998;21(6):343-351. doi: 10.1046/j.1365-2605.1998.00134.x
14. Siervo GE, Vieira HR, Ogo FM, et al. Spermatic and testicular damages in rats exposed to ethanol: influence of lipid peroxidation but not testosterone. *Toxicology*. 2015;330:1-8. doi: 10.1016/j.tox.2015.01.016
15. Jt J, Eo O, Od I. Effects of various alcoholic bitters on the haematological parameters of albino wistar rats. *Asian Journal of Biomedical and Pharmaceutical Sciences*. 2021;11(80):1-5.
16. Adias TC, Egerton E, Erhabor O. Evaluation of coagulation parameters and liver enzymes among alcohol drinkers in Port Harcourt, Nigeria. *Int J Gen Med.* 2013;6:489-494. doi: 10.2147/IJGM.S43472
17. Quaye I, Nyame PK, Dodoo D, Gyan B, Adjei AA. Biochemical and haematological markers of alcohol intake in Ghanaians. *West Afr J Med.* 1992;11(3):199-202.
18. Shair RM, Maskat MY, Ayob MK, Kasran R. Protective effect of cocoa extract on ethanol induced liver injury in sprague-dawley rats. *Sains Malaysiana*. 2020;49(1):93-101. doi: 10.17576/jsm-2020-4901-11
19. Iluz-Freundlich D, Zhang M, Uhanova J, Minuk GY. The relative expression of hepatocellular and cholestatic liver enzymes in adult patients with liver disease. *Ann Hepatol*. 2020;19(2):204-208. doi: 10.1016/j.aohep.2019.08.004
20. Sato KK, Hayashi T, Nakamura Y, et al. Liver enzymes compared with alcohol consumption in predicting the risk of type 2 diabetes: the Kansai Healthcare Study. *Diabetes Care*. 2008;31(6):1230-1236. doi: 10.2337/dc07-2184
21. Stranges S, Freudenheim JL, Muti P, et al. Differential effects of alcohol drinking pattern on liver enzymes in men and women. *Alcohol Clin Exp Res*. 2004;28(6):949-956. doi: 10.1097/01.alc.0000128229.23396.42
22. Hashimoto E, Riederer P, Hesselbrock V, et al. Consensus paper of the WFSBP task force on biological markers: Biological markers for alcoholism. *World J Biol Psychiatry*. 2013;14(8):549-564. doi: 10.3109/15622975.2013.838302
23. Mostafa H, Amin AM. Arif NH, Ibrahim B. Nuclear magnetic resonance spectroscopy-based metabolomics to identify novel biomarkers of alcohol-dependence. *Songklanakarin Journal of Science and Technology.* 2017;39(2):153-162.
24. Buyco D, Martin J, Jeon S, Hooks R, Lin CY, Carr RM. Experimental models of metabolic and alcoholic fatty liver disease. *World J Gastroenterol*. 2021;27(1):1-18. doi: 10.3748/wjg.v27.i1.1
25. Bellentani S, Saccoccio G, Costa GC, et al. Drinking habits as cofactors of risk for alcohol induced liver damage. *Gut*. 1997;41(6):845-850. doi: 10.1136/gut.41.6.845
26. Chalmers DM, Rinsler MG, MacDermott S, Spicer CC, Levi AJ. Biochemical and haematological indicators of excessive alcohol consumption. *Gut*. 1981;22(12):992-996. doi: 10.1136/gut.22.12.992
27. Petterino C, Argentino-Storino A. Clinical chemistry and haematology historical data in control Sprague-Dawley rats from pre-clinical toxicity studies. *Exp Toxicol Pathol*. 2006;57(3):213-219. doi: 10.1016/j.etp.2005.10.002
28. Ola-Davies OE, Olukole SG, Amoo O. Haematological and Serum Biochemical Variables in rats Treated with Ethanol Extract of the Root of Moringa oleifera. *African Journal of Biomedical Research*. 2014;17(1):31.
29. O'Malley SS, Gueorguieva R, Wu R, Jatlow P. Acute alcohol consumption elevates serum bilirubin: An endogenous antioxidant. *Drug Alcohol Depend*. 2015;149:87-92. doi: 10.1016/j.drugalcdep.2015.01.023
30. Tanaka M, Budhathoki S, Hirata A, et al. Behavioral and clinical correlates of serum bilirubin concentrations in Japanese men and women. *BMC Endocr Disord*. 2013;13(1):39. doi: 10.1186/1472-6823-13-39
31. Foti R, Fisher MB. Assessment of UDP-glucuronosyltransferase catalyzed formation of ethyl glucuronide in human liver microsomes and recombinant UGTs. *Forensic Sci Int*. 2005;153(2-3):109-116. doi: 10.1016/j.forsciint.2004.12.003
32. Palmer RB. A review of the use of ethyl glucuronide as a marker for ethanol consumption in forensic and clinical medicine. *Semin Diagn Pathol*. 2009;26(1):18-27. doi: 10.1053/j.semdp.2008.12.005
33. Perumal E, Sundari BT. Erythrocyte glutathione depletion impairs resistance to haemolysis in women consuming alcohol. *J Clin Biochem Nutr*. 2008;42(1):14-20. doi: 10.3164/jcbn.2008003
34. Waribo HA, Edamisan E, Elekima I, Bartimaeus ES. Effect of oral consumption of action bitters on renal indices of apparently healthy subjects in Port Harcourt Metropolis. *Asian Journal of Biochemistry, Genetics and Molecular Biology*. 2021;9(3):14-19. doi: 10.9734/ajbgmb/2021/v9i330217
35. Delwatta SL, Gunatilake M, Baumans V, et al. Reference values for selected hematological, biochemical and physiological parameters of Sprague-Dawley rats at the Animal House, Faculty of Medicine, University of Colombo, Sri Lanka. *Animal Model Exp Med*. 2018;1(4):250-254. doi: 10.1002/ame2.12041
36. Alemán C, Más R, Rodeiro I, et al. Reference database of the main physiological parameters in Sprague-Dawley rats from 6 to 32 months. *Lab Anim*. 1998;32(4):457-466. doi: 10.1258/002367798780599802
37. Ogoun TR. Ogidi OI, Aye T. Toxicity Studies of Yoyo Cleanser Bitters Poly-Herbal Formulation in Albino Rats. *World Journal of Pharmaceutical Research*. 2022;11(1):1-11.
38. Fanton S, Cardozo LFMF, Combet E, et al. The sweet side of dark chocolate for chronic kidney disease patients. *Clin Nutr*. 2021;40(1):15-26. doi: 10.1016/j.clnu.2020.06.039