BMJ Open Gastroenterology

Fortification of alcoholic beverages (12% v/v) with tea (*Camellia sinensis*) reduces harmful effects of alcohol ingestion and metabolism in mouse model

S O Ochanda,[1,2] K Rashid,[3] J K Wanyoko,[1] M Ngotho,[4] A K Faraj,[2] C A Onyango,[5] F N Wachira,[6] D N Maranga[7]

To cite: Ochanda SO, Rashid K, Wanyoko JK, *et al*. Fortification of alcoholic beverages (12% v/v) with tea (*Camellia sinensis*) reduces harmful effects of alcohol ingestion and metabolism in mouse model. *BMJ Open Gastro* 2016;**3**:e000058. doi:10.1136/bmjgast-2015-000058

Received 16 July 2015
Revised 18 November 2015
Accepted 23 November 2015

For numbered affiliations see end of article.

Correspondence to
Dr S O Ochanda;
simonochanda@gmail.com

ABSTRACT

Background: An animal model was used to study the health benefits inherent in tea fortified alcoholic beverages fed to laboratory mice.

Objectives: An investigation of the effects of tea fortified alcoholic beverages 12% alcohol (v/v) on antioxidant capacity and liver dysfunction indicators in white Swiss mice including packed cell volume (PCV), albumin, total protein, alkaline phosphatase (ALP) and glutathione (GSH) was carried out.

Methods: Plain, black, green and purple tea fortified alcohols were developed with varying tea concentrations of 1, 2 and 4 g/250 mL in 12% v/v. Control alcoholic beverages without teas were also developed. A permit (number IRC/13/12) was obtained for the animal research from the National Museums of Kenya, Institute of Primate Research prior to the start of the study. Alcoholic beverages were orally administered every 2 days for 4 weeks at 1 mL per mouse, and thereafter animals were euthanised and liver and blood samples harvested for analyses. Assays on body weight (*bwt*), packed cell volume (PCV) albumin, total protein, ALP and GSH were performed. Results were statistically analysed using GraphPad statistical package and significant differences of means of various treatments determined.

Results: Consumption of tea fortified alcohols significantly decreased ($p=0.0001$) *bwt* at 0.32–9.58% and PCV at 5.56–22.75% for all teas. Total protein in serum and liver of mice fed on different tea fortified alcohols ranged between 6.26 and 9.24 g/dL and 2.14 and 4.02 g/dL, respectively. Albumin, ALP and GSH range was 0.92–2.88 µg/L, 314.98–473.80 µg/L and 17.88–28.62 µM, respectively. Fortification of alcoholic beverages lowered liver ALP, replenished antioxidants and increased liver albumin, improving the nutritional status of the mice.

Conclusions: The findings demonstrate tea's hepatoprotective mechanisms against alcohol-induced injury through promotion of endogenous antioxidants. The beneficial effects of tea in the fortified alcoholic beverages could be used to develop safer alcoholic beverages.

Summary box

What is already known about this subject?

- Tea contains polyphenols, which are also present in some alcoholic beverages such as wine.
- Polyphenols are potent antioxidants which are used to boost blood antioxidants to reduce inflammation, damage and abnormal proliferation in animal cells.
- Fortification of alcoholic beverages with tea will increase the polyphenolic compounds and reduce their harmful effects.
- Alcohol consumption and metabolism leads to generation of acetaldehyde and formation of free radicals in the body, which in turn leaves the liver vulnerable to damage from these by-products.

What are the new findings?

- Alcoholic beverage fortification helps reduce the harmful health effects associated with alcohol consumption such as depletion of antioxidants in blood, increase in body weight and liver cirrhosis.
- Blood and liver antioxidant biomarkers showed that antioxidants are boosted; hence, there is less damage to body tissue. This shows that teas can be used in alcohol fortification to produce safer alcoholic drinks.
- Tea fortification of alcoholic beverages increases antioxidant biomarkers in the blood, when compared with alcoholic beverages without tea.

How might it impact on clinical practice in the foreseeable future?

- Tea fortified alcohols can be used in alcohol rehabilitation centres since tea fortified alcoholic beverages can ameliorate the harmful effects of alcohols as the addicts are gradually stopped from using harmful alcoholic beverages.
- For moderate alcoholic beverage consumers, this brand of alcoholic beverages is safer than the ordinary ones and can be used for health benefits for people with stomach disorders and as food for people who consume alcohol as part of their meals.

INTRODUCTION

Alcohol is a beverage produced and consumed by people from all walks of life for cultural, social and religious reasons. Owing to its wide usage availability and affordability, alcohol abuse is common.[1 2] Moderate consumption has some health benefits, which have been recommended by some medical practitioners.[3 4] These include boosting antioxidant activities,[5 6] antimicrobial properties[7] and treatment of stomach ailments,[8] to mention but a few.

On the other hand, alcohol abuse causes psychological and physical health injuries.[9–12] Alcohol consumption increases free radicals, namely superoxide ($O_2^{\bullet -}$), peroxide (O_2^{2-}), and the hydroxyl radical ($^{\bullet}OH$), which exist in cells and deplete antioxidant enzymes, viz. glutathione (GSH) peroxidase, superoxide dismutase, catalase and methionine reductase by causing cell injury through hepatocyte damage, inflammatory cell activation and increased intestinal permeability, fatigue, disease and death.[13–16] It is against these challenges that tea fortified alcoholic beverages with potent antioxidant properties were developed and an investigation made on the effects of their consumption using mice.[17 18]

Tea (*Camellia sinensis*) is ranked as the second most widely consumed beverage after water.[19] Tea is rich in phytochemicals with antioxidant properties.[20 21] Researchers have used and recommended it in value addition.[22–25] Studies have demonstrated the effectiveness of tea in detoxifying harmful chemicals.[22 26 27]

Prior to this research, no studies had been carried out to evaluate tea and alcohol combinations on immune/oxidative stress activity; therefore, this study is the first one of its kind to attempt to investigate the effects of tea and alcohol on the immune system of the body.

METHODOLOGY

Tea samples

Teas used in alcohol fortification were sourced from the Kenya Agricultural and Livestock Research Organization, Tea Research Institute (KALRO-TRI), Kericho, Kenya. Non-aerated green and aerated black tea were processed from variety Tea Research Foundation of Kenya (TRFK) 6/8 while non-aerated purple tea was processed from variety TRFK 306.[24 28] The tea variety TRFK 6/8 developed by TRI is used in Kenya as the standard black tea variety of high polyphenol content and high yield. The variety TRFK 306 is a purple coloured tea rich in anthocyanins developed by TRI.

Tea processing

About 2 kg of two leaves and a bud of freshly plucked tea were processed into aerated and non-aerated Cut, Tear and Curl (CTC) teas (500 g each). The yield ratio of green leaf to processed tea is 20%. Aerated and non-aerated teas were processed by the method described by Ochanda *et al*[24] and Kilel *et al*,[28] respectively.

Raw materials for alcoholic beverages production

Ingredients included aerated black and non-aerated green and purple teas, sugar, citric acid, raisins, yeast (*Saccharomyces cerevisiae* var. *ellipsoideus*) and potable water.

Development of alcoholic beverages

Alcoholic beverages were produced at the food processing unit of KALRO-TRI, Kericho, Kenya. Sugar (340 g), raisins (56 g), citric acid (0.5 g), water (1000 mL), yeast (0.8 g) and tea (4, 8, and 16 g) were mixed and fermented for 14 days, then filtered and stored at 20°C. Controls had identical ingredients excluding tea. Variation of the alcoholic beverages resulted from the type of tea, viz. non-aerated green and purple teas and aerated black teas, and their quantities in the brews.

Ethical clearance

Mice protocols approved by the Institutional Animal Care and Use Committee (IACUC) were adhered to and a permit (number IRC/13/12) obtained from the National Museums of Kenya, Institute of Primate Research (NMK-IPR), Karen, Kenya.

Experimental animals

Fifty-five male and female, 8 weeks old adult white Swiss mice, weighing 26–32 g were acquired from rodent breeding colony of NMK-IPR. The animals were housed in groups of five (separated according to sex) under conventional animal housing conditions within standard mice cages at 21–28°C. Ad libitum potable water and standard mice cubes obtained from Unga Feeds Ltd, Kenya, were supplied. Sterile wood chippings were provided as bedding material. Although none of the mice used in the study showed any signs or symptoms of parasite infestation, all mice were treated with 0.02 mL of Ivermectin (Ivermectin, Anupco, Suffolk, England) injected subcutaneously into each animal to eradicate possible endoparasite infestation as a precautionary measure. Euthanisation was done with Carbon dioxide (CO_2) at the end of the experiment as described by Close *et al*.[29]

Experimental design

Mice were randomly selected and allocated to 11 groups prior to the start of the experiment (table 1). Each animal served as a replicate in a completely randomised design. Plain, black, green and purple tea fortified alcohols (12% v/v) developed at KALRO-TRI were orally administered to the mice using a gavage needle at 1 mL/mouse every 2 days at around 09:30 for 28 days and the animals were euthanised 24 h after the last dosage. Body weight (*bwt*) was assayed[30] every 2 days and packed cell volume (PCV) on a weekly basis while liver and blood samples were harvested at the end of the experiment period, processed and stored at −80°C to await assay.

Liver and blood sample preparation

Frozen livers were homogenised at 4°C in a buffer of 0.25 M sucrose, 5 mM Hepes-Tris, pH 7.4, 1 mM EDTA

Table 1 Mice groups with and without supplementation with tea fortified alcoholic beverages

Group	Level of tea fortification				Treatment
	0 g	1 g	2 g	4 g	
1	✓	–	–	–	Water only
2	✓	–	–	–	Plain alcohols
3	–	✓	–	–	Black tea alcohols
4	–	–	✓	–	
5	–	–	–	✓	
6	–	✓	–	–	Green tea alcohols
7	–	–	✓	–	
8	–	–	–	✓	
9	–	✓	–	–	Purple tea alcohols
10	–	–	✓	–	
11	–	–	–	✓	

The levels of fortification are weights of tea added in grams per 250 mL of 12% v/v alcohol. The water only group are mice administered water only. Plain alcohols group are mice administered plain alcohols. Black tea alcohols group are mice administered black tea fortified alcohols. Green tea alcohols group are mice administered green tea fortified alcohols. Purple tea alcohols group are mice administered purple tea alcohols.

with protease inhibitor cocktail to a final concentration of 10% (w/v) using a tissue homogeniser (Stuart homogenizer SHM2/UK, Bibby Scientific Ltd, USA). The homogenate was aliquoted and stored at −80°C.[31]

Blood was drawn in 1 mL falcon tubes and kept for 1 h at room temperature (trp) to clot. The resulting serum was centrifuged at 1000*g* using 1.5 mL microfuge tubes (Heraeus Labofuge 400R, Hanau, Germany) for 15 min. Clarified serum was aliquoted into 1.5 mL microfuge tubes and stored at −80°C.[32]

PCV and bwt

Determination of PCV was done every 1 week as described by researchers.[33 34] The *bwt* was determined every 2 days using a digital analytical balance (Mettler PM2000 balance, Ohio, USA).

GSH assay

A modified GSH assay method described by Rahman *et al*[35] was used. In total, 200 µmoL/L of GSH standard solution was prepared and concentrations of 100, 50, 25, 12.5, 6.25, 3.13, 1.56 and 0.78 µmoL/L made using 0.5% sulphosalicylic acid (SSA). Ellman's reagent (5,5'-dithiobis 2-nitrobenzoic acid (DTNB)) was prepared by dissolving in 0.1 M K_2PO_4 buffer with 5 mM EDTA disodium salt, pH 7.5 (KPE buffer) to a final concentration of 0.6 mg/mL. About 50 µL of liver homogenate and serum was mixed with 50 µL of 5% SSA and 0.25 mM EDTA. The mixture was centrifuged (8000*g*) at 4°C for 10 min and 25 µL of the supernatant loaded on a 96-well microtitre plate. Approximately 25 µL of standards and blank was also loaded. Freshly prepared DTNB (100 µL) was added and absorbance measured (405 nm) at 30 s intervals using a multidetection microtitre plate reader (DYNEX MRX, Vancouver, USA).

Total proteins, albumin and alkaline phosphatase (ALP) assay

Total protein, albumin and ALP in serum and liver homogenates were assayed using a clinical biochemical analyser (Humalyzer 2000, Wiesbaden, Germany) utilising reagent kits (Human Diagnostics, Wiesbaden, Germany) as described by Heikal *et al*.[36]

Data analysis

Recording of data for the various assays were done in replicate values in Microsoft Excel sheets and exported to Prism GraphPad V.5.0 statistical analysis programme. Analysis of variance (ANOVA) and Turkey post hoc test were used to evaluate mean differences for *bwt*, PCV, GSH, total proteins, albumin and liver ALP. Significant differences were considered at $p \leq 0.05$. Data were expressed as mean±SEM.[37]

RESULTS

PCV and *bwt*

The PCV recorded for mice administered with black, green and purple tea alcoholic beverages showed initial increments of 2.83%, 1.04% and 4.68%, respectively, before reducing. Black, green and purple tea alcohols at 1, 2 and 4 g/250 mL recorded reductions of 22.75%, 18.34% and 13.19%; 5.56%, 7.69% and 7.42%, and 14.68%, 7.01% and 17.51%, respectively (figure 1A–C).

The *bwts* of control mice on water only and plain alcohols groups were higher than those on tea fortified alcohols, except for some black and green tea groups. Notable is the comparison of the water only and plain alcohol groups which gained *bwt* by 1.27% and 1.69%, respectively, while black tea alcohols at 1 and 2 g/250 mL lost *bwt* by 9.58% and 1.92% with the exception of the 4 g/250 mL group which gained 4.80%, respectively (figure 2A).

The green tea group also recorded similar results. Controls on water only and plain alcohols gained 1.57%

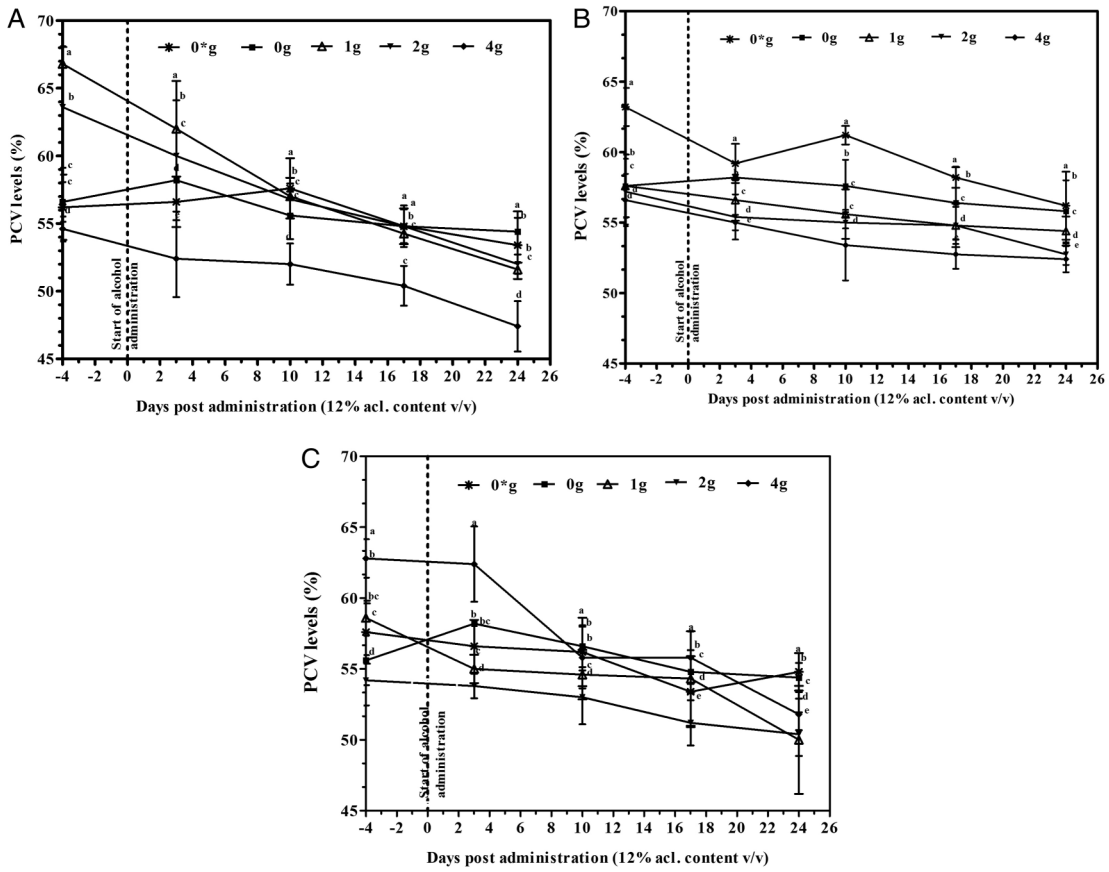

Figure 1 Packed cell volume (PCV) of mice supplemented with (A) black, (B) green and (C) purple tea fortified alcoholic beverages (12% v/v) at 0, 1, 2 and 4 g/250 mL and water only 0*.

and 1.69%, respectively, while the group on green tea alcohol at 1 and 2 g/250 mL lost 0.32% and 1.03% *bwt*. On the other hand, the 4 g/250 mL group gained *bwt* by 4.10% (figure 2B). The purple tea group gained *bwt* by 1.27% and 1.69% for water and plain alcohols and lost 8.76%, 2.42% and 2.28% for 1, 2 and 4 g/250 mL, respectively (figure 2C).

Effects of tea fortified alcoholic beverages on total protein

The water only group had significantly higher (p=0.0457) total protein in serum compared with those which consumed tea fortified alcohols (figure 3A) from the two-way ANOVA. The mice which were supplemented with plain alcohols recorded the least (6.26 g/dL) values. Mice administered concentrations of black tea alcohols of 1 and 2 g registered 7.36 and 7.44 g/dL, respectively, while 4 g/250 mL registered significantly (p=0.0457) the highest at 8.80 g (figure 3A). In the green tea alcohols mice group, plain alcohols registered the least total protein in serum at 7.44 g/dL followed by 1 g/250 mL at 8.22 g/dL. Mice supplemented with fortified beverages at 2 and 4 g/250 mL recorded high total proteins values of 8.66 and 9.12 g/dL but were not significantly different (p=0.3743) with the water only group. Mice which were administered purple tea alcohols at 1, 2 and 4 g/250 mL recorded 7.88, 8.74 and 8.78 g/dL, respectively, of total protein, which were significantly different (p=0.0457) from the plain alcohols but not from the water only group (figure 3A).

Total proteins in liver of mice administered plain alcohols were least followed by water only in all the tea alcohol groups. However, for all the groups, the total proteins increased with tea concentration. Black, green and purple tea alcohol groups at 1, 2 and 4 g/250 mL recorded 2.14, 3.30 and 4.02 g/dL; 3.52, 3.74 and 3.98 g/dL, and 3.28, 3.72 and 3.96 g/dL, respectively, of total proteins which were significantly (p=0.0001) higher than that of controls (figure 3B).

Effects of tea fortified alcohols on albumin

Serum albumin of mice administered water only was least at 1.60 µg/L in all mice groups. The mice administered black tea alcohols at 1, 2 and 4 g/250 mL recorded 2.30, 2.48 and 2.70 µg/L while the plain mice group registered 2.62 µg/L albumin, which was not significantly different (p=0.0001). Mice administered green tea alcohols at 1, 2 and 4 g/250 mL recorded 2.30, 2.44 and 2.88 µg/L albumin, respectively, while the plain alcohol group recorded 2.62 µg/L albumin. The purple tea alcohols administered mice exhibited a similar trend. At 1, 2 and 4 g/250 mL, albumin contents were 2.50, 2.56 and 2.58 µg/L, respectively, while plain alcohol mice recorded 2.62 µg/L (figure 4A).

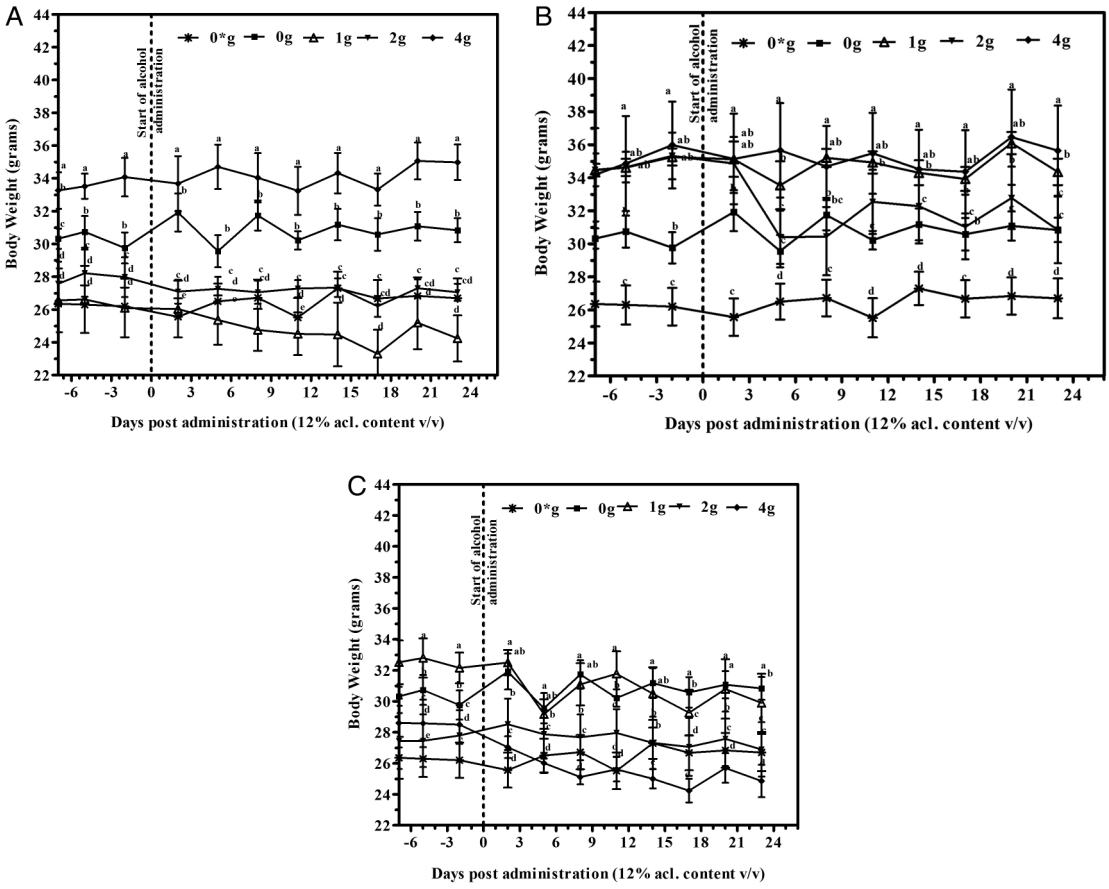

Figure 2 Body weight of mice supplemented with (A) black, (B) green and (C) purple tea fortified alcoholic beverages (12% v/v) at 0, 1, 2 and 4 g/250 mL and water only 0*.

Liver albumin for mice administered water only significantly exceeded (p=0.0169) the plain and tea fortified alcohols group. Mice fed on plain and black tea alcohols at 0, 1, 2 and 4 g/250 mL recorded 1.30, 1.02, 1.02 and 1.38 µg/L albumin, respectively. This trend was exhibited by mice groups administered green and purple tea alcohols. At 0, 1, 2 and 4 g/250 mL, the mice administered green tea recorded 1.30, 0.92, 0.92 and 1.64 µg/L, respectively. Mice in the purple tea group at 0, 1, 2 and 4 g/250 mL recorded 1.30, 1.20, 1.34 and 1.90 µg/L, respectively (figure 4B).

Effects of tea fortified alcohols on ALP

Mice supplemented with plain alcohols had significantly (p=0.03995) higher liver ALP (450.88 µg/L) compared with black (314.98 µg/L) and green tea alcohols (435.64 µg/L) or water only (393.76 µg/L) groups (figure 5). On the other hand, mice administered purple tea at 4 g/250 mL recorded ALP values of 473.80 µg/L, which was significantly higher (p=0.03995) than that of animals administered plain alcohol alcohols while the mice administered purple tea alcohols at 1 and 2 g/250 mL had lower ALP values than the plain

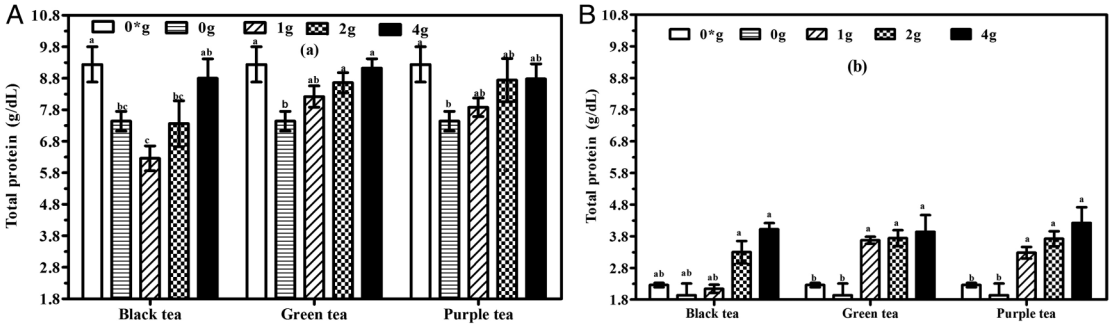

Figure 3 Total protein in (A) serum and (B) liver homogenates of mice supplemented with black, green and purple tea fortified alcoholic beverages (12% v/v) at 0, 1, 2 and 4 g/250 mL and water only 0*.

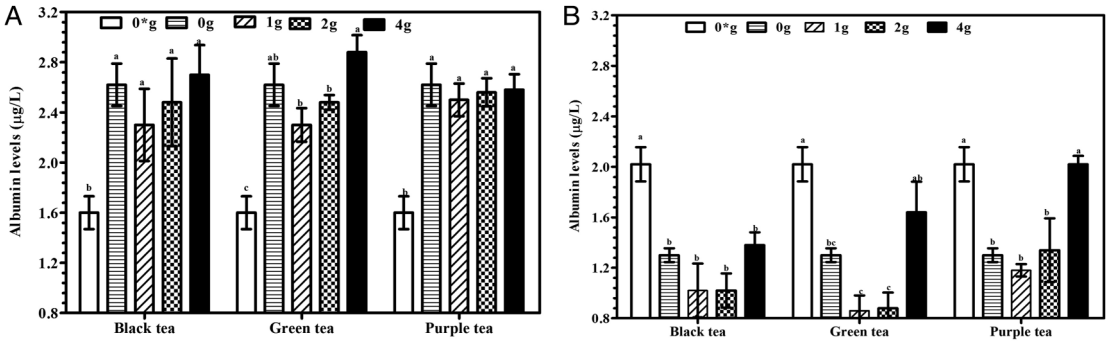

Figure 4 Albumin levels in (A) serum and (B) liver homogenates of mice supplemented with black, green and purple tea fortified alcoholic beverages (12% v/v) at 0, 1, 2 and 4 g/250 mL and water only 0*.

alcohol group at 320.00 and 425.00 µg/L ALP, respectively.

Effects of tea fortified alcohols on GSH

Serum GSH of the plain and black tea alcohol groups was significantly (p=0.0001) decreased compared with that of the water only group (figure 6A). However, green and purple tea groups at all concentrations recorded increasing serum GSH. Black tea at 4 g/250 mL and plain alcohol groups had comparable serum GSH.

Liver GHS for mice administered black, green and purple tea alcohols recorded higher GHS compared with controls of water only and plain alcohol groups. However, there were three outliers which included mice administered black and green tea alcohols at 1 g/250 mL and the purple tea alcohol group at 2 g/250 mL (figure 6B). The GHS values included 17.88, 24.75 and 27.41 µM for the black tea alcohol; 18.88, 25.89 and 22.41 µM for the green tea alcohol and 23.25, 23.25 and 28.62 µM for the purple tea alcohol administered mice groups corresponding to 1, 2 and 4 g/250 mL of tea, respectively. Mice administered water only and plain alcohols recorded 21.82 and 22.62 µM GHS, respectively.

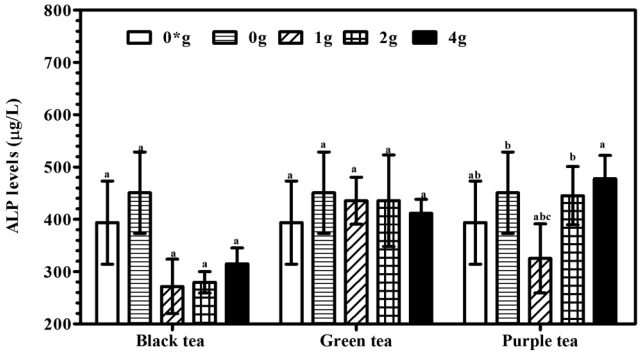

Figure 5 Alkaline phosphatase (ALP) in liver homogenates of mice supplemented with black, green and purple tea fortified alcoholic beverages (12% v/v) at 0, 1, 2 and 4 g/250 mL and water only 0*.

DISCUSSION

Heavy consumption of alcohol may result in tissue and organ injuries.[38–40] This was demonstrated by the elevated liver ALP in the plain alcohol group (positive controls) compared with the water only group (negative controls). High ALP is attributed to malfunctioning of hepatocytes from alcohol ingestion.[41–43] Moreover, plain alcohol-induced oxidative stress manifested by a significant decrease in serum GSH. Metabolism of alcohol depletes the body's antioxidants, damaging tissue and organs.[16 44 45] Studies have shown that continuous alcohol consumption generated free radicals leading to the reduction of GSH.[26 46] Thus, oxidative stress leads to alcohol toxicity by generating free radicals which damage cells and organs;[47] therefore, neutralising free radicals could protect against alcohol toxicity.[48]

The groups of mice administered tea fortified alcohols had liver ALP comparable to water only controls. In addition, tea ingestion in alcoholic beverages prevented GSH drop. The different types of teas used, viz. black, green and purple, had varying antioxidant activities due to the different processing methods and leaf biochemical components.[23] The black and green tea used in the alcohol fortification were processed from the tea variety clone TRFK 6/8 by aeration and non-aeration protocols, respectively, while purple tea was processed from clone TRFK 306 by non-aeration protocol.[28 49] The variation in leaf raw material and processing methods gave rise to a variation of antioxidants in the tea alcoholic beverages.[50] The results demonstrated that the hepatoprotective effects of teas on alcohol-induced toxicity could be similar to effects of plants such as *Bauhinia purpurea* and red grape.[1 13] These findings provide new information on the ability of teas to ameliorate alcohol toxicity and boost antioxidant defences. It is possible that the teas provided needed antioxidants at varied potencies in serum GSH, liver albumin and total protein and this can be attributed to the polyphenolic composition of the teas used in the fortification.[23 51 52]

The study also showed that plain and tea fortified alcohols reduced and increased liver albumin, respectively.[11] This is because the liver metabolises alcohol and continued alcohol consumption causes hypoalbuminaemia due to hepatocyte injury and liver dysfunction.[43] The

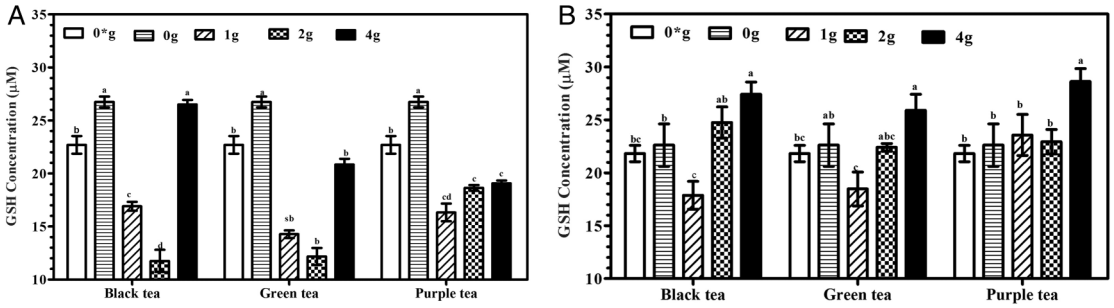

Figure 6 Total glutathione (GSH) in (A) serum and (B) liver homogenates of mice supplemented with black, green and purple tea fortified alcoholic beverages (12% v/v) at 0, 1, 2 and 4 g/250 mL and water only 0*.

increase in serum total protein in tea alcohol groups indicated an improved functional and secretory mechanism of hepatic cells and the hepatoprotective activity of tea.[21 53] The findings suggest that tea fortification of alcohols may have protective effects on liver diseases such as fatty liver, chronic alcoholic liver disease, viral hepatitis, cirrhosis, etc, since there was a reduction of oxidative effects on the liver cells.[21 30 54] However, mice in plain and tea fortified alcohol groups had high serum albumin compared with water controls. Alcohol, being antidiuretic, dehydrates the body by promoting urine production and preventing water reabsorption.[55] Dehydration decreases plasma volume, and therefore serum albumin may have been inaccurately amplified.

The tea fortified alcohols group recorded decreasing PCV with increasing tea consumption. Tea inhibits iron absorption, forming insoluble complexes in the lumen, thus decreasing haemoglobin (Hb).[56–58] Therefore, the low PCV observed could be attributed to insufficient Hb in the body causing a reduction in red blood cells synthesis. However, this needs to be investigated further.

Mice fed on plain and tea fortified alcohols had increased *bwts*. This could be attributed to the calorific value of the alcohols as opposed to the water only controls. This shows that alcohol consumption leads to energy accumulation in the body and hence an increase in *bwt*; thus, alcohols should be consumed in moderation.[55]

The study did not look at the aspects of varying the alcohol dosage administered to the mice. The authors therefore recommend that a study be conducted to determine effects of varying the tea fortified alcohol dosage on the serum and liver antioxidant biomarkers in mice. This could help determine the dosage at which the protective effects of tea could be lost during the consumption of the tea alcoholic beverages.

CONCLUSION

The findings of this study provide further evidence that oxidative stress plays an important role in ethanol toxicity. Further, the observation that the ingestion of tea fortified alcoholic beverages ameliorated alcohol toxicity, as is evident by decreased albumin and GSH coupled with reduced ALP activity, shows the antioxidant and hepatomodulatory properties of tea. The study has shown that tea can thus be used in the development of functional foods to boost the body's antioxidants which may have the potential to achieve the desired protective effects and add health benefits.

Author affiliations
[1]Tea and Health Unit, Tea Processing and value addition Programme, Kenya Agricultural and Livestock Research Organization, Tea Research Institute (KALRO-TRI), Kericho, Kenya
[2]Department of Dairy and Food Science and Technology, Egerton University, Egerton, Kenya
[3]University of Cologne, Cologne, Germany
[4]Mount Kenya University, Thika, Kenya
[5]Taita Taveta University College, Voi, Kenya
[6]Association for Strengthening Agricultural Research in Eastern and Central Africa (ASARECA), Entebbe, Uganda
[7]Department of Animal Sciences, National Museums of Kenya, Institute of Primate Research (NMK-IPR), Nairobi, Kenya

Acknowledgements The authors thank the National Commission for Science Technology and Innovation (NACOSTI) and Kenya Agricultural and Livestock Research Organization, Tea Research Institute (KALRO-TRI) for their financial support. The National Museums of Kenya, Institute of Primate Research (NMK-IPR) is also acknowledged for its technical support.

Contributors SOO contributed to the project design and biochemical and data analysis and paper writing. KR contributed by biochemical and statistical assay. JKW sought collaboration and interpreted data. MN contributed to project design, and permit acquisition. AKF contributed by sourcing of funds and data interpretation. CAO helped in designing the experiment and proof reading the draft paper. FNW coordinated the work, and paper reading. DNM contributed though analysis of samples and data.

Funding Tea Research Institute, Kenya and National Commission for Science Technology and Innovation of Kenya.

Competing interests None declared.

Provenance and peer review Not commissioned; externally peer reviewed.

Data sharing statement Additional material are available to authors to be published in a future article.

REFERENCES

1. Hassan HMM. Hepatoprotective effect of red grape seed extracts against ethanol-induced cytotoxicity. *Glob J Biotechnol Biochem* 2012;7:30–7.
2. Martin SE, Bachman R. The relationship of alcohol to injury in assault cases. In: Galanter M, eds. *Recent developments in alcoholism. Vol. 13. Alcohol and violence: epidemiology, neurobiology, psychology, and family issues*. New York, NY: Plenum Press, 1997:41–56.
3. Baum-Baicker C. The health benefits of moderate alcohol consumption: a review of the literature. *Drug Alcohol Depend* 1985;15:207–27.
4. Rimm EB, Klatsky A, Grobbee D, *et al*. Review of moderate alcohol consumption and reduced risk of coronary heart disease: is the effect due to beer, wine, or spirits. *BMJ* 1996;312:731–6.
5. Muselík J, Alonso MG, Martín-López MP, *et al*. Measurement of antioxidant activity of wine catechins, procyanidins, anthocyanins and pyranoanthocyanins. *Int J Mol Sci* 2007;8:797–809.
6. Stratil P, Kubán V, Fojtová J. Comparison of the phenolic content and total antioxidant activity in wines as determined by spectrophotometric methods. *Czech J Food Sci* 2008;26:242–53.
7. Papadopoulou C, Soulti K, Roussis IG. Potential antimicrobial activity of red and white wine phenolic extracts against strains of *staphylococcus aureus*, Escherichia coli and Candida albicans. *Food Technol Biotechnol* 2005;43:41–6.
8. Heinrich H, Goetze O, Menne D, *et al*. Effect on gastric function and symptoms of drinking wine, black tea, or schnapps with a Swiss cheese fondue: randomised controlled crossover trial. *BMJ* 2010;341:c6731.
9. Bode C, Bode JC. Alcohol's role in gastrointestinal tract disorders. *Alcohol Health Res World* 1997;21:76–83.
10. Das SK, Vasudevan DM. Alcohol-induced oxidative stress. *Life Sci* 2007;81:177–87.
11. Mitzner S, Williams R. Albumin dialysis MARS 2003: what evidence, how to proceed? *Liver Int* 2003;23:3–4.
12. Harford EM, Grant BF, Hasin DS. The effect of average daily consumption and frequency of intoxication on the occurrence of dependence symptoms and alcohol related problems. In: Clark WB, Hilton ME, eds. Alcohol in America: drinking practices and problems. Albany, NY: State University of New York Press, 1991:212–37.
13. Chaturvedi P, Pipedi-Tshekiso M, Moseki B, *et al*. Hepatoprotective potentials of water extract of Bauhinia purpurea bark against alcohol induced toxicity. *Sci Res Essays* 2011;6:4347–53.
14. Dahiru D, Obidoa O. Evaluation of the antioxidant effects of Ziziphus mauritiana lam. leaf extracts against chronic ethanol-induced hepatotoxicity in rat liver. *Afr J Tradit Complement Altern Med* 2007;5:39–45.
15. Szabo G, Satishchandran A. "MicroRNAs in alcoholic liver disease." University of Massachusetts Medical School Faculty Publications. Paper 625. *Semin Liver Dis* 2015;35:36–42.
16. Wu D, Cederbaum AI. Alcohol, oxidative stress, and free radical damage. *Alcohol Res Health* 2003;27:277–84.
17. Dubey NK, Kumar R, Tripathi P. Global promotion of herbal medicine: India's opportunity. *Curr Sci* 2004;86:37–41.
18. Kerio LC, Wachira FN, Wanyoko JK, *et al*. Total polyphenols, catechin profiles and antioxidant activity of tea products from purple leaf coloured tea cultivars. *Food Chem* 2013;136:1405–13.
19. Willson KC, Clifford MNN. Tea: cultivation to consumption. 1991:769. http://www.cabdirect.org/abstracts/19920311043.html;jsessionid=6E0E 2C4EFA900 F98B428AFAE994F14E4
20. El-Beshbishy HA. Hepatoprotective effect of green tea (Camellia sinensis) extract against tamoxifen-induced liver injury in rats. *J Biochem Mol Biol* 2005;38:563–70.
21. Sengottuvelu S, Duraisami S, Nandhakumar J, *et al*. Hepatoprotective activity of Camellia sinensis and its possible mechanism of action. *Iran J Pharmacol Ther* 2008;7:9–14.
22. Heikal TM, Mossa A-TH, Rasoul MAA, *et al*. The ameliorating effects of green tea extract against cyromazine and chlorpyrifos induced liver toxicity in male rats. *Changes* 2013;5:9.
23. Kerio LC, Bend JR, Wachira FN, *et al*. Attenuation of t-Butylhydroperoxide induced oxidative stress in HEK 293 WT cells by tea catechins and anthocyanins. *J Toxicol Environ Health Sci* 2011;3:367–75.
24. Ochanda SO, Wanyoko JK, Onyango CA, *et al*. Screening of suitable clones for Un-aerated tea production. *Afr J Hort Sci* 2012;6:118–35.
25. Ochanda SO. A review on tea manufacture, tea types and tea products in the Kenyan tea industry. *Tea J* 2010;31:38–48.
26. Dongare PP, Shah PR, Dhande SR, *et al*. Antihepatotoxic activity of *Pogostemon patchouli* against alcohol-induced hepatotoxicity in rats. *Int J Adv Res* 2013;1:225–34.
27. Issabeagloo E, Ahmadpoor F, Kermanizadeh P, *et al*. Hepatoprotective effect of green tea on hepatic injury due to leflunomide in rat. *Asian J Exp Biol Sci* 2012;3:136–41.
28. Kilel EC, Faraj AK, Wanyoko JK, *et al*. Green tea from purple leaf coloured tea clones in Kenya—their quality characteristics. *Food Chem* 2013;141:769–75.
29. Close B, Banister K, Baumans V, *et al*. Recommendations for euthanasia of experimental animals: part. *Lab Anim* 1996;30:293–316.
30. Rashid K, Wachira FN, Nyabuga JN, *et al*. Kenyan purple tea anthocyanins ability to cross the blood brain barrier and reinforce brain antioxidant capacity in mice. *Nutr Neurosci* 2014;17:178–85.
31. Tripathi S, Srivastav AK. Liver profile of rats after long-term ingestion of different doses of chlorpyrifos. *Pest Bioch Physiol* 2010;97:60–5.
32. Heikal TM, Mossa ATH, Marei GIK, *et al*. Cyromazine and chlorpyrifos induced renal toxicity in rats: the ameliorating effects of green tea extract. *J Environ Anal Toxicol* 2012;2:146.
33. Hoff J, Rlatg LVT. Methods of blood collection in the mouse. *Lab Animal* 2000;29:10.
34. Parasuraman S, Raveendran R, Kesavan R. Blood sample collection in small laboratory animals. *J Pharmacol Pharmacother* 2010;1:87–93.
35. Rahman I, Kode A, Biswas SK. Assay for quantitative determination of glutathione and glutathione disulfide levels using enzymatic recycling method. *Nat Protoc* 2006;1:3159–65.
36. Heikal TM, Ghanem HZ, Soliman MS. Protective effect of green tea extracts against dimethoate induced DNA damage and oxidant/antioxidant status in male rats. *Biohealth Sci Bull* 2011;3:1–11.
37. Motulsky HJ. *Analyzing data with GraphPad Prism*. San Diego, CA: GraphPad Software Inc., 1999:379. http://www.graphpad.com
38. Babor TF, Higgins-Biddle JC, Saunders JB, *et al*. *The alcohol use disorders identification test: guidelines for use in primary care*. Geneva: World Health Organization, 2001:1–40.
39. Gopumadhavan S, Rafiq M, Azeemuddin M, *et al*. Ameliorative effect of PartySmart in rat model of alcoholic liver disease. *Indian J Exp Biol* 2008;46:132–7.
40. Muthulingam M, Mohandoss P, Indra N, *et al*. Antihepatotoxic efficacy of *Indigofera tinctoria* (Linn.) on paracetamol induced liver damage in rats. *Int J Pharm Biomed Res* 2010;1:13–18.
41. Ho WY, Yeap SK, Ho CL, *et al*. Hepatoprotective activity of Elephantopus scaber on alcohol-induced liver damage in mice. *Evid Based Complement Alternat Med* 2012;2012:417953.
42. Panda V, Ashar H, Srinath S. Antioxidant and hepatoprotective effect of Garcinia indica fruit rind in ethanol-induced hepatic damage in rodents. *Interdiscip Toxicol* 2012;5:207–13.
43. Priya N, Venkatalakshmi P. The impact of heavy alcohol consumption and cigarette smoking on liver function—a clinical survey. *Int J Pharm Pharm Sci* 2013;4:82–5.
44. Butterworth RF. Hepatic encephalopathy—a serious complication of alcoholic liver disease. *Alcohol Res Health* 2003;27:143–5.
45. Lieber CS. Role of oxidative stress and antioxidant therapy in alcoholic and nonalcoholic liver diseases. *Adv Pharmacol* 1997;38:601–28.
46. Esmaeili MA, Sonboli A, Kanani MR, *et al*. Salvia sahendica prevents tissue damages induced by alcohol in oxidative stress conditions: effect on liver and kidney oxidative parameters. *J Med Plant Res* 2009;3:276–83.
47. Cook C, Thomson A. The Wernicke Korsakoff syndrome can be treated. *Alcoholis* 2000;19:4.
48. Matés JM, Pérez-Gómez C, Núñ ez de Castro I. Antioxidant enzymes and human diseases. *Clin Biochem* 1999;32:595–603.
49. Kilel EC. *Chemical and sensory quality evaluation of newly developed tea clones in Kenya*. Njoro, Kenya: Board of Postgraduate.Thesis, Egerton University, 2013.
50. Ochanda SO, Wanyoko JK, Ruto HK. Effect of spices on consumer acceptability of purple tea (Camellia sinensis). *Food Nutr Sci* 2015;6:703–11.
51. Karori SM, Wachira FN, Wanyoko JK, *et al*. Antioxidant capacity of different types of tea products. *Afr J Biotechnol* 2007;6:2287–96.
52. Luczaj W, Welerowicz T, Skrzydlewska E, *et al*. Chromatographic examinations of tea's protection against lipid oxidative modifications. *Toxicol Mech Methods* 2008;18:483–90.
53. Hua Li, Xiaoyu Wang, Yong Li, *et al*. Polyphenolic compounds and antioxidant properties of selected China wines. *Food Chem* 2009;112:454–60.
54. Micallef M, Lexis L, Lewandowski P. Red wine consumption increases antioxidant status and decreases oxidative

stress in the circulation of both young and old humans. *Nutr J* 2007;6:27.
55. Swift R, Davidson D. Alcohol hangover: mechanisms and mediators. *Alcohol Health Res World* 1998;22:54–60.
56. Hunt JR, Roughead ZK. Adaptation of iron absorption in men consuming diets with high or low iron bioavailability. *Am J Clin Nutr* 2000;71:94–102.
57. Kaltwasser JP, Werner E, Schalk K, *et al*. Clinical trial on the effect of regular tea drinking on iron accumulation in genetic haemochromatosis. *Gut* 1998;43:699–704.
58. Thankachan P, Walczyk T, Muthayya S, *et al*. Iron absorption in young Indian women: the interaction of iron status with the influence of tea and ascorbic acid. *Am J Clin Nutr* 2008;87:881–6.