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Original Research Article

Gross morphometric and histological effect of turmeric and vitamin C on alcohol induced liver toxicity among albino rats

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ABSTRACT

Background: Turmeric has curcumin, as active constituent that improves ethanol-induced hepatotoxicity, prevents accumulation of Reactive Oxygen Species (ROS) and suppression of systems of anti-oxidation in the liver.
Methods: A total of 25 albino rats of species Rattus Norvegicus were simpled randomly picked and grouped into five groups. They were exposed to alcohol, turmeric and Vitamin C at calculated doses. Thereafter, the animals were sacrificed, hepatotoxicity was confirmed and histological study was conducted.
Results: Negative control group (food and water ad-libitum), where the central vein, hepatocytes, hepatic triad and capillary sinusoid appeared normal as shown in Figure 1a. Group 2 (alcohol 3g/kgbwt) that showed the Sinusoids had marked dilatation and the central vein had also dilated and congested, Focal points of hemorrhagic necrosis were observed and patches of macrovascular steatosis were observed shown in Figure 1b. Liver sections from Group III (alcohol 3 g/kgbwt and turmeric 0.187 mg/kg/day) showed the central vein was slightly dilated as compared to control and smaller to group that only received alcohol. Sections from group IV (alcohol 3 g/kgbwt and vitamin C 0.3 mg/kg/day) showed the central vein was slightly dilated and not congested as compared to the alcohol only group. The sinusoids and the hepatocytes were normal shown in Figure 2b. Sections from group V (alcohol 3 g/kgbwt and vitamin C 0.3 mg/kg/day and turmeric 0.187mg/kg/day) the central vein, sinusoids and hepatocytes had almost similar histological characteristics as the negative control group (Group 1) as shown in Figure 2c.
Conclusions: Turmeric and vitamin C have histomorphological protective effects on alcohol induced liver toxicity among albino rats.

Keywords: Hemorrhage, Hepatocytes, Hepatotoxicity, Histological, Histomorphological

INTRODUCTION

Study on turmeric reveal that curcumin, a main active constituent, improves ethanol-induced hepatotoxicity, accumulation of reactive oxygen species (ROS) and suppression of systems of anti-oxidation in the liver of mice (Balb/c) on chronic alcohol administration over a period of 6 weeks.[1]

Other studies show that treatment with curcumin reversed increased serum enzymes markers (ALT, AST) caused by hepatotoxicity.[2] More studies illustrate that curcumin supplementation reduces macro-vesicular steatosis induced by ethanol, lipid droplet in liver and lowers activities of ALT and AST, showing protection of the liver from ethanol toxicity.[1] Curcumin prevented hepatotoxicity due to ethanol, involving lipid accumulation prevention and stress due to oxidation.[1]

Well known pathological changes in the liver tissue of methotrexate treated subjects including infiltration by inflammatory cells, severe centrilobular or intermediate zone or periportal degeneration, bile duct hyperplasia, hyperemia and necrosis are observed. In most cases turmeric betters the histological changes in hepatocytes induced by methotrexate including the decrease in

periportal degeneration, necrosis, hyperemia and inflammatory cells infiltration prevention.[3] Other studies suggested that the turmeric has shown antioxidant, hepatoprotective nature as well as anti-carcinogenic activity better than garlic.[4]

Administration of curcumin, an extract from turmeric which is known to be a strong antioxidant reduced the ASA induced hepatic toxicity according to histopathology assessment of liver sections of the turmeric treatment groups, liver tissue appeared normal and they lacked histopathological alterations as demonstrated in pericentral zone and periportal zone with H&E, in low magnification.[3,5]

Vitamin C in its active form Ascorbic acid is a powerful antioxidant.[5] It is involved in many body functions, specifically in the liver. Humans get it either as Ascorbic acid or as dehydroascorbic acid its oxidized form.[5,6] Oxidative stress plays a role in the pathophysiology of acute and chronic liver diseases.

This happens when Reactive oxygen species produced from either endogenous or exogenous sources (e.g. cytochrome P-450 enzymes) and cellular functions (e.g. mitochondrial metabolism) are not balanced by a similar rate of neutralization by antioxidant. Results of stress oxidation include those of cellular component oxidation, for example proteins, DNA and lipids which causes cell damage and death.

Vitamin C scavenges free radicals, radicals trapping and bio membranes protection from damage by peroxide. It scavenges effectively singlet oxygen, hydroxyl, superoxide, water-soluble peroxyl radical and hypochlorous acid. It is also is an excellent source of electrons and can also donate electrons to free radicals, for example hydroxyl and superoxide radicals quench their activities.[6]

Most of above-mentioned studies focused on the histopathology and mode of protection of turmeric and vitamin C individually. This study is set to focus on the synergistic effects of turmeric and vitamin C on the histomorphological of the liver on alcohol induced liver toxicity which has not been exploited before.

METHODS

Experimental animals

Adult albino rats were bred in a condition which is microbiologically controlled for all the experimental and control groups. They were obtained from University of Nairobi. The albino rats were bred in cages which hold a maximum of 6 rats per cage.

The rats were acclimatized for 1 week in an animal house (26±2°C) with 12 h light and dark cycles in the animal house in zoology department, Maseno University.

Inclusion criteria

All healthy albino rats in the cage, animals with average weight of 150-250 g and animals between 6-8 weeks of age were included in this study animals between 6-8 weeks of age.

Exclusion criteria

Sick animals in the cage.

Animal ethics

All procedures were performed in line with the guide for the care and use of laboratory animals.

Study period

The study duration was from March 2023 to March 2024.

Dose determination and administration

Dosage of turmeric was 40 mg/ml (0.187 mg/kg/day) adopted from previous study (Karamalakova et al, 2019). Dosage of vitamin C was at 200 mgs (0.3 mg/kg) (Tawfik and Al Badr, 2012). Dosage of alcohol (ethanol) was at 3 g/kg b.w (Boby et al, 2021).

Drugs were administered by use of gastric gavage needle i.e., the rats were wrapped with the tablecloth and carefully held from the neck region by the researcher. The assistant researcher then turned the mouth of the rat to face forward. Gently, the gavage needle inserted into the mouth the drug delivered and the gavage needle then gently removed.

Harvesting and determination of gross morphological parameters

Before the animals were sacrificed their final body weight was determined using an electronic weighing scale. Animals were killed in a humane way by use of a chloroform-soaked cotton gauze put in a bell jar. A thoracoabdominal pubosymphic cut was made to identify the liver.

Microscopic and photographic examination of histological section

Fixation of tissues was done using the formaldehyde solution for 24 hours. Dehydration of samples was done using ethanol that had been prepared in an ascending concentration to a maximum of 100% with each concentration lasting one hour.

Clearance of liver sample was done using xylene. Sample tissues were infiltrated with wax for twelve hours at 56 degrees Celsius. Tissue orientation was then done by making longitudinal cuts from the apex to base. Samples were embedded in paraffin wax. To properly expose the whole liver tissue, excess wax was trimmed off from the

blocks. A rotary microtome was used to cut prepared sections into 5 µm thick longitudinal sections. To properly spread the tissue, the cut sections were allowed to float in water at thirty-seven degrees. The sections were then put on top of the glass slides and a thin film will be applied over it by a micro- dropper. Using an oven, the slides were dried at thirty-seven degrees for 24 hours. Staining of the slides was done using hematoxylin and eosin (H&E). 32 megapixels' Digital camera of BP Olympus microscope was used to take photomicrographs. Slides were mounted on the stage of the microscope.

Appropriate field magnification was done. Photomicrographs of the slides viewed under the best focus were taken transferred to a computer then uploaded on adobe fireworks program for labelling. Liver biochemical assays to ascertain toxicity occurred, liver biochemical assays were done before and after administration of alcohol.

Statistical analysis

The gross morphometric data was entered into excel sheet and then analysis done through SPPS version 25 (IBM). The results are expressed as a mean value±standard error of mean (SEM). One-way ANOVA was used to compare the data obtained from experimental and control groups. Significance levels was P value less than or equal to 0.05 ($p \leq 0.05$) at 95% confidence level. Histological features were observed microscopically and comparison made between groups.

Ethical approval

The proposal was initially presented and cleared by the School of Medicine and then the School of Graduate Studies (SGS), Maseno University (SGS=MSC/SM/00034/020). Animal ethical approval was obtained from Institutional Scientific Ethical Review Committee (ISERC) of University of Eastern Africa Baraton (B0419012023). The study research license was obtained from national commission for science technology and innovation (NACOSTI-#804422).

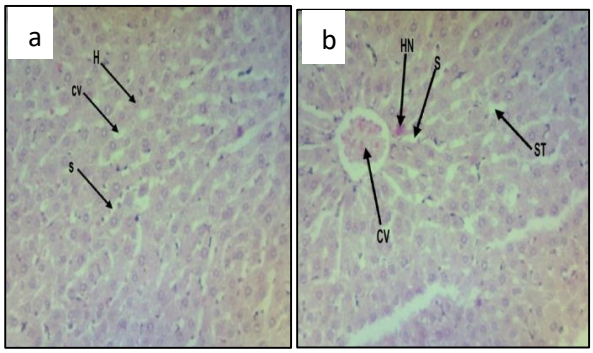

Figure 1: (a) Group I show a photomicrographs of negative control group (food +water ad libitum). (b) Shows photomicrographs of group II (alcohol 3 g/kgbwt).

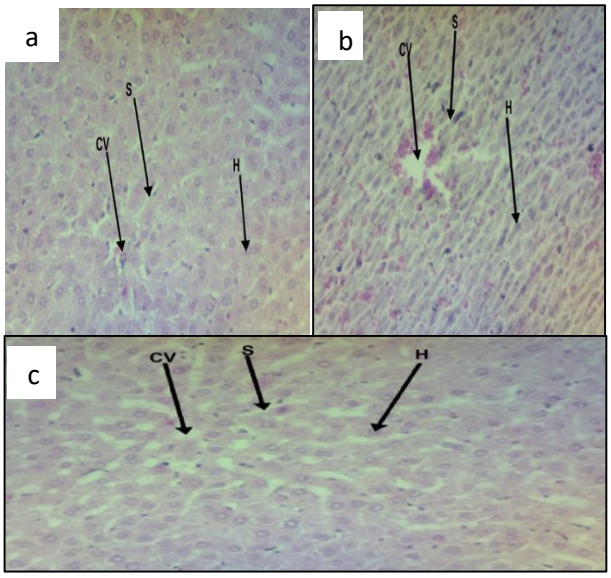

Figure 2: (a) Group III (alcohol 3 g/kgbwt +turmeric 0.187 mg/kg/day). (b) group IV (alcohol 3 g/kgbwt +vitamin C 0.3 mg/kg/day). (c) group V (alcohol 3 g/kgbwt +vitamin C 0.3 mg/kg/day+ turmeric 0.187 mg/kg/day).

RESULTS

Comparison histomorphological findings of liver in the negative control group (group I) and alcohol only group (group II)

Normal liver histomorphological features were observed in the negative control group (food and water ad-libitum), where the central vein, hepatocytes, hepatic triad and capillary sinusoid appeared normal as shown in Figure 1a. that were different from those of group 2 (alcohol 3g/kgbwt) that showed the Sinusoids had marked dilatation and the central vein had also dilated and congested, Focal points of hemorrhagic necrosis were observed and patches of macrovascular steatosis were observed shown in figure 1b. These features were absent in the negative control group thus suggesting alcohol-induced hepatotoxicity.

Comparison histomorphological features between negative control group and the hepatoprotective groups

Liver sections from Group III (alcohol 3 g/kgbwt+turmeric 0.187 mg/kg/day) showed the central vein is slightly dilated as compared to the control and smaller to the group that was only administered with alcohol. The hepatocytes and the sinusoids appeared normal as shown in Figure 2a. Sections from group IV (alcohol 3 g/kgbwt+vitamin C 0.3 mg/kg/day) showed the central vein was slightly dilated and not congested as compared to the alcohol only group. The sinusoids and the hepatocytes were normal shown in Figure 2b. Sections from group V (alcohol 3 g/kgbwt+vitamin C 0.3 mg/kg/day+ turmeric 0.187 mg/kg/day) the central vein, sinusoids and hepatocytes had

almost similar histological characteristics as the negative control group (Group 1) as shown in Figure 2c.

Table 1: Liver biochemical parameters (AST, ALT, GGT and albumin).

liver biochemical parameters (u/l)	Group 1 (food+water)	Group2 (Alcohol 3 g/kgbwt)
AST	29.41±0.77	60.38±3.07#
ALT	64.18±4.16	103.0±4.97#
GGT	2.54±.34	14.5±1.8#
Albumin	4.4±.34	9.1±.91#

Values are expressed as the means ± SEM; $n = 5$. #P < 0.0001 vs Normal control; *P<0.0001 vs alcohol; **P < 0.0001 vs alcohol. AST=aspartate transferase, ALT=alanine transaminase, GGT=gamma glutamate

DISCUSSION

Liver injury caused by alcohol is a major threat to the functioning the liver, therefore it is necessary to evaluate the safety of natural products to provide hepatotoxicity.[7] Vitamin C exhibits anti-inflammatory and antioxidant properties.[8] It induces decreased levels of C-reactive proteins which decreases pro-inflammatory mediator levels. Similarly, turmeric has been proven to contain antioxidant properties which function to scavenge reactive oxygen species, free radicals and reactive oxygen species.[9,10]

In the current study, significant derangement in histomorphological features was observed in alcohol treated group. The liver sinusoid appeared dilated, central vein disrupted, focal points of hemorrhagic necrosis and patches of macro vascular steatosis were observed.[11] Established similar observations in a study on rat model. In another study by, these histological changes involve series of mechanism ranging from oxidation of ethanol, cytochrome P450 induction and oxidative stress, anti-oxidant defenses and lipid peroxidation. The effect of liver steatosis has been attributed to disruption of lipid metabolism in the hepatocytes.[12,13]

The metabolism of alcohol generates hydrogen peroxide which induces hypoxia. These findings have also been reported by other studies.[14] However, co-treatment of alcohol with Turmeric and vitamin C independently (group III and IV) showed mild to moderate derangement in relation to alcohol treatment group. The central veins appeared slightly disrupted and liver sinusoids moderately dilated. Although focal necrosis was observed, the hepatocytes were observed to be normal. Combined treatment with turmeric and vitamin C demonstrated normal histomorphological features of the liver parenchyma. The hepatocytes appeared normal; liver sinusoids showed no dilation while central veins were normal. This was significantly different relative to the alcohol treated group which showed marked derangement. The combined treatment provided synergistic hepatoprotective effect which exceeds that of vitamin c or turmeric alone.

Although synergism effect of turmeric and vitamin C against alcohol has not been reported, the synergism effect against other toxic agents has been demonstrated.[15] reported similar findings in a combined treatment of paracetamol, vitamin C and α-tocopherol against their individual pre-treatment. He observed normal histological findings in the liver in combined vitamin C and α-tocopherol pre-treatment groups in relation to groups where the pre-treatment therapy is administered alone. Phenolic compounds in the turmeric possesses the ability to destroy and penetrate bacterial cell wall hence disrupting its metabolism.[16] This mechanism is due to prevention of phenolic compound oxidation.[17-19] In another study, combination of turmeric and Rosemary's extract in co administration with paracetamol ameliorated necrosis, absence of central vein disruption and normal sinusoids. The combination produces a synergistic effect which potentiate the anti-inflammatory, anti-microbial and anti-oxidant properties activities thereby preventing hepatic parenchymal injury.[20]

The present studies confirm the findings of the liver biochemical parameters changes exhibited in the combination group (alcohol, turmeric and vitamin C) compared to the groups that were treated with either turmeric or vitamin C. The changes demonstrated that the combination effect induces a marked significant difference compared to the alcohol treatment groups. These findings are attributed to the hepatoprotective effects of both turmeric and vitamin C such that their combination therapy potentiate the ameliorative activities.

Some of the limitations encountered in the study were that a number of rats did not achieve the required weight while some died in the process of drug administration.

CONCLUSION

It can be concluded that Turmeric and vitamin C have histomorphological protective effects on alcohol induced liver toxicity among albino rats. This is very vital in patient with alcohol hepatotoxicity as when adopted could provide an alternative management modality since turmeric is readily available in the market.

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Ethical approval: The study was approved by the Institutional Ethics Committee

REFERENCES

1. Rong S, Zhao Y, Bao W, Xiao X, Wang D, Nussler AK, Yan H, Yao P, Liu L. Curcumin prevents chronic alcohol-induced liver disease involving decreasing ROS generation and enhancing antioxidative capacity. Phytomedicine. 2012;19(6):545-50.

2. Naik A, Belič A, Zanger UM, Rozman D. Molecular interactions between NAFLD and xenobiotic metabolism. Frontiers in genetics. 2013;4:2.
3. Moghadam AR, Tutunchi S, Namvaran-Abbas-Abad A, Yazdi M, Bonyadi F, et al. Pre-administration of turmeric prevents methotrexate-induced liver toxicity and oxidative stress. BMC complementary and alternative medicine. 2015;15:1-3.
4. Thompson MD, Wickline ED, Bowen WB, Lu A, Singh S, Misse A, Monga SP. Spontaneous repopulation of β-catenin null livers with β-catenin-positive hepatocytes after chronic murine liver injury. Hepatology. 2011;54(4):1333-43.
5. Mohapatra TK, Nayak RR, Subudhi BB. Exploration of anti-inflammatory and hepatoprotective effect of curcumin on co-administration with acetylsalicylic acid. J Pharm Pharmacogn Res. 2019;7(5):310-22.
6. Marin JJ, Perez MJ, Serrano MA. Chemoprotective Role of Vitamin C in Liver Diseases. In The Liver Elsevier. 2018: 133-153.
7. Li J, Zou B, Yeo YH, Feng Y, Xie X, Lee DH, et al. Prevalence, incidence, and outcome of non-alcoholic fatty liver disease in Asia, 1999–2019: a systematic review and meta-analysis. The lancet Gastroenterol & Hepatol. 2019;4(5):389-98.
8. Zadeh FA, Jasim SA, Atakhanova NE, Majdi HS, Jawad MA, Hasan MK, et al. Drug delivery and anticancer activity of biosynthesised mesoporous Fe2O3 nanoparticles. IET Nanobiotechnol. 2022;16(3):85.
9. Sindhu M, Rajkumar V, Annapoorani CA, Gunasekaran C, Kannan M. Functionalized nanoencapsulated Curcuma longa essential oil in chitosan nanopolymer and their application for antioxidant and antimicrobial efficacy. International J Bio Macr. 2021;251:126387.
10. He YJ, Kuchta K, Lv X, Lin Y, Ye GR, Liu XY, et al. Curcumin, the main active constituent of turmeric (Curcuma longa L.), induces apoptosis in hepatic stellate cells by modulating the abundance of apoptosis-related growth factors. Zeitschrift für Naturforschung C. 2015;70(11-12):281-5.
11. Chaudhry H, Sohal A, Iqbal H, Roytman M. Alcohol-related hepatitis: a review article. World journal of gastroenterology. 2023;29(17):2551.
12. Metha P. Mechanisms of alcohol induced liver injury in rats and treatments. International J Adv Res in Biol Sciences. 2016;2:72-84.
13. Cederbaum AI. Cytochrome P450 2E1-dependent oxidant stress and upregulation of anti-oxidant defense in liver cells. J of Gastroenterol and Hepatol. 2006;21:22-5.
14. Fang TJ, Wei QK, Liao CW, Hung MJ, Wang TH. Microbiological quality of 18 C ready-to-eat food products sold in Taiwan. Int J food Microbiol. 2003;80(3):241-50.
15. Khan SI, Begum M, Chowdhury R. Synergistic Hepatoprotective Interaction between α-Tocopherol and Ascorbic Acid on Paracetamol Induced Liver Damage in Rats. 2020.
16. Brown BG, Zhao XQ, Chait A, Fisher LD, Cheung MC, Morse JS, et al. Simvastatin and niacin, antioxidant vitamins, or the combination for the prevention of coronary disease. New England J of Med. 2001;345(22):1583-92.
17. Sabir SM, Zeb A, Mahmood M, Abbas SR, Ahmad Z, Iqbal N. Phytochemical analysis and biological activities of ethanolic extract of Curcuma longa rhizome. Brazilian Journal of Biology. 2020;81(3):737-40.
18. Antunes SA, Robazza WD, Schittler L, Gomes GD. Synergistic and antimicrobial properties of commercial turmeric (Curcuma longa) essential oil against pathogenic bacteria. Food Science and Technol. 2012;32:525-30.
19. Naz S, Jabeen S, Ilyas S, Manzoor F, Aslam F, Ali A. Antibacterial activity of Curcuma longa varieties against different strains of bacteria. Pak J Bot. 2010;42(1):455-62.
20. Mohamed ME, Younis NS, El-Beltagi HS, Mohafez OM. The Synergistic hepatoprotective activity of rosemary essential oil and curcumin: the role of the mek/erk pathway. Molecules. 2022;27(24):8910.

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