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Original Article

Zingerone mitigates hepatic injury associated with Dimethylnitrosamine exposure in male adult Wistar rats

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Abstract

BACKGROUND AND AIM: Dimethyl nitrosamine (DMN) burden compromises liver function due to its complicated pathophysiology. Zingerone (ZG), is a phenolic compound in ginger rhizome with pharmacological potential against chemo toxin mediated hepatic damage. This study investigated the therapeutic efficacy of Zingerone against DMN in the liver

METHODOLOGY: Thirty-six rats were randomly divided into groups of 6 rats each. Group A (control), Group B (given 10mg/kg DMN for 4 weeks). Group C, D, E, F (Pre-treated with oral doses of 25mg/kg, 50mg/kg, 75mg/kg body weight of ZG and 50mg/kg of silymarin respectively for 4weeks followed thereafter with 10mg of DMN for 4weeks). At the end of experiment, the animals (fasted) were sacrificed; blood samples obtained for assay of Gamma Glutamyl Transferase (GGT) activity, Total bilirubin concentration (Tbil) and the liver harvested for histological and histochemical evaluations. Data obtained were analysed using graph pad prism, 8.0 and Image J for quantifying staining intensity.

RESULTS: ZG pre-treated rats showed significantly ($p<0.05$) decreased GGT activity and Tbil concentration compared to DMN-alone exposed rats. Furthermore, necrotic changes with inflammatory cell infiltrations, vascular ulceration, pyknotic hepatocytes with sinusoidal distortions in the histology of DMN alone treated rats were minimal in ZG pre-administered rats. Despite fasting, DMN-alone exposed rats still showed strong PAS reaction, associated with impaired glycogenolysis and unregulated glycogenesis. However, moderate intrahepatic glycogen store depicted by mild PAS reaction were seen in ZG pre-treated rats. These were particularly visible at 75mg/kg, comparing favorably to control and silymarin pre-treated rats.

CONCLUSION: Zingerone is a promising hepatoprotective agent against DMN hepatic injury

Keywords:
Liver; Dimethylnitrosamine; Zingerone; histology; PAS reaction

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INTRODUCTION

The critical role of the liver in xenobiotic metabolism exposes it to reactive toxic metabolites, with vulnerability to hepatic injuries that lead to liver pathologies (Pei *et al.,* 2023). Liver pathologies contribute to global mortality rate and life expectancy gap across regions (United Nations, 2010; Cheemerla and Balakrishnan 2021). Dimethylnitosamine (DMN) is a liver specific toxin, potentially present in cured meat and other processed dietary food, tobacco condensate, contaminated ground and recycled water, leached during water treatment by chlorination or anion exchange resins (Gushgari and Halden, 2018). Although, allowable levels in different countries exist such as in the United state of California and Canadian province of Ontario set as 10ng/L and 9ng/L respectively, adherence to minimal safe level remains a global problem particularly among tropical dwellers due to poor environmental practices, a predisposing risk for adverse health conditions (Sach, 2000). DMN endogenous burden triggers inflammatory signals and increased reactive oxygen species leading to oxidative stress mediated damage (Adeleke and Adaramoye, 2017). Plant antioxidants may play an important in role drug incorporation, since they offer variable therapeutic benefit owing to their relative safety, efficacy, economy and availability (Veeresham, 2012; Yuan *et al.,* 2016.). Zingerone (ZG) is the chief bioactive in dry ginger rhizome (Zhang *et al.,* 2012) with anti-oxidative, anti-cancer and anti-inflammatory properties (Ahmad *et al.,* 2016). Though knowledge of its pharmacological properties abounds in literatures,

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there is a dearth of information pertaining to the effect of ZG in mitigating DMN-mediated hepatic injury using histological and PAS histochemical approaches, as well as evaluating the implicated role of Gamma Glutamyl Transferase (GGT) and Total bilirubin in liver metabolism. Providing scientific research evidences in this light was therefore the bane of the present study.

MATERIALS AND METHODS

Ethical approval

The ethical clearance for the study was obtained from the Research Ethics committee of College of Medical Sciences, University of Benin, Benin City (Ethical no: CMS/REC/2023/453) in compliance to guidelines by the Nigeria National Health Research Ethics Committee on control and supervision of experimental animal adopted from Guidelines of National Institute of Health (NIH Publications No. 8023, USA, 1978) for the Care and Use of Laboratory Animals

Materials

DMN (European Pharmacopoeia Reference Standard) was purchased from Sigma Aldrich, Germany (CAT No:62-75-9). Zingerone (CAT No: 2490-9) was purchased and shipped from Cayman Chemical Company in Ann Arbor, Michigan, United States. Silymarin (SIL) was purchased under the trade name Silybon-140, and manufactured by Micro Lab Pharmaceutical Company Ltd, Bengaluru, India. All other reagents were of analytic grade

Experimental design

Thirty-six (36) adult Wistar rats (weighing 160-200 g) were purchased from the Animal house of the Department of Anatomy, College of Medicine, University of Benin, Benin City, Edo state, Nigeria. The animals were housed in well-ventilated wired cages and were allowed to acclimatize for two weeks in the Animal house of the Department of Anatomy, University of Benin. They were fed with rat pellet (a product of Top Feed Ltd, Nigeria) and water *ad libitum.* The rats were randomly divided into six (6) groups of six (6) rats each. Group A served as control; group B received 10 mg/kg body weight of DMN only (dissolved in sterile saline) 3 times per week for 4 consecutive weeks (Choi *et al.,* 2017). Group C, D, E were given oral doses of 25 mg/kg, 50 mg/kg and 75 mg/kg of ZG, respectively once daily for 4 weeks (Ahmad *et al.*, 2015; Bashir *et al.*, 2021;) prior to induction of hepatic injury with 10mg of DMN for the last 4 weeks of the 8weeks' experimental period. Groups F was administered with daily oral dose of 50 mg/kg of silymarin for 4 consecutive weeks and later given 10mg DMN for the remaining 4weeks of the experiment duration.

Animal sacrifice and sample collection

At the end of the experiment, all the animals were fasted overnight, anaesthetized with chloroform. Blood samples were collected via a syringe punctured into the apex of the heart, emptied into plain sample bottles and samples centrifuged at 3000 rpm for 10 minutes. The serum obtained was stored at -20^{O}C for assay of Gamma Glutamyl Transferase (GGT) activity and total bilirubin. After sacrifice via cervical dislocation, the liver was harvested following an abdominal incision and rapidly blotted on ash-free filter paper before fixing in 10% phosphate buffered formal saline for histological assessment. Tissue was processed by dehydrating in 70% alcohol, 90% alcohol and via three changes of absolute alcohol for two hours each. The tissue was cleared in three changes of xylene, followed by infiltration and embedding in paraffin wax. Thin sections of 5microns were then obtained using a rotary microtome, ribbons were floated in a warm water bath, collected with clean glass slide, stained with H and E dyes prior to mounting and cover slipping. This procedure follows descriptive method by Dury and Wallington (1980). The PAS reaction technique for hepatic glycogen was done following the method of Mcmanus (1948). Sections of the liver containing 1, 2 glycol groupings in the unsubstituted form was deparaffinized in in two changes of xylene for 5 minutes, and hydrated in distilled water. Slides were placed in 0.5% Periodic acid for 5 minutes, rinsed in distilled water before being placed in Schiff reagent at room temperature for 30mins. This was then washed in running tap water for 5 minutes. It was then counterstained in hematoxylin for 3 minutes, washed in tap water, blue hematoxylin, rinsed in distilled water before finally dehydrating in alcohol, cleared and cover slipped. Photomicrographs were taken after evaluation of slides under Eakins Microscope Camera (2307su, UK) mounted on Lasec microscope (UB 200i). The staining depth and area of PAS reaction for glycogen in each rat micrograph of groups A-F was quantified by Image J software. Each micrograph was copied to the software, scaled and converted to micrometer using the Analyse-set scale dialog box before proceeding to split the image into red, green and blue channels. The green channel is selected and manually adjusted to threshold image. Then the (*Analyse>Measure*) command is entered, the area and percent area is displayed in the results window with mean values and standard deviations of stained areas of slices. The scale bar was erased to prevent it from being included in the calculated area. The values were furthered analysed using graph pad prism 8.0.

Data Analysis

The data generated from the study were analysed using GraphPad Prism 8.1 Statistical Software package (GraphPad Software Inc.). Values were expressed as mean ± Standard Error of Mean (SEM). The differences in the mean were compared between groups using one-way ANOVA followed by Tukey *post hoc* test for multiple comparisons. p-value < 0.05 was considered significant.

RESULTS

Figure 1 shows elevated GGT activity in the group given 10mg of DMN only (group B) when mean values were compared to control (group A) at $p < 0.05$. Contrastingly, only groups pre-treated with 75mg of ZG and 50mg SIL showed significantly decreased activity of GGT in the rats when mean values were compared with DMN alone treated rats.

Figure 2 shows increased concentration of total bilirubin in the group given 10mg of DMN only (group B) when mean values were compared to control (group A) at $p < 0.05$. However, all ZG and SIL pre-treated groups (C-F) showed significant ($p < 0.05$) reduction in T Bil concentration in the rats when their mean values were compared with DMN alone treated rats.

Micrographs of PAS Liver Sections in control, toxicity and pre-treated groups

PAS liver sections of the control group of wistar rats were compared with that given DMN only. Comparisons were also made between liver sections of all groups pre-treated with ZG (C, D and E) and SIL (group F) before exposure of the rats to DMN toxicity. Assessment of hepatic impairment for glycogen metabolism and prophylaxis were based on the degree of positive reaction for glycogen within the hepatic tissue.

Quantification of PAS histochemical reaction

The mean stained area of hepatic tissue were calculated and presented in bar-charts to buttress strong to mild positive reactions when comparisons were made between experimental groups (A-F)

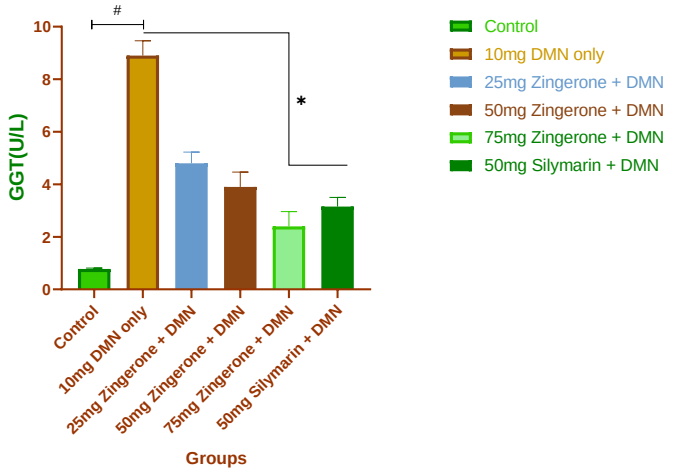

Figure 1: Gamma Glutamyl Transferase activity in experimental groups
The values are the Mean ± SEM. N = 6 rats in each group
#significant increase when comparing Control and DMN alone group at $p < 0.05$
*significant decrease when comparing DMN alone group and Pre-treated groups (Groups C, D, E and F) at $p < 0.05$

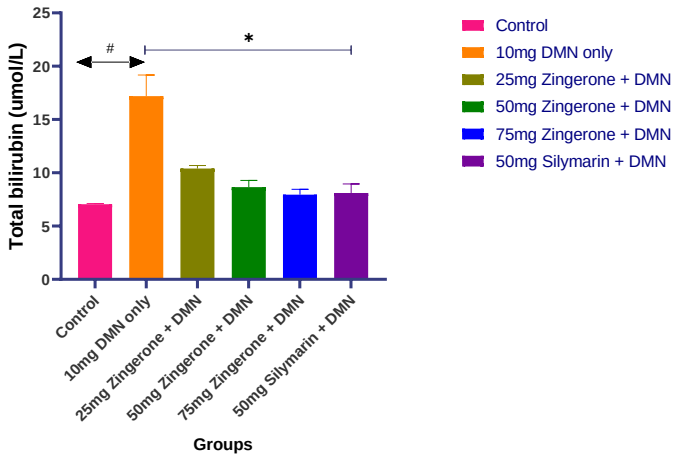

Figure 2: Concentration of Total Bilirubin in experimental groups
The values are the Mean ± SEM. N = 6 rats in each group
#significant increase when comparing Control and DMN alone group at $p < 0.05$
*significant decrease when comparing DMN alone group and Pre-treated groups (Groups C, D, E and F) at $p < 0.05$

Micrographs of liver sections stained with H and E in experimental groups

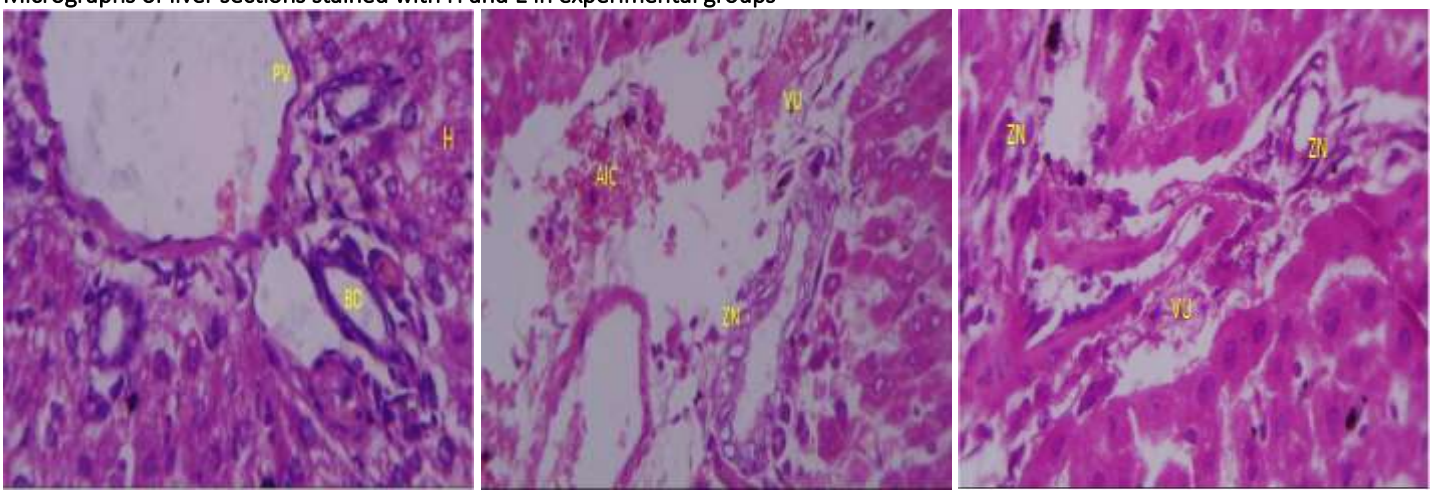

Plate 1A: From Left to right, Control liver (group A) showed normal tissue architecture: Hepatocytes (H), Sinusoids (SI) and Portal tract. Group B given 10mg of DMN only revealed Zonal necrosis (ZN), Aggregation of Inflammatory cells (AIC) and Vascular ulceration (VU) which were also visible in rat liver of group C pre-administered with 25mg ZG. H and E 400x

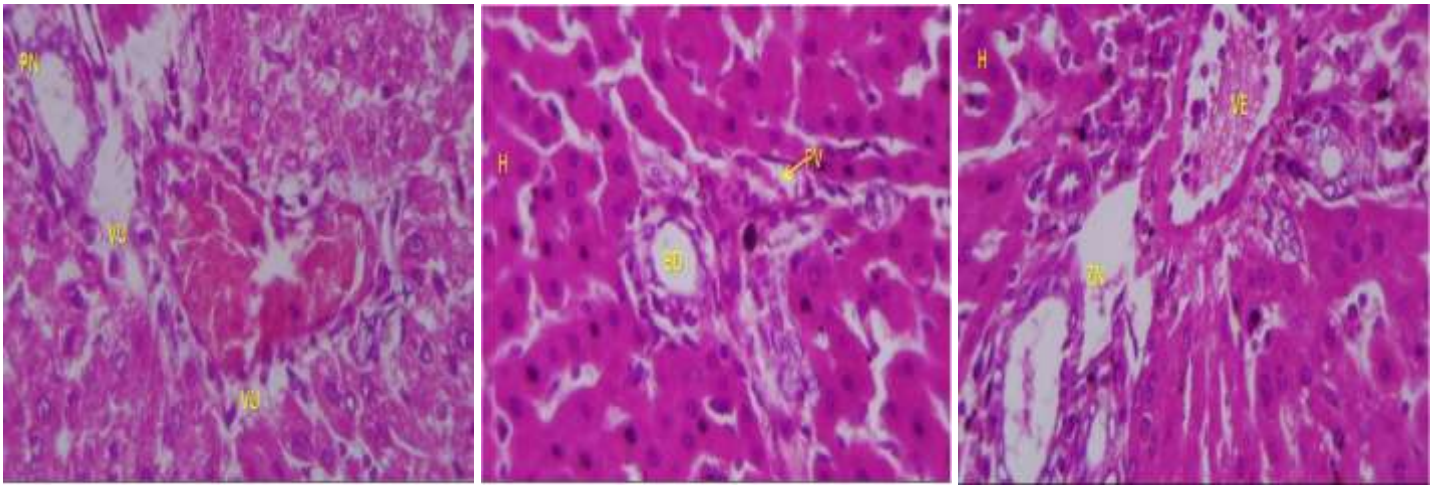

Plate 1B: From Left to right, 50mg ZG pretreated liver showed areas of Patchy necrosis (PN) with pyknosis and moderate vascular ulceration (VU). 75mg ZG Pre-administration revealed fairly normal Hepatocytes (H), Bile duct (BD) and Portal vein (PV) comparably better than liver of rats pre-treated with 50mg SIL (group F) with necrotic hepatocytes and vascular Erosion (VE). H and E 400x

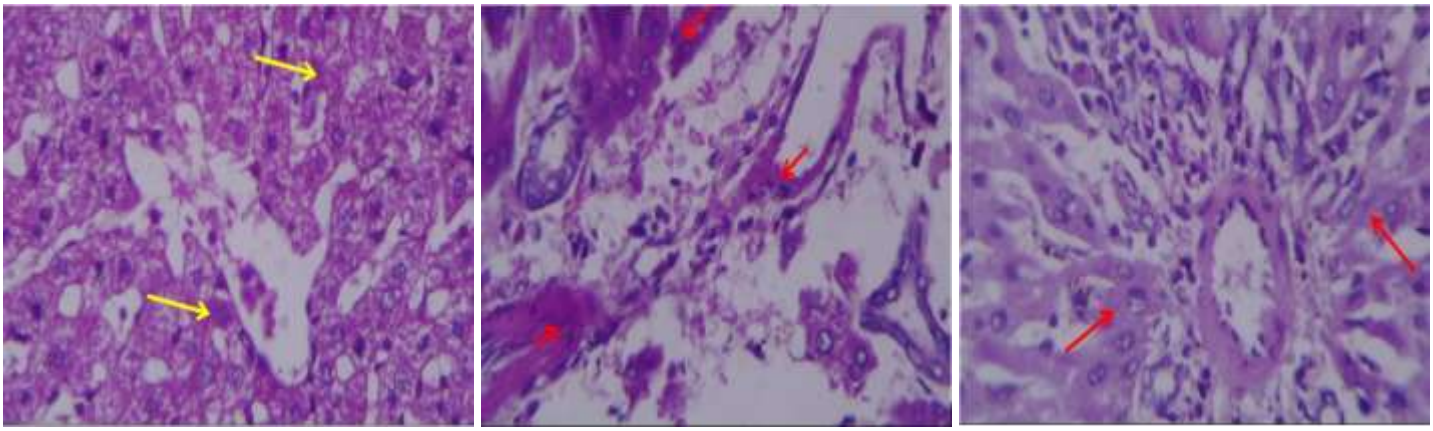

Plate 2A: From Left to right, Control liver shows mild positive reaction (+) for hepatic glycogen in different areas (long yellow arrow) compared to rat liver given 10mg of DMN only showing strongly positive reaction (+++) for glycogen in necrotic areas (short red arrow), while group C given 25mg ZG before DMN showed slightly weak reaction for glycogen. PAS 400x

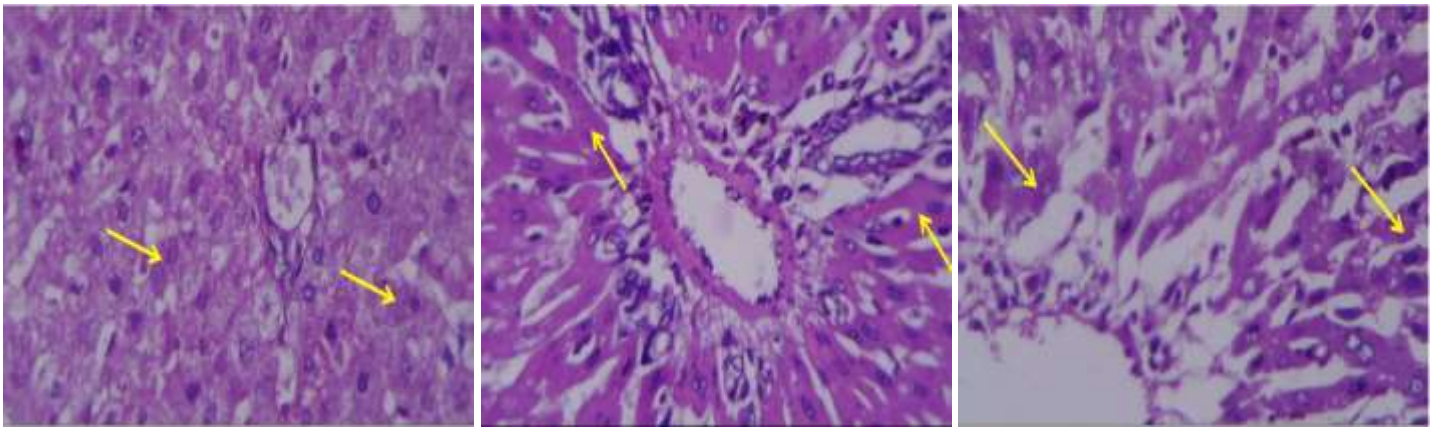

Plate 2B: From Left to right, micrograph of rat liver given 50mg ZG, 75mg ZG and 50mg SIL before DMN (Groups D, E and F) shows moderate positive reaction (+) intrahepatic glycogen (long yellow arrow) comparably similar to control liver. PAS 400x

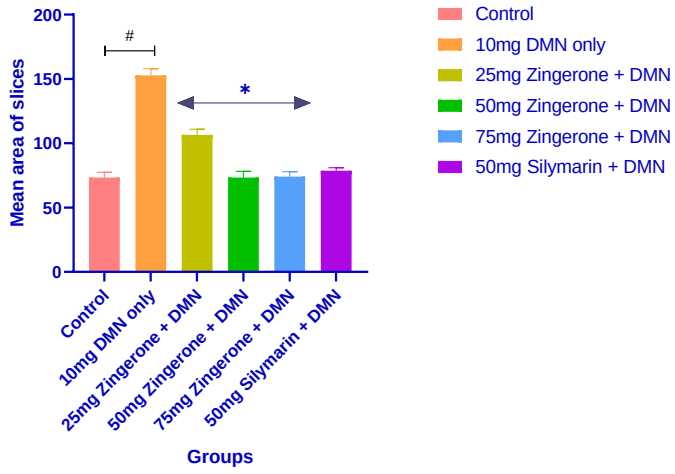

Figure 3: PAS reaction in liver sections of all experimental groups
[#]Significant increase comparing Control and DMN only group at $p < 0.05$; [*]Significant decrease comparing DMN only group and Pre-treated groups (Groups C, D, E and F) at $p < 0.05$

DISCUSSION

An imbalance in redox homeostasis affects hepatocyte and may provoke hepatic dysfunction (Mello et al., 2016; Chaudhary et al., 2023). Hepatic conjugation of bilirubin with glucuronic acid by uridine diphosphoglucuronate-glucuronyltransferase (UDP-GT) for subsequent excretion may be impaired when confronted with a burden of chemotoxin leading to hyperbilirubinemia. This may lead to jaundice, a condition reinforced by related factors that increases the rate of bilirubin formation and its conjugation such as premature rapid destruction of erythrocytes (VanWagner and Green, 2015). DMN only exposed rats showed elevated levels of bilirubin in the serum compared to ZG pre-treatment which mitigated DMN associated Hyperbilirubinemia. This finding is comparable to the report by Amin *et al.* (2020) who investigated the effect of Zingerone on lead induced hepatic function. Gamma glutamyl transferase (GGT) is a hepatic enzyme that catalyzes intracellular glutathione needed for oxidative cellular defense (Cooper and Hanigan, 2018). Although estimation of GGT activity is often ignored in clinical diagnosis due to its low specificity, it is quite a sensitive index for evaluation of hepatic damage than

aminotransferases due to its vast presence in the microsomes and the plasma membranes of hepatocytes (Rosalki, 1975). Its increase is a pointer for oxidative stress mediated hepatocellular injury and cholestasis (Xing *et al.,* 2022). GGT level was elevated in DMN only exposed rats. The rise in GGT level is a reflection of DMN induced hepatobiliary damage which may shed insight on progressive onset of non-alcoholic liver disease. In this present study, only pre-treatment with 75mg/kg of ZG assuaged the surge in GGT level comparable to SIL pre-treatment. It is believed that ZG enhances adherens junctions of hepatocyte on its canalicular and sinusoidal surfaces preventing enzymatic leakage into the blood stream. ZG may have also supressed biliary epithelial cell proliferation, promote increase secretion of bile and influence biosynthesis as well as catabolism of gluthathione. ZG protective effect is comparably similar to reports by Priya *et al.* (2011) on hepatoprotective properties of N-acetylcysteine (NAC) on DMN and Mohamed *et al.* (2014) on Alleviation of DMN-Induced Liver Injury and Fibrosis by Supplementation of *Anabasis articulata* extract.

Although biochemical based evidences give clues to hepatic injuries and diseases, histopathological evaluations of liver biopsies appear to be the most accepted, sensitive and direct means of diagnosing diseases regardless of aetiology, prognosis as well as treatment responses (Chowdhury and Mehta, 2022). In contrast, to the Control, the hepatocyte-sinusoidal distortions in DMN only exposed rat liver may suggest intrinsic structural defects in the fenestrated endothelium of the sinusoids running through the hepatic cords tasked with ensuring efficient nutrient uptake for hepatocyte cell survival. Furthermore, vascular ulcerations affect blood flow promoting necrotic changes around the portal area and periportal hepatocytes with infiltration of inflammatory cells within the hepatic stroma. These features are consistent with hepatic damage (Nwafor *et al.,* 2020). Administration of graded doses of ZG before DMN exposure suggests ZG efficacy to mitigate DMN associated histological alterations, thereby maintaining physiological processes. This is perhaps linked to ZG ability to provoke cascade of signalling molecules involved in angiogenesis, hepatocyte cell survival and apoptosis. However, only 75mg of ZG offered a robust hepato-protective effect against DMN toxicity and compared favourably to SIL pre-treatment. These findings align with similar but earlier reported hepatoprotective effects of zingerone by Al-suhaimi *et al.* (2015) and Gungor *et al.* (2020).

Periodic Acid Schiff reaction is a histochemical test for polysaccharides. It is an investigative tool for assessing liver function in glycogenesis and glycogenolysis with PAS positive substances appearing purplish red within the cytoplasm (Shu *et al.,* 2021). Under relatively narrow range of fasting periods, glucagon signals through binding receptors on the hepatic cell surface, the stimulation of hepatic glucose output required for cellular metabolism and survival. This is via activation of glycogen phosphorylation for glycogen breakdown (Sharabi *et al.,* 2015). DMN only exposed rats revealed a strong PAS reaction for glycogen, particularly in the necrotic areas compared to control liver section. This may inadvertently suggest that reactive metabolites of DMN could interfere with glucagon mediated hormonal regulations involved in glycogenolysis and the possible risk of glycogen storage disease. In contrast, 25mg, 50mg and 75mg of ZG showed moderate glycogen presence. It is hypothesized that ZG may promote metabolism of intra hepatic store of glycogen in events of glucose depletion, possibly by ensuring minimal conformational changes in glucagon receptors bind site on hepatocyte membranes likely due to DMN reactive species. Furthermore, even in the presence of fewer glucagon receptors, ZG administration may modulate glycogen metabolism via upregulation of cAMP levels, although mechanisms are yet to be fully understood.

In conclusion, our findings from the present study revealed that pre-supplementation with ZG could mitigate DMN mediated hepatobiliary toxicity. It is interesting to note that ZG efficacy is dose dependent with the 75mg ZG comparing favorably with control and SIL pre-treated rats.

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Conflict of Interest

The Authors declare that there was no conflicting interest.

REFERENCES

Adeleke GE and Adaramoye O.A. (2017). Betulinic acid protects against N-nitrosodimethylamine-induced redox imbalance in testes of rats. *Redox Reproduction*, 22: 556–562.

Ahmad B, Rehman MU, Amin I, Arif A, Rasool S, Bhat SA, Afzal I, Hussain I, Bilal S, and Mir MU. (2016). A Review on Pharmacological Properties of Zingerone (4-(4-Hydroxy-3-methoxyphenyl)-2-butanone). *Scientific World Journal*, 81: 63-64.

Al-Suhaimi EA, Al-Riziza NA, and Al-Essa RA (2015). Physiological and therapeutic effects of ginger and its active constituents in the gastrointestinal tract: A review. *Food and Function,* 6(8): 2481-2493.

Amin I, Hussain I, Rehman MU, Mir BA, Ganaie SA, Ahmad SB, Manzoor URM, Syed S, Muzamil S, Arafah A, Ahmad P. (2020). Zingerone prevents lead-induced toxicity in liver and kidney tissues by regulating the oxidative damage in Wistar rats. J Food Biochem.1-14

Bashir N, Ahmad SB, Rehman MU, Muzamil S, Bhat RR, Mir MUR, Shazly GA, Ibrahim MA, Elossaily GM, Sherif AY, Kazi M. (2021).

Zingerone (4-(four-hydroxy-3-methylphenyl) butane-two-1) modulates adjuvant-induced rheumatoid arthritis by regulating inflammatory cytokines and antioxidants. *Redox Rep*, 26(1): 62-70.

Chaudhary P, Janmeda P, Docea AO, Yeskaliyeva B, Abdull RAF, Modu B, Calina D, Sharifi-Rad J. (2023). Oxidative stress, free radicals and antioxidants: potential crosstalk in the pathophysiology of human diseases. *Frontiers in Chemistry*, 11:1-24

Cheemerla S and Balakrishnan M. (2021). Global Epidemiology of Chronic Liver Disease. Clin Liver Dis (Hoboken), 17(5): 365-370.

Choi JH, Jin SW, Choi CY, Kim HG, Lee GH, Kim YA, Chung YC, and Jeong HG. (2017). Capsaicin inhibits dimethylnitrosamine-induced hepatic fibrosis by inhibiting the TGF-β1/Smad pathway via peroxisome proliferator-activated receptor gamma activation. J Agric Food Chem, 65: 317−326

Chowdhury AB and Mehta KJ. (2022). Liver biopsy for assessment of chronic liver diseases: a synopsis. Clinical and Experimental Medicine. 2022, 1-14. .

Cooper AJL, Hanigan MH. (2018). Metabolism of Glutathione *S*-Conjugates: Multiple Pathways. Comprehensive Toxicology. 2363–406.

Drury RAB, and Wallington E.A (1980) Carleton's histological technique, 5th edition Oxford University Press, Oxford New York Toronto, pp 188–189, 237–240, 290–291

Gungor H, Ekici M, Onder-Karayigit M, Turguz NH, Karaz H and Arslanbas E. (2020). Zingerone ameliorates oxidative stress and inflammation in bleomycin-induced pulmonary fibrosis: modulation of the expression of TGF-β1 and iNOS. *Naunyn-Schmiedeberg's Archives of Pharmacology.* 1-13

Gushgari AJ, and Halden RU. (2018). A critical review of major sources of human exposure to N-nitrosamines. *Chemosphere*, *210*, 1124–1136

Mello T, Zanieri F, Ceni E and Galli A. (2016). Oxidative Stress in the Healthy and Wounded Hepatocyte: A Cellular Organelles Perspective. *Oxidative Medicine and Cellular Longevity*, 1-15

Mohamed AM, Abdalla MS, Rizk MZ, Mahdy el-SM, Farrag AR, El-Sharabasy FS, Aly HF, Mohamed MR. Alleviation of Dimethylnitrosamine-Induced Liver Injury and Fibrosis by Supplementation of Anabasis articulata Extract in Rats. *Indian J Clin Biochem.* 2014, 29(4):418-29

Mcmanus JFA. (1948). Histological and Histochemical uses of Periodic acid. Stain Technology, 23: 99-108.

Nwafor JA, Obikili EN, Akunna GG, Anyanwu GE, Esom E (2020). The role of Aqueous Extract of Citrus Sinensis peel in Cadmium mediated hepatotoxicity. *International Journal of Pharmaceutical Science and Research*, 11(5): 2042-2051

Pei Q, Yi Q, Tang L.(2023). Liver fibrosis resolution: From molecular mechanisms to therapeutic opportunities. *Int J Mol Sci,*24: 9671.

Priya S, Vijayalakshmi P, Vivekanandan P and Karthikeyan S.(2011). Influence of N-acetylcysteine against dimethylnitrosamine induced hepatotoxicity in rats. *Toxicology and Industrial Health*. 27(10):914-922.

Rosalki SB, Rau D, Lehmann D, Prentice M. (1970). Determination of Serum γ-Glutamyl Transpeptidase Activity and its Clinical Applications. *Annals of Clinical Biochemistry*. 7(6):143-147.

Sachs J. (2000). Tropical Underdevelopment. CID Working Paper No. 57

Sharabi K, Tavares CD, Rines AK, Puigserver P. (2015). Molecular pathophysiology of hepatic glucose production. Mol Aspects Med. 46:21-33

Shu, Y, Faizule H, Ostrowski MC and Mehta KD. (2021). Role of hepatic PKCβ in nutritional regulation of hepatic glycogen synthesis. Journal of Clinical investigation insight. 6(19):1-16

United Nations. (2010). Estimates based on United Nations, World Population Prospects, the 2010 Revision and census data. (WPP2010). New York (NY): United Nations DESA, Population Division

VanWagner LB and Green RM. (2015). Evaluating elevated bilirubin levels in asymptomatic adults. *JAMA*. 3;313(5):516-7.

Veeresham C. (2012). Natural products derived from plants as a source of drugs. *Journal of Advanced Pharmaceutical Technology and Research,* 3(4): 200-1.

Xing M, Gao M, Li J, Han P, Mei L, Zhao L. (2022). Characteristics of peripheral blood Gamma-glutamyl transferase in different liver diseases. *Medicine (Baltimore).* 7, 101(1):e28443

Yuan H, Ma Q, Ye L and Piao G (2016). The Traditional Medicine and Modern Medicine from Natural Products. *Molecules*. 21(5):559

Zhang YX, Li JS, Chen LH, Peng WW and Cai BC. (2012). Simultaneous determination of five gingerols in raw and processed ginger by HPLC. *Chinese Pharmaceutical Journal,* 47(6): 471–474.