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REVIEW PAPER

Comparative therapeutic role of ascorbic acid, α-tocopherol and riboflavin in mitigating hepatotoxicity induced by drugs and chemical toxins – a review

Dayo Omotoso , Divine Joseph

Department of Human Anatomy, Faculty of Basic Medical Sciences, Redeemer's University, Ede, Nigeria

ABSTRACT

Introduction and aim. The liver plays a central role in the metabolism of drugs, xenobiotics, and nutrients, making it highly susceptible to exposure to toxicity due to drugs and chemical toxins (DCT). DCT-induced hepatotoxicity (DIH), remains one of the most common causes of acute liver failure, and potential therapeutic agents such as ascorbic acid, α-tocopherol and riboflavin have been explored to mitigate DIH. This review summarizes the current knowledge in the experimental model.

Material and methods. This review was based in publications available on scientific databases such as PubMed, Scopus, Web of Science, and Google Scholar. After the abstract evaluation, the relevant articles were selected for further analysis.

Analysis of the literature. The vital role of oxidative stress and inflammation in mediating DIH has been demonstrated. Hence, the most effective therapeutic intervention includes agents that exhibit potent antioxidant and anti-inflammatory properties such as ascorbic acid, α-tocopherol and riboflavin.

Conclusion. The comparative therapeutic role of ascorbic acid, α-tocopherol and riboflavin against DIH involves the reparation of hepatic histomorphological impairments and modulation of biochemical and molecular alterations that characterized the onset and progression of DIH.

Keywords. hepatotoxicity, hepatotoxins, vitamins hepatoprotection

Introduction

The liver is considered the largest visceral organ which performs various functions and produces various physiological secretions.[1] It also plays a vital role in the metabolism of nutrients, drugs, and xenobiotics, which makes it prone to toxicity due to exposure to drugs and chemical toxins (DCT). Furthermore, exposure to DCT can cause liver damage and disrupt physiological function leading to organ failure through a direct deleterious effect or through alteration of the hepatic vasculature.[2] Basically, liver damage can be categorized into acute and chronic categories, with the acute damage easily ameliorated through rapid elimination of hepatotoxins, leading to restoration of liver physiological function. However, untreated acute damage could lead to chronic liver pathologies such as cirrhosis, fibrosis, encephalopathy, and liver cancer.[3,4]

DCT-induced hepatotoxicity (DIH), which results from the abuse of drugs and chemical substances, is a common cause of acute liver failure, thereby constituting a public health challenge globally.[5] DCT such as acetaminophen, carbon tetrachloride (CCl_4), isoniazid, aflatoxin B1, methotrexate, and isotretinoin possess different molecular structures (Fig. 1) that form the basis of their distinct mechanisms of action.[6] Essentially, DIH is a major cause of acute liver failure which often results

Corresponding author: Dayo Omotoso, e-mail: dayohmts@gmail.com

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in the issuance of warnings about the application or the complete withdrawal of some drugs and chemical substances by a regulatory agency such as Food and Drug Administration (FDA).[4,7]

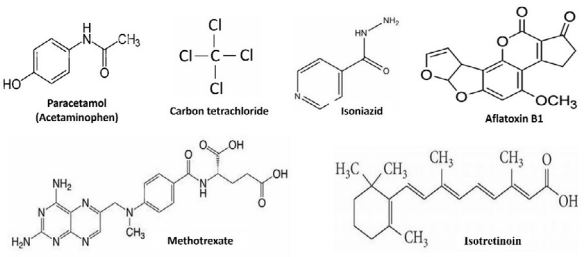

Fig. 1. Molecular structures of the selected DCT

Hepatotoxicity induced by DCT occurs as intrinsic or idiosyncratic with intrinsic hepatotoxicity usually results from the activity of DCT or its metabolites, especially due to excessive dose exposure, thereby making it highly reproducible.[8] On the idiosyncratic hepatotoxicity represents a subset of the broader idiosyncratic adverse drug reactions, which usually occurs at recommended therapeutic doses and is relatively rare.[8,9]

Furthermore, the pathogenesis of DIH have been described to involve multiple phases with the initial phase of tissue injury occurring due to direct cellular stress, mitochondrial dysfunction, activation of inflammatory and immune responses. Initial tissue injury initiates downstream events that include the death receptor-mediated pathways that are characterized by mitochondrial permeability disruption and result in apoptosis or necrosis in the liver tissue.[10] Experimental studies and a model have been explored to exploit the potential of therapeutic agents in modulating the associated pathways, thus mitigating DIH. In this regard, experimental studies have demonstrated the role of essential vitamins (including ascorbic acid, α-tocopherol and riboflavin) as prophylactic or therapeutic agents with potency in mitigating the onset and pathogenesis of DIH.

Aim

This review aimed at summarizing the current findings on the therapeutic activity of ascorbic acid, α-tocopherol and riboflavin in mitigating DIH using an experimental model. The review also explored the mechanisms of hepatoprotective activity of the selected essential vitamins.

Material and methods

Research articles that are relevant to the review were searched across scientific databases including Google Scholar, PubMed, Scopus, and Web of Science. Search keywords included 'hepatoprotective effect of ascorbic acid, 'anti-inflammatory effect of ascorbic acid,' anti-apoptotic effect of ascorbic acid, 'hepatoprotective effect of -tocopherol, 'anti-inflammatory effect of α-tocopherol, 'anti-inflammatory effect of α-tocopherol, 'anti-apoptotic effect of α-tocopherol, 'hepatoprotective effect of riboflavin', 'anti-inflammatory effect of riboflavin', 'anti-apoptotic effect of riboflavin'. The critical assessment of the search results was conducted to identify articles that contain relevant information regarding therapeutic potential of ascorbic acid, α-tocopherol and riboflavin against DIH in the experimental model. The eligibility criteria included articles with findings that are relevant to the purpose of the review, articles published in English, articles published in peer-reviewed journals, while the criteria for exclusion included duplicate articles and articles containing irrelevant information with regard to the aim of the review.

Analysis of the literature

The pivotal role of oxidative stress and inflammation, as anchor cellular events that drive the onset and progression of DIH, has made antioxidant and anti-inflammatory agents the most effective therapeutic intervention. Therefore, the antioxidant and anti-inflammatory properties of essential vitamins such as ascorbic acid, α-tocopherol and riboflavin could delineate their role as potent hepatoprotective agents against DIH.

Hepatotoxic effect of selected DCT and associated mechanisms in the experimental model

The exposure to acetaminophen (a known analgesic) could induced hepatotoxicity, characterized by prominent hepatic morphological impairments such as necrosis, sinusoidal congestion, inflammatory cell infiltration.[11] Other indicators of acetaminophen-induced hepatotoxicity included significantly elevated serum level of liver enzymes such as alanine aminotransferase (ALT) and aspartate aminotransferase (AST).[11-13] Furthermore, the hepatic and serum levels of oxidative stress, inflammatory and apoptotic factors such as malondialdehyde (MDA), tumor necrosis factor-alpha (TNF-α), nuclear factor kappa B (NF-kB), interleukin-6 (IL-6), IL-1β,), interferon-gamma (INF-γ), inducible nitric oxide synthase (iNOS), myeloperoxidase, caspase-3 and Bax were significantly elevated.[11-14] Conversely, the hepatic levels of antioxidant, anti-inflammatory and anti-apoptotic markers such as glutathione (GSH), IL-10, Bcl-2 were significantly decreased.[11-15]

Furthermore, the hepatotoxicity induced by CCl_4 exposure was characterized with impaired liver function indicated by increased levels of markers such as ALT, AST, and alkaline phosphatase (ALP), induction of oxidative damage due to generation and accumulation of reactive oxygen species (ROS), induction of autophagy in hepatic tissue, and observable hepatic histopathological changes which include necrosis, steatosis, and inflammation.[16-18] CCl_4-induced hepatotoxicity was fur-

Table 1. Characterization of selected hepatotoxic drugs and chemical toxins

Selected DCT (other name)	IUPAC NAME (Structural formula)	Molecular weight	Pharmacological/ general properties	Mechanisms of hepatotoxic effect of selected DCT	References
Acetaminophen (paracetamol)	N-(4-hydroxyphenyl) acetamide ($C_8H_9NO_2$)	151.165 g/mol	- used as an analgesic and antipyretic agent	- hepatic morphological impairments - increased serum levels of liver enzymes - elevated inflammatory and apoptotic markers - reduced antioxidant and anti-apoptotic markers	Chariyakornkul et al.[11] Koshak et al.[12] Akgun et al.[13] Ahmed Mohammed and Fadheel[14] Mohamed Kamel et al.[15]
Carbon tetrachloride	Tetrachloromethane (CCL_4)	153.823 g/mol	- used as industrial solvent in dry cleaning, insecticide dispersant, fumigant, extinguisher, and production of chlorofluorocarbons	- increased serum levels of liver enzymes - elevated inflammatory and apoptotic markers - induction of oxidative stress and autophagy in hepatic tissue - hepatic histopathological changes	Shaban et al.[16] Omotoso and Amakhabi[17] Sharma et al.[18] Abdelgalil et al.[19] Rabey et al.[20]
Isoniaziad (isonicotinic acid hydrazide)	Pyridine-4-carbohydrazide ($C_6H_7N_3O$)	137.142 g/mol	- used as antibiotic singly or with other drugs to treat tuberculosis	- elevated serum levels of liver enzymes - reduction of antioxidant enzymes - hepatic histopathological changes - elevated levels of inflammatory and apoptotic markers	Ruan et al.[21] Patel et al.[22] Metushi et al.[23]
Aflatoxin B1	(3S,7R)-11-methoxy-6,8,19-trioxapentacyclo[10.7.0.02,9.03,7.013,17] nonadeca-1,4,9,11,13(17)-pentaene-16,18-dione ($C_{17}H_{12}O_6$)	312.27 g/mol	- a carcinogenic mycotoxin that contaminates food, and crops	- decreased level of oxidative stress and apoptotic markers in hepatic tissue - down-regulation of antioxidant enzymes and anti-inflammatory markers	Moloi et al.[24] Xu et al.[25] Xu et al.[26] Wang et al.[27] Yılmaz et al.[28]
Methotrexate	(2S)-2-[[4-[(2,4-diaminopteridin-6-yl)methyl-methylamino]benzoyl]amino] pentanedioic acid ($C_{20}H_{22}N_8O_5$)	454.447 g/mol	- used as an immuno-suppressant to treat psoriasis, rheumatoid arthritis, Crohn's disease; used as anti-metabolite during chemotherapy for various tissue cancers	- elevated serum levels of liver enzymes - elevated oxidative stress and apoptotic markers - decline in antioxidant enzymes - hepatic histopathological changes - depletion of hepatic folate leading to hepatocyte death	Alorabi et al.[3] Ezhilarasan et al.[6] Mahmoud et al.[29] Kalantari et al.[30] Yanasoglu et al.[31] Almalki et al.[32]
Isotretinoin (13-cis-retinoic acid or Accutane)	(2Z,4E,6E,8E)-3,7-dimethyl-9-(2,6,6-trimethyl-1-cyclohexenyl)nona-2,4,6,8-tetraenoic acid ($C_{20}H_{28}O_2$)	300.44 g/mol	- retinoic acid used to treat skin disease such as acne vulgaris and ichthyosis	- increased serum levels of liver enzymes - elevated oxidative stress markers - decline in antioxidant enzymes	Saied et al.[34] Taziki et al.[35] Daye et al.[36] Xu.[37]

ther marked by significantly elevated levels of inflammatory, apoptotic and autophagy-related proteins such as TNF-α, transforming growth factor-beta (TGF-β), IL-6, Bax, Beclin-1 and LC38.[16,19,20]

Furthermore, exposure of isoniazid (a common antituberculosis agent) usually resulted into oxidative stress, energy metabolism, and osmotic imbalance which culminated in toxicity of body tissues including liver tissue.[21] In particular, isoniazid-induced hepatotoxicity was characterized by impairment of hepatic tissue function highlighted by elevated serum levels of ALT, AST, ALP and decreased antioxidant enzyme such as superoxide dismutase (SOD) and catalase (CAT). Further profiling of isoniazid-induced hepatotoxicity revealed hepatic histopathological changes including necrosis and steatosis, as well as up-regulation of markers of oxidative damage and inflammation such as MDA, NO, TNF-α, IL-6, and IL-1β.[22,23]

Regarding aflatoxin B1-induced hepatotoxicity, the primary cellular events included mitochondrial dysfunction, oxidative stress, activation of inflammatory signaling, apoptosis and pyroptosis.[24,25] These events were further characterized by reduced mitochondrial membrane potential (MMP), elevated expression of cytoplasmic cytochrome c, Bax, p53, caspase-3 and caspase-9 proteins.[26] Aflatoxin B1-induced pyroptosis in hepatic tissue occurred via upregulation of NOD-like receptor protein 3 (NLRP3), caspase-1 and GSDMD, IL-1β and IL-18.[27] Aflatoxin B1 exposure further caused oxidative stress which was characterized with increased MDA levels and reduced levels of nuclear factor erythroid 2-related factor 2 (Nrf2) and antioxidant markers including SOD, CAT, glutathione peroxidase (GPx), heme oxygenase-1 (HO-1), NAD (P)H quinone oxidoreductase 1 (NQO1) in hepatic tissue.[27,28]

Hepatotoxicity that resulted from methotrexate treatment was characterized with endoplasmic reticulum (ER) stress, oxidative stress and induction of inflammation and apoptosis. The treatment further resulted into impairment of physiological function of liver characterized with elevated serum levels of liver enzymes such as ALT, AST, and ALP; induction of oxidative stress indicated by elevated hepatic MDA and decline in activity of anti-oxidative enzyme such as SOD, CAT, GSH and GPx.[3,29,30] This was further characterized by prominent derangement of hepatic tissue histomorphology as indicated by necrosis and inflammation.[32] Other cellular markers of methotrexate-induced hepatotoxicity included significant elevation of apoptosis and inflammatory factors including caspase-3, NF-kB, TNF-α, IL-1β, IL-6, IL-12 and depletion of hepatic folate leading to hepatocyte death.[29,32,33]

Furthermore, isotretinoin which is a widely used to treat skin diseases especially acne has been reported to cause oxidative damage of body tissues including the liver. Its mechanisms of hepatotoxicity included increased serum levels of liver enzymes (AST, ALT, AST), cholesterol, triglycerides and MDA with a concurrent decline in hepatic antioxidant markers (GSH, SOD, CAT).[34-36] Furthermore, isotretinoin-induced hepatotoxicity was characterized by a significant elevation of NO and protein carbonyl (PC).[37,38]

Therapeutic effect of ascorbic acid against DIH

Ascorbic acid (or vitamin C) is a water-soluble solid compound, composed of a five-member near-planar ring having two chiral centers that resolve into four stereoisomers (Fig. 2).[38] It is abundantly available in many natural sources, including fresh fruits such as oranges, lemons, grape and leafy green vegetables and exists in the form of ascorbate within the body.[39-41] It acts as an antioxidant which scavenges free radicals to abrogate oxidative stress and also mitigate the inflammatory response that follows exposure to tissue toxins.[41-44] Therefore, the therapeutic potential of ascorbic acid against DIH has been explored in experimental animal model with satisfactory outcomes.

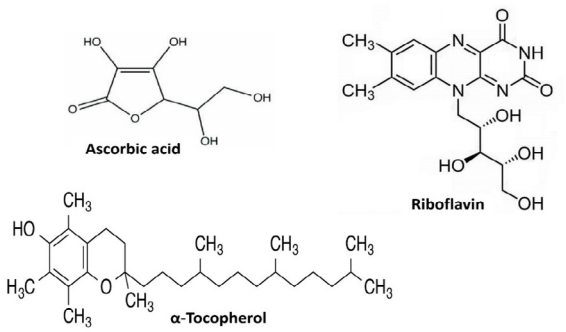

Fig. 2. Molecular structures of ascorbic acid, α-tocopherol and riboflavin

Treatment with ascorbic acid has been shown to exhibit protective effect against isoniazid-mediated hepatotoxicity through its radical scavenging and cytokine suppression activity, which was characterized with increased levels of SOD and CAT, as well as concurrent decreased TNF-α, IL-1β, IL-6, MDA, and NO levels.[22] Moreover, pretreatment with ascorbic acid has been reported to mitigate acetaminophen-induced hepatotoxicity with amplification of the hepatoprotective effect demonstrated when combined with silymarin and alpha lipoic acid.[45] Previous study has reported hepatoprotective activity of ascorbic acid alone or in combination with omega-3 against methotrexate toxicity that was characterized with improvement of liver enzymes and oxidative stress markers such as ALT, ALP, MDA, and lactate dehydrogenase (LDH) as well as liver histomorphology.[3] Based on similar mechanisms, ascorbic acid alone or in combination with natural product (curcumin) or niclosamide have exhibited potent therapeutic effect.

In a previous comparative study, hepatoprotective role of ascorbic acid against acetaminophen (paracetamol)-induced toxicity has been used to assess the therapeutic potential of medicinal plant – *Phoenix dactylifera* L. based on the effect on liver function parameters (AST, ALT, ALP, LDH, total bilirubin, and total protein), antioxidants (SOD, CAT and GPx), and GSH.[48] The antioxidative stress activity of ascorbic acid or in combination with other essential vitamins like α-tocopherol (vitamin E) and cobolamin (vitamin B_{12}) have been reported to drive the hepatoprotective effect against acetaminophen-induced hepatotoxicity through attenuation of oxidative stress and lipid peroxidation, and reduction of serum levels of liver enzymes (ALP, ALP, AST).[49]

Therapeutic effect of α-tocopherol against DIH

α-tocopherol is a lipophilic vitamin, one of the four homologs of tocopherols which together with the four tocotrienols constitute the eight essential compounds of vitamin E. Essentially, all eight compounds contain one chromane ring with one hydroxyl group and side chain; with tocopherols having saturated side chain (Fig. 2) while tocotrienols possess unsaturated isoprenoid side chain with double bonds at 3rd, 7th, and 11th carbon.[50,51] α-tocopherol functions as a potent antioxidant that protects cellular membranes and lipoproteins from peroxidation thereby preventing tissue damage due to free radicals.[52-54]

α-Tocopherol has demonstrated a protective effect against aflatoxin B1-induced toxicities, including hepatotoxicity.[28] The mechanisms of hepatoprotective activity of α-tocopherol against aflatoxin B1 included significantly decreased serum levels of ALP, AST, ALT and LDH, amelioration of associated hepatic histopathological changes, and suppression of oxidative stress makers in haptic tissue.[28] Furthermore, α-tocopherol has shown a therapeutic effect against hepatotoxicity due to methotrexate exposure through mitigation of oxidative stress and reparation of associated hepatic histopathological changes such as hepatocyte degeneration, sinusoidal enlargement, mononuclear cell infiltration, vacuolization and pyknotic nucleus.[52] Moreover, α-tocopherol exhibited potent ameliorative effect against acetaminophen-induced hepatotoxicity based on its antioxidant activity and characterized by reduction of ROS and lipid peroxidation indicated by reduced levels of MD.[55]

Therapeutic effect of riboflavin against DIH

Riboflavin (or vitamin B_2) is a member of B-vitamin family composed of isoalloxazin ring, that possesses three six-carbon rings: benzoic, pyrazine and pyrimidine (Fig. 2). As a water-soluble vitamin, it plays vital

Table 2. General and therapeutic properties of the ascorbic acid, α-tocopherol and riboflavin

Selected Vitamins	IUPAC NAME (Structural formula)	Molecular weight	Common sources	General properties and uses	Mechanisms of therapeutic effect against DIH	References
Ascorbic acid (vitamin C)	(2R)-2-[(1S)-1,2-dihydroxyethyl]-3,4-dihydroxy-2H-furan-5-one ($C_6H_8O_6$)	176.124 g/mol	orange, tomato, guava, potato, mango, lemon, pepper, strawberries, grape fruits	- water soluble and heat-stable. - used to treat scurvy, acne, gum infection, stomach ulcers caused by *Helicobacter pylori*; plays vital role in formation of collagen fibres and to prevent gallbladder disease	- decreased oxidative stress, inflammatory, apoptotic markers - increase in antioxidant enzymes - decreased serum levels of liver enzymes - reparation of hepatic histopathological changes	Alorabi et al.[3] Metushi et al.[23] Abdulrazzaq et al.[45] Khudair and Al-Gareeb[46] Zeki and Al-Gareeb[47] Bouhlali et al.[48] Abdulkhaleq et al.[49]
α-Tocopherol (vitamin E)	(2R)-2,5,7,8-tetramethyl-2-[(4R,8R)-4,8,12-trimethyltridecyl]-3,4-dihydrochromen-6-ol ($C_{29}H_{50}O_2$)	430.71 g/mol	almonds, nuts, butter, spinach, vegetable oils, cereals, meat, egg, avocado, fruits, wheat	- fat-soluble, viscous pale yellow liquid, insoluble in water; - used to prevent skin or hair damage and reduce risk of eye disorder; used as immune booster and for treatment of atherosclerosis, eczema	- reduction of serum levels of liver enzymes - amelioration of hepatic histopathological changes - suppression of oxidative stress makers	Yilmaz et al.[28] Akman et al.[52] Torquato et al.[53] Sudheesh et al.[55]
Riboflavin (vitamin B_2)	7,8-dimethyl-10-[(2S,3S,4R)-2,3,4,5-tetrahydroxypentyl] benzo[g]pteridine-2,4-dione ($C_{17}H_{20}N_4O_6$)	376.369 g/mol	meat, eggs, almonds, nuts, spinach, apple, kidney bean, spinach, banana, potato, avocado, tomato, oatmeal	- orange-yellow crystals, soluble in water, heat-stable. - used for development of lining of digestive tract, blood cells and normal body growth; used to treat migraine and to lower blood level of homocysteine	- restoration of serum levels of liver enzymes - increased tissue antioxidants - declined oxidative stress markers - amelioration of hepatic histopathological changes	Saedisomeolia and Ashoori[58] Wang et al.[59] Al-Harbi et al.[60]

roles in different biological pathways or processes and functions as a precursor to flavin mononucleotide and flavin adenine dinucleotide (which regulates the activity of flavoprotein enzymes).[56,57] Riboflavin is also an essential nutrient for normal health status; therefore, dietary intake is essential. However, it is present in sources such as milk, calf liver, eggs, fish, fruits, nuts, and legumes, green leafy vegetables, wild rice, mushrooms, yeast, cheese, and dietary products.[56,58]

Furthermore, riboflavin exhibits antioxidant properties and plays a vital role in lipid metabolism by preventing lipid peroxidation.[58,59] The therapeutic role of riboflavin against CCl_4-induced hepatotoxicity has been reported and associated with its antioxidant or anti-inflammatory effects.[60] In essence, the hepatoprotective effect of riboflavin against DCT was characterized by restoration of serum levels of liver enzymes (ALT, AST, ALP), oxidant parameter (MDA).[60] Other indicators of hepatoprotective effect of riboflavin included amelioration of liver histopathological changes due to exposure to CCl_4 exposure and down-regulation of inflammatory makers such as TNF-α.[60]

Conclusion

Generally, the imbalance in the cellular redox status in hepatic tissue that leads to hepatotoxicity can be caused by different mechanisms depending on the specific DCT. However, the induction of oxidative damage and inflammation in liver tissue are regarded as the main mechanisms that trigger the onset and progression of DIH. In contrast, the counteracting potential of essential vitamins such as ascorbic acid, α-tocopherol and riboflavin, against the mechanisms that drive the onset and progression of DIH, highlighted their comparative prophylactic and therapeutic effect against the deleterious impact of DCT on liver tissue.

Declarations

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Author contributions

Conceptualization, D.O., D.J.; Methodology, D.O.; Software, D.J.; Validation, D.O., D.J.; Formal Analysis, D.O.; Investigation, D.O., D.J.; Resources, D.O.; Data Curation, D.O.; Writing – Original Draft Preparation, D.J.; Writing – Review & Editing, D.O; Visualization, D.O., D.J.; Supervision, D.O.

Conflicts of interest

Authors do not declare any conflict of interest.

Data availability

The data that support the findings of this study have been included in the article.

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