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Curcumin protects against ketoconazole-induced hepatotoxicity in Wistar rats

Nwakaego Omonigho EBONG [1], Bamiwuye Similoluwa DANIEL[1], Elias ADIKWU[2,*]

[1]Department of Pharmacology /Toxicology, Faculty of Pharmacy, Madonna University, Rivers State, Nigeria
[2]Department of Pharmacology /Toxicology, Faculty of Pharmacy, Niger Delta University, Bayelsa State, Nigeria

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Abstract

Ketoconazole (KZ) is a broad-spectrum antifungal drug that can cause hepatotoxicity. Curcumin (CM), an isolate of *Curcuma longa L.* is used in folk medicine to treat various ailments. This study assessed whether CM can protect against a rat model of KZ-induced hepatotoxicity. Thirty healthy adult Wistar rats (220-240g) were randomly grouped (n=5/group) and supplemented orally with CM (50, 100 and 200 mg/kg/day) prior to the oral administration of KZ (200 mg/kg/day) for 14 days. On day 15, the rats were weighed and anesthetized. Blood samples were collected, and sera were extracted for biochemical analyses. Liver samples were excised, weighed and processed for oxidative stress markers and histology. KZ significantly ($p < 0.01$) decreased body weight and significantly ($p< 0.001$) decreased liver glutathione, superoxide dismutase, catalase, and glutathione peroxidase levels when compared to control. KZ significantly ($p < 0.01$) increased liver weight and significantly ($p < 0.001$) increased serum gamma-glutamyl transferase, alkaline phosphatase, amino transferases, lactate dehydrogenase, total bilirubin and liver malondialdehyde levels when compared to control. KZ caused hepatocyte necrosis. However, CM supplementation significantly restored body and liver weights at 50 mg/kg ($p < 0.05$), 100 mg/kg ($p < 0.01$) and 200 mg/kg ($p < 0.01$) when compared to KZ. CM supplementation restored serum biochemical and liver oxidative stress markers at 50 mg/kg ($p < 0.05$), 100 mg/kg ($p < 0.01$) and 200 mg/kg ($p < 0.001$) when compared to KZ. CM restored liver histology. CM was effective against KZ-induced hepatotoxicity in a dose-related fashion.

Keywords: curcumin, ketoconazole, liver, prevention, toxicity

1. Introduction

Hepatotoxicity is a serious adverse drug reaction that occurs as a result of liver damage caused by toxic chemical substances or drugs leading to liver dysfunction, after ruling out other potential causes (1). Antifungals are commonly associated with hepatotoxicity. The risk of antifungal drug induced hepatotoxicity is complex and can be influenced by pre-existing liver diseases as well as various factors such as patient demographics, drug-drug interactions, comorbidities, environmental and genetic factors and chemical properties of the drug (2). Hepatotoxicity from antifungal drugs typically manifest as increased serum aminotransferase levels, but the clinical significance of these changes is not always clear. The incidence of hepatotoxicity from antifungal drugs varies widely with higher rates seen in patients taking azole antifungal drugs (2).

Ketoconazole (KZ) is a broad-spectrum azole-based antifungal drug. It works by inhibiting the biosynthesis of ergosterol in fungal cell which increases the permeability of mycetes, ultimately causing death (3). It is commonly used to treat patients with systemic fungal infections. Recently, clinical data from China has linked KZ with severe cases of hepatotoxicity (3). Additionally, based on clinical data, the United States issued safety information urging caution when using KZ due to the risk of potentially fatal hepatotoxicity. In humans. It has been associated with hepatitis, elevated transaminases, and hepatic necrosis (4). Literatures have shown that it may cause acute and severe liver failure, especially in individuals with pre-existing liver diseases. In addition, preclinical experiments have shown that KZ can altered liver structure (5).

Natural products serve as a repository for the identification and discovery of chemicals that can be processed and used for the treatment of ailments. Most drugs used clinically were sourced from natural products or their derivatives (6). Curcumin (CM) is a natural bioactive polyphenolic compound extracted from the rhizome of *Curcuma longa* Linn. It is an orange-red powder that is tasteless, and insoluble in water which has received significant recognition as a dietary supplement with potential therapeutic activities on a wide range of ailments (7, 8). These potential therapeutic activities

*Correspondence: adikwuelias@gmail.com

include anticancer, anti-inflammatory, antiviral and antibacterial. Additionally, it has shown therapeutic potential in mental cognitive disorders, tumors, cerebrovascular and cardiovascular diseases (7). It has shown strong antioxidant activity through the scavenging of free radicals and the upregulation of antioxidant proteins. Its anti-inflammatory effect includes the suppression of proinflammatory cytokines (6,9). Aside from the aforementioned activities, it has shown promising liver protective activity in animal studies against ethanol and paracetamol-induce hepatotoxicity (10, 11). It also exhibited protective activity against liver oxidative stress and inflammation caused ochratoxin and carbon tetra chloride (9,12). In light of the aforementioned, this research novelty assessed the protective ability of CM against KZ-induced hepatotoxicity in Wistar rats.

2. Materials and Methods

2.1. Drugs, chemicals and animals

Thirty healthy adult Wistar rats of both sexes weighing 180-200g were randomly grouped into 6 groups of n=5 per group in metallic cages. The rats were purchased from the Animal handling units of the Faculty of Pharmacy, Madonna University, Nigeria. They were kept under standard temperature (25-30ºC) with 12-h light/dark cycle and had access to food and water freely. The rats were acclimated to laboratory conditions before the experiment began. The European Communities Council Directive of 24 November 1986 (86/609/EEC) for animal handling was followed (13). Ethical approval was obtained from the research ethics committee of the Department of Pharmacology/toxicology, Faculty of Pharmacy, Madonna University. Modified doses of KZ (200 mg/kg/day) (14) and CM (50-200 mg/kg/day) (15) were used. Piperine (20 mg/kg/day) was added to CM to increase bioavailability (15). KZ (2) and CM (15) were suspended in normal saline

2.2. Administration of drug and chemicals.

The rats were orally administered with the chemical agents daily for 14 days as follows; Groups I (Control) and II were administered with normal saline (0.2mL) and CM (200 mg/kg), respectively whereas group III was administered with KZ (200 mg/kg). Groups IV-VI were supplemented with CM; 50 mg/kg, CM; 100 mg/kg, and CM 200 mg/kg prior to the administration of KZ (200 mg/kg).

2.3. Animal sacrifice and sample collection

On day 15, the rats were euthanized with thiopental sodium (40 mg/kg). Blood samples were obtained from the heart in heparinized containers and evaluated for serum total bilirubin (TB), alanine amino transferase (ALT), lactate dehydrogenase (LDH), alkaline phosphatase (ALP), gamma-glutamyl transferase (GGT), conjugated bilirubin (CB) and alanine amino transferase (AST). Liver tissues were excised, rinsed in saline (0.9% NaCl) and preserved in neutral buffered formalin (10%) for histological analysis. Liver tissues were also processed to produce homogenates in ice-cold 20 mM tris hydroxymethyl aminomethane buffer. Thereafter, the homogenates were centrifuged (3000 X g for 30min at 4ºC), the supernatants obtained and evaluated for oxidative stress markers.

2.4. Evaluation of biochemical markers

Serum total bilirubin, AST, GGT, ALT, TB, ALP, LDH and CB were evaluated using an auto chemistry analyzer.

2.5. Assay of liver oxidative stress markers

Glutathione (GSH) and glutathione peroxidase (GPx) activities were analyzed as described by Rotruck *et al.,* 1973 (16) and Sedlak and Lindsay, 1968 (17), respectively. Superoxide dismutase (SOD) and catalase (CAT) activities were evaluated as documented by Sun and Zigman, 1978 (18) and Aebi, 1974 (19), respectively. Malondialdehyde (MDA) was analyzed as explained by Buege and Aust, 1978 (20).

2.6. Liver histology

Liver tissues were fixed for 24 h in neutral formalin (10%) solution and thereafter, dehydrated in ascending ethanol concentrations. The tissues were processed and fixed in paraffin wax. The processed tissues were sectioned (five-micrometer-thick), stained with hematoxylin and eosin and viewed with a Nikon Eclipse E200-LED microscope (Tokyo, Japan).

2.7. Data analysis

Results as mean ± standard error of mean (SEM) of five replicates. Two-way analysis of variance (ANOVA) and Duncan's Multiple Range Test were used for data analysis with the aid of Graph Pad Prism (Version 5.0, Graph Pad Software Inc., La Jolla, California, U.S.A.). *P* values < 0.05, < 0.01 and < 0.00 were used to express significance.

3. Results

3.1. Protective effect of curcumin on the body and liver weights of ketoconazole- administered rats

CUM (200 mg/kg) had no significant ($p>0.05$) effects on the body and liver weights of rats when compared to the control. However, KZ (200 mg/kg) decreased body weight and increased liver weight significantly ($p<0.01$) in rats when compared to the control (Table 1). Interestingly, body and liver weights were restored significantly by CM; 50 mg/kg ($p<0.05$), CM; 100 mg/kg ($p<0.01$) and CM; 200 mg/kg ($p<0.01$) supplementation when compared to KZ (Table 1).

3.2. Protective effect of curcumin on the serum liver biochemical markers of ketoconazole- administered rats

Serum ALT, LDH, ALP, GGT, CB, AST and TB were normal ($p>0.05$) in CM (200 mg/kg) administered rats when compared to the control. The aforementioned biochemical markers were significantly ($p<0.001$) elevated in KZ (200 mg/kg) administered rats when compared to the control (Table 2). But the aforementioned serum biochemical markers were significantly restored in a dose-related fashion in CM; 50 mg/kg ($p<0.05$), CM; 100 mg/kg ($p<0.01$), and CM; 200 mg/kg ($p<0.001$) supplemented rats when compared to KZ (Table 2).

Table 1. Effect of curcumin on body and liver weights of ketoconazole- administered rat

Groups	Dose (mg/kg)	FBW (g)	ALW(g)	RLW (%)
Group I	NS	261.6±20.1	6.31±0.22	2.41±0.08
Group II	CUM 200	255.0±18.7	6.30±0.17	2.47±0.01
Group III	KZ 200	140.7±10.2*	14.24±0.45*	10.12±0.08*
Group IV	CUM 50 + KZ 200	200.9±19.3 [a]	11.51±0.43 [a]	5.73±0.25 [a]
Group V	CUM 100 + KZ 200	249.3±20.2 [b]	7.42±0.24 [b]	2.98±0.43 [b]
Group VI	CUM 200 + KZ 200	255.8±19.7 [b]	6.24 ±0.36 [b]	2.44±0.21 [b]

Data as mean ± SEM, n = 5. NS: Normal saline, KZ: Ketoconazole, CUM: Curcumin, *p < 0.01 Significant difference when compared to control, a p < 0.05; b p < 0.01 Significant difference when compared to KZ (ANOVA).

Table 2. Effect of curcumin on serum biochemical markers of ketoconazole- administered rats

Groups	Dose (mg/kg)	AST (U/L)	ALT (U/L)	ALP (U/L)	TB (g/dL)	CB (g/dL)	LDH (U/L)	GGT (U/L)
Group I	NS	40.11±2.00	42.51±6.00	35.83±4.32	5.72±0.21	3.20=±0.21	22.82±2.00	0.31±0.05
Group II	CUM 200	38.80±3.42	40.42±4.21	34.04±2.11	5.43±0.17	3.11±0.33	22.47±3.21	0.29±0.09
Group III	KZ 600	130.01±17.1*	135.70±15.0*	99.23±9.01*	17.74±1.00*	14.63±1.51*	87.16±10.5*	1.01±0.01*
Group IV	CUM50+KZ 200	97.62±10.2[a]	99.21±10.1[a]	77.15±7.16[a]	12.90±0.92[a]	11.25±0.62[a]	60.65±5.22[a]	0.76±0.02[a]
Group V	CUM 100+KZ200	65.91±8.66[b]	69.45±5.22[b]	55.96±3.21[b]	7.99±0.41[b]	7.66±0.77[b]	35.14±3.00[b]	0.56±0.01[b]
Group VI	CUM 200+KZ200	42.13±6.68[c]	47.24±6.71[c]	36.54±4.55[c]	5.97±0.66[c]	3.57±0.431[c]	23.09±2.11[c]	0.35±0.07[c]

Data as mean ± SEM, n = 5. NS: Normal saline, KZ: Ketoconazole, CUM: Curcumin, AST: Aspartate aminotransferase, GGT: Gamma glutamyl transferse, ALT: Alanine aminotransferase, LDH: Lactate dehydrogenase, TB: Total bilirubin, ALP: Alkaline phosphatase. n=5, *p < 0.001 Significant difference when compared to control. a p < 0.05; b p < 0.01 and c p < 0.001 Significant difference when compared to KZ. (ANOVA).

3.3. Protective effect curcumin on liver oxidative stress markers of ketoconazole- administered rats

Liver SOD, GSH, MDA, CAT, and GPx levels did not change significantly (p>0.05) in CM (200 mg/kg) administered rats when compared to the control. KZ (200 mg/kg) significantly decreased liver SOD, GSH, CAT, and GPx and significantly elevated liver MDA levels at p<0.001 when compared to the control (Table 3). But liver SOD, GSH, MDA, CAT, and GPx levels were restored in a dose-related fashion by supplementation with CM; 50 mg/kg (p<0.05), CM;100 mg/kg (p<0.01), and CM; 200 mg/kg (p<0.001) when compared to KZ (Table 3).

3.4. Protective effect curcumin on the liver histology of ketoconazole- administered rats

The liver of rats in the control group (Fig.1.a) and CM (200 mg/kg/day) (Fig.1.b) administered group showed normal histology. In contrast, the liver of KZ (200 mg/kg) administered group showed severe hepatocytes necrosis and central vein congestion (Fig.1.c). Furthermore, the liver of rats in the group supplemented with CM; 50 mg/kg showed mild hepatocytes necrosis and inflammatory cells (Fig.1.d). The liver of rats in the group supplemented with CM; 100 mg/kg (Figure e), and CM; 200 mg/kg (Fig.1.f) showed congested central vein.

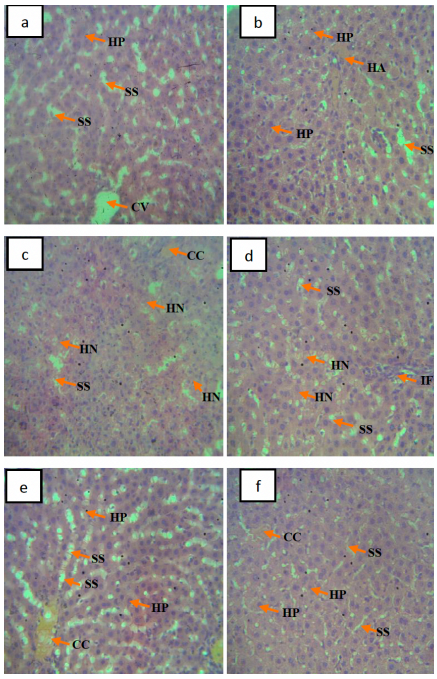

Fig.1. (a) and (b) are the liver of the control and CM (200 mg/kg) administered rats, respectively. (c) is the liver of KZ (200 mg/kg) administered rats. (d), (e) and (f) are the liver of CM; 50 mg/kg, 100 mg/kg, and 200 mg/kg supplemented rats, respectively. HP: Normal hepatocytes, CV: Normal central vein, HN: Hepatocytes necrosis, IF: Inflammatory cells, SS: Sinusoids, HA: Hepatic artery, CC: Congested central vein

Table 3. Effect of curcumin on liver oxidative stress markers of ketoconazole - administered rats

Group	Dose (mg/kg)	SOD (u/mg protein)	CAT (u/mg protein)	GSH (µg/mg protein)	GPX (u/mg protein)	MDA (nmol/mg protein)
Group I	NS (0.2mL)	43.20 ± 4.00	40.56± 4.01	23.62 ± 3.20	28.16 ± 3.00	0.13 ± 0.01
Group II	CUM 200	42.32 ± 3.45	40.72 ± 5.22	24.01 ± 3.35	29.19 ± 2.74	0.10 ± 0.05
Group III	KZ 200	17.65 ± 2.21*	18.32 ± 2.51*	7.73 ± 0.34*	9.69 ± 0.51*	0.78 ± 0.07*
Group IV	CUM 50+KZ 200	22.02 ± 2.00a	22.30 ± 2.37a	11.41 ± 1.62a	13.57 ± 1.22a	0.47 ± 0.01a
Group V	CUM 100+KZ 200	30.33 ± 3.71b	30.31 ± 3.56b	15.32 ± 1.44b	18.47 ± 2.31b	0.21 ± 0.06b
Group VI	CUM 200+KZ 200	39.21± 3.44c	38.55± 4.02c	21.22 ± 3.72c	27.23 ± 2.51c	0.15 ± 0.04c

Data mean ± SEM, n = 5. Normal saline, KZ: Ketoconazole, CUM: Curcumin, SOD: Superoxide dismutase, CAT: Catalase, GSH: Glutathione, MDA: Malondialdehyde, GPX: Glutathione peroxidase, * $p < 0.001$ Significant difference when compared to control. a $p < 0.05$; b $p < 0.01$; c $p < 0.001$ Significant difference when compared to KZ. (ANOVA).

4. Discussion

The essential role of the liver in drug biotransformation makes it a primary target for toxicity (21). Metabolites, or parent drugs, can elicit different types of biochemical changes such as oxidative stress and covalent binding leading to signal transduction, endoplasmic reticulum and, mitochondrial stress which can cause cell death. Alternatively, these stress responses may indicate the occurrence of idiosyncratic drug associated hepatotoxicity (22). Oral KZ use has been linked to both acute and chronic hepatotoxicity (23). Studies have shown that CM is a naturally occurring substance with numerous biologic activities of medicinal importance (24). This study aims to explore the protective benefit of CM against KZ-induced hepatotoxicity in Wistar rats.

Body and organ weights are important factors in assessing test article-associated toxicities. Studies haves shown that changes in body and organ weights may be as a result of treatment-related effects (25). In this study the rats administered with KZ showed a decrease in body and an increase in liver weights. This aligns with decrease in body and increase in liver weights in rats administered with KZ (200 mg/kg/day) for 15 days (26). The increase in liver weight may indicate changes such as hepatocellular hypertrophy possibly due to peroxisome proliferation or enzyme induction (25, 26). On the other hand, the decrease in body weight could be due to decrease in body mass. Interestingly, supplementation with CM was able to restore both liver and body weights.

In clinical practice, the traditionally used biochemical markers to detect liver injury measures alterations in serum liver function indexes, and changes in liver tissues. The evaluation of liver function depends on serum biomarkers like total bilirubin, ALT, GGT AST, LDH and ALP (27, 28). In this study, KZ administration impaired liver function significantly by elevating serum ALT, GGT AST, ALP, LDH, TB and CB levels. This observation correlates with the elevated serum biochemical markers reported in rats administered with KZ (200 mg/kg/day) for 15 days (26). The elevation in TB level shows incapacitated liver activity, increased AST and ALT activities reflect hepatocellular necrosis, and increased ALP levels reflect damage to biliary epithelial cells or canalicular membrane (29, 30). Elevated TB may be a consequence of excess synthesis, poor liver absorption, altered metabolism, conjugation errors, or biliary excretion errors (31). The damage to the liver hepatic cell membrane is evidenced by increased AST and ALT circulation (32). However, this study observed that CM supplementation normalized liver function marked by restored serum liver biochemical markers in a dose-related fashion.

Exposure of the liver to reactive oxygen species (ROS) during metabolic functions and xenobiotics biotransformation can disrupt the redox balance causing oxidative stress. This can impair liver function, and modulate proinflammatory pathways contributing to diseases. Oxidative stress has been associated with the pathogenesis and progression of acute and chronic liver diseases (33). The liver possesses robust antioxidant defense mechanisms such as SOD, GPx, CAT and GSH which help maintain ROS at physiological levels. CAT and SOD protect the liver from superoxide radicals and hydrogen peroxide (34), while GSH, acts as a detoxifying agent removing toxic compounds and heavy metals (34). However excessive liver ROS activity can overwhelm these antioxidants leading to oxidative stress (35). In the current study, KZ caused significant liver oxidative stress characterized by low antioxidant levels and elevated MDA level, a primary marker of lipid peroxidation. This supports previous findings of rats administered with KZ (14). Similar results were reported with KZ (200 mg/kg/day) administration for 15 days (26). Furthermore, CM supplementation inhibited KZ-induced liver oxidative stress by stabilizing antioxidant and MDA levels in a dose-dependent manner. The observation may be attributed to CM's ability to scavenge or inhibits ROS, thereby preventing KZ-induced liver oxidative stress.

In addition to the elevated serum biochemical markers, this study observed altered liver histology marked by hepatocyte necrosis in rats administered with KZ. This is consistent with

the findings reported by Hamza *et al.,* in rats given 100 mg/kg/day of KZ for 5 days (14). It also agrees with similar observations reported in rats administered with KZ (100 mg/kg/day) for 15 days (26). The altered liver histology observed in this study, might be due to the induction of oxidative stress via ROS production by KZ causing damage to liver biomolecules (14). Nonetheless, supplementation with various doses of CM restored liver histology.

The mechanism by which KZ causes hepatotoxicity is yet to be established, but might be idiosyncratic or immune mediated as speculated by some studies (36). Additionally, studies have suggested a disruption in liver homeostasis through the generation of ROS leading to liver oxidative stress (14). Excessive ROS activity within the liver can damage proteins, lipids, and DNA, resulting in structural and functional liver impairment and potentially progressing to various liver diseases (37). The evident protective activity of CM observed in this study may due to its antioxidant effect through free radical scavenging. CM has the ability to scavenge free radicals, such as reactive oxygen and nitrogen species (7,38,39) and can enhance the activities of GSH, CAT, and SOD in neutralizing free radicals. Furthermore, it can inhibit ROS-producing enzymes like xanthine oxidase/hydrogenase and cyclooxygenase/lipoxygenase. As a chain-breaking antioxidant, CM is a lipophilic compound that efficiently scavenges peroxyl radicals and can also inhibit inflammation through various mechanisms (39, 40).

CM supplementation restored biochemical and liver histological changes-induced by KZ in a dose-related fashion. CM may have clinical benefit in KZ associated hepatotoxicity.

Conflict of interest

Authors declare that there was no conflict of interest.

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None to declare.

Authors' contributions

Concept: E.A., N.O.E., B.S.D., Design: E.A., N.O.E., B.S.D., Data Collection or Processing: E.A., N.O.E., B.S.D., Analysis or Interpretation: E.A., N.O.E., B.S.D., Literature Search: E.A., N.O.E., Writing: E.A., N.O.E., B.S.D.

Ethical Statement

Ethical approval was obtained from the research ethics committee of the Department of Pharmacology/toxicology, Faculty of Pharmacy, Madonna University.

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