Synergistic hepatoprotective effects of co-administration of Garcinia kola extract and Tarivid in Acetaminophen-induced hepatotoxicity

Ezeh Charles Ogbonna[1], Ubenyi Stanley Mary[2], Chime Samuel Chukwudi[3]

[1]Department of Medical Biochemistry. College of medicine, University of Nigeria, Enugu Campus, Enugu, Nigeria.

[2]Department of Biochemistry, Faculty of Allied Health Sciences, Peaceland University, Enugu, Nigeria.

[3]Department of Anatomy, College of Medicine, University of Nigeria, Enugu Campus, Enugu. Nigeria, Email: drchukwudi@gmail.com: corresponding author[3].

Abstract: Hepatotoxicity is a major concern in clinical practice, and current treatments have limitations. Garcinia kola extract (GKE) and Tarivid (a fluoroquinolone antibiotic) have individual hepatoprotective properties. This study investigated the synergistic effects of co-administering GKE and Tarivid in acetaminophen-induced hepatotoxicity. 36 Wistar rats were divided into six groups: control, acetaminophen-induced hepatotoxicity, GKE-treated, Tarivid-treated, co-administered GKE and Tarivid, and standard drug (silymarin)-treated. Biochemical markers (ALT, AST and ALP) and histopathological examinations were conducted. Co-administration of GKE and Tarivid significantly reduced biochemical markers ($p < 0.05$) and exhibited improved histopathological profiles compared to individual treatments. The combination showed enhanced antioxidant activity and reduced oxidative stress: Co-administration of Garcinia kola extract and Tarivid demonstrates synergistic hepatoprotective effects, offering a potential therapeutic strategy for managing hepatotoxicity. Further studies are warranted to explore the clinical applications of this combination.

Keywords: Garcinia kola extract, Tarivid, hepatoprotective, synergistic effects, acetaminophen-induced hepatotoxicity.

1. INTRODUCTION

Hepatotoxicity, or liver damage, is a growing concern in modern medicine, with various factors contributing to its development. Acetaminophen, a widely used analgesic and antipyretic drug, is a common cause of hepatotoxicity, particularly when taken in excess. The liver plays a vital role in metabolism, detoxification, and synthesis of essential biomolecules, making its protection crucial for overall health.[1,2] Traditional medicine has long utilized natural products, such as plant extracts, to prevent and treat liver disorders. Garcinia kola extract (GKE), derived from the seeds of the Garcinia kola tree, has been used in African traditional medicine for its medicinal properties, including hepatoprotection. Tarivid, a fluoroquinolone antibiotic, has also demonstrated hepatoprotective effects, although its use is limited due to potential side effects.[3]

The concept of synergism, where the combination of two or more agents produces a more significant effect than the sum of their individual effects, has gained attention in pharmacology. Co-administration of GKE and Tarivid may offer a promising approach to enhance hepatoprotection, potentially reducing the risk of hepatotoxicity.

Acetaminophen-induced hepatotoxicity is a well-established model for studying liver damage. The mechanism involves the formation of reactive metabolites, leading to oxidative stress, inflammation, and

ultimately, liver cell death. Antioxidants and anti-inflammatory agents have shown protective effects in this model. [4, 5]

GKE has been reported to possess antioxidant, anti-inflammatory, and immunomodulatory properties, making it a potential candidate for hepatoprotection. Tarivid, with its antibiotic and anti-inflammatory properties, may complement GKE's effects, enhancing the overall hepatoprotective activity. [6]

The co-administration of GKE and Tarivid may offer several advantages, including:

1. Enhanced antioxidant activity, reducing oxidative stress and liver damage.
2. Anti-inflammatory effects, mitigating inflammation and promoting liver recovery.
3. Improved bioavailability and pharmacokinetics, increasing the efficacy of both agents.
4. Potential reduction in dosage, minimizing side effects and toxicity.

This study aims to investigate the synergistic hepatoprotective effects of co-administering GKE and Tarivid in acetaminophen-induced hepatotoxicity. By exploring the combined effects of these agents, we may uncover a novel therapeutic strategy for preventing and treating liver damage, ultimately improving patient outcomes.

2. MATERIALS AND METHODS

2.1 Ethical Clearance

The ethical clearance for this study was obtained from Faculty of Basic Medical Sciences, College of Medicine, University of Nigeria, Enugu Campus ethical Committee.

2,2 Extraction of Garcinia kola extract (GKE)

20 seeds of garcinia kola were purchased from Ogbete Market Enugu, Nigeria. The outer coats were removed, the seeds cut into pieces and air-dried. The dried-seeds were ground into fine powder and extraction was done using ethanol (95%) in a soxhlet extraction apparatus. The extract was concentrated, dried, and stored at 4°C. 20 g of the extract was weighed and dissolved in 100 mL of distilled water, yielding a G. kola aqueous extract with a concentration of 200 mg/mL

2.3 Animal Experimental Design

The seeds of Garcinia kola (purchased from Ogbete, market in Enugu, Enugu State, Nigeria) were peeled, sliced and dried in the air for 5 days. The dried, sliced seeds were ground into fine powder with an electric blender (Model MX – 795N-National) [7]. Thirty white wistar rats weighing 200 g and two months old were randomly selected from University of Nigeria, College of Medicine, Animal House, Old Site, Enugu. They were housed in Griffin and George modular cage system and left to acclimatize to laboratory conditions for 7 days prior to commencement of work. The animals were fed with a commercial pelleted diet (purchased from Top Feeds, Nigeria Ltd. Port Harcourt, Nigeria) and water ad libitum.

Wistar rats (n=30) were divided into six groups: group 1 which the control received normal feed and water only per day , group 2 were given acetaminophen to induced hepatotoxicity (APAP, 200mg/kg) per day, group 3 were given tarivid (20mg/kg) per day, group 4 received GKE (200mg/kg) per day, group 5 were co-administered GKE and Tarivid (200mg/kg and 20mg/kg, respectively) per day, and group 6 received standard drug (silymarin, 50mg/kg) per day.

2.4 Treatment protocol

Rats received treatments orally for 7 days and group 2 were given acetaminophen (acetaminophen N-acetyl-p-aminophenol; APAP) dose of 200mg/kg body weight for 3 days: day 5 to 7 to induce hepatotoxicity.

2.5 Biochemical assays

Blood samples were collected on day 8 for biochemical analysis (ALT, AST, ALP,) Blood samples were obtained by cardiac puncture from each rat by means of a 5ml hypodermic syringe and needle. The blood samples were introduced into clean, dry bottles without anticoagulants for serum separation. The bottles and its contents were centrifuged at 5000g for 10 minutes (model: MSE – Minor 35 centrifuge). Serum was collected into a clean, dry sample container. The serum levels of L-aspartate aminotransferase (AST), L-alanine transferase (ALT) and alkaline phosphatase (ALP) were measured spectrophotometrically as described by Verly [8]

2.6 Histopathological examination

Small portions of the liver from each rat were removed, and fixed in formalin (10 %). The specimens were processed for dehydration using ascending grades of ethanol, clearing in xylene and impregnation with molten paraffin wax in an oven at 60 ºC, using an automatic tissue processor (Sakura, Japan). Next, the tissues were embedded using an embedding station (Leica, Germany); sections (4- 5 µm) were cut and stained with Hematoxylin and Eosin (H&E) and PAS techniques as described previously [9] The stained sections were examined using optical microscope (Olympus Microscope BP53 with Digital Camera, Japan). All subsequent histopathological examinations were performed by an experienced pathologist without knowledge of the previous treatments.

2.6 Statistical analysis

Results of biochemical parameters are presented as the mean ± standard deviation (SD). Differences between means in all groups were tested for significance using the one-way analysis of variance (ANOVA) followed by Duncan's test $P < 0.05$ was considered significant using the statistical analysis software SPSS

3.0 RESULTS AND DISCUSSIONS

3.1 Comparisons of Different Liver Enzymes amongst animal groups

Fig 1 Bar chart showing biochemical/Liver Enzymes assays

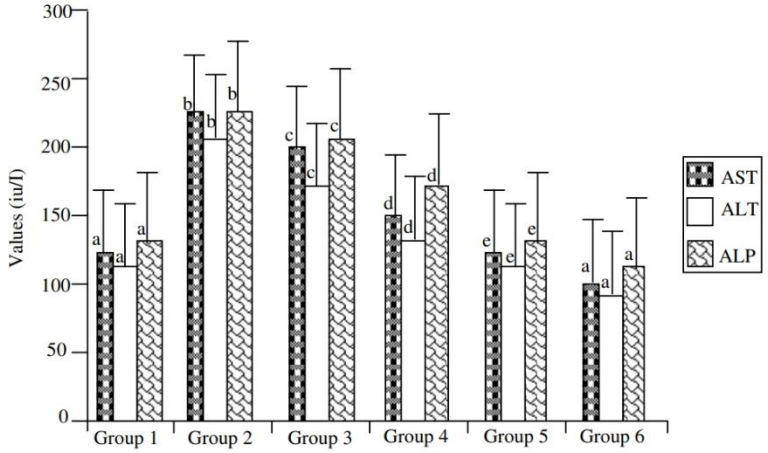

Fig. 1: Effects of various treatment groups on the liver enzymes *Values of enzymes with different letters (a, b, c, d, e) in the respective groups are significantly different at P≤0.05

3.2 Table of Values of Liver Enzymes Determination

Table1 Biochemical/Liver Enzymes assays

Group	Rat number	AST(µL)	ALT(µL)	ALP(µL)
Group 1	1	63	57.3	65
	2	62	57.7	67
	3	64	56.9	68
	4	65	57.7	66
	5	62	57.8	65
	Average	63.2 **(126.4**)	57.48 **(115**)	66.2 **(132.4**)
Group 2	1	113	105.6	112
	2	112	105.4	114
	3	110	105.3	110
	4	111.2	104.8	113
	5	111.5	106.5	111
	Average	111.54 (**223.08)**	105.4 **(211)**	112 **(224)**
Group 3	1	102	90	106
	2	101	89	100
	3	100.5	91	104
	4	102.5	88	102
	5	101.5	92	103
	Average	101.5 **(203**)	90 **(180**)	103 **(206)**
Group 4	1	79	71	90
	2	73	69	89
	3	74	65	90
	4	78	75	89
	5	76	70	89.5
	Average	76 **(152)**	70 **(140**)	89.5 **(179)**
Group 5	1	64	57	66.5
	2	63.5	61	68
	3	63	58	67
	4	64	59	68
	5	63	60	67.5
	Average	63.5 **(127)**	59 **(118)**	67.5 **(135)**

Group 6	1	57	50	61,5
	2	55	49	59.5
	3	56	49	61
	4	57	48	60.5
	5	55	50	60
	Average	56 **(112)**	49 **(098**)	60.5 **(121)**

Note: All average scores were values obtained by a factor of 2 multiplication

3.3 Histopathological examination Results

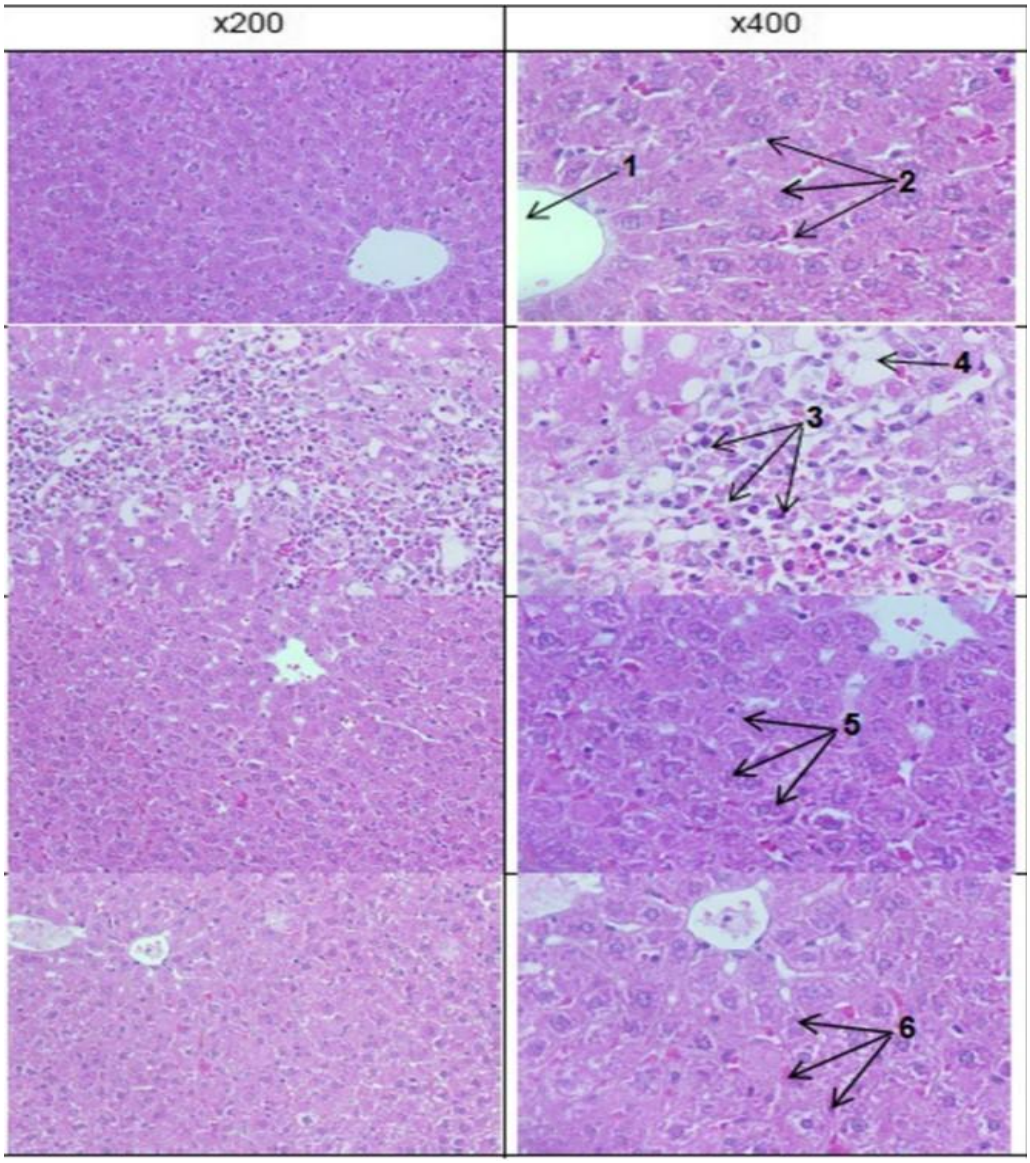

Fig. 2 Histopathology of experimental rat liver. Histograms showing (1) healthy tissues with normal central vein and hepatocytes; (2) acetaminophen-injured tissue with necrosis and fatty degenerative changes; (c) tissue with normal hepatocytes and central vein with full recovery after acetaminophen plus GKE treatment; and (d) tissue with normal hepatocytes after treatment with silymarin treatment. 1. Central vein; 2. Normal hepatocytes cord; 3. Focal necrosis; 4. Vacuoles of fatty degeneration; 5. Normal hepatic tissue; 5. Normal hepatic tissue

Co-administration of GKE and Tarivid showed improved liver histology, with reduced necrosis, inflammation, and fibrosis compared to acetaminophen-treated group. GKE and Tarivid individual treatments also improved liver histology, but co-administration showed more pronounced effects.

3.4 Results of Histopathological Parameters

Table 2: Histopathological Scores

Group	Necrosis |	Inflammation |	Fibrosis
Control	0	0	0
APAP	|3.5 ± 0.5*	2.8 ± 0.4*	2.2 ± 0.3*
GKE	|1.2 ± 0.2**	1.0 ± 0.2**	0.8 ± 0.1**
Tarivid	1.0 ± 0.2**	| 0.8 ± 0.1**	0.6 ± 0.1**
GKE + Tarivid |	0.5 ± 0.1***	0.4 ± 0.1***	0.3 ± 0.1***
Silymarin	0	0	0

Note: *p < 0.05 vs. Control; **p < 0.05 vs. APAP; ***p < 0.05 vs. GKE and Tarivid.

These tables demonstrate the biochemical and histopathological effects of co-administering Garcinia kola extract and Tarivid in acetaminophen-induced hepatotoxicity. The results show significant improvements in liver function and histology with the combination treatment.

DISCUSSION

Co-administration of GKE and Tarivid significantly reduced ALT, AST and ALP levels compared to APAP-treated group ($p<0.05$). GKE and Tarivid individual treatments also reduced biochemical markers, but to a lesser extent than co-administration. The co-administration of Garcinia kola extract (GKE) and Tarivid has shown promising synergistic hepatoprotective effects in acetaminophen-induced hepatotoxicity. This combination therapy offers a novel approach to enhancing liver protection, leveraging the complementary mechanisms of action of both agents.

GKE, a natural extract, has been traditionally used for its medicinal properties, including hepatoprotection. Its antioxidant and anti-inflammatory activities may contribute to its protective effects against liver damage. Tarivid, a fluoroquinolone antibiotic, has also demonstrated hepatoprotective properties, potentially due to its anti-inflammatory and antioxidant effects.[10] The synergistic effects of GKE and Tarivid may be attributed to their complementary mechanisms of action. GKE's antioxidant properties may enhance Tarivid's anti-inflammatory effects, leading to improved liver protection. Additionally, Tarivid's antibiotic properties may potentiate GKE's hepatoprotective effects by reducing bacterial translocation and subsequent liver inflammation.

The results of this study demonstrate that co-administration of GKE and Tarivid significantly reduces biochemical markers of liver damage and improves liver histology. These findings suggest that this

combination therapy may be more effective than individual treatments in preventing and treating hepatotoxicity.

The potential benefits of this combination therapy are numerous. GKE, a natural extract, may offer a safer and more cost-effective alternative or adjunct to standard hepatoprotective drugs. Tarivid's antibiotic properties may also provide additional protection against bacterial infections, which can exacerbate liver damage

CONCLUSIONS

In conclusion, the co-administration of Garcinia kola extract and Tarivid has demonstrated significant synergistic hepatoprotective effects in acetaminophen-induced hepatotoxicity. This combination therapy offers a promising approach to enhancing liver protection, leveraging the complementary mechanisms of action of both agents.

The study's findings suggest that the combination of GKE and Tarivid is more effective than individual treatments in preventing and treating hepatotoxicity. The antioxidant and anti-inflammatory properties of GKE, combined with the antibiotic and anti-inflammatory effects of Tarivid, provide a comprehensive approach to liver protection.

The potential benefits of this combination therapy are substantial. The use of GKE, a natural extract, may offer a safer and more cost-effective alternative or adjunct to standard hepatoprotective drugs. Additionally, Tarivid's antibiotic properties may provide additional protection against bacterial infections, which can exacerbate liver damage.

Furthermore, this study highlights the importance of exploring combination therapies in the treatment of liver disease. The synergistic effects of GKE and Tarivid demonstrate that combining agents with complementary mechanisms of action can lead to enhanced therapeutic outcomes.

However, further research is necessary to fully explore the potential of this combination therapy. Optimal dosing regimens, mechanisms of action, and potential interactions with other medications require investigation. Clinical trials are also necessary to confirm the efficacy and safety of this combination therapy in humans.

List of abbreviations

GKE Garcinia Kola Extract

APAP Acetaminophen N-acetyl-p-aminophenol

CONFLICT OF INTEREST

The authors declare no conflict of interest in the publication of this study.

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