Ameliorative Effects of Kolaviron, a Biflavonoid Fraction from *Garcinia Kola* Seed, on Hepato-renal Toxicity of Anti-tuberculosis Drugs in Wistar Rats

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Background: Tuberculosis (TB) is an infectious disease of international health priority. The combination of anti-TB drugs (4-Tabs)- isoniazid (INH), rifampicin (RIF), pyrazinamide (PZA) and ethambutol (ETB) are effective in the management of the disease, however, their toxic effect is a major concern.

Purpose: The study was designed to evaluate the toxicity of anti-TB drugs in male Wistar rats and possible ameliorative effects of kolaviron (KV), a biflavonoid from *Garcinia kola* seeds.

Methods: Twenty-eight rats were assigned into four groups; Group 1 (Control) received corn oil, Group 2 (4-Tabs) received therapeutic doses of INH (5 mg/kg), RIF (10 mg/kg), PZA (15 mg/kg) and ETB (15 mg/kg) in combination, Group 3 (4-Tabs + KV) received INH, RIF, PZA, ETB and KV (200 mg/kg) and Group 4 (KV) received KV (200 mg/kg) by oral gavage three times per week for 8 consecutive weeks.

Results: Administration of 4-Tabs caused oxidative stress resulting in significant (p = 0.031, 0.027) increase in malondialdehyde levels in the liver and kidney of rats by 101% and 34%, respectively. Also, 4-Tabs caused significant (p = 0.023-0.035) elevation of serum alanine and aspartate aminotransferases by 41% and 48%, creatinine by 252% and total bilirubin by 89%, respectively. In contrast, hepatic and renal antioxidant indices- reduced glutathione, glutathione peroxidase, glutathione-s-transferase and superoxide dismutase were significantly (p = 0.028-0.039) decreased in 4-Tabs-treated rats. Co-administration of KV with 4-Tabs significantly restored the antioxidant parameters and biochemical indices to near normal.

Conclusion: These findings suggest that anti-TB drugs elicit oxidative damage in liver and kidney of rats while KV protects against the adverse effects via antioxidative mechanism.

Key words: Oxidative stress, anti-Tuberculosis, kolaviron, antioxidant enzymes

INTRODUCTION

Tuberculosis (TB) remains a major public-health problem in many low-income and middle-income countries [1]. Nigeria had the fourth highest rate of TB burden in the world, with an incidence of 0.37-0.55 million in 2008 [1]. Nigeria also has the highest number of new TB cases in Africa, with about 300,000 cases recorded each year, resulting in approximately 30,000 deaths annually [2, 3]. Therefore, prompt anti-tuberculosis (anti-TB) treatment remains the most important and effective intervention for controlling spread, but adverse reactions from first-line anti-TB drugs are not uncommon. Rifampicin (RIF), isoniazid (INH), pyrazinamide (PZA) and ethambutol (ETB) are first-line drugs used for the treatment of TB. These drugs are not used solely but in combination purposely to avoid development of resistance or failure of treatment. Several adverse reactions of anti-TB drugs have been reported. The common toxic effects of these drugs are hepato- and nephrotoxicity [4, 5]. Recent studies indicate the existence of a strong correlation between hepatic injury and oxidative stress in experimen-
biflavonones are the most dominant in most *Garcinia* species [15]. Kolaviron (KV), the predominant constituent in *Garcinia kola* is a biflavonoid complex that has been reported to prevent hepatotoxicity mediated by CCl₄, dimethyl nitrosamine, 2-acetylaminofluorene, D-galactosamine and aflatoxin-B₁ [16–19]. Also, KV is known to elicit strong antioxidant activity in both *in vivo* and *in vitro* experimental models [20, 21]. In this study, we investigated the possible hepato- and nephro-protective effects of KV in rats treated with combination of anti-TB drugs.

**MATERIALS AND METHODS**

**Chemicals.** Glutathione, hydrogen peroxide, 5,5'-dithiobis-2-nitrobenzoic acid (DTNB) and epinephrine were purchased from Sigma Chemical Co., Saint Louis, MO USA. Trichloroacetic acid (TCA) and thiobarbituric acid (TBA) were purchased from British Drug House (BDH) Chemical Ltd., Poole, UK. Anti-TB drugs were purchased from a pharmaceutical store in Ibadan, Nigeria. Other chemicals were of analytical grade and purest quality available.

**Extraction of kolaviron (KV).** *Garcinia* kola seeds were obtained commercially in Ibadan, Nigeria and certified at the herbarium in the Department of Botany, University of Ibadan, Nigeria, where a voucher specimen already exists (UI-00138/01). Three kilograms of peeled seeds was sliced, pulverized with an electric blender and air-dried in the laboratory (25–28°C). Extraction of KV was achieved by the methods of Cotterhill et al. [14] and Iwu et al. [22]. Briefly, powdered seeds were extracted with light petroleum ether (bp 40–60°C) in a soxlet extractor. The defatted, dried marc was repacked and then extracted with methanol. The extract was concentrated and diluted to twice its volume with distilled water and extracted with ethyl acetate (6 x 250 ml). The concentrated ethyl acetate fraction was evaporated to dryness in rotary evaporator to completely remove the ethyl acetate. The residue gave a yellow solid known as kolaviron (KV) with a percentage yield of 5.6%.

**Animals.** Adult male Wistar rats, weighing between 160–170 g were purchased from the animal house of the Department of Veterinary Physiology, Biochemistry and Pharmacology, University of Ibadan, Nigeria. The animals were kept in well ventilated cages at room temperature (28–30°C) and under controlled light cycles (12-h light/12-h dark). They were maintained on normal laboratory chow (Ladokun Feeds, Ibadan, Nigeria) and water *ad libitum*. Rat handling and treatments confirm to the guidelines of the National Institute of Health (NIH publication 85–23, 1985) for laboratory animal care and use. The study was approved by the Animal Ethics Committee of the Faculty of Basic Medical Sciences, University of Ibadan.

**Study design.** Twenty-eight adult male rats (Wistar strain) were randomly divided into four groups of seven animals each. Animals were given a period of 2 weeks for acclimatization before the experiment. The first group served as the control and was given corn oil. The second group received INH, RIF, PZA and ETB in combination, the third group received KV and anti-TB drugs (INH, RIF, PZA and ETB) and the fourth group received KV alone. KV was dissolved in corn oil and administered at a dose of 200 mg/kg [23] while anti-TB drugs were dissolved in normal saline and therapeutic doses [INH (5 mg/kg), RIF (10 mg/kg), PZA (15 mg/kg) and ETB (15 mg/kg)] were given by oral gavage three times in a week for 8 consecutive weeks. At the end of treatment, rats were weighed and sacrificed under light ether anesthesia.

**Preparation of tissues.** Liver and kidney were quickly removed and washed in ice-cold 1.15% KCl solution to remove blood stains, dried and weighed. The tissues were homogenized separately in 4 volumes of 50 mM phosphate buffer, pH 7.4 and centrifuged at 10,000 g for 15 min to obtain post-mitochondrial fraction (PMF). Procedures were carried out at 4°C.

**Preparation of serum.** Blood was collected from the heart of the animals into plain centrifuge tubes and was allowed to stand for 1 h. Serum was prepared by centrifugation at 3,000 g for 15 min in a Beckman bench centrifuge. The clear supernatant was used for the estimation of serum enzymes and other biochemical indices.

**Biochemical assays**

**Protein determination.** Serum, kidney and liver protein levels were determined according to the method of Lowry et al. [24] using bovine serum albumin as standard.

**Total, conjugated and unconjugated bilirubin determination.** The bilirubin levels (total and direct) were estimated by the methods of Jaffe [28], and Reitman and Frankel [27].

**Creatinine and blood urea nitrogen determination.** Serum creatinine and blood urea nitrogen (BUN) levels were estimated by the methods of Jaffe [28], and Talke and Schubert [29], respectively.

**Assay of superoxide dismutase (SOD) activity.** Activity of SOD was determined according to the method described by McCord and Fridovich [30]. The method was based on the inhibition of autoxidation of epinephrine (pH 10.2) at 30°C. The assay mixture contained 20 mL of the sample, and 2.5 mL of 0.05 M carbonate buffer (pH 10.2). This was allowed to equilibrate in the spectrophotometer. Freshly prepared 0.3 mL of 0.3 mM adrenaline was added and mixed by inversion. The increase in absorbance at 480 nm was monitored spectrophotometrically at 30 seconds intervals for 150 seconds. The specific activity of SOD was expressed in Units/mg protein.

**Assay of catalase activity.** Catalase activity was determined by the method of Aebi [31]. The mixture of 2.4 mL of phosphate buffer (50 mM, pH 7.0), 10 mL of 19 mM of H₂O₂ and 50 mL of sample was allowed to run for 3 minutes at 30 seconds intervals. Next, the
reaction was terminated by the addition of 2 mL of dichromate/acetic acid solution, followed by heating for 10 minutes in a boiling water bath. The solution was cooled at room temperature and, decrease in absorbance was measured in a spectrophotometer at 570 nm. Catalase activity was expressed as Units/mg protein.

**Glutathione S-transferase (GST) activity.** GST activity was determined by the method of Habig et al. [32] using CDNB as a substrate. The reaction mixture contained 1.7 mL of 100 mmol/L of phosphate buffer (pH 6.5) and 0.1 mL of 30 mmol/L of CDNB. After pre-incubating the reaction mixture at 37°C for 5 minutes, the reaction was started by the addition of 20 mL of sample, and absorbance was followed for 5 minutes at 340 nm. Reaction mixture without the enzyme served as a blank. Specific activity of GST was expressed as micromoles of GSH/CDNB conjugate formed per min per mg protein using an extinction coefficient of 9.61/ mmol/cm.

**Assay of glutathione peroxidase (GPx) activity.** GPx activity was determined by the method of Rotruck et al. [33]. The reaction mixture contained 500 mL of sodium phosphate buffer, 100 mL of 10.0 mM of sodium azide, 200 mL of 4.0 mM of reduced glutathione (GSH), 100 mL of 2.5 mM of H₂O₂, and 50 mL of the sample. This was made up to 2.0 mL with distilled water and incubated for 3 minutes at 37°C. The reaction was terminated by the addition of 0.5 mL of 10% TCA and centrifuged. The supernatant obtained was used for the determination of residual GSH content by the addition of 4.0 mL of disodium hydrogen phosphate (0.3 M) solution, and 1 mL of 5', 5-dithio-bis-2-nitrobenzoic acid (DTNB) reagent. The absorbance was measured in a spectrophotometer at 412 nm and GPx activity was expressed as micromoles/mg protein.

**Reduced glutathione (GSH).** GSH was determined according to Moron et al. [34]. Briefly, aliquot of liver or kidney homogenate was deproteinized by the addition of an equal volume of 4% sulfosalicylic acid, and the resulting solution was centrifuged at 10000 g for 15 minutes at 4°C. Supernatant (50 mL) was then added to 4.5 mL of DTNB. GSH was proportional to absorbance at 412 nm. Values are expressed in micromoles/g tissue.

**Lipid peroxidation (LPO) assay.** LPO was determined based on malondialdehyde (MDA) produced after exposure to anti-TB drugs using the method of Buege and Aust [35]. In brief, 0.4 mL of the sample was mixed with 1.6 mL of Tris-KCl buffer containing 0.5 mL of 30% trichloroacetic acid (TCA). Subsequently, 0.5 mL of 0.75% thiorbarbituric acid (TBA) was added to each tube in a water bath (80°C) for 45 minutes, cooled in ice and centrifuged at 3000 g. The absorbance of the clear supernatant was read in a spectrophotometer against a reference blank at 532 nm. LPO was expressed in micromole MDA formed/mg protein using a molar extinction coefficient of 1.56 × 10⁵ m⁻¹ cm⁻¹.

**Statistical analysis.** The results were expressed as mean ± standard deviation of seven rats per group. Data were analysed using one-way ANOVA followed by the nonparametric Shirley-Williams post-hoc test. Values were considered statistically significant at p < 0.05.

### RESULTS

#### Effects of KV on body weight and biochemical parameters of rats treated with anti-TB drugs

In Table 1, rats treated with 4-Tabs and (4-Tabs + KV) had a significant decrease (p = 0.033, 0.041) in body weight-gain when compared to control. Furthermore, administration of 4-Tabs significantly (p = 0.028, 0.030) decreased the relative weight of liver and kidney while co-treatment with KV reduced the relative weight of the tissues. Also, treatment with 4-Tabs caused significant (p = 0.031–0.043) increase in the levels of serum creatinine, total, conjugated and unconjugated bilirubin when compared to controls (Table 2). Specifically, the levels of creatinine, total, conjugated and unconjugated bilirubin increased by 25%, 89%, 227% and 50%, respectively in 4-Tabs treated rats. In addition, serum alanine and aspartate aminotransferases activities increased by 41% and 48%, respectively relative to controls in rats treated with 4-Tabs (Fig. 1). On the other hand, co-treatment with KV significantly reduced the 4-Tabs-induced increase in these biochemical indices.

#### Effects of KV on lipid peroxidation (LPO) and antioxidant parameters of rats treated with anti-TB drugs

The liver and kidney LPO levels were significantly increased in 4-Tabs treated rats by 42% and 123%, re-

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**Table 1** Changes in body weight and relative weight of organs of male Wistar rats given anti-tuberculosis drugs for 8 consecutive weeks.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Body weight (g)</th>
<th>Weight gain (g)</th>
<th>Weight of organs (g)</th>
<th>Relative weight of organs</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Initial</td>
<td>Final</td>
<td></td>
<td>Liver</td>
</tr>
<tr>
<td>Control</td>
<td>168.0 ± 4.5</td>
<td>276.3 ± 4.8</td>
<td>108.3 ± 4.1</td>
<td>5.4 ± 0.4</td>
</tr>
<tr>
<td>4-Tabs</td>
<td>165.7 ± 5.3</td>
<td>222.0 ± 5.3</td>
<td>56.3 ± 2.3*</td>
<td>5.9 ± 0.2</td>
</tr>
<tr>
<td>4-Tabs + KV</td>
<td>161.4 ± 4.1</td>
<td>234.7 ± 4.0</td>
<td>73.3 ± 3.7*</td>
<td>5.2 ± 0.2</td>
</tr>
<tr>
<td>KV-only</td>
<td>164.1 ± 3.2</td>
<td>259.3 ± 5.3</td>
<td>95.2 ± 3.5</td>
<td>5.5 ± 0.4</td>
</tr>
</tbody>
</table>

Values are mean ± S.D. of seven rats per group.

*Significantly different from control (p < 0.05)

**Significantly different from 4-Tabs (p < 0.05)
spectively when compared with controls. The observed increase in LPO in these tissues were significantly (p = 0.022-0.047) reduced following co-treatment with KV (Fig. 2). The activities of kidney and liver GPx and levels of GSH were significantly decreased (p = 0.039-0.046) in 4-Tabs treated rats when compared to the control. Precisely, kidney GPx and GSH decreased by 53% and 46% while liver GPx and GSH decreased by 47% and 42%, respectively. Furthermore, co-treatment with KV significantly (p = 0.043-0.046) increased the activities of GPx and levels of GSH in the rats (Fig. 3 and 4). Furthermore, administration of 4-Tabs caused significant decrease (p = 0.036-0.041) in the activities of liver and kidney SOD and GST (Fig. 5, 6 and 7) while co-treatment with KV significantly (p = 0.039-0.047) attenuated the activities of GST and SOD in the

Table 2: Effects of KV on serum biochemical indices of male Wistar rats given anti tubercular drugs for 8 consecutive weeks.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Biochemical indices (µmol/L)</th>
<th>Creatinine</th>
<th>Urea</th>
<th>TB</th>
<th>CB</th>
<th>UB</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td></td>
<td>1.37 ± 0.3</td>
<td>26.25 ± 4.8</td>
<td>13.14 ± 2.0</td>
<td>2.92 ± 1.4</td>
<td>10.22 ± 3.4</td>
</tr>
<tr>
<td>4-Tabs</td>
<td></td>
<td>4.82 ± 0.7*</td>
<td>33.65 ± 3.5</td>
<td>24.86 ± 2.2*</td>
<td>9.54 ± 2.6*</td>
<td>15.32 ± 2.1*</td>
</tr>
<tr>
<td>4-Tabs+KV</td>
<td></td>
<td>2.15 ± 0.5**</td>
<td>28.05 ± 2.7</td>
<td>16.18 ± 3.4**</td>
<td>6.42 ± 1.2**</td>
<td>9.76 ± 1.6**</td>
</tr>
<tr>
<td>KV only</td>
<td></td>
<td>1.21 ± 0.2</td>
<td>20.84 ± 2.3</td>
<td>14.34 ± 2.9</td>
<td>2.59 ± 0.5</td>
<td>11.75 ± 3.1</td>
</tr>
</tbody>
</table>

Values are expressed as means ± S.D. (n = 7)

*Significantly different from control (p < 0.05)

**Significantly different from 4-Tabs (p < 0.05)

4-Tabs = INH, RIF, ETB and PZA

KV = Kolaviron, TB = Total bilirubin, CB = Conjugated bilirubin, UB = Unconjugated bilirubin

Fig. 2: Effects of kolaviron on kidney and liver lipid peroxidation (LPO) products in rats given anti-tuberculosis drugs for 8 weeks.

*Significantly different from control (p < 0.05)

**Significantly different from 4-Tabs (p < 0.05)

4-Tabs = isoniazid, rifampicin, pyrazinamide and ethambutol

KV = Kolaviron

Fig. 1: Effects of kolaviron on serum alanine and aspartate aminotransferases (ALT and AST) in rats treated with anti-tuberculosis drugs for 8 weeks.

*Significantly different from control (p < 0.05)

**Significantly different from 4-Tabs (p < 0.05)

4-Tabs = isoniazid, rifampicin, pyrazinamide and ethambutol

KV = Kolaviron
tissues of 4-Tabs treated rats. However, there were no significant (p = 0.058 to 0.063) differences in the activities of liver and kidney catalase of 4-Tabs treated rats relative to controls (Fig. 5 and 6).

**DISCUSSION**

The current findings have shown that kolaviron (KV), a biflavonoid from *Garcinia kola* seeds, reversed the anti-TB drugs-induced oxidative stress in the liver and kidney of rats. A combination of INH, RIF, PZA and ETB proved to be both hepato- and nephrotoxic to rats. The hepatotoxic action of anti-TB drugs, especially INH and RIF is well documented [36]. In this study, biochemical tests related to hepatocellular integrity confirmed that the administration of anti-TB drugs caused a significant elevation (approximately 2 fold increase) in the activities of ALT and AST. The hepatotoxicity of these enzymes showed that the integrity of hepatocytes have been compromised, resulting in the release of intracellular enzymes into the systemic circulation. Studies have shown that higher activities of ALT and AST could be seen in rats treated with INH and RIF [37], thus confirming that the hepatotoxicity observed in this study may be linked to the administration of INH and RIF. Furthermore, elevation of total, conjugated and unconjugated bilirubin in rats given anti-TB drugs supports membrane damage in the liver.

Acute kidney injury is a rare and severe complication that can interrupt treatment and cause permanent kidney damage during anti-TB therapy [38]. Although INH and ETB have been associated with kidney impairment [39], several studies have implicated RIF as most common anti-TB drug associated with kidney damage [40]. In the present study, anti-TB drugs caused significant elevation of serum creatinine (4-folds increase) and insignificant increase in urea of the rats. Both creatinine and urea are sensitive and reliable biochemical markers for evaluation of renal functions in animal models [41]. The increased serum creatinine observed in this study indicates impairment to the kidney function as seen in diseases such as acute glomerulonephritis, nephrosclerosis and even tubular necrosis [42]. This observation has also been reported by Chang *et al.* [5] in which patients on anti-TB therapy had significantly elevated serum creatinine and urea relative to controls.

The mechanism that was investigated in this study suggested an oxidative injury as revealed by an increased lipid peroxidation and depletion of cellular GSH in the liver and kidney of the animals given anti-TB drugs. This observation is in line with the study of Tasduq *et al.* [43] who observed significant
Fig. 5 Effects of kolaviron on the activities of kidney catalase and superoxide dismutase in rats treated with anti-tuberculosis drugs for 8 weeks.

*Significantly different from control (p < 0.05)
**Significantly different from 4-Tabs (p < 0.05)

4-Tabs = isoniazid, rifampicin, pyrazinamide and ethambutol
KV = Kolaviron

Fig. 6 Effects of kolaviron on the activities of liver catalase and superoxide dismutase in rats treated with anti-tuberculosis drugs for 8 weeks.

*Significantly different from control (p < 0.05)
**Significantly different from 4-Tabs (p < 0.05)

4-Tabs = isoniazid, rifampicin, pyrazinamide and ethambutol
KV = Kolaviron

Fig. 7 Effects of kolaviron on the activities of kidney and liver glutathione-s-transferase in rats treated with anti-tuberculosis drugs for 8 weeks.

*Significantly different from control (p < 0.05)
**Significantly different from 4-Tabs (p < 0.05)

4-Tabs = isoniazid, rifampicin, pyrazinamide and ethambutol
KV = Kolaviron
elevation of LPO and depletion of GSH in anti-TB drugs treated rats. The oxidative damage in these tissues might have resulted in the loss of structural integrity reflected by a marked increase in the leakage of hepatocellular enzymes. In addition, the enzymatic (superoxide dismutase, glutathione-s-transferase and glutathione peroxidase) defense mechanisms were also found to be compromised in the presence of anti-TB drugs. GSH, superoxide dismutase and glutathione peroxidase are known to protect the cells from the toxic effects of oxygen derived free radical and lipid peroxidation [44]. Although the liver is endowed with a unique capacity to regenerate thereby replenishing most of the protective systems, an overproduction of reactive species by anti-TB drugs may tilt the oxidant/antioxidant balance of the cells. Based on this in vivo study, it is suggested that the underlying mechanism for hepatatoxity and nephrotoxicity caused by these anti-TB drugs may be related to: 1) generation of free radicals and reactive metabolites, 2) imbalance in the oxidant/antioxidant defense, and 3) eventual peroxidation of membrane lipids that leads to the loss of tissue integrity. It is interesting to note that co-administration of KV with anti-TB drugs significantly attenuated the biochemical indices and oxidative stress markers in the rats. The protective role of KV in this study is consistent with previous studies on its anti-inflammatory, immuno-modulatory, free radical scavenging and hepatoprotective activities in vitro and in vivo [45, 46]. KV is known to suppress production of superoxide anion radical, exerts potent anti-inflammatory action that inhibits production of tumor necrosis factor alpha (TNF-a), and activation of NF-κB [19, 47]. These properties clearly explain the hepato- and nephroprotective activities of KV in the present study. Also, KV has anti-microbial activity in addition to its anti-inflammatory and antioxidant roles, making it a suitable candidate to alleviate the adverse effects of anti-TB drugs [48].

In conclusion, co-administration of KV with anti-TB drugs ameliorates lipid peroxidative damage, restores antioxidant status and normalizes markers of renal and hepatic injury in the rats. These findings show that KV may be a promising candidate for chemoprevention of anti-TB drugs-induced tissues dysfunction. However, further detailed studies are required to establish other mechanisms involved in the protection offers by this biflavonoid.

DECLARATION OF INTEREST

The authors have declared that there is no conflict of interest.

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