Protective Roles of Kolaviron Extract from *Garcinia kola* seeds against Isoniazid-Induced Kidney Damage in Wistar rats

Abstract

Background: The study investigated the protective effect of kolaviron extract obtained from the seed of *Garcinia kola* against isoniazid-induced kidney damage.
Methodology*:* Kolaviron was extracted from fresh seeds of *Garcinia kola* (2 kg) using soxhlet extractor and partitioned with chloroform. Nephrotoxicity was induced in wistar rats by oral administration of isoniazid (20 mg/kg bwt) while kolaviron was administered on wistar rats an hour before isoniazid administration and lasted for 30 days. Protective effect of kolaviron was measured in the plasma of wistar rats by estimating the levels of key metabolites used as kidney biomarkers which are total protein, creatinine, urea and uric acid concentration.
Results: The result showed a significant ($p < 0.05$) decrease in total protein concentration of the isoniazid-treated group (3.57 ± 0.12) while kolaviron-treated group showed a remarkable increase (6.15 ± 0.96), compared to the standard drug treated group (7.59 ± 0.54). Also, there was a significant ($p < 0.05$) increase in urea, uric acid and creatinine concentrations of the isoniazid-treated group only with values of 70.30 ± 4.77, 55.71 ± 11.15 and 18.04 ± 5.33 (mg/dl) respectively. However, treatment with kolaviron showed a protective effect of the plant extract by preventing toxic affront of isoniazid, thereby lowering the very high concentration of urea, uric acid and creatinine to a concentration of 45.25 ± 2.29, 35.60 ± 11.01 and 13.28 ± 4.41 (mg/dl) respectively.
Conclusion: The results showed that kolaviron extract obtained from *Garcinia kola* seeds exhibited a remarkable protective effect against kidney damage caused by isoniazid by regulating renal biomarkers and functions. Thus, it may be relatively safe when used therapeutically at this dose in the treatment and management of diseases associated with kidney damage.

Keywords

Kolaviron
Isoniazid
Kidney damage
Garcinia kola
Xenobiotics

1. Introduction

Isoniazid is a pro-drug used in the treatment of tuberculosis [1,2,3,4,,5]. It acts as a mild monoamine inhibitor by blocking the cytochrome p450 system, thereby releasing free radicals which is bactericidal to the microorganism [6]. Despite its exceptional anti-tuberculosis effects, simple-to-severe side effects have been reported with chronic injuries like peripheral neuropathy and liver failure [7,8,9,10,11,12,13]. This is associated with different metabolites released during isoniazid metabolism such as acetylhydrazine, hydrazine and acetylisoniazid which have been implicated in hepatic necrosis,

macrovesicular degeneration, steatosis [12,14,15] mitochondrial complex I and II inhibition and hepatocyte death [16]. In addition, the kidney which has been recognised as a probable site of extrarenal toxification of drugs [17] may likely be affected. In fact, Emeigh-Hart *et al*. [18] reported that some less toxic compounds can become toxic within the kidney through biotransformation resulting from the activities of xenobiotic metabolising enzymes [19]. Consequently, this leads to drug-induced kidney damage such as interstitial nephritis [20,21] or hepatorenal dysfuction [22]. However, most synthetic drugs available for the management of kidney damage show limited efficacy coupled with side effects leading to the interest in medicinal plants as possible alternative.

Numerous findings have revealed the protective effects of *Garcinia kola* seeds against carbon tetrachloride (CCL_4) and paracetamol-induced liver damage [23,24,25]. Likewise, the anti-diabetic, anti-lipidemic, anti-atherogenic properties of the seeds have been evaluated and found to have remarkable results [26]. Furthermore, Onasanwo *et al*. [27] also recommended its use as a potent anti-ulcer agent after using different ulcer models. Apparently, most beneficial properties of plants have been attributed to flavonoids and related phytoconstituents [28]. However, the effect of kolaviron on isoniazid-induced kidney damage has not been studied nor substantiated with experimental data. Hence, this study is aimed at evaluating the protective roles of kolaviron on key kidney parameters such as creatinine, urea, uric acid and total protein in kidney damage caused by isoniazid in wistar rats.

2. Materials

2.1. Chemicals

Methanol, n-Hexane, Chloroform, Normal saline and Tween-20 were obtained from Sigma Chemical Company (St Louis, MO, USA). Bovine serum albumin (BSA), Urea, Uric acid, and Creatinine (diagnostic kits) were obtained from Randox Laboratories Ltd, United Kingdom.

2.2.Plant collection and authentication

Fresh seeds of *Garcinia kola* were purchased from a local market in South-West Nigeria and was authenticated at IFE Herbarium, Department of Botany, Obafemi Awolowo University, Ile-Ife. Specimen identification number was also obtained (IFE-17733).

2.3.Experimental animals

Twenty five male wistar rats (150–250 g) were used in the study and were obtained from Faculty of Biological Sciences Animal Breeding House, University of Ibadan, Oyo state, Nigeria. The animals were maintained under standard laboratory condition (12-h light/dark cycle). They were fed with standard pellet diet and water ad libitum. The animals were acclimatized to laboratory condition for two weeks prior to experimentation. The principle of laboratory animal care (National Institute of Health Publication No. 85-23) guidelines and procedures were followed in the study (NIH publication revised, 1985).

3. Methods

3.1.Extract preparation

Kolaviron was isolated from *Garcinia kola* according to the method of Ademola *et al*. [29]. Five kilograms of peeled seeds of *Garcinia kola* were sliced and air-dried in the laboratory

for four weeks after which it was ground to coarse powder. Two kilograms of the powdered seeds were extracted with n-hexane in the Soxhlet extractor. The defatted, dried marc was repacked and then extracted with methanol. Thereafter, the extract was concentrated using a rotary evaporator and diluted to twice its volume with distilled water, followed by partitioning with chloroform. The concentrated chloroform fraction gave a brownish-yellow gel known as kolaviron.

3.2. Animal grouping and treatments

Twenty-five male wistar rats were divided into five (5) groups of five (5) animals each and were given orally the following treatment for thirty (30) days: Group 1 received 0.2% (v/v) tween 20 and served as the normal control; Group 2 received 100mg/kg b. wt Kolaviron only; Group 3 received 20 mg/kg b. wt Isoniazid only as the toxic dose for inducing nephrotoxicity; Group 4 received Kolaviron extract (100mg/kg b. wt) + Isoniazid (20 mg/kg b. wt); Group 5 received Vitamin C (100 mg/kg b. wt.) + Isoniazid (20 mg/kg b. wt). Vitamin C served as positive control. Pre-treatment with Kolaviron was done 1 hr before administering isoniazid for Groups 4 and 5 while tween-20 served as vehicle for administration. On the 30^{th} day, food and water were withdrawn from the animals for 24 hrs and decapitated.

3.3. Collection of blood samples and homogenates

Blood samples were collected into heparinized bottles and centrifuged at 4000 rpm for 10 min. Collection of Plasma was done using Pasteur pipette and was used for protein estimation. Likewise, kidney was removed and prepared by homogenizing the kidney 10% (w/v) separately in phosphate buffer solution (pH 7.4) using Potter–Elvejhem glass homogenizer. The homogenates were centrifuged at 4000 rpm for 15 min and the supernatant was collected as a source for the assessment of kidney function parameters.

3.4. Biochemical parameters

Total protein concentration was estimated according to the method of Lowry *et al*. [30] while creatinine, urea and uric acid concentrations were estimated using standard Randox diagnostic kits.

3.5. Statistical analysis

Data presented as mean ± SEM. Relationships between groups were carried out using one way analysis of variance (ANOVA) followed by Tukey–Kramer multiple comparisons test using Graphpad Prism. A probability level of less than 0.05 ($p < 0.05$) was accepted as statistically significant.

4. Results and discussion

4.1. Results

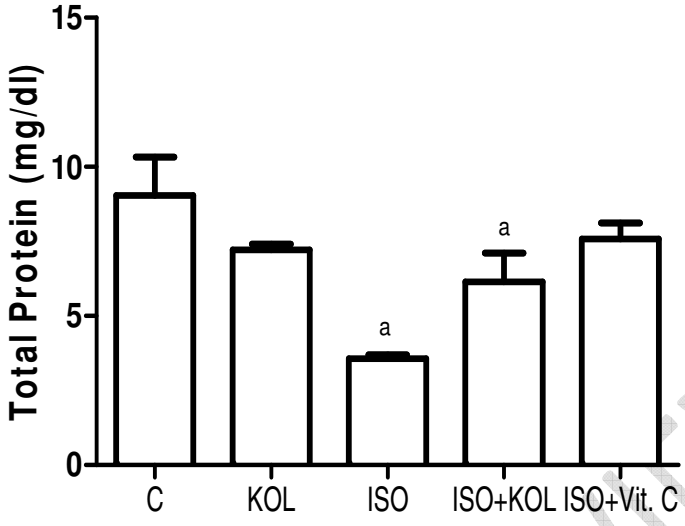
The percentage yield of kolaviron from 2k g of powdered *Garcinia kola* was 147.68 g representing 7.38 % of the starting material.

The effect of kolaviron extract from *Garcinia kola* seed on plasma total protein concentration is shown in Figure 1. Oral administration of 20 mg/kg body wt. of isoniazid caused decreased ($p < 0.05$) level of total protein compared to normal control and kolaviron-treated group. There was significant improvement in inhibition of nephrotoxicity as observed in the kolaviron + isoniazid group and vitamin C + isoniazid treated group when compared to toxin (isoniazid) treated group. This remarkable increase in the level of total protein in the kolaviron treated group indicated the protective effect of kolaviron.

Effect of isoniazid-induced toxicity and treatment with kolaviron extract on kidney uric acid concentration is shown in Figure 2. The group treated with isoniazid has relatively high level of uric acid when compared to the control and kolaviron-treated group. There was however significant ($p < 0.05$) decrease in the plasma level of uric acid in kolaviron + isoniazid group and Vitamin C + isoniazid treated group when compared to isoniazid only. Treatment with Kolaviron revealed more potent efficacy in the modulation of kidney function parameters.

Administration of isoniazid at 20 mg/kg body wt caused a significant ($p < 0.05$) increase in urea concentration as shown in figure 3. This however became lowered on administration of 100 mg/kg b.wt of kolaviron as compared with the group treated with vitamin C. A higher efficacy was observed in the kolaviron-treated group when compared with the group treated with standard drug vitamin c and the normal control.

Effect of isoniazid-induced toxicity and treatment with kolaviron extract on plasma level of creatinine is shown in Figure 4. The plasma creatinine in the isoniazid-treated group was higher than the control and the treated groups. However, there was significant ($p < 0.05$) decrease in creatinine concentration of the group treated with kolaviron + isoniazid and vitamin C + isoniazid when compared to the group treated with Isoniazid only. Commendably, both kolaviron at 100 mg/kg b.w. regimen resulted in significant protective effect against isoniazid-induced kidney damage and this observable effect compared well with vitamin C which was employed for the study.



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160 Figure 1. Effect of kolaviron extract from *Garcinia kola* seed on plasma total protein
161 concentration (n= 5, mean ± SEM). Superscript on each bar denotes significant difference (p
162 < 0.05) from Control group (C). C: Control (20% tween 20), KOL: kolaviron (100 mg/kg
163 b.w.), ISO: Isoniazid (20 mg/kg b.w.), ISO+KOL: Isoniazid (20 mg/kg b.w.) + Kolaviron
164 (100 mg/kg b.w.), ISO + Vit. C: Isoniazid (20 mg/kg b.w.) + Vitamin C (100 mg/kg b.w.).

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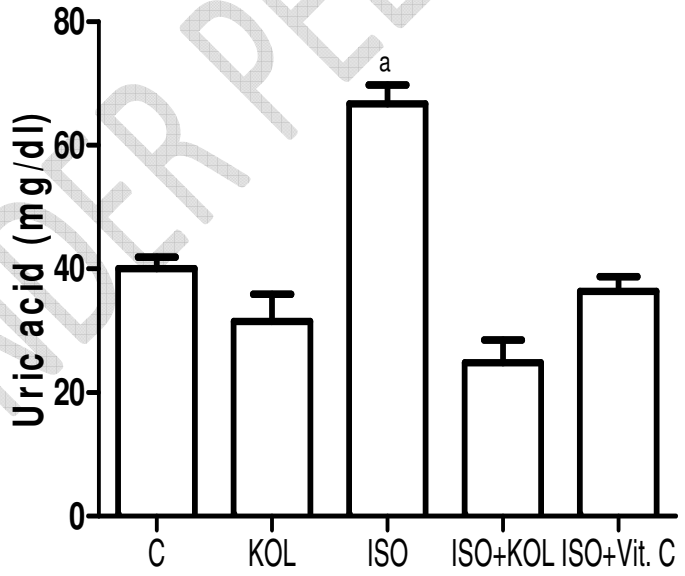

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167 Figure 2. Effect of kolaviron extract from *Garcinia kola* seed on uric acid concentration (n=
168 5, mean ± SEM). Superscript on each bar denotes significant difference (p < 0.05) from
169 Control group (C). C: Control (20% tween 20), KOL: kolaviron (100 mg/kg b.w.), ISO:
170 Isoniazid (20 mg/kg b.w.), ISO+KOL: Isoniazid (20 mg/kg b.w.) + Kolaviron (100 mg/kg
171 b.w.), ISO + Vit. C: Isoniazid (20 mg/kg b.w.) + Vitamin C (100 mg/kg b.w.).

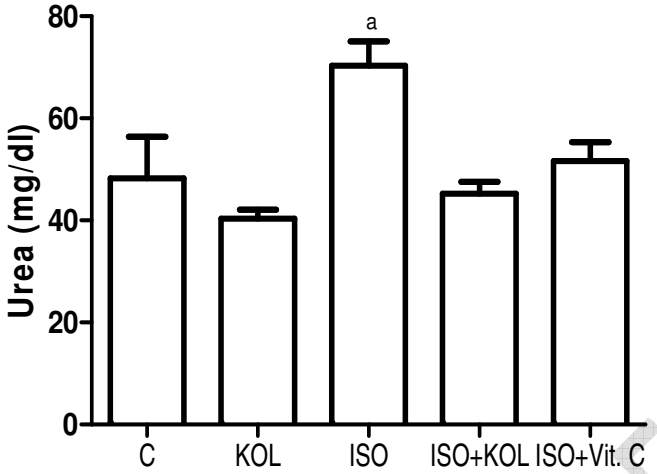

Figure 3. Effect of kolaviron extract from *Garcinia kola* seed on urea concentration (n= 5, mean ± SEM). Superscript on each bar denotes significant difference ($p < 0.05$) from Control group (C). C: Control (20% tween 20), KOL: kolaviron (100 mg/kg b.w.), ISO: Isoniazid (20 mg/kg b.w.), ISO+KOL: Isoniazid (20 mg/kg b.w.) + Kolaviron (100 mg/kg b.w.), ISO + Vit. C: Isoniazid (20 mg/kg b.w.) + Vitamin C (100 mg/kg b.w.).

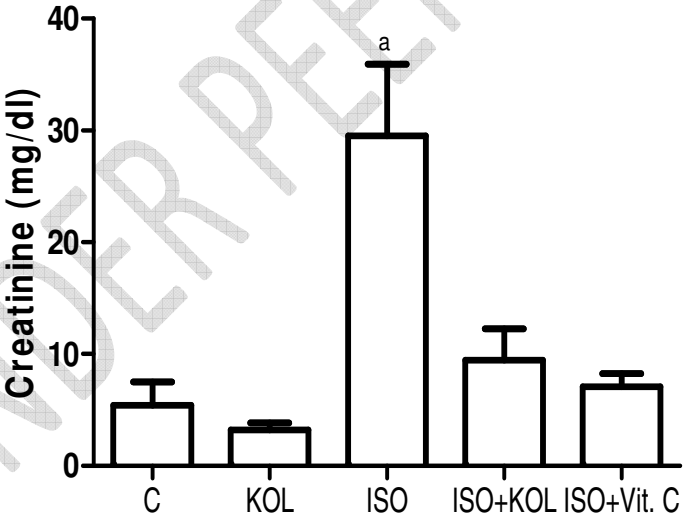

Figure 4. Effect of kolaviron extract from *Garcinia kola* seed on creatinine concentration (n= 5, mean ± SEM). Superscript on each bar denotes significant difference ($p < 0.05$) from Control group (C). C: Control (20% tween 20), KOL: kolaviron (100 mg/kg b.w.), ISO: Isoniazid (20 mg/kg b.w.), ISO+KOL: Isoniazid (20 mg/kg b.w.) + Kolaviron (100 mg/kg b.w.), ISO + Vit. C: Isoniazid (20 mg/kg b.w.) + Vitamin C (100 mg/kg b.w.).

4.2. Discussion

Biochemical processes involved in drug transformation and activation have been implicated in cellular damage leading to kidney dysfunction [31,32]. This is due to the substantial amount of blood supply, ensuring a high level of xenobiotic delivery over a period of time to the kidney and predisposes it to nephrotoxicity which therefore enhances its vulnerability to developing various forms of injury [33,34]. Several studies have implicated increased total protein excretion in renal diseases. Dietary protein can modulate renal function and thus, consumption of dietary protein in excess of recommended amounts promotes chronic renal disease through increased glomerular pressure and hyper-filtration [35]. When kidneys are not functioning properly, protein may escape from the blood into the urine. The high concentration level of total protein excreted is accompanied by simultaneous reduction in plasma total protein concentration [36]. The study revealed the protective effect of kolaviron in upregulating total protein concentration which has become impaired by administration of isoniazid.

Until recently, uric acid relevance in chronic kidney diseases (CKD) has been viewed with less interest. It has however revived as a contributory risk factor in the pathogenesis and progression of CKD. It has been reported that high level uric acid suggests CKD while lowering the uric acid level slows down the progression of chronic kidney diseases [37]. The study showed that isoniazid increased the uric acid concentration thereby indicating renal damage. Administration of kolaviron however lowered uric acid level, even more than the standard drug, vitamin C. Although, vitamin C also showed some protective effect, since it is an antioxidant, but kolaviron showed a more observable change indicating that it is more potent than the vitamin C.

Urea serves as nitrogen pool which prevents nitrogen in circulating proteins. The synthesis and release of nitrogen changes in response to the level of both dietary and endogenous proteins [38,39]. Hence, functional role of urea includes the metabolism of nitrogen-containing compounds by animals and it serves as the major nitrogen-containing substance in the urine. As a result of this, the body uses it in many processes, most notably for nitrogen excretion. However, elevated kidney urea concentration indicates a dysfunctional kidney [40]. From the study, isoniazid administration caused a significant increase ($p < 0.05$) in urea concentration. As previously suggested by Mitchell and Kline [41], the relationship between renal function and urea serum level is implicated in increased blood urea nitrogen-creatinine ratio in acute renal failure and pre renal condition. Several pathological conditions including kidney disease, blockage of the urinary tract (kidney stone), congestive heart failure, dehydration, fever, shock and bleeding in the digestive tract have been attributed to increased blood urea nitrogen [42]. Conversely, administration of kolaviron protected the kidney from affront caused by isoniazid, by bringing down the concentration of urea as a result of reabsorption of nitrogen in the blood.

Serum creatinine has been reported to be an important kidney function test used to monitor the progression of renal disease. As a by-product of muscle metabolism which is excreted unchanged through the kidney, whenever there is kidney damage, filtration fails and creatinine blood level rises [43,44]. From the study, isoniazid was observed to cause lethal kidney damage which resulted to a high level of creatinine. According to Edmund and David [45], renal failure is usually speculated when there is a higher level of creatinine than the

upper normal control limit. Pretreatment with kolaviron however attenuated the increase resulting in a drop in creatinine concentration. The clearance of creatinine as indicated in kolaviron-treated group showed the protective effect of kolaviron against isoniazid-induced kidney damage. Conversely, the effect of kolaviron itself on the kidney showed that this extract has no harmful effect on the kidney as the total protein, creatinine, urea and uric acid levels of the group treated with kolaviron only (KOL) compared reasonably well to that of the control group. In addition, the pretreatment of the animals with kolaviron before inducing kidney damage with isoniazid (ISO + KOL) showed the protective effect of the plant extract in preventing renal disease or damage.

5. Conclusion

Elevated kidney function biomarkers such as urea, uric acid and creatinine in addition to decreased total protein levels indicate kidney damage caused by isoniazid administration. Administration of kolaviron (100 mg/kg b.w) however revealed the efficacy of the plant in protecting the kidney against isoniazid-induced damage. However, further study is needed to unravel its mechanism of protection. The study therefore concluded that kolaviron extract obtained from *Garcinia kola* seeds exhibited a protective effect against isoniazid-induced kidney toxicity and it may be relatively safe when used therapeutically at this dose in the treatment and management of diseases associated with kidney damage.

Ethical Approval:

As per international standard or university standard written ethical approval has been collected and preserved by the authors.

Conflict of interest

Authors have declared that no competing interests exists.

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