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Comparative Effects of Vitamin E and Kolaviron (a biflavonoid from *Garcinia kola*) on Carbon Tetrachloride-Induced Renal Oxidative Damage in Mice

O.A. Adaramoye
Drug Metabolism and Toxicology Research Laboratories, Department of Biochemistry,
College of Medicine, University of Ibadan, Ibadan, Nigeria

Abstract: It became evident in this study that carbon tetrachloride (CCl_4), can induce renal oxidative damage. The hepatoprotective effects of vitamin E (Vit. E) and kolaviron (KV), a biflavonoid complex from the seeds of *Garcinia kola* are well documented. The present study was designed to investigate and compare the renal protective effects of Vit. E and KV in mice given CCl_4 (1.2 g kg^{-1}) intra-peritoneally thrice a week for two weeks. CCl_4 caused a marked increase in serum and renal lipid peroxidation (LPO) by 106 and 225%, respectively. Treatment with KV at 100 and 200 mg kg^{-1} and Vit. E at 100 mg kg^{-1} significantly ($p<0.05$) decreased the CCl_4-mediated increase in LPO. Furthermore, CCl_4-intoxication decreased the levels of renal reduced glutathione (GSH), superoxide dismutase (SOD) and catalase (CAT) by 44, 56 and 43%, respectively. Treatment with KV and Vit. E significantly ($p<0.05$) ameliorated the GSH and SOD levels. Specifically, KV at 100 and 200 mg kg^{-1} increased GSH by 32 and 27% and SOD levels by 50 and 53%, respectively. Likewise, treatment with Vit. E increased GSH and SOD levels by 31 and 53%, respectively. Effects on markers of renal functions showed that CCl_4-intoxication significantly ($p<0.05$) elevated serum urea and creatinine by 287 and 186%, respectively. While treatment with Vit. E decreased serum urea and creatinine by 60 and 55%, respectively, KV produced insignificant ($p>0.05$) effect on these parameters. This study found KV unable to protect against CCl_4-induced renal damage but confirmed the potency of Vit. E to enhance recovery from renal oxidative damage.

Key words: Carbon tetrachloride, *Garcinia kola*, kolaviron, oxidative stress, renal damage, vitamin E

INTRODUCTION

Drug exposure, ionizing radiations and environmental pro-oxidant pollutants are known to induce free radicals formation (Tirkey *et al*., 2005). Lipid peroxidation initiated by free radicals is deleterious for cell membranes and has been implicated in a number of pathologies. Carbon tetrachloride (CCl_4), an industrial solvent, is a well-established hepatotoxin (Tung *et al*., 2009). Studies demonstrated that liver is not the only target organ of CCl_4 since it causes free radical generation in other tissues such as the kidneys, lungs, testis and blood (Jayakumar *et al*., 2008). It has also been reported that exposure to CCl_4 induces acute and chronic renal injuries (Ogeturk *et al*., 2005). Case control studies increasingly establish that hydrocarbon solvents produce kidney diseases in humans (Brüning *et al*., 2003). Extensive evidence shows that as a result of the metabolic activation of CCl_4, trichloromethyl and chloro free radicals are formed which initiate lipid peroxidation (McCay *et al*., 1984).

The ability of vitamin E and antioxidants from plants to protect against CCl_4-induced liver injury supported the role of oxidative stress in this model (Adaramoye *et al*., 2008). Studies show that antioxidants such as *N*-acetylcysteine, betaine and hesperidin (a biflavonoid) protect against the CCl_4-induced increase in lipid peroxide levels and impairment to renal tissues (Tirkey *et al*., 2005). Furthermore, the hepatoprotective effect of vitamin E has been reported in experimental animal models intoxicated with halothane, diazinon, doxorubicin, isoniazid and rifampicin (Gokcimen *et al*., 2007; Beştaş *et al*., 2008). Likewise, vitamin E has been shown to reduce the injuries induced by ROS in a rat model of renal ischemia-reperfusion and also protects against renal damage in animals challenged with potassium dichromate (Arreola-Mendoza *et al*., 2006; Aktoz *et al*., 2007). However, the effect of vitamin E on CCl_4-induced renal damage remains unknown.

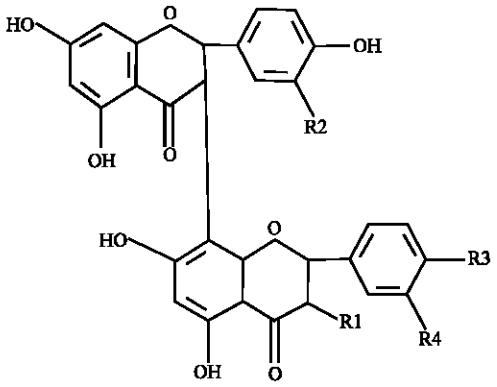
Garcinia kola seeds (Family: Guttiferae) are eaten in West and Central Africa and are known to contain high amount of biflavonoids. The seeds have been used for the treatment of catarrh, pains and liver disorders. Kolaviron (KV), a biflavonoid complex from the seed, contains *Garcinia biflavanone* (GB) 1, GB 2 and kolaflavanone in an approximate ratio of 2: 2: 1 (Cotterhill *et al*., 1978). KV has been reported to modulate the hepatotoxicity of several toxins (Adaramoye and Adeyemi, 2006a).

Likewise, Adaramoye *et al.* (2005a) and Adaramoye and Adeyemi (2006b) reported the anti-atherogenic effect of KV in hypercholesterolemic rats and its hypoglycemic effect in streptozotocin-diabetic rats, respectively. Since, the liver is the main target organ of CCl_4 metabolism and the kidney as its main site of accumulation (Shi *et al.*, 2006), the present study was designed to investigate and compare the effect of vitamin E and KV on CCl_4-induced renal oxidative damage in mice.

MATERIALS AND METHODS

Chemicals: Carbon tetrachloride, trichloroacetic acid and thiobarbituric acid were purchased from BDH Chemicals Limited, Poole, UK. Alpha-tocopherol acetate (Vitamin E), kits for creatinine and urea were procured from Sigma Chemical Company, Saint Louis, MO, USA. Other reagents were of analytical grade and the 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. Three kilogram 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 method of Iwu *et al.* (1990). Briefly, powdered seeds were extracted with petroleum ether (bp 40-60°C) in a soxhlet extractor. The defatted, dried marc was repacked and extracted with methanol. The extract was concentrated and diluted to twice its volume with distilled water and extracted with ethyl acetate. The concentrated ethyl acetate fraction gave a yellow solid known as kolaviron (KV) with a percentage yield of 6% (Fig. 1).



	R1	R2	R3	R4
GB1	OH	H	OH	H
GB2	OH	H	OH	OH
Kolaflavanone	OH	H	OMe	OH

Fig. 1: Structure of kolaviron

Study design: Twenty five male mice (TO strain) weighing between 30-35 g were randomly distributed into five groups of five animals each and, were given free access to feed and water for a period of two weeks for acclimatization before the commencement of the experiment. The study was conducted between October 2002 to March 2003 at the Department of Internal Medicine, UAE University Al Ain, UAE. The Animal Care Ethics Committee of Faculty of Medicine and Health Sciences, UAE University Al Ain, UAE approved the design of the experiment and the protocol conforms to the guidelines of the National Institute of Health (NIH publication 85-23, 1985). Group A received the drug vehicle (Corn oil), while groups B, C and D received 0.2 mL preparation of KV (100 mg kg^{-1} b.wt.), KV (200 mg kg^{-1} b.wt.) and α-tocopherol (Vitamin E) (100 mg kg^{-1} b.wt.), respectively for the first two weeks and were simultaneously given these drugs and CCl_4 for another two consecutive weeks. Furthermore, animals in group E were given CCl_4 alone for two consecutive weeks. KV and Vitamin E were administered orally five times in a week for the four weeks of the study, while CCl_4 was given intraperitoneally at a dose of 1.2 g kg^{-1} (Fumio *et al.*, 1989) thrice (consecutively) in a week for two weeks. Researchers, wore hand gloves and face masks to protect from accidental discharge of carbon tetrachloride during the experiment. Animals were fasted overnight and sacrificed by cervical dislocation 24 h after the last dose of the drugs.

Preparation of serum: Mice were sacrificed by cervical dislocation and dissected. Blood was collected from the heart into plain centrifuge tubes and then allowed to stand for 1 h. Serum was prepared by centrifugation at 3,000 g for 15 min in a bench centrifuge. The clear supernatant was used for analysis.

Preparation of kidney homogenate: Kidney samples were removed from the dissected animals and washed in ice-cold 1.15% KCl solution, dried and weighed. The kidney samples were homogenized in 4 volumes of 5 mM phosphate buffer, pH 7.4 and centrifuged at 10,000 g for 15 min to obtain a clear supernatant which was stored at -80°C until use.

Determination of protein and glutathione: Protein level was determined by the method of Lowry *et al.* (1951), briefly, the method involved the reduction of phospho-18 molybdic tungstic complex by phenolic groups present in amino acids to blue complex at alkaline pH. The absorbance of the complex was read at 720 nm. The reduced glutathione (GSH) level was assayed by measuring the rate of formation of chromphoric product in

a reaction between 5,5[1]-dinitrobis-2-nitrobenzoic acid (DTNB) and free sulphydryl groups (such as GSH) at 412 nm as described by Moron *et al.* (1979). To the homogenate, 10% trichloroacetic acid was added and centrifuged. 1.0 mL of the supernatant was treated with 0.5 mL Ellman's reagent (19.8 mg of 5,5[1]-dinitrobis-2-nitrobenzoic acid in 100 mL of 0.1% sodium nitrite) and 3.0 mL of 0.2 M phosphate buffer (pH 8.0). The absorbance of the colour formed was read at 412 nm.

Determination of Lipid Peroxidation (LPO): LPO level was assayed spectrophotometrically by the thiobarbituric acid reactive substances (TBARS) method, as described by Walls *et al.* (1976). The 1.0 mg mL^{-1} final concentration of sample was incubated for 6 hours at 37°C with or without 1 mM FeSO4; 1 mM ascorbate and 0.2 M H_2O_2 (final concentration). The 0.5 mL of 0.75% TBA in 0.1 M HCl was added to 0.5 mL of the incubation mixture already quenched with 0.5 mL of 10% TCA. The mixture was heated at 90-95°C for 25 min in a boiling water bath and then cooled. The mixture was then centrifuged at 3,000 rpm for 10 min and the absorbance of supernatant read at 532 nm.

Determination of creatinine: Serum creatinine was assayed using Sigma diagnostic kit. The kit employed the method of Jaffe (1886).

Determination of Blood Urea Nitrogen (BUN): The level of BUN was estimated with Sigma diagnostic kit. The kit employed the procedure of Talke and Schubert (1965) with slight modification.

Determination of superoxide dismutase and catalase activities: Superoxide dismutase activity (SOD) was measured by the nitro blue tetrazolium (NBT) reduction method of McCord and Fridovich (1969), briefly, 0.5 mL of tissue homogenate was mixed with ethanol and chloroform mixture and then centrifuged. To the supernatant, 0.025 M sodium pyrophosphate buffer (pH 8.3), phenazine methosulphate, nitroblue tetrazolium and NADH were added and incubated at 30°C for 90 sec. The reaction was stopped by the addition of glacial acetic acid and mixed with n-butanol. The intensity of the chromogen in the butanol was measured at 560 nm. Catalase (CAT) activity was assayed by measuring the rate of decomposition of hydrogen peroxide at 240 nm as described by Aebi (1974). The reaction mixture contained phosphate buffer (0.01 M, pH 7.0), tissue homogenate and 2 M H_2O_2. The reaction was stopped by the addition of dichromate-acetic acid reagents (5% potassium dichromate and glacial acetic acid were mixed in a ratio of 1:3).

Statistical analysis: All values were expressed as the Mean±S.D. of five animals per group. Data were analyzed using one-way ANOVA followed by the post-hoc Duncan multiple range test for analysis using spss (10.0) statistical software. Values were considered statistically significant at $p<0.05$.

RESULTS

Effect of vitamin E and kolaviron (KV) (a biflavonoid complex from *Garcinia kola* seeds) on the levels of serum protein, urea and creatinine in CCl_4-intoxicated mice is shown in Table 1. CCl_4-intoxication caused marked increases in serum urea and creatinine levels by 287 and 186%, respectively when compared to the control. Furthermore, there were no significant ($p>0.05$) differences in the levels of serum urea and creatinine of mice given KV + CCl_4 when compared to the CCl_4-intoxicated mice. However, pretreatment with vitamin E at 100 mg kg^{-1} significantly ($p<0.05$) ameliorated the CCl_4-induced increases in urea and creatinine levels of the mice. In addition, there were no significant ($p>0.05$) differences in the levels of serum protein of the test groups when compared to the control.

Figure 2 shows the effect of vitamin E and kolaviron on the levels of lipid peroxidation (LPO) in CCl_4-intoxicated mice. CCl_4-intoxication increased the serum and renal lipid peroxidation (LPO) levels as assessed by the formation of thiobarbituric acid reactive substances (TBARS). Precisely, serum and renal LPO were elevated by 106 and 225%, respectively when compared to the control (Fig. 2). However, pretreatment with kolaviron at 100 and 200 mg kg^{-1} and vitamin E at 100 mg kg^{-1} significantly ($p<0.05$) decreased the CCl_4-induced increase in serum LPO by 39, 44 and 50%, while renal LPO were also reduced by 43, 56 and 46%, respectively.

In Fig. 3, CCl_4-intoxication caused a marked decrease in the renal GSH level by 44%. Pretreatment with kolaviron at 100 and 200 mg kg^{-1} and vitamin E at 100 mg kg^{-1} meliorated the GSH levels by 41, 48 and 68%, respectively.

Table 1: Effect of vitamin E and kolaviron (a biflavonoid complex from *Garcinia kola* seeds) on the levels of serum protein, urea and creatinine of CCl_4-intoxicated mice

Treatments	Protein (mg mL^{-1})	Urea (mmol L^{-1})	Creatinine (mmol L^{-1})
Control	1.78±0.32	0.15±0.06	0.07±0.02
KV1 + CCl_4	1.63±0.51	0.55±0.20[a,b]	0.18±0.08[a,b]
KV2 +CCl_4	1.41±0.67	0.53±0.19[a,b]	0.19±0.06[a,b]
Vit. E + CCl_4	1.57±0.41	0.23±0.25[c]	0.09±0.01[c]
CCl_4	1.32±0.48	0.58±0.13[a]	0.20±0.09

Values are the Means±SD of five mice in each group. [a]Significantly different from the control ($p<0.05$), [b]No significant difference from CCl_4-group ($p>0.05$), [c]No significant difference from the control ($p>0.05$). KV1: Kolaviron administered at 100 mg kg^{-1} b.wt., KV2: Kolaviron administered at 200 mg kg^{-1} b.wt., Vit. E: Vitamin E, CCl_4: Carbon tetrachloride

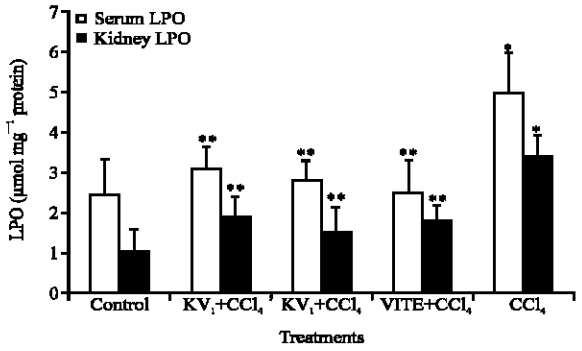


Fig. 2: Effect of vitamin E and kolaviron (a biflavonoid complex from *Garcinia kola* seeds) on the levels of serum and kidneys lipid peoxidation in CCl_4-intoxicated mice. *Significantly difference from other ($p<0.05$), **Significantly different from CCl_4 group ($p<0.05$). KV_1: Kolaviron at 100 mg kg^{-1} b.wt., KV_2: Kolaviron at 200 mg kg^{-1} b.wt., Vit. E: Vitamin E, CCl_4: Carbon tetrachloride, LPO: Lipid peroxidation

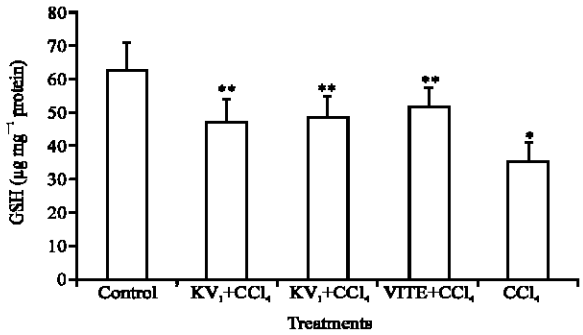


Fig. 3: Effect of vitamin E and kolaviron (a biflavonoid complex from *Garcinia kola* seeds) on kidney reduced glutathione levels in CCl_4-intoxicated mice. *Significantly difference from other ($p<0.05$), **Significantly different from CCl_4 group ($p<0.05$). KV_1: Kolaviron at 100 mg kg^{-1} b.wt., KV_2: Kolaviron at 200 mg kg^{-1} b.wt., Vit. E: Vitamin E, CCl_4: Carbon tetrachloride, GSH: Reduce glutathione

Also, CCl_4-intoxication decreased renal superoxide dismutase (SOD) and catalase (CAT) activities by 56 and 43%, respectively (Fig. 4). While, kolaviron at 100 and 200 mg kg^{-1} significantly ($p<0.05$) attenuated the CCl_4-induced decrease in SOD activities, the flavonoid produced no significant effect ($p>0.05$) on renal CAT activities. In contrast, pretreatment with vitamin E at 100 mg kg^{-1} significantly ($p<0.05$) elevated both renal SOD and CAT in the mice by 53 and 38%, respectively, relative to CCl_4-intoxicated group.

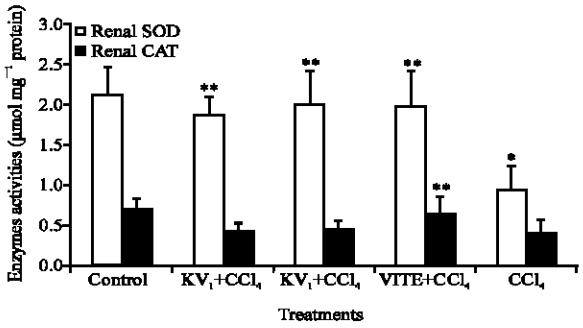


Fig. 4: Effect of vitamin E and kolaviron (a biflavonoid complex from *Garcinia kola* seeds) on renal SOD and CAT activities of CCl_4-intoxicated mice. *Significantly difference from other ($p<0.05$), **Significantly different from CCl_4 group ($p<0.05$). KV_1: Kolaviron at 100 mg kg^{-1} b.wt., KV_2: Kolaviron at 200 mg kg^{-1} b.wt., Vit. E: Vitamin E, CCl_4: Carbon tetrachloride, SOD: Superoxide dismutase, CAT: Catalase

DISCUSSION

The major finding of this study was that carbon tetrachloride (CCl_4) administered at a dose of 1.2 g kg^{-1} thrice in a week for two consecutive weeks caused severe renal oxidative stress in the mice. The study found that vitamin E exhibited better effectiveness than kolaviron in certain parameters, especially serum urea and creatinine and thus attenuated the adverse effect of CCl_4.

CCl_4 induces acute renal failure in experimental animals where glomerular haemodynamic and more specifically proximal tubules are affected (Tietz, 1976). The pathogenic mechanism also induces oxidative damage and so antioxidant intake could attenuate or prevent this toxicity. The involvement of free radical-mediated oxidative processes in the development of CCl_4 toxicity in liver and kidney of animals is well established (Shi *et al*., 2006). It is also known that the impairment of hepatic and renal antioxidant status is associated with a substantial cellular damage in these tissues following CCl_4 intoxication (Dwivedi *et al*., 2006).

Reduced glutathione (GSH) is a crucial determinant of tissue susceptibility to oxidative damage and the depletion of GSH content has been shown to be associated with an enhanced toxicity to chemicals including CCl_4, (Randle *et al*., 2008) and was confirmed in this study. Furthermore, the renal protection afforded by vitamin E, an inhibitor of lipid peroxidation indicates that both the sustained renal GSH level and the inhibition of lipid peroxidation are important factors involved in protecting against CCl_4-induced toxicity. The accumulation of lipid peroxidation products by CCl_4 in

renal tissues of the studied animals is in consonance with the findings of Soni *et al.* (2008) who reported increased levels of TBARS in rats after exposure to CCl_4 and the ability of cyanobacterial phycoerythrin to attenuate the elevation. The concomitant decrease in the GSH level (a non-enzymic antioxidant), SOD and CAT activities (enzymic antioxidants) and the accumulation of LPO products observed in this study implies an increase susceptibility of the tissue to radical species generated by CCl_4. These could have serious implication on renal functions of animals on short or long term treatment with the drug.

In the present study, it is note worthy that CCl_4-intoxication for two consecutive weeks caused significant elevation of serum urea and creatinine. This observation has also been reported in several animal models (Jaramillo-Juárez *et al.*, 2008). Serum creatinine and urea levels are sensitive and reliable biochemical indices for the evaluation of renal function in animal model (El Daly, 1996). The increased serum urea levels indicate impairment to the kidney function such as acute glomerulonephritis, nephrosclerosis and even tubular necrosis (Jaramillo-Juárez *et al.*, 2008). Although, KV at 100 and 200 mg kg^{-1} inhibited the lipid peroxidation process and enhanced the recovery of superoxide dismutase in renal tissues of CCl_4-intoxicated mice. The role of KV as an *in vivo* and *in vitro* anti-lipoperoxidative agent has been confirmed in several studies (Adaramoye *et al.*, 2005b). The inability of KV to attenuate serum urea and creatinine levels indicates a lack of protection against CCl_4-induced renal injury in this study. In contrast to the findings of Farombi *et al.* (2002) which reported that KV ameliorated potassium bromate ($KBrO_3$)-induced nephrotoxicity in the rats. The difference observed in the two studies may be two folds. First, $KBrO_3$ is known to accumulate and is metabolized mainly in the liver, unlike CCl_4 that can accumulate appreciably in the kidneys. Second, the active radical generated by $KBrO_3$ is less potent than trichloromethyl and chloro free radicals produced by CCl_4 during metabolism. Furthermore, vitamin E pretreated mice had significantly lower serum urea and creatinine levels when compared to the CCl_4-intoxicated mice. Vitamin E therefore acts as a better antioxidant, capable of scavenging free radicals derived from CCl_4 biotransformation in the renal tissue. The possession of a chromen moiety, a phenolic hydroxy group and a shorter side chain by vitamin E has been linked to its strong antioxidative activity (Terashima *et al.*, 2002).

In conclusion, this study demonstrates that CCl_4 induced marked oxidative stress in renal tissues of the mice and an elevation of serum urea and creatinine, which is amenable to attenuation by vitamin E and not kolaviron.

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