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The *Garcinia kola* biflavonoid kolaviron attenuates experimental hepatotoxicity induced by diclofenac

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ABSTRACT

This study sought to investigate the effects of kolaviron on diclofenac-induced hepatotoxicity in rats. Sixty male Wistar rats were divided into 6 groups of 10 rats each as follows: a control group that received oral propylene glycol and treatment groups that received diclofenac alone, diclofenac followed by Livolin Forte (a reference drug), or diclofenac followed by kolaviron at three different doses. At the end of the study period, five rats per group were sacrificed under ketamine hydrochloride anesthetic, 24 h after treatment, while the other 5 rats in the group were allowed to recover for 2 weeks before being sacrificed. Liver enzyme activities, total bilirubin levels, and the concentrations of several pro-inflammatory cytokines were determined using plasma samples, while liver tissue samples were used for antioxidant analysis and histopathological examination.

Compared with the control group, plasma liver enzyme activities, along with bilirubin levels, were higher in the groups that received diclofenac alone or diclofenac + the highest dose of kolaviron, respectively. These groups had higher plasma concentrations of pro-inflammatory cytokines than did the control group. However, the administration of Livolin Forte and kolaviron (at the lower doses) ameliorated diclofenac-induced hepatic injury by improving antioxidant status, preventing an increase in inflammatory mediators, decreasing malondialdehyde, and attenuating the adverse effect of diclofenac on hepatic tissues. In addition, there was a significant difference in the histological scores between the groups that received either diclofenac alone or diclofenac followed by the highest dose of kolaviron when compared with the other three groups (Livolin Forte or lower doses of kolaviron). In conclusion, kolaviron appears to be as effective as Livolin in attenuating DCLF-induced hepatotoxicity in rats. However, high doses of kolaviron seem to cause damage to the liver.

1. Introduction

Diclofenac is a member of the group of drugs known as non-steroidal anti-inflammatory drugs (NSAIDs). It is a phenylacetic acid derivative well-known for its analgesic and anti-inflammatory properties [1]. In addition, it has been reported to have anti-pyretic and anti-bacterial effects [2,3]. It is a selective COX-2 inhibitor that impairs the synthesis of prostaglandins from arachidonic acid [4].

Although the United States Food and Drug Administration (FDA) has approved the use of cyclooxygenase-2 inhibitors, some of these drugs have already been removed from the market owing to their adverse effects on liver function [5]. The hepatotoxicity of diclofenac has been attributed to its reactive metabolites [6]. The liver metabolizes diclofenac to 4-hydroxydiclofenac and other hydroxylated forms. These metabolites undergo glucoronidation and sulfation before they are ultimately excreted in the urine (65%) and bile (35%) [7,8].

Previous studies have established the following hepatotoxic effects of diclofenac: mitochondrial damage [9], generation of oxidative stress [10], and alteration of the integrity of covalent proteins by reactive metabolites [11]. Therefore, any potential ther-

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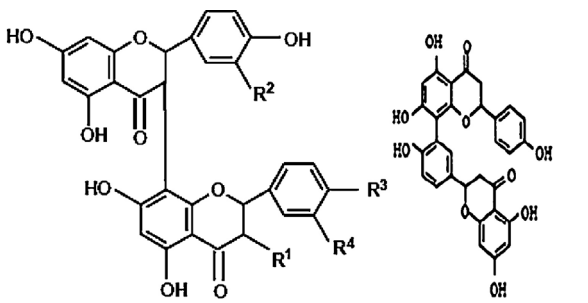

binaringenin

	R1	R2	R3	R4
GB1	OH	H	OH	H
GB2	OH	H	OH	OH
Kolaflavone	OH	H	OH	----
Kolaflavanone	OH	H	OCH_3	OH

Fig. 1. Chemical structures of the bioactive compounds present in kolaviron.

apeutic agent that would attenuate the cytotoxic effect of this drug has to reverse or arrest the progress of its adverse effects.

Kolaviron (Fig. 1) is a biflavonoid complex isolated from *Garcinia kola* seeds that has been noted for its therapeutic actions. This biflavonoid complex consists of a variety of compounds, which include: Garcinia biflavonoids 1 and 2 (GB-1, GB-2), kolaflavone, kolaflavanone, and binaringenin [12,13]. Kolaviron has also been reported to have anti-inflammatory [14], antioxidant [15], hypoglycemic, and antigenotoxic [16] actions. Also, its hepatoprotective effects with respect to toxins such as carbon tetrachloride have been reported [17]. However, the effects of kolaviron on diclofenac-induced hepatotoxicity remain to be elucidated. Therefore, this study was designed to investigate the protective effects of kolaviron on diclofenac-induced hepatotoxicity in rats. The effect of kolaviron was also compared with that of Livolin Forte, a hepatoprotective drug used in the treatment of liver disease.

2. Materials and methods

2.1. Drugs and chemicals

Diclofenac sodium (DCLF) was purchased from Wuhan Grand Pharmaceutical Company (Wuhan, China); Livolin Forte (LIV) (batch number 107050) was purchased from Mega Lifesciences (Pakenham, VIC, Australia); propylene glycol and ketamine hydrochloride were purchased from Biovision (Milpitas, CA, USA) and Rotexmedica (Trittau, Germany); and petroleum ether, acetone, and ethyl acetate were purchased from Sigma (St. Louis, MO, USA). Other reagents used were of analytical grade.

2.2. Extraction of kolaviron

Garcinia kola seeds were purchased from Oja Oba, in Ikere-Ekiti, Nigeria and certified by a taxonomist at the herbarium of the Department of Botany, Obafemi Awolowo University, with the voucher number IFE 17540. Kolaviron (KV) was isolated according to the method of Iwu et al. [19] and modified by that of Farombi et al. [18] (Fig. 2).

2.3. Stock solutions of livolin Forte® and kolaviron

Livolin Forte® (366 mg) was dissolved in 20 mL propylene glycol, after which 0.04 mL of the solution (equivalent to 0.78 mg LIV) was administered orally to rats weighing 150 g. This dosage is equivalent to 5.2 mg/kg, which is the therapeutic dose of the drug in humans [20]. The kolaviron extract's stock solution of 100 mg/kg was prepared by dissolving 1 g kolaviron in 20 mL propylene glycol. Consequently, every 100 g rat received 0.2 mL kolaviron to prevent the deleterious effects of extract overload. Accordingly, stock solutions of kolaviron (200 mg/kg and 400 mg/kg) were prepared by dissolving 2 g or 4 g of the extract, respectively, in 20 mL propylene glycol.

2.4. Animal care

Sixty (60) male Wistar rats (110–150 g) obtained from the Animal House of the College of Health Sciences, Obafemi Awolowo University, Ile-Ife were used for this study. The rats were housed in plastic cages for two weeks under normal laboratory conditions with a natural light/dark cycle before the commencement of the study, and they were allowed access to standard rodent pellet (Ace Feed PLC, Ibadan) and water *ad libitum*. All of the animals received humane care according to the criteria outlined in the Guide for the Care and Use of Laboratory Animals prepared by the National Academy of Science (NAS) [21] and approved by the Institutional Research Committee.

2.5. Experimental design

The rats were divided into 6 groups (n = 10 per group) as follows: Group 1 (the control group) received 2 mL/kg propylene glycol orally for 28 days; Group 2 (DCLF alone) received 10 mg/kg DCLF (via intramuscular route) for 7 consecutive days; and Group 3 were pre-treated with 10 mg/kg DCLF and then with 5.2 mg/kg LIV orally for 28 consecutive days. The rats in groups 4, 5, and 6 were pre-treated with 10 mg/kg DCLF and then with KV orally at 100, 200, or 400 mg/kg, respectively, for 28 consecutive days. At the end of the study period, five rats per group were sacrificed under ketamine hydrochloride anesthetic (10 mg/kg/body weight via intramuscular route) 24 h after treatment, while the remaining 5 rats in the group were allowed to recover for 2 weeks before sacrifice. Blood samples from all the rats were drawn via cardiac puncture and collected into separate heparinized tubes. The samples were then centrifuged at 4000 rpm for 15 min at −4 °C using a cold centrifuge (Centurium Scientific, Model 8881) to separate out the plasma. The plasma was then collected into separate plain tubes for the assessment of biochemical parameters and tumor necrosis factor-α (TNF-α) and interleukin-1β (IL-1β) concentrations with the aid of a DNM 9602 Microplate Reader (China) using an ELISA kit (Wkea Med Supplies Corp, Changchun, China).

2.6. Measurement of body and organ weight

The rats in all groups were weighed weekly using a Hanson digital weighing balance (Hanson, China) to assess any changes in body weight over time. However, the weights of the organs were determined using a Camry sensitive weighing balance (Camry, China).

2.7. Biochemical analysis

2.7.1. Liver marker enzymes and bilirubin analyses

Plasma aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), and total bilirubin (TB)

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