ABSTRACT

Garcinia kola, a medicinal herb, commonly used in folk medicine to treat many diseases including hepatitis, jaundice, ulcer, stomach ache, high fever etc. The aim of the present study is to evaluate the hepatoprotective activity of kolaviron (Garcinia kola extract) against DMH (1,2-dimethylhydrazine) experimentally induced liver injury. The respective doses of kolaviron 100mg/kg and DMH 30 mg/kg were administered accordingly; Male albino wistar rats were randomly divided into four groups. Group 1 served as control, received 1mM EDTA-saline injection subcutaneous (s.c) once a week for 4 weeks. Group 2 rats served as kolaviron (KV) control received 100 mg/kg bodyweight of kolaviron per oral (p.o.) every day. Group 3 served as carcinogen control, received 30 mg/kg bodyweight of DMH subcutaneous injection once a week for 4 weeks to induce liver injury. Group 4 rats received DMH injection and kolaviron 100 mg/kg bodyweight. The hepatotoxicity assays were measured by monitoring the levels of alanine aminotransferase (ALT), aspartate transaminase (AST), alkaline phosphatase (ALP). The livers were collected and subjected to biochemical and antioxidant analysis. Garcinia kola extract (kolaviron) possessed strong antioxidant activity in vivo. The results of DMH-induced livertoxicity experiment showed significant decrease in the levels and activities of GSH, SOD, GST, GPxand CAT. On the other hand the activities of ALT, AST and ALP were increased in DMH alone treated rats (p < 0.05). The results observed after administration of 100mg/kg kolaviron elevated the levels and activities of GSH, SOD, GST, GPx and CAT (p < 0.05). Also the elevated activities of ALT, AST and ALP in DMH treated rats alone were also decreased significantly (p < 0.05) due to the co-treatment of kolaviron. These results seem to support the traditional use of Garcinia kola in pathologies involving hepatotoxicity, and the possible mechanism of this activity may be due to strong free radical scavenging and antioxidant activities of kolaviron.

Key words: Garcinia kola, medicinal herb.

INTRODUCTION

Liver is the largest organ in the human body and is a key organ of metabolism (Starr and Hand, 2002). Despite its considerable regenerative capacity, continuous and various exposures to xenobiotics, environmental pollutants, and chemotherapeutic agents could suppress and possibly overcome the natural protective mechanisms of the liver, leading to liver malfunction and later if it is not treated properly leads to injury. Despite the remarkable progresses in conventional medical therapies in the last 20 years, drugs available for the treatment of liver diseases were often limited in efficacy and could have triggered various unwanted side effects when compared to other medical therapies for liver diseases which were often difficult to handle (Arhoghro et al 2009). Despite tremendous advances in modern medicine, hepatic disease remains a worldwide health problem; thus the search for new medicines is still ongoing. Numerous formulations of medicinal plants are used to treat liver disorders in traditional medicine. Many of
these treatments act as radical scavengers, whereas others are enzyme inhibitors or mitogens (Fadhel and Amran, 2002; He et al., 2002).

Garcinia kola seeds widely cultivated in West and Central Africa has been reported as a remedy for many ailments. Garcinia kola belongs to the family of plants called Guttiferae and the genus Garcinia. Kolaviron (KV) is an extract from the seeds of Garcinia kola, containing a complex mixture of biflavonoids and polyphenols namely Garciniabiflavonoid 1 (GB1), Garcinia biflavonoid 2 (GB2) and Kolaflavanone (Iwu, 1985). Many studies have confirmed the antioxidative, anti-lipid peroxidation, chemoprevention in colon carcinogenesis and anti-inflammatory effects of kolaviron in chemically-induced toxicity, animal models of diseases and in cell culture (Abarikwu et al., 2012, Adedara et al., 2013, Farombi et al., 2013, Eboh et al., 2015).

Research and epidemiological studies show that hepatoprotective effects have been associated with plant extracts that are rich in phenolic compounds (De et al., 1996). However, to the best of our knowledge, the hepatoprotective effects of Garcinia kola, against DMH-induced liver injury in rats has not been demonstrated. The present study focused on valuating the potential hepatoprotective effects of methanolic extracts from Garcinia kola on DMH-induced liver injury in rats.

**MATERIALS AND METHODS**

**Chemicals**

5,5'-Dithio-bis (2-nitrobenzoic acid) (DTNB), 1-chloro 2,4-dinitrobenzene (CDNB), reduced glutathione (GSH), 1,2-Dimethylhydrazine dihydrochloride was purchased from Sigma Chemical Company, St. Louis, MO, USA. All other chemicals used were of analytical grade.

**Extraction of kolaviron**

Garcinia kola seeds were purchased from a local market in Yenagoa, Bayelsa State, Nigeria. Peeled seeds were sliced and air dried powdered seeds were extracted with n-hexane in a soxhlet for 24 h. The defatted dried marc was repacked and extracted with methanol. The extract was concentrated with chloroform. The concentrated chloroform yielded kolaviron as a golden yellow solid (Iwu et al., 1990).

**Animals and experimental design**

3-week-old male albino Wistar rats weighing 100–140 g (n = 5 per group) were obtained and maintained at the Pharmacology Animal House, Niger Delta University, Wilberforce Island, Bayelsa State. Rats were housed in aluminium cages under the standard environmental conditions of a 12 h light/dark cycle, water and standard rodent diet was made available ad libitum. These animals were maintained under specific pathogen-free conditions. The total experimental period was 8 weeks, and the detailed experimental protocol is as follows:

**Treatment schedule**

Group 1: Control animals received 1mM EDTA-saline (pH 7.0 adjusted with 1mM NaOH) injection (s.c) once a week for 4 weeks.

Group 2: kolaviron (KV) control animals received 100 mg/kg b.w of kolaviron dissolved in water intubation for 8 weeks.

Group 4: 1, 2 Dimethyl hydrazine dihydrochloride (DMH) carcinogen group received 30 mg/kg b.w DMH injection (subcutaneous in the right thigh) dissolved in 1mM EDTA-saline (pH 7.0 adjusted with 1mM NaOH) once a week for 4 weeks.

Group 5: DMH + kolaviron animals received 30 mg/kg b.w DMH injection (s.c) dissolved in 1mM EDTA-saline once a week for 4 weeks plus 100 mg/kg b.w of kolaviron dissolved in water intubation for 8 weeks (Kanwar et al., 2008).

Animals were sacrificed 24 h after the last treatment. Liver was quickly removed, and homogenized in 9 volumes of ice NaCl (0.9%). The homogenates were centrifuged at 8000rpm for 10 min to yield a clear supernatant fraction that was used for all the biochemical and antioxidant analysis.

**Biochemical determinations**
Biochemical parameters were assayed according to standard methods. Activity of the following liver enzymes was measured: alanine transaminase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP) using the method of King (1965). Assay kits were obtained from the Institute of Biological Engineering of Nanjing Jianchen (Nanjing, China).

**Determination of Protein**

The protein concentration in all samples was determined by the method of Lowry et al., 1951 using BSA as standard.

**Determination of antioxidant enzymes**

**Determination of Reduced Glutathione (GSH)**

The GSH content in liver was determined by the method of Jollow et al., 1974 in which 1.0 ml of liver post mitochondrial fraction was mixed with 1.0 ml of sulphosalicylic acid (4%). The samples were incubated at 4ºC for at least 1 h and then subjected to centrifugation at 1200 x g for 15 min at 4ºC. The assay mixture contained 0.4 ml filtered aliquot, 2.2 ml phosphate buffer (0.1 M, pH 7.4) and 0.4 ml DTNB (10 mM) in a total volume of 3.0 ml. The yellow color developed was read immediately at 412 nm on spectrophotometer. The GSH content was calculated as nmol of DTNB conjugate formed/mg protein using molar extinction coefficient of 13.6 x 10^3 M^−1 cm^−1.

**Determination of Glutathione Peroxidase (GPx)**

The GPx activity was measured spectrophotometrically according to the method described by Leopold and Wolfgang, 1984. The reaction mixture consisted of 50 mM potassium-phosphate buffer (pH 7.0) containing 1 mM EDTA, 1.125 M NaN3, 0.2 mM NADPH, 0.3 mM GSH, 12 m Mcumene hydroperoxide and an appropriate amount of the liver cytosol sample in a total volume of 1.0 ml. The reaction was started by adding NADPH. The change in absorbance of system at 340 nm was monitored. One unit of enzyme activity is expressed as nmoles NADPH consumed/min/mg protein related to an extinction coefficient of 6.22 mM^−1 cm^−1.

**Determination of Malondialdehyde (MDA)**

The assay for membrane lipid peroxidation was done by the method of Wright et al. 1981 with some modifications. The reaction mixture in a total volume of 3.0 ml contained 1.0 ml liver homogenate, 1.0 ml of TCA (10%), and 1.0 ml TBA (0.67%). All the test tubes were placed in a boiling water bath for a period of 45 min. The tubes were shifted to ice bath and then centrifuged at 2500 x g for 10 min. The amount of malondialdehyde (MDA) formed in each of the samples was assessed by measuring the optical density of the supernatant at 532 nm. The results were expressed as the nmol MDA formed/mg protein by using a molar extinction coefficient of 1.56 x10^5 M^−1 cm^−1.

**Determination of Glutathione-S-transferase (GST) Activity**

The GST activity was measured by the method of Habig et al. 1974. The reaction mixture consisted of 2.4 ml phosphate buffer (0.1 M, pH 6.5), 0.2 ml reduced glutathione (1.0 mM), 0.2 ml CDNB (1.0 mM) and 0.2 ml of liver cytosolic fraction in a total volume of 3.0 ml. The changes in absorbance were recorded at 340 nm and the enzyme activity was calculated as nmol CDNB conjugate formed/min/mg protein using a molar extinction coefficient of 9.6 x10^3 M^−1 cm^−1.

**Determination of Catalase Activity**

The activity of catalase was assayed by the method described by Sinha 1972. The reaction was started by the addition of 0.4 mL of H₂O₂ to the reaction mixture containing 1 mL of phosphate buffer and 0.1 mL of enzyme solution. The reaction was stopped at 30 s by the addition of 2 mL dichromate acetic acid reagent. The tubes were kept in a boiling water bath for 10 min and cooled. The utilization of H₂O₂ by the enzyme was read at 620 nm. Values are
expressed in micromoles of H₂O₂ utilized per minute per milligram protein.

**Determination of Superoxide Dismutase (SOD) Activity**

The SOD activity was measured by the method of Marklund and Marklund 1974. The reaction mixture consisted of 2.875 ml Tris–HCl buffer (50 mM, pH 8.5), pyrogallol (24 mM in 10 mM HCl) and 100 µL liver mitochondrial fraction in a total volume of 3 ml. The enzyme activity was measured at 420 nm and was expressed as units/mg protein. One unit of enzyme is defined as the enzyme activity that inhibits auto-oxidation of pyrogallol by 50%.

**RESULTS**

**Effect of kolaviron on biochemical parameters of DMH induced liver injury in control and experimental rats**

<table>
<thead>
<tr>
<th>Treatments</th>
<th>AST (U/L)</th>
<th>ALT (U/L)</th>
<th>ALP (U/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>55.78 ± 8.28*</td>
<td>59.44 ± 6.88*</td>
<td>94.00 ± 7.60*</td>
</tr>
<tr>
<td>KV control</td>
<td>58.72 ± 7.45*</td>
<td>68.41 ± 12.95*</td>
<td>101.86 ± 9.03*</td>
</tr>
<tr>
<td>DMH control</td>
<td>108.40 ± 13.63*</td>
<td>114.7 ± 7.99*</td>
<td>208.83 ± 13.89*</td>
</tr>
<tr>
<td>DMH + KV control</td>
<td>60.69 ± 5.74*</td>
<td>68.67 ± 6.24</td>
<td>106.66 ± 8.99</td>
</tr>
</tbody>
</table>

Values are mean ± SD for groups of five rats each. Values are statistically significant at P < 0.05. Values in a column not sharing a common superscript letter (a–c) differ significantly.

**Effect of kolaviron on antioxidant parameters of DMH induced liver injury in control and experimental rats**

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control</th>
<th>KV control</th>
<th>DMH control</th>
<th>DMH + KV</th>
</tr>
</thead>
<tbody>
<tr>
<td>SOD (U/mg protein)</td>
<td>6.81 ± 0.49*</td>
<td>6.74 ± 0.28*</td>
<td>2.09 ± 0.26*</td>
<td>5.04 ± 1.12*</td>
</tr>
<tr>
<td>CAT (molecules H₂O₂ utilized/mg protein)</td>
<td>128.30±3.76*</td>
<td>127.62±5.55*</td>
<td>80.81±4.96*</td>
<td>108.30±1.27*</td>
</tr>
<tr>
<td>GST nmol CDNB conjugate/mg protein</td>
<td>62.17±4.71*</td>
<td>65.92±4.6*</td>
<td>59.91±3.88*</td>
<td>59.44±7.10*</td>
</tr>
<tr>
<td>GPnmol NADPH consumed/mm/mg protein</td>
<td>26.97±3.68*</td>
<td>29.01±4.37*</td>
<td>17.45±3.77*</td>
<td>25.40±6.11*</td>
</tr>
<tr>
<td>GSH nmol DTNB conjugate/mg protein</td>
<td>21.92±3.12*</td>
<td>24.62±2.88*</td>
<td>10.39±2.98*</td>
<td>22.30±1.16*</td>
</tr>
<tr>
<td>MDA nmol MDA formed/mg protein</td>
<td>7.72±0.39*</td>
<td>7.17±0.63*</td>
<td>14.36±0.73*</td>
<td>8.38±0.47*</td>
</tr>
</tbody>
</table>

Values are mean ± SD for groups of five rats each. Values are statistically significant at P < 0.05. Values in a column not sharing a common superscript letter (a–c) differ significantly.

**DISCUSSION**

DMH can lead to hepatic damage that histologically resembles carbon tetrachloride damage because both proceed through free radical mechanisms. Loss of metabolic enzymes that are located in intracellular structures results from changes in the endoplasmic reticulum (Tanaka et al., 1997). Therefore, hepatoprotective activity was evaluated by measuring activity of the enzymes AST, ALT, and ALP. In our studies, DMH-treated rats showed a significant increase in liver AST, ALT and ALP, which could reflect a pathological damage in liver function. Kolaviron treatment reduced the levels of AST, ALT, and ALP suggesting that it was able to stabilize DMH-induced dysfunction in the liver. To a large extent, kolaviron administration reversed the hepatocellular damage caused by DMH toxicity these results are in line with the recent findings of Bo et al., 2010 who also reported that the extract of Haleniaelliptica protected against carbon tetrachloride induce liver damage in wistar rats.

GSH is a major non-protein thiol, which plays a significant role in protecting cells against cytotoxic and chemicals by scavenging reactive oxygen species. GSH and its oxidized counterpart GSSG represent a major redox buffer system of the cell ((Khalid et al., 2011). The GSH levels in rats treated with DMH in liver showed significant decrease in our study and previous studies (Khan and Sultana, 2011 and Odayet al., 2012), and the co-treatment with Kolaviron restored the normal levels of GSH significantly indicating the hepatoprotective efficacy of KV. The decrease in the levels of GSH in liver may be due to the insult of DMH which increases and multiply free radicals there by decreasing or over stressing the cells of GSH stores to combat free radicals generated by DMH insult in Liver.

Superoxide dismutase (SOD) and catalase (CAT) forms the backbone of the
primary cellular defense that involved in the inactivation of environmental carcinogen and direct elimination of toxic free radicals and electrophiles which may result in oxidative injury. CAT is a heme protein in the peroxisomes that catalyses the direct degradation of H₂O₂ into H₂O and O₂ also helps to discard H₂O₂ accelerated by the action of oxidases in these organelles. GPx catalyses the reduction of peroxides (H₂O₂ and lipid peroxides) to non-toxic products and scavenges the highly reactive lipid peroxides in the aqueous phase of cell membranes using reduced glutathione as a cofactor (Eboh, 2014). GSTs are an important phase II enzyme that catalyzes the reaction between GSH and the hydrophobic or electrophilic compound (diazonium ion). (i) Reducing the toxicity and biological activities of carcinogens and (ii) facilitating their removal as water-soluble compounds. (Oday et al., 2012, Ragunath et al., 2013 and Oday et al., 2014). The present study shows a decrease in the activities of SOD, CAT, GPx, and GST in DMH treated rats alone, this decrease could be due to the fact that DMH produces free radicals that overwhelm the antioxidant activities of the liver enzymes. In same vein the DMH and kolaviron co-treatment increases the activities of these enzymes this could be due to the fact that kolaviron acts as a free radical scavenger and modulator of antioxidant enzymes. These results are in accordance with the research reports of Eboh et al 2015 and Oday et al 2014.

Lipid peroxidation involves the formation and propagation of lipid radicals, the uptake of oxygen, a rearrangement of the double bonds in unsaturated lipids and the eventual destruction of membrane lipids, with the production of a variety of breakdown products, including alcohols, ketones, alkanes, aldehydes and ethers (Dianzani & Barrera, 2008). In the present study, the levels of malondialdehyde protein liver are in accordance with the recent reports of Venkatachalam et al (2010) who also reported an increase in the levels of MDA in the DMH treated groups. These levels were decreased significantly in the kolaviron supplemented group.

Based on these results, it can be concluded that them ethanolic extracts of Garcinia kola seems to have hepatoprotective effects in rats. These results support the traditional use of this plantin hepatotoxic disorders, hepatitis, jaundice, ulcer, stomach ache, high fever etc.

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