An investigation of the hepatoprotective potential of *Garcinia kola* seed extract in an anti-tubercular treatment model

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Hepatoprotective effect of *Garcinia kola* seed extract on oxidative stress and hepatotoxicity biomarkers of rats exposed to antitubercular drugs was investigated to evaluate its potential to ameliorate drug-induced hepatotoxicity. Six groups of five animals each were used. Combination of Isoniazid (7.5 mg/kg bodyweight) and Rifampicin (10 mg/kg bodyweight) was administered intraperitoneally to a group. Drug combination was co-administered with *G. kola* seed extracts (40, 60 and 80 mg/kg bodyweight) to three other groups for 35 days. Control group received saline and the last group received *G. kola* alone (60 mg/kg bodyweight). Plasma oxidative stress biomarkers (superoxide dismutase, glutathione, glutathione peroxidase, catalase and malondialdehyde) and hepatotoxicity biomarkers (alanine aminotransferase, aspartate aminotransferase, aspartate aminotransferase and alkaline phosphatase) were assayed after treatment. The antitubercular drugs significantly increased levels of hepatotoxicity biomarkers. The increase was mitigated by treatment with *G. kola*, with reduction in levels of the hepatotoxicity biomarkers upon *G. kola* co-administration. Similarly, the antitubercular drugs reduced the activities of oxidative stress biomarkers. Histomorphological analysis of the liver showed that the *G. kola* extract administered at 60 mg/kg offered significant protection from exposure to the hepatotoxic drugs. The study concluded that *G. kola* extract at 60 mg/kg significantly protected the animals from the damages occasioned by exposure to hepatotoxic antitubercular drugs.

Key words: Isoniazid, rifampicin, tuberculosis, hepatotoxicity, hepatoprotection, histomorphology.

INTRODUCTION

Tuberculosis (TB) remains a major health concern worldwide. Nearly a third of the world’s population is infected with TB, and in 2012, there were around 1.3 million TB-related deaths worldwide (Center for Disease Control and Prevention, 2014).
The causative organism *Mycobacterium tuberculosis* is an ancient pathogen in humans, having been associated with humans since antiquity (Leung et al., 2013). *M. tuberculosis* attacks the lungs mainly, but can also affect other parts of the body when the microbes gain entry into the bloodstream from an area of damaged tissue. TB – a contagious and air borne disease affecting young adults mostly in their productive years, ranks the second leading cause of death from an infectious disease worldwide (World Health Organization (WHO), 2012). The bacteria spread through the air when people who have an active TB infection cough, sneeze or otherwise transmit their saliva through the air (Konstantinos, 2010).

Social factors that are known to be driving the global increase in TB cases include: overcrowding (especially in urban centers), late reporting and diagnosis, non-compliance to treatment schedule, lack of commitment on the part of National control programmes in developing countries, lack of education, health care infrastructure and poverty (Collins and Ahorlu, 2013). The bacteria multiply in organs that have oxygen pressure such as the upper lobe of the lungs, the kidneys, the central nervous system (in tuberculous meningitis) etc. (Kabra et al., 2013).

TB treatment is difficult and requires administration of multiple antibiotics over a long period of time. Recommended standard treatment for adult respiratory TB is a regimen of Isoniazid (INH), Rifampicin (RIF), Pyrazinamide and Ethambutol for 2 months followed by 4 months INH and RIF (Lian et al., 2013). The mechanism is generally attributed to the formation of the highly reactive oxygen species (ROS) which act as stimulators of lipid peroxidation and the source for destruction and damage to the cell membrane (Lian et al., 2013). Asymptomatic transaminase elevations are common during antituberculosis treatment but hepatotoxicity can be fatal when not recognized early and when therapy is not interrupted in time (Kaona et al., 2004). Adverse effects diminish treatment effectiveness because they significantly contribute to non-adherence, eventually contributing to treatment failure, relapse or the emergence of drug-resistance (Kaona et al., 2004). Adherence to the prescribed treatment is crucial for curing patients with active TB (Wares et al., 2003).

*Garcinia kola* (Heckel), commonly called bitter kola, is an angiosperm, belonging to the family Guttiferae. Bitter kola is a highly valued ingredient in African ethnomedicine because of its varied and numerous uses which include the social and medicinal; thus making the plant an essential ingredient in folk medicine. Medicinal plants such as *G. kola* are believed to be an important source of new chemical substances with potential therapeutic benefits (Adesuyi et al., 2012). The seed is commonly used as a masticatory and is a major kola substitute offered to guests at home and shared at social ceremonies. The seeds are used in folk medicine and in many herbal preparations for the treatment of ailments such as laryngitis, liver disorders and bronchitis (Farombi, 2003). Some of the phytochemicals compounds that have been isolated from *G. kola* include: oleoresin, tannin, saponins, alkaloids, cardiac glycoside, biflavonoids such as kolaflavonone and 2-hydroxyflavonoids, and kolavirions (Adesuyi et al., 2012).

Tuberculosis is a health risk for a large number of people. The current treatment regimens are long and pose adverse effects, among which is hepatotoxicity. This has increased the level of non-compliance to treatment in patients. *G. kola* has been shown to possess hepatoprotective and antioxidative properties. This study was designed to evaluate the possibility of utilizing the extracts of this popularly eaten plant to alleviate the hepatotoxicity associated with these antitubercular drugs, thus encouraging compliance to tuberculosis treatment.

**MATERIALS AND METHODS**

**Drugs, reagents and chemicals**

All drugs used in the study were procured from the Anti-Tuberculosis Clinic of the Obafemi Awolowo University Teaching Hospitals, Ile-Ife. All the reagents and chemicals used were of analytical grade and were obtained from Sigma, BDH or Randox Laboratories Limited, UK.

**Plant**

The dried seeds of *G. kola* were obtained from a local market in Ile-
Kits and reagents were made by Randox Laboratories Limited, UK.

bilirubin levels in the plasma

aminotransferase (AST), alkaline phosphatase (ALP) and the freezer (-20°C) and assayed within 48 h. This was immediately prepared with a Potter-Elvehjem homogenizer from a homogenate of each liver in chilled 250 mM-sucrose was collected and plasma obtained by centrifugation (3000 rpm for 10 min). This was immediately stored in the refrigerator (4°C) and collected within 48 h of collection. The whole liver was also excised upon dissection, and the wet weight obtained. A 10% (w/v) homogenate of each liver in chilled 250 mM-sucrose was immediately prepared with a Potter-Elvehjem homogenizer from a portion of the liver. The homogenates were centrifuged (5000 rpm for 10 min) and the supernatant immediately stored wet-frozen in the freezer (-20°C) and assayed within 48 h.

Estimation of alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP) and bilirubin levels in the plasma

ALT activity levels in the plasma

AST Activity Levels in the Plasma

Sacrificing of animals

Estimation of protein concentration in the plasma and liver

Histomorphological studies

Statistical analysis

RESULTS

ALT activity levels in the plasma

AST Activity Levels in the Plasma

Sacrificing of animals

Estimation of total protein in the plasma and liver

Histomorphological studies

Statistical analysis

RESULTS

ALT activity levels in the plasma

ALT activity levels are as shown in Table 1. There was a higher level (up to 130.9%) of ALT in the group that was administered INH and RIF only (Group III) than the control group (Group I). ALT levels, however, decreased when G. kola was taken in combination with the antitubercular drugs. The difference was significant (p < 0.05) in those groups given dosages of 40 mg/kg (Group IV) and 60 mg/kg (Group V) body weight (bw) of G. kola seed extracts.

AST Activity Levels in the Plasma

As shown in Table 1, there was significantly (p < 0.001) lower levels of AST in the plasma of animals in the group administered the drug combination together with various doses of G. kola extract than in the group administered the drug combinations only.

ALP activity levels in the plasma

There were significant (p < 0.001) reduction of ALP level in the groups administered with the drug combination of INH and RIF, together with 40 mg/kg bw (45.43%), 60 mg/kg bw (67.96%) and 80 mg/kg bw (50.64%) doses of G. kola extract when compared with the group administered only the drug combination INH and RIF (116.328 ± 13.336 U/l) as shown in Table 1.

Bilirubin concentration in the plasma

As is shown in Table 1, the group administered the anti-
Table 1. Effect of treatment with Isoniazid (INH) and Rifampicin (RIF) alone or with *Garcinia kola* seed extracts at various doses on liver antioxidant biomarkers, liver function biomarkers, and the total protein concentrations in the plasma and liver.

<table>
<thead>
<tr>
<th>Groups parameter</th>
<th>I (Control)</th>
<th>II (G. kola only)</th>
<th>IIIa (INH + RIF)</th>
<th>IVa (INH + RIF + G. kola 40 mg/kg)</th>
<th>Vb (INH + RIF + G. kola 60 mg/kg)</th>
<th>VIb (INH + RIF + G. kola 80 mg/kg)</th>
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<tbody>
<tr>
<td>Oxidative stress biomarkers</td>
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<tr>
<td>CAT (μmol of H2O2 consumed/min/mg protein)</td>
<td>15.83 ± 0.05</td>
<td>27.50 ± 2.78</td>
<td>11.53 ± 2.57</td>
<td>28.38 ± 1.12***</td>
<td>22.91 ± 2.99*</td>
<td>19.53 ± 1.41</td>
</tr>
<tr>
<td>GPx (μmol/mg protein)</td>
<td>0.220 ± 0.075</td>
<td>0.377 ± 0.208</td>
<td>0.190 ± 0.097</td>
<td>0.388 ± 0.145</td>
<td>0.303 ± 0.123</td>
<td>0.460 ± 0.215</td>
</tr>
<tr>
<td>SOD (units/mg protein)</td>
<td>1.570 ± 0.188</td>
<td>1.157 ± 0.523</td>
<td>0.568 ± 0.245</td>
<td>2.830 ± 0.496</td>
<td>1.812 ± 0.305</td>
<td>1.466 ± 0.71</td>
</tr>
<tr>
<td>GSH (μmol/mg protein)</td>
<td>0.012 ± 0.001</td>
<td>0.017 ± 0.001</td>
<td>0.008 ± 0.004</td>
<td>0.019 ± 0.002</td>
<td>0.013 ± 0.001</td>
<td>0.016 ± 0.002</td>
</tr>
<tr>
<td>MDA (μmol)</td>
<td>0.194 ± 0.009</td>
<td>0.199 ± 0.019</td>
<td>0.262 ± 0.003</td>
<td>0.181 ± 0.026</td>
<td>0.190 ± 0.025</td>
<td>0.208 ± 0.027</td>
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<tr>
<td>Total protein (plasma; mg/ml)</td>
<td>8.328 ± 0.891</td>
<td>7.612 ± 0.521</td>
<td>6.587 ± 0.462</td>
<td>7.900 ± 0.627</td>
<td>7.938 ± 0.246</td>
<td>8.325 ± 0.542</td>
</tr>
<tr>
<td>Total Protein (liver; mg/ml)</td>
<td>7.289 ± 0.223</td>
<td>4.156 ± 0.353</td>
<td>1.725 ± 0.582**</td>
<td>4.133 ± 0.157</td>
<td>5.274 ± 0.781</td>
<td>6.050 ± 0.362</td>
</tr>
<tr>
<td>Liver function biomarkers</td>
<td></td>
<td></td>
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<tr>
<td>ALT (U/L)</td>
<td>7.35 ± 2.85</td>
<td>10.84 ± 1.30</td>
<td>17.56 ± 3.35</td>
<td>7.04 ± 1.75*</td>
<td>6.40 ± 3.09*</td>
<td>7.84 ± 1.80</td>
</tr>
<tr>
<td>ALP (U/L)</td>
<td>86.94 ± 11.41</td>
<td>69.00 ± 9.91</td>
<td>116.33 ± 13.34</td>
<td>63.48 ± 9.03*</td>
<td>37.26 ± 12.77***</td>
<td>57.41 ± 3.07**</td>
</tr>
<tr>
<td>AST (U/L)</td>
<td>437.75 ± 13.16</td>
<td>477.00 ± 13.30</td>
<td>535.20 ± 26.75</td>
<td>434.80 ± 15.14**</td>
<td>362.00 ± 20.94***</td>
<td>412.60 ± 11.63***</td>
</tr>
<tr>
<td>Bilirubin (mg/dl)</td>
<td>0.169 ± 0.033</td>
<td>0.226 ± 0.010</td>
<td>0.380 ± 0.025***</td>
<td>0.139 ± 0.023</td>
<td>0.127 ± 0.009</td>
<td>0.125 ± 0.025</td>
</tr>
</tbody>
</table>

Each value represents mean ± SEM, n = 5. *p value < 0.05, **p value < 0.01, ***p value < 0.001. CAT = Catalase; SOD = Superoxide dismutase; GPx = Glutathione peroxidase; GSH = Glutathione; MDA = Malondialdehyde; ALT = Alanine aminotransferase; ALP = Alkaline phosphatase; AST = Aspartate aminotransferase; a = Group III was compared to Group I for statistical significance; b = Groups IV, V and VI were compared to Group III for statistical significance.

Protein concentration in the plasma and liver

Protein concentration levels of the plasma and liver of the rats in the group administered the drug combination were lower by 20.91 and 76.33%, respectively, than in the control group. In both the plasma and the liver, the groups administered the drug combination with *G. kola* seed extracts had higher protein levels than the group administered the drug combination only, with protein levels comparable to that of the control group in the rats in the group co-administered with 80 mg/kg bw *G. kola* seed extract.

GSH, CAT, GPx and SOD activities in the liver

GSH, CAT and SOD activities in the liver of the rats were highest in the groups administered the anti-tubercular drugs together with 40 mg/kg bw *G. kola* extract. Highest GPx activity was found in the group administered the anti-tubercular drugs in combination with 80 mg/kg dosage of *G. kola* seed extract. Lowest activity was found in the group that had only INH and RIF administered. Groups fed with various doses of *G. kola* extract had significantly higher levels of activity of the various enzyme biomarkers. This is illustrated in Table 1.

MDA levels in the liver

MDA levels, as shown in Table 1, were higher in the group administered only the anti-tubercular drugs INH and RIF than in every other group including the control group and those administered with the antitubercular drugs together with doses of *G. kola* seed extracts.

Liver histomorphology

Images of the hepatocytes as observed under a microscope are shown in Plate 1. The hepatocytes of the rats of the control group showed a normal lobular architecture and normal hepatic cords of the liver. The hepatocytes are arranged in a regular pattern around the portal triad (portal vein, hepatic artery and bile duct) with sinusoids between excessive plates. Hepatocytes of the group administered only *G. kola* seed extracts also showed normal architecture, arranged normally around the portal triad. In the INH + RIF only treated group, the hepatocytes showed swelling, congestion and feathery degeneration. The arrangement of the hepatocytes in...
this group was seen to be scattered, showing sign of
degeneration, necrosis and vascular damage. The INH +
RIF + G. kola (40 mg/kg) group showed hepatocyte
congestion to some degree, but lobular architecture was
more ordered on comparison with those of the control
group. The hepatocytes of the INH + RIF + G. kola (60
mg/kg) group showed less congestion and more ordered
lobular architecture than the INH + RIF + G. kola
(40mg/kg) group, more similar to that of the control
group. Hepatocytes of the INH + RIF + G. kola (80
mg/kg) group showed minimal changes with moderate
portal triaditis, and their lobular architecture was normal.
They showed tighter vascular congestion in the central
vein, portal area and sinusoidal spaces than in the control
group.

**DISCUSSION**

As can be deduced from Table 1, treatment with the
antitubercular drugs alone elicited depression of CAT
(27%), GPx (19%), SOD (63.80%) and GSH (33%) when
compared to the control group. There was a concurrent
elevation of MDA (35%) in the antitubercular drug group.
These changes were reversed to different degrees by co-
treatment with the different doses of G. kola. A
consideration of the liver function biomarkers showed that
there was significant elevation of ALT, ALP and AST in the
INH + RIF group when compared to controls. As was
observed with the oxidative stress biomarkers, co-
treatment with G. kola lowered the levels of all these
biomarkers. In similar manner, there was a very
significant reduction in total protein in the liver in the INH +
RIF group when compared to the control. As was
observed with oxidative stress biomarkers, co-treatment
with G. kola ameliorated this reduction.

Toxic metabolites are suggested to play a central role
in the development of anti-tuberculosis drug-induced
hepatotoxicity. Formation and detoxification of toxic
metabolites can be affected by the inhibition, induction or
polymer polymorphism in drug-metabolizing enzymes
(Somasundaram et al., 2014). In this study, the ability of
a 70% ethanol extract of G. kola seeds to protect against
hepatotoxicity induced by anti-tubercular drugs, INH and
RIF, was investigated. Metabolism of chemicals takes
place largely in the liver, which accounts for the organ's
susceptibility to metabolism-dependent, drug induced
injury. The drug metabolites can be electrophilic
chemicals or free radicals that undergo or promote a
variety of chemical reactions, such as depletion of
reduced glutathione; covalently binding to proteins, lipids
or nucleic acids; or inducing lipid peroxidation (Kaplowitz,
2001). These destroy the integrity of the liver.

Lower level of activity, in the liver, of oxidative stress
biomarkers (GSH, SOD, CAT and GPx) in the group
administered INH and RIF only compared to the control
group is indicative of an inhibition of these enzymes by
toxic metabolites of the drugs. This makes them unable
to scavenge and mop up free radicals generated by the
anti-TB drugs used. Higher levels in the groups co-
administered with various doses of G. kola extract
indicates the protective effect of the G. kola extract, either
by inducing the elevation of activities of these enzymes or
its active principles also acting as free radical
scavengers, thus overwhelming the inhibition activity of
the anti-tubercular drug metabolites.

Liver enzyme biomarkers are enzymes specialized and
concentrated mainly in the liver which upon injury to the
liver, seep into other bodily fluids. A measure of their
levels in those bodily fluids is an indication of the extent
of liver damage (if any). Examples of these hepatic
biomarkers are ALP, AST, and in particular ALT.

In this study, hepatotoxicity in Wistar rats was produced
by the administration of the anti-tubercular drug
combination INH (7.5 mg/kg) and RIF (10 mg/kg) every
day for 35 days. This is indicated by the higher plasma
levels of ALP, AST and ALT on comparison with the
control group. Mitigation of the toxicity of the anti-
tubercular drugs is affected by co-administration with
various doses of ethanol extract of G. kola seeds. This is
indicated by the lower levels of ALT, ALP and AST in the
group of animals administered the drugs together with
doses of G. kola seed extracts. This is due to the inability
of the liver to synthesize proteins. In
result of the inability of the liver to conjugate bilirubin with glucoronide, thereby
generating an accumulation of unconjugated bilirubin in
the blood. Co-administration of G. kola seed extracts with
the anti-tubercular drugs mitigated hepatic damage,
hence improved bilirubin-glucoronide conjugation in the
liver.

Total protein in both the plasma and liver were seen to
be lowered in the INH-RIF only treated group. This is as a
result of the inability of the liver to synthesize proteins. In
the INH-RIF and G. kola seed extract treated groups, the
G. kola extract mitigated the damage done by the anti-
tubercular drugs, thus, resulting in higher protein level in
the plasma and liver. A particularly important
consequence of free radical damage in many cells is the
peroxidation of lipids in the membranes, which results in
the formation of lipid peroxides and aldehydes. MDA is
one of the major products of peroxidation in cells and is
usually quantitatively measured by its reaction with
thiobarbituric acid to measure level of oxidation. MDA
levels were observed to be higher in the INH-RIF only
treated group than the control group and the groups co-
administration with anti-tubercular drugs and various
doses of ethanol extract of G. kola seeds. This higher
level could be as a result of damage imposed by the
increased free radicals generated during the
detoxification of the drugs. The resulting lower levels of
MDA in the groups co-administered with anti-tubercular
drugs and the G. kola seed extracts indicate a lower level
of peroxidation in the liver.
The Rifampicin induces Cytochrome P450 which is a drug metabolizing enzyme. By inducing this enzyme, it acts by metabolizing more of the INH, thereby producing more toxic hydrazine (metabolite of INH). These hydrazines act by inhibiting or reducing the drug detoxifying enzymes, thereby making the detoxifying enzymes unable to scavenge these free radicals been produced, causing oxidative stress which eventually leads to liver toxicity or liver damage (Figure 1). Studies indicate that there is no clear evidence that INH proves much more injuries than RIF and in this connection, they consider that it is the combination of these two drugs that confers the additive or even synergistic potential of liver toxicity than either agent alone, as conjectured. INH is metabolized in the liver primarily by acetylation and hydrolysis, and it is these acetylated metabolites that are thought to be hepatotoxic. Reports on rats suggest that the hydrazine metabolite of INH which has subsequent effect on CYP2E1 induction is involved in the development of INH-induced hepatotoxicity, and also oxidative stress as one of the mechanism for INH + RIF induced hepatic injury (Saleem et al., 2008; Yue et al., 2004).

\[ G. \ kola \] with its chemical constituent such as the kolaviron is believed to protect and elevate the activities of the drug detoxifying enzymes, thereby protecting the liver against liver damage (Figure 1). The varied chemical composition of \[ G. \ kola \] seed extract makes it difficult to assign its hepatoprotective property to any one of its constituent chemicals. Kolaviron has been reported to significantly prevent hepatotoxicity induced by several hepatotoxic agents such as phalloidin, thioacetamide and paracetamol (Iwu et al., 1987; Akintowa and Essien, 1990). The hepatoprotective property of \[ G. \ kola \] seed extract may also be because of its other properties like anti-inflammatory property which may prevent inflammatory hepatic damage, immune-modulating property and antioxidant property thereby reducing the oxidative stress imposed by the drugs; this antioxidant mechanism seems to be important as \[ G. \ kola \] extract has been shown to reduce oxidative stress and oxidative stress has been found to be the most important mechanism in hepatotoxicity of anti-tubercular drugs (Iwu et al., 1987; Akintowa and Essien, 1990).

To examine the extent of hepatic damage, by the anti-tubercular drugs, and mitigation, by the \[ G. \ kola \] seed extract, histomorphological examinations were carried out. Degeneration of cells, vascular congestion and necrosis were observed in the INH-RIF only treated group (Plate 1C). Varying levels of recovery of the cells were seen in the INH-RIF and \[ G. \ kola \] treated groups at different concentrations of the \[ G. \ kola \] seed extract. However, at 40 mg/kg bw \[ G. \ kola \] extract co-administered with INH-RIF, the hepatocytes’ arrangement is closer to normal than the INH-RIF only treated group, with a lesser degree of congestion (Plate 1D). The 60 mg/kg bw \[ G. \ kola \] extract co-administered with INH-RIF, the hepatocytes’ arrangement is closer to normal than the INH-RIF only treated group, with a lesser degree of congestion (Plate 1D).
Plate 1. Liver histomorphology of animals treated with isoniazid (INH), rifampicin (RIF) and G. kola seed extracts (H&E ×400).

*kola* group’s hepatocytes showed very minor congestion and close to normal lobular architecture (Plate 1E). The hepatocytes of the 80 mg/kg bw *G. kola* group had normal cellular arrangement, however, the tighter vascular congestions in the central vein, portal area and sinusoidal spaces indicate a higher concentration of the *G. kola* extract and could not protect the liver significantly by recovering from the damage done by the anti-TB drugs. Plate 1D, E and F showed the potency of the *G. kola* extract in protecting the liver against serious damage by the anti-TB drugs induce hepatotoxicity.

**Conclusion**

This study demonstrates the hepatoprotective effect of *G. kola* seed extracts when co-administered with antitubercular drugs. Optimum mitigation was achieved at the 60 mg/kg bw dosage of the ethanol extracts of the *G. kola* seeds. This hepatoprotective effect could be due to its high content of flavonoids. Although this study was done with rats, the findings offer a way of combating the harmful side-effects of antitubercular treatment. The study clearly highlights the possibility of mitigating
hepatotoxicity of antituberculosis drugs with a simple extract from G. kola, a readily accessible plant. After larger studies and further fractionation to identify the protective compounds, its co-administration could make treatment easier for the patients. This in turn would improve patient compliance and reduce the risk of developing resistance or spreading the disease.

Conflict of interest

Authors declare that there are no conflicts of interests

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