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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 mk/kg bodyweight). Plasma oxidative stress biomarkers (superoxide dismutase, glutathione, glutathione peroxidase, catalase and malondialdehyde) and hepatotoxicity biomarkers (alanine 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

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Control (CDC), 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., 2006).

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 Isoniazid and Rifampicin alone (WHO, 2003). The most frequent adverse effects of antituberculosis treatment are hepatotoxicity, skin reactions, gastrointestinal and neurological disorders.

A previous study in our laboratories showed that combined INH/RIF administration for 15 days in adult Wister rats produced astrocytic aggregation and hyperactivity in the cerebral cortex (Fakoya et al., 2013). Hepatotoxicity, however, is the most serious adverse effect (Frieden et al., 2003). INH hepatotoxicity is a common complication of antituberculosis therapy that ranges in severity, from asymptomatic elevation of serum transaminases to hepatic failure requiring liver transplantation. INH is metabolized to monoacetyl hydrazine, which is further metabolized to a toxic product by cytochrome P450 leading to hepatotoxicity.

The combined use of RIF and INH has been associated with an increased risk of hepatotoxicity (Tostmann et al., 2008). Rifampicin induces isoniazid hydrolase, increasing hydrazine production when rifampicin is combined with isoniazid (especially in slow acetylators), which may explain the higher toxicity of the combination. Rifampicin also interacts with anti-retroviral drugs and affects the plasma levels of these drugs as well as risk of heaptotoxicity (Tostmann et al., 2008). Antituberculosis drug-induced hepatotoxicity (ATDH) causes substantial morbidity and mortality and diminishes treatment effectiveness. Peroxidation of in the cytotoxic action of 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 ethno-medicine 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 kolavirons (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-

Ife. They were identified and authenticated at the Ife Herbarium. The seeds were dehusked, chopped into pieces and oven dried at 40°C for 48 h and then ground to powder. The powdered material (120 g) was suspended in 1.2 L of 70% (v/v) ethanol for 72 h and filtered. The residue was extracted thrice using ethanol and followed by filtration. The combined filtrate was concentrated *in vacuo* at 45°C in a rotary evaporator to a semi-solid mass. The extract was suspended in normal saline and administered at different doses.

Animal treatment

Thirty adult albino rats (Wistar strain) of either sex (weights between 180 to 220 g) reared from the same colony were used for the experiment. They were maintained under standard laboratory conditions, fed with standard mouse chow (Ladokun Feeds, Ibadan, Nigeria) and provided with water *ad libitum*. The animals were randomly assigned into six groups (n = 5). Hepatotoxicity was induced using a combined dose of INH + RIF (7.5 mg/kg body weight of INH and 10 mg/kg body weight of RIF) administered intraperitonially (i.p.). The following treatments were administered daily for 35 days:

Group I (Control): Saline only (0.5 ml, i.p.).
Group II: *Garcinia kola* only (60 mg/kg, p.o).
Group III: INH + RIF only.
Group IV: INH + RIF and *G. kola* seed extract (40 mg/kg, p.o).
Group V: INH + RIF and *G. kola* seed extract (60 mg/kg, p.o).
Group VI: INH + RIF and *G. kola* seed extract (80 mg/kg, p.o).

Sacrificing of animals

After 35 days of treatment, all animals were anaesthetized with diethyl ether and then dissected. Intra-cardiac blood samples were collected and plasma obtained by centrifugation (3000 rpm for 10 min). This was immediately stored in the refrigerator (4°C) and assayed 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

ALP, ALT, AST and bilirubin levels in the plasma were assayed using assay kits and reagents utilizing principles based on methods as described by German Society for Clinical Chemistry (DGKC) (1972) (ALP), Reitman and Frankel (1957) (ALT and AST) and Jendrassik and Grof (1938) (bilirubin), all with slight modifications. Kits and reagents were made by Randox Laboratories Limited, UK.

Estimation of total protein in the plasma and liver

This was done using the Bradford method (Bradford, 1976) with slight modifications.

Estimation of glutathione (GSH), catalase (CAT), glutathione peroxidase (GPx), superoxide dismutase (SOD) and malondialdehyde (MDA) activity levels in the liver

Estimation of activity of oxidative stress biomarkers, GSH, CAT, GPx, SOD and MDA were carried out according to methods as described by Moron et al. (1979), Sinha (1972), Rotruck et al. (1973), Misra and Fridovich (1972) and Parihar and Taruma (2003), respectively, all with slight modifications.

Histomorphological studies

Histomorphological investigation was performed by fixing the representative liver tissues from each group in 10% formal saline by total immersion for 48 h, after which they were processed via paraffin wax embedding according to standard methods (Drury and Wallington, 1980). The staining procedure of Haemotoxylin and Eosin as described by Drury and Wallington (1980) was also adopted.

Statistical analysis

All values were expressed as mean ± SEM (n = 5 in each group). One-way analysis of variance (ANOVA) was applied to test for significance of biochemical data of the different groups.

RESULTS

ALT activity levels in the plasma

ALT activity levels are as shown in Table 1. There was a higher level (up to 139%) 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) bodyweight (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.

Groups parameter		I (Control)	II (*G. kola* only)	III[a] (INH + RIF)	IV[b] (INH + RIF + *G. kola* 40 mg/kg)	V[b] (INH + RIF + *G. kola* 60 mg/kg)	VI[b] (INH + RIF + *G. kola* 80 mg/kg)
Oxidative stress biomarkers	CAT (µmol of H_2O_2 consumed/min/mg protein)	15.83 ± 0.05	27.50 ± 2.78	11.53 ± 2.57	28.38 ± 1.12***	22.91 ± 2.99*	19.53 ± 1.41
	GPx (µmol/mg protein)	0.220 ± 0.075	0.377 ± 0.208	0.190 ± 0.097	0.388 ± 0.145	0.303 ± 0.123	0.460 ± 0.215
	SOD (units/mg protein)	1.570 ± 0.188	1.157 ± 0.523	0.568 ± 0.245	2.830 ± 0.496	1.812 ± 0.305	1.466 ± 1.071
	GSH (µmol/mg protein)	0.012 ± 0.001	0.017 ± 0.001	0.008 ± 0.004	0.019 ± 0.002	0.013 ± 0.001	0.016 ± 0.002
	MDA (µmol)	0.194 ± 0.009	0.199 ± 0.019	0.262 ± 0.003	0.181 ± 0.026	0.190 ± 0.025	0.208 ± 0.027
	Total protein (plasma; mg/ml)	8.328 ± 0.891	7.612 ± 0.521	6.587 ± 0.462	7.900 ± 0.627	7.938 ± 0.246	8.325 ± 0.542
	Total Protein (liver; mg/ml)	7.289 ± 0.223	4.156 ± 0.353	1.725 ± 0.582***	4.133 ± 0.157	5.274 ± 0.781	6.050 ± 0.362
Liver function biomarkers	ALT (U/L)	7.35 ± 2.85	10.84 ± 1.30	17.56 ± 3.35	7.04 ± 1.75*	6.40 ± 3.09*	7.84 ± 1.80
	ALP (U/L)	86.94 ± 11.41	69.00 ± 9.91	116.33 ± 13.34	63.48 ± 9.03*	37.26 ± 12.77***	57.41 ± 3.07**
	AST (U/L)	437.75 ± 13.16	477.00 ± 13.30	535.20 ± 26.75	434.80 ± 15.14**	362.00 ± 20.94***	412.60 ± 11.63***
	Bilirubin (mg/dl)	0.169 ± 0.033	0.226 ± 0.010	0.380 ± 0.025***	0.139 ± 0.023	0.127 ± 0.009	0.125 ± 0.025

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 peroxide; 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.

tubercular drugs alone, had a significantly (p < 0.001) higher bilirubin concentration than the control group. There were reductions in concentration of bilirubin in the groups after *G.* kola extracts were added with the drug combination.

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 together 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 de-generation. 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* ameloriated 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 genetic 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 Table 1. Bilirubin concentration was significantly ($p < 0.001$) higher in the INH-RIF only treated group. This is due to 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.

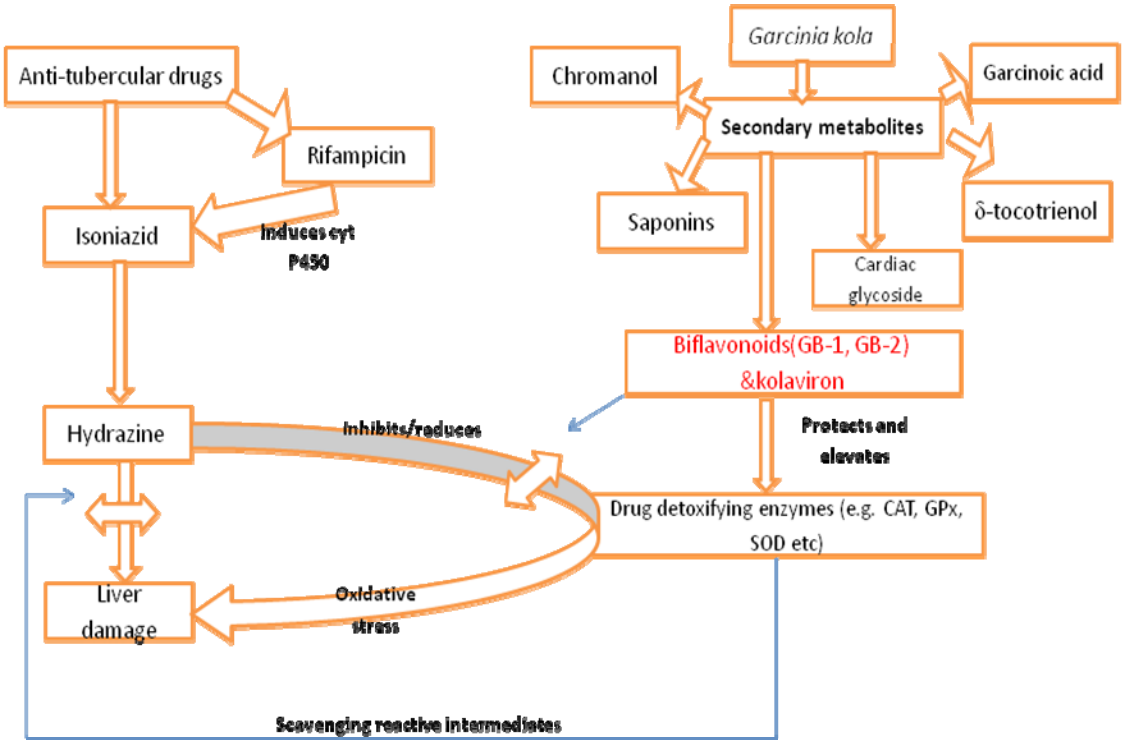

Figure 1. Possible actions of *G. kola* as a hepatoprotective agent.

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.*

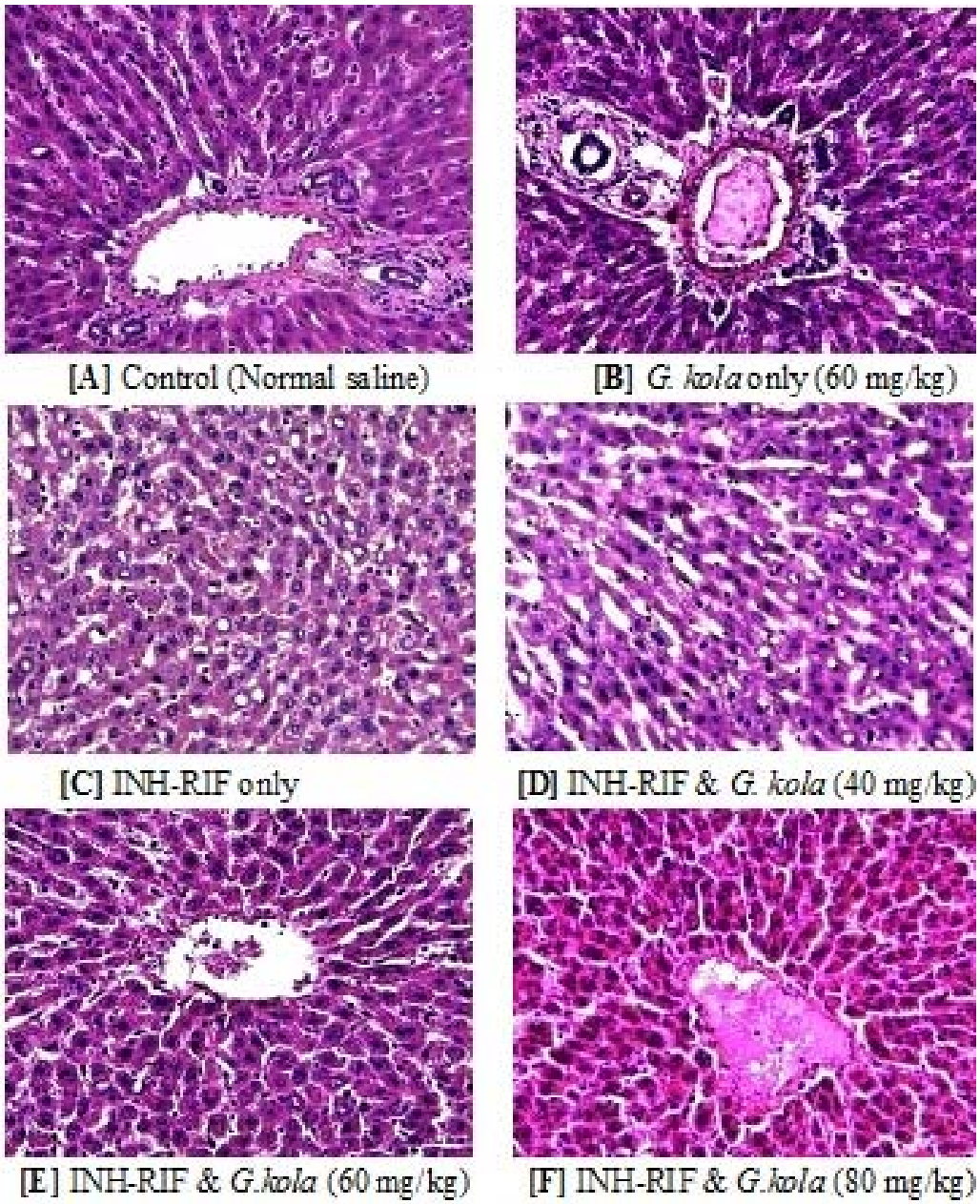

Plate 1. Liver histomorphology of animals treated with isoniazid (INH), rifampicin (RIF) and *G. kola* seed extracts (H&E ×400).

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 anti-tubercular 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

REFERENCES

Akintowa A, Essien AR (1990). Protective effects of *Garcinia kola* Seed extract against Paracetamol Induced hepatotoxicity in rats. J. Ethnopharmacol. 29(2):207-211.

Adesuyi AO, Elumm IK, Adaramola FB, Nwokocha AGN (2012). Nutritional and Phytochemical Screening of *Garcinia kola*. Adv. J. Food Sci. Technol. 4(1):9-14.

Bradford MM (1976). A rapid and sensitive method for the quantification of microgram quantities of protein, utilizing the principle of protein-dye binding. Anal. Biochem. 72:248-254.

Centers for Disease Control and Prevention (CDC) (2014). Tuberculosis-Data and Statistics.

Collins K, Ahorlu FB (2013). Factors affecting TB case detection and treatment in the Sissala East District, Ghana. J. Tuberc. Res. 1:29-36.

Deutsche Gesellschaft fur KlinischeChemie (DGKC) (1972). Standardization of methods for measurement of enzymatic activities in biological fluids. Z. Klin. Chem. Klin. Biochem.10:182-192.

Drury AA, Wallington EA (1980). Carleton's Histological Technique, 5th Edition. Oxford University Press, New York.

Fakoya FA, Osoniyi O, Obuotor EM (2013). Analysis of Combined anti-tuberculous drug induced neurotoxicity in the cerebral cortex of adult wister rats by immune histochemistry. J. Neurosci. Behav. Health 5(2):20-29.

Farombi EO (2003). African indigenous plants with chemotherapeutic potentials and biotechnological approach to the production of bioactive prophylactic agents. Afr. J. Biotechnol. 2(12):662-671.

Frieden TR, Sterling TR, Munsiff SS, Watt CJ, Dye C (2003). Tuberculosis. Lancet 362:887-899.

Iwu MM, Igboko OA, Onwuchekwa U, Okunji CO (1987). Evaluation of theantihepatotoxicity of the biflavanoids of *Garcinia kola* seeds. J. Ethnopharmacol 21:127-142.

Jendrassik L, Grof P (1938). Vereinfachte photometrische methoden zur bestimmung des bilirubin. Biochem. Z. 297:81-89.

Kaona FA, Tuba M, Siziya S, Sikaona L (2004). An assessment of factors contributing to treatment adherence and knowledge of TB transmission among patients on TB treatment. BMC Public Health 4:68.

Kaplowitz N (2001). Drug-induced liver disorders: Implications for drug development and regulation. Drug Saf. 24(7):483-490.

Konstantinos A (2010). Testing for tuberculosis. Aust. Prescr. 33(1):12-18.

Leung CC, Lange C, Zhang Y (2013). Tuberculosis: current state of knowledge; an epilogue. Respirology 18:1047-1055.

Lian Y, Zhao J, Xu P, Wang Y, Zhao J, Jia L, Fu Z, Jing L, Liu G, Peng S (2013) Protective Effects of Metallothionein on Isoniazid and Rifampicin-Induced Hepatotoxicity in Mice. PLoS ONE 8(8):e72058.

Misra HP, Fridovich I (1972). The role of superoxide anion in the autoxidation of epinephrine and a simple assay for superoxide dismutase. J. Biol. Chem. 247:3170-3175.

Moron MS, Depierre JW, Mannervik B (1979). Levels of glutathione, glutathione reductase and glutathione S-transferase activities in rat lung and liver. Biochim. Biophys. Acta 582(1):67-78.

Parihar MS, Taruma H (2003). Phenolic anti-oxidant attenuate hippocampal neuronal cell damage against Kinic acid induced excitotoxicity. J. Biol. Sci. 28:121-128.

Reitman S, Frankel S (1957). A calorimetric method for the determination of serum glutamic oxaloacetic acid and glutamic pyruvic transaminases. Am. J. Clin. Pathol. 28:56-63.

Rotruck JT, Pope AL, Ganther HE, Swanson AB, Hafeman DG, Hoekstra WG (1973). Selenium: biochemical role as a component of glutathione peroxidase. Science 179(73):588-590.

Saleem TSM, Christina AJM, Chidambaranathan N, Ravi V, Gauthaman K (2008). Hepatoprotective activity of Annonasquamosa Linn. on experimental animal model. Int. J. App. Res. Nat. Prod. 1(3):1-7.

Sinha AK (1972). Colorimetric assay of catalase. Anal. Biochem.47:389-394.

Tostmann A, Boeree MJ, Aarnoutse RE, de Lange WC, van der Ven AJ, Dekhuijzen R (2008). Antituberculosis drug-induced hepatotoxicity: concise up-to-date review. J. Gastroen. Hepatol. 23(2):192-202.

Wares DF, Singh S, Acharya AK, Dangi R (2003). Non-adherence to tuberculosis treatment in the eastern Tarai of Nepal. Int. J. Tuberc. Lung D. 7:327-335.

World Health Organization (WHO) (2003). Treatment of Tuberculosis: Guidelines for National Programmes, 3rd edn. (WHO/CDS/TB/2003.13). Geneva: World Health Organization.

World Health Organisation (WHO) (2012). Global tuberculosis report 2012. Geneva, World Health Organization. WHO/HTM/TB/2012.6.

Yue J, Peng RX, Yang J, Kong R, Liu J (2004). CYP2E1 mediated isoniazid-induced hepatotoxicity in rats. Acta Pharm. Sin. 25(5):699-704.