Isoniazid-Induced Kidney Damage: Protective Roles of Kolaviron Extract from *Garcinia Kola* seed

Abstract

The study investigated the protective effect of kolaviron extract obtained from the seed of *Garcinia kola* against isoniazid-induced kidney damage. Fresh seed of *Garcinia kola* (2 kg) was extracted using soxhlet extractor and partitioned with chloroform to obtain kolaviron using standard procedure. Treatment of wistar rats lasted for 30 days in which toxicity was induced by oral administration of isoniazid (20 mg/kg bwt). Protective effect of kolaviron was measured in the plasma of wistar rats by estimating the levels of some metabolites used as kidney biomarkers such as total protein, creatinine, urea and uric acid concentration. The result showed a significant ($p < .05$) decrease in total protein concentration while there was a significant ($p < .05$) increase in urea, uric acid and creatinine concentrations. However, treatment with kolaviron prevented kidney damage by increasing concentration of total protein, while there was significant decrease in urea, uric acid and creatinine concentrations. The results therefore concluded that kolaviron extract obtained from *Garcinia kola* seeds exhibited a protective effect against isoniazid-induced kidney toxicity and it may be relatively safe when used therapeutically at this dose in the treatment and management of diseases associated with kidney damage.

Keywords

Kolaviron
Isoniazid
Kidney damage
Garcinia kola
Xenobiotics

1. Introduction

One of the most effective drugs in tuberculosis prevention and management is isoniazid [1]. Tuberculosis (TB) is a communicable disease caused by mycobacterium tuberculosis. Its lethality has been implicated in a mortality rate of 1.3 million HIV-negative people and additional 300000 mortality among HIV-positive people in 2017 while about 10 million people are latently infected worldwide [2]. Isoniazid has been reportedly used alone for TB prevention [1] as well with other drugs like rifampicin [3]. Isoniazid is a pro-drug which upon activation stops the growth of rapidly dividing mycobacteria [4,5]. It acts as a mild monoamine inhibitor by blocking the cytochrome p450 system, thereby releasing free radicals which is bactericidal to the mycobacterium [6]. Despite its exceptional anti-tuberculosis effects, simple-to-severe side effects have been reported with chronic injuries like peripheral neuropathy and liver failure [7,8,9,10,11,12,13]. This is associated with different metabolites released during isoniazid metabolism such as acetylhydrazine, hydrazine and acetylisoniazid which have been implicated in hepatic necrosis, macrovesicular degeneration, steatosis [12,14,15] mitochondrial complex I and II inhibition and hepatocyte death [16]. In addition, the kidney which has been recognised as a probable

site of extrarenal toxification of drugs [17] may likely be affected. In fact, Emeigh-Hart et al. [18] reported that some less toxic compounds can become toxic within the kidney through biotransformation resulting from the activities of xenobiotic metabolising enzymes [19]. Consequently, this leads to drug-induced kidney damage such as interstitial nephritis [20,21] or hepatorenal dysfuction [22]. However, most synthetic drugs available for the management of kidney damage show limited efficacy coupled with side effects.

Numerous findings have revealed the protective effects of *Garcinia kola* seeds against carbon tetrachloride (CCL_4) and paracetamol-induced liver damage [23,24,25]. Likewise, the anti-diabetic, anti-lipidemic, anti-atherogenic properties of the seeds have been evaluated and found to have remarkable results [26]. Furthermore, Onasanwo et al. [27] also recommended its use as a potent anti-ulcer agent after using different ulcer models. Apparently, most beneficial properties of plants have been attributed to flavonoids and related phytoconstituents [28]. However, the effect of kolaviron on isoniazid-induced kidney damage has not been studied nor substantiated with experimental data. Hence, this study is aimed at evaluating the protective roles of kolaviron on key kidney parameters such as creatinine, urea, uric acid and total protein in kidney damage caused by isoniazid in wistar rats.

2. Materials

2.1. Chemicals

Methanol, n-Hexane, Chloroform, Normal saline and Tween-20 were obtained from Sigma Chemical Company (St Louis, MO, USA). Bovine serum albumin (BSA), Urea, Uric acid, and Creatinine (diagnostic kits) were obtained from Randox Laboratories Ltd, United Kingdom.

2.2.Plant collection and authentication

Fresh seeds of Garcinia kola were purchased from a local market in South-West Nigeria and was authenticated at IFE Herbarium, Department of Botany, Obafemi Awolowo University, Ile-Ife. Specimen identification number was also obtained (IFE-17733).

2.3.Experimental animals

Twenty five male wistar rats (150–250 g) were used in the study and were obtained from Faculty of Biological Sciences Animal Breeding House, University of Ibadan, Oyo state, Nigeria. The animals were maintained under standard laboratory condition (12-h light/dark cycle). They were fed with standard pellet diet and water ad libitum. The animals were acclimatized to laboratory condition for two weeks prior to experimentation. The principle of laboratory animal care (National Institute of Health Publication No. 85-23) guidelines and procedures were followed in the study (NIH publication revised, 1985).

3. Methods

3.1.Extract preparation

Kolaviron was isolated from garcinia kola according to the method of Ademola et al. [29]. Five kilograms of peeled seeds of Garcinia kola were sliced and air-dried in the laboratory for four weeks after which it was ground to coarse powder. Two kilograms of the powdered seeds were extracted with n-hexane in the Soxhlet extractor. The defatted, dried marc was repacked and then extracted with methanol. Thereafter, the extract was concentrated using a

rotary evaporator and diluted to twice its volume with distilled water, followed by partitioning with chloroform. The concentrated chloroform fraction gave a brownish-yellow gel known as kolaviron.

3.2. Animal grouping and treatments

Twenty-five male wistar rats were divided into five (5) groups of five (5) animals each and were given orally the following treatment for thirty (30) days: Group 1 received 0.2% (v/v) tween 20 and served as the normal control; Group 2 received 100mg/kg b. wt Kolaviron only; Group 3 received 20 mg/kg b. wt Isoniazid only as the toxic dose for inducing nephrotoxicity; Group 4 received Kolaviron extract (100mg/kg b. wt) + Isoniazid (20 mg/kg b. wt); Group 5 received Vitamin C (100 mg/kg b. wt.) + Isoniazid (20 mg/kg b. wt). Vitamin C served as positive control. Pre-treatment with Kolaviron was done 1 hr before administering isoniazid for Groups 4 and 5 while tween-20 served as vehicle for administration. On the 30^{th} day, food and water were withdrawn from the animals for 24 hrs and decapitated.

3.3. Collection of blood samples and homogenates

Blood samples were collected into heparinized bottles and centrifuged at 4000 rpm for 10 min. Collection of Plasma was done using Pasteur pipette and was used for protein estimation. Likewise, kidney was removed and prepared by homogenizing the kidney 10% (w/v) separately in phosphate buffer solution (pH 7.4) using Potter–Elvejhem glass homogenizer. The homogenates were centrifuged at 4000 rpm for 15 min and the supernatant was collected as a source for the assessment of kidney function parameters.

3.4. Biochemical parameters

Total protein concentration was estimated according to the method of Lowry et al. [30] while creatinine, urea and uric acid concentrations were estimated using standard Randox diagnostic kits.

3.5. Statistical analysis

Data presented as mean ± SEM. Relationships between groups were carried out using one way analysis of variance (ANOVA) followed by Tukey–Kramer multiple comparisons test using Graphpad Prism. A probability level of less than 0.05 ($p < .05$) was accepted as statistically significant.

4. Results and discussion

4.1. Results

The percentage yield of kolaviron from 2k g of powdered Garcinia kola was 147.68 g representing 7.38 % of the starting material.

The effect of kolaviron extract from Garcinia kola seed on plasma total protein concentration is shown in Figure 1. Oral administration of 20 mg/kg body wt. of isoniazid caused decreased ($p < .05$) level of total protein compared to normal control and kolaviron-treated group. There

was significant improvement in inhibition of nephrotoxicity as observed in the kolaviron + isoniazid group and vitamin C + isoniazid treated group when compared to toxin (isoniazid) treated group. This remarkable increase in the level of total protein in the kolaviron treated group indicated the protective effect of kolaviron.

Effect of isoniazid-induced toxicity and treatment with kolaviron extract on kidney uric acid concentration is shown in Figure 2. The group treated with isoniazid has relatively high level of uric acid when compared to the control and kolaviron-treated group. There was however significant ($p < .05$) decrease in the plasma level of uric acid in kolaviron + isoniazid group and Vitamin C + isoniazid treated group when compared to isoniazid only. Treatment with Kolaviron revealed more potent efficacy in the modulation of kidney function parameters.

Administration of isoniazid at 20 mg/kg body wt caused a significant ($p < .05$) increase in urea concentration as shown in figure 3. This however became lowered on administration of 100 mg/kg b.wt of kolaviron as compared with the group treated with vitamin C. A higher efficacy was observed in the kolaviron-treated group when compared with the group treated with standard drug vitamin c and the normal control.

Effect of isoniazid-induced toxicity and treatment with kolaviron extract on plasma level of creatinine is shown in Figure 4. The plasma creatinine in the isoniazid-treated group was higher than the control and the treated groups. However, there was significant ($p < .05$) decrease in creatinine concentration of the group treated with kolaviron + isoniazid and vitamin C + isoniazid when compared to the group treated with Isoniazid only. Commendably, both kolaviron at 100 mg/kg b.w. regimen resulted in significant protective effect against isoniazid-induced kidney damage and this observable effect compared well with vitamin C which was employed for the study.

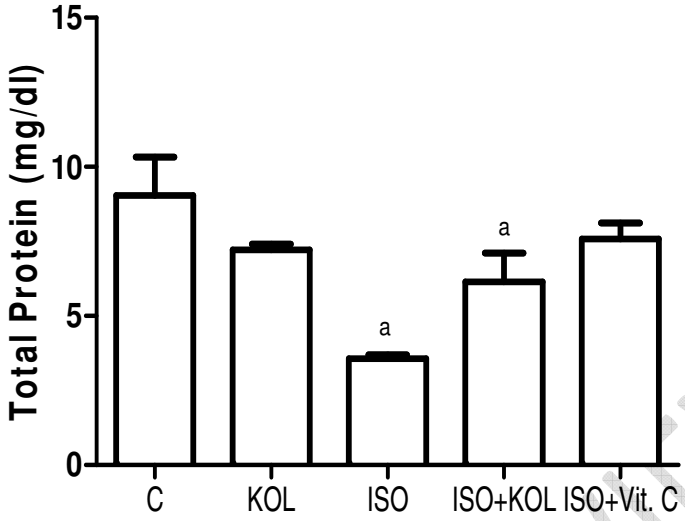


Figure 1. Effect of kolaviron extract from Garcinia kola seed on plasma total protein concentration (n= 5, mean ± SEM). Superscript on each bar denotes significant difference ($p < .05$) from Control group (C). C: Control (20% tween 20), KOL: kolaviron (100 mg/kg b.w.), ISO: Isoniazid (20 mg/kg b.w.), ISO+KOL: Isoniazid (20 mg/kg b.w.) + Kolaviron (100 mg/kg b.w.), ISO + Vit. C: Isoniazid (20 mg/kg b.w.) + Vitamin C (100 mg/kg b.w.).

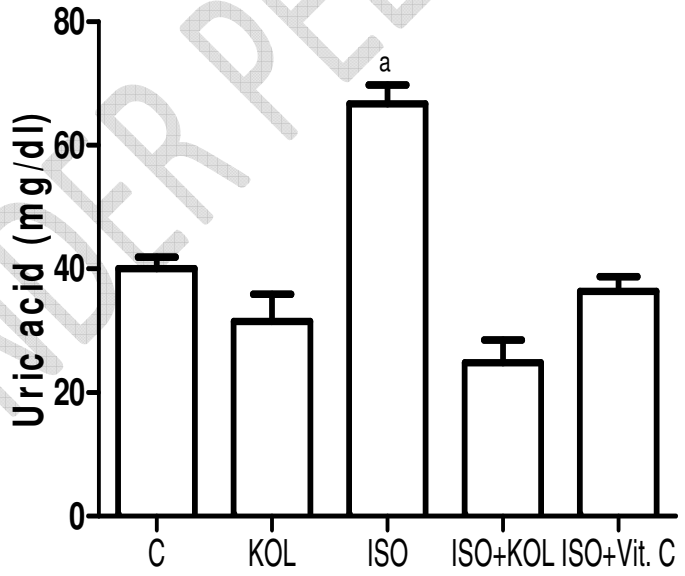
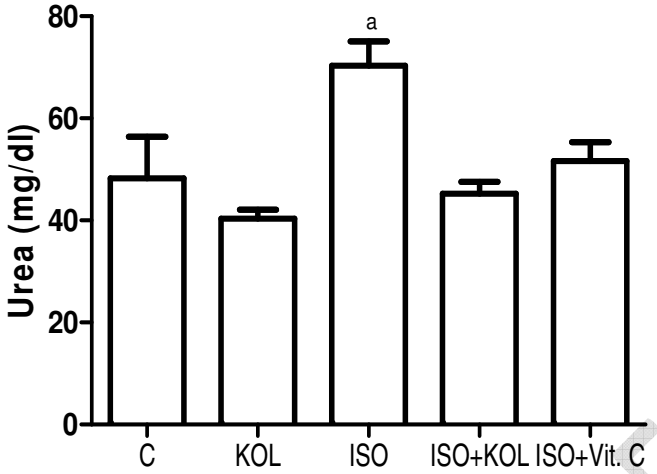


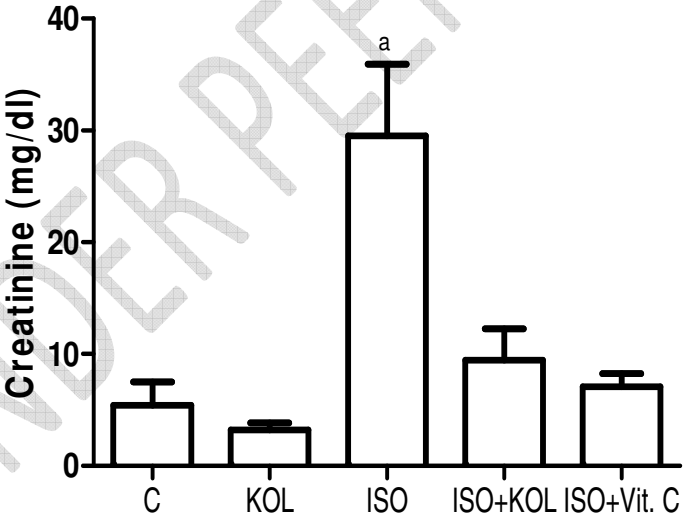
Figure 2. Effect of kolaviron extract from Garcinia kola seed on uric acid concentration (n= 5, mean ± SEM). Superscript on each bar denotes significant difference ($p < .05$) from Control group (C). C: Control (20% tween 20), KOL: kolaviron (100 mg/kg b.w.), ISO: Isoniazid (20 mg/kg b.w.), ISO+KOL: Isoniazid (20 mg/kg b.w.) + Kolaviron (100 mg/kg b.w.), ISO + Vit. C: Isoniazid (20 mg/kg b.w.) + Vitamin C (100 mg/kg b.w.).



169

170 Figure 3. Effect of kolaviron extract from Garcinia kola seed on urea concentration (n= 5,
171 mean ± SEM). Superscript on each bar denotes significant difference ($p < .05$) from Control
172 group (C). C: Control (20% tween 20), KOL: kolaviron (100 mg/kg b.w.), ISO: Isoniazid (20
173 mg/kg b.w.), ISO+KOL: Isoniazid (20 mg/kg b.w.) + Kolaviron (100 mg/kg b.w.), ISO + Vit.
174 C: Isoniazid (20 mg/kg b.w.) + Vitamin C (100 mg/kg b.w.).

175



176

177 Figure 4. Effect of kolaviron extract from Garcinia kola seed on creatinine concentration (n=
178 5, mean ± SEM). Superscript on each bar denotes significant difference ($p < .05$) from
179 Control group (C). C: Control (20% tween 20), KOL: kolaviron (100 mg/kg b.w.), ISO:
180 Isoniazid (20 mg/kg b.w.), ISO+KOL: Isoniazid (20 mg/kg b.w.) + Kolaviron (100 mg/kg
181 b.w.), ISO + Vit. C: Isoniazid (20 mg/kg b.w.) + Vitamin C (100 mg/kg b.w.).

182

183

184 4.2. Discussion

185 Singhal et al. [31] and Lee et al. [32] indicated that drug metabolites excreted during and after
186 drug transformation and activation in the body are responsible for cellular damage which lead
187 to kidney dysfunction. This is due to the substantial amount of blood supply, ensuring a high
188 level of xenobiotic delivery over a period of time to the kidney and predisposes it to
189 nephrotoxicity which therefore enhances its vulnerability to developing various forms of
190 injury [33,34]. Serum creatinine has been reported to be an important kidney function test
191 used to monitor the progression of renal disease. As a by-product of muscle metabolism
192 which is excreted unchanged through the kidney, whenever there is kidney damage, filtration
193 fails and creatinine blood level rises [35,36]. From the study, isoniazid was observed to cause
194 lethal kidney damage which resulted to a high level of creatinine. According to Edmund and
195 David [37], renal failure is usually speculated when there is a higher level of creatinine than
196 the upper normal control limit. Pretreatment with kolaviron however attenuated the increase
197 resulting in a drop in creatinine concentration. The clearance of creatinine as indicated in
198 kolaviron-treated group showed the protective effect of kolaviron against isoniazid-induced
199 kidney damage.

200 The importance of urea has been identified for several decades [38]. Urea serves as nitrogen
201 pool which prevents nitrogen in circulating proteins. The synthesis and release of nitrogen
202 changes in response to the level of both dietary and endogenous proteins [39]. Hence,
203 functional role of urea includes the metabolism of nitrogen-containing compounds by animals
204 and it serves as the major nitrogen-containing substance in the urine. As a result of this, the
205 body uses it in many processes, most notably for nitrogen excretion. However, elevated
206 kidney urea concentration indicates a dysfunctional kidney [40]. From the study, isoniazid
207 administration caused a significant increase ($p < .05$) in urea concentration. Mitchell and
208 Kline [41] has previously suggested the relationship between renal function and urea serum
209 level and implicated increased blood urea nitrogen-creatinine ratio in acute renal failure and
210 pre renal condition. Several pathological conditions including kidney disease, blockage of the
211 urinary tract (kidney stone), congestive heart failure, dehydration, fever, shock and bleeding
212 in the digestive tract have been attributed to increased blood urea nitrogen [42]. Conversely,
213 administration of kolaviron protected the kidney from affront caused by isoniazid, by
214 bringing down the concentration of urea as a result of reabsorption of nitrogen in the blood.

215 Until recently, uric acid relevance in chronic kidney diseases (CKD) has been viewed with
216 less interest. It has however revived as a contributory risk factor in the pathogenesis and
217 progression of CKD [43]. It has been reported that high level uric acid suggests CKD while
218 lowering the uric acid level slows down the progression of chronic kidney diseases. The
219 study showed that isoniazid increased the uric acid concentration thereby indicating renal
220 damage. Administration of kolaviron however lowered uric acid level, even more than the
221 standard drug, vitamin C. Although, vitamin C also showed some protective effect, since it is
222 an antioxidant, but kolaviron showed a more observable change indicating that it is more
223 potent than the vitamin C.

224 Several studies have implicated increased total protein excretion in renal diseases. Dietary
225 protein can modulate renal function and thus, consumption of dietary protein in excess of
226 recommended amounts promotes chronic renal disease through increased glomerular pressure
227 and hyper-filtration [45]. When kidneys are not functioning properly, protein may escape

from the blood into the urine. The high concentration level of total protein excreted is accompanied by simultaneous reduction in plasma total protein concentration [46]. The study revealed the protective effect of kolaviron in upregulating total protein concentration which has become impaired by administration of isoniazid. Conversely, the effect of kolaviron itself on the kidney showed that this extract has no harmful effect on the kidney as the total protein, creatinine, urea and uric acid levels of the group treated with kolaviron only (KOL) compared reasonably well to that of the control group. In addition, the pretreatment of the animals with kolaviron before inducing kidney damage with isoniazid (ISO + KOL) showed the protective effect of the plant extract in preventing renal disease or damage.

5. Conclusion

Elevated kidney function biomarkers such as urea, uric acid and creatinine in addition to decreased total protein levels indicate kidney damage caused by isoniazid administration. Administration of kolaviron however revealed the efficacy of the plant in protecting the kidney against isoniazid-induced damage. Hence, kolaviron extract obtained from *Garcinia kola* seeds exhibited a protective effect against isoniazid-induced kidney toxicity and it may be relatively safe when used therapeutically at this dose in the treatment and management of diseases associated with kidney damage.

Conflict of interest

Authors have declared that no competing interests exists.

References

1. Sia IG, Wieland ML. Current concepts in the management of tuberculosis. Mayo Clin Proc. 2011;86:348-61.
2. World Health Organization. Global Tuberculosis Report. 2018:12
3. Zumla A, Raviglione M, Hafner R, Von Reyn C. Current concepts Tuberculosis. New Engl J M,ed 2013.
4. Singh R, Manjunatha U, Boshoff HI). PA-824 kills non-replicating Mycobacterium tuberculosis by intracellular NO release. Science. 2008;322 (590): 1392-1395.
5. Suarez J, Ranguelova K, Jarzecki AA. An oxyferrous heme/protein-based radical intermediate is catalytically competent in the catalase reaction of Mycobacterium tuberculosis catalase-peroxidase (KatG). J Biol Chem. 2009;284 (11): 7017-7029.
6. Judd FK, Mijch AM, Cockram A, Norman TR. Isoniazid and antidepressants: is there cause for concern. Int Clin Psychopharmacol. 1994;9 (2): 123-125.
7. Nolan CM, Goldberg SV, Buskin SE. Hepatotoxicity associated with isoniazid preventive therapy: a 7-year survey from a public health tuberculosis clinic. JAMA. 1999;281:1014-8.
8. Metushi IG, Cai P, Zhu X, Nakagawa T, Uetrecht JP. A fresh look at the mechanism of isoniazid-induced hepatotoxicity. Clin Pharmacol Ther. 2011;89:911-4.
9. Boelsterli UA, Lee KK. Mechanisms of isoniazid-induced idiosyncratic liver injury: emerging role of mitochondrial stress. J Gastroenterol Hepatol. 2014;29:678-87.

10. Carlson HB, Anthony EM, Russell WF Jr, Middlebrook G. Prophylaxis of isoniazid neuropathy with pyridoxine. N Engl J Med. 1956;255:118-22.
11. Centers for Disease Control and Prevention (CDC). Severe isoniazid- associated liver injuries among persons being treated for latent tuberculosis infection-United States, 2004-2008. MMWR Morb Mortal Wkly Rep. 2010;59:224-9.
12. Black M, Mitchell JR, Zimmerman HJ, Ishak KG, Epler GR. Isoniazid-associated hepatitis in 114 patients. Gastroenterology. 1975;69:289-302.
13. Maddrey WC, Boitnott JK. Isoniazid hepatitis. Ann Intern Med. 1973;79:1-12.
14. Metushi IG, Nakagawa T, Uetrecht J. Direct oxidation and covalent binding of isoniazid to rodent liver and human hepatic microsomes: humans are more like mice than rats. Chem Res Toxicol. 2012;25:2567-76.
15. Richards VE, Chau B, White MR, McQueen CA. Hepatic gene expression and lipid homeostasis in C57BL/6 mice exposed to hydrazine or acetylhydrazine. Toxicol Sci 2004;82:318-32.
16. Lee KK, Fujimoto K, Zhang C, Schwall CT, Alder NN, Pinkert CA, et al. Isoniazid-induced cell death is precipitated by underlying mitochondrial complex I dysfunction in mouse hepatocytes. Free Radic Biol Med. 2013;65:584-94.
17. Greaves P. Histopathology of Preclinical Toxicity Studies: Interpretation and Relevance in Drug Safety Evaluation. 4th Ed. Elsevier Science; 2011.
18. Emeigh-Hart SG, Kinter LP. Assessing Renal Effects of Toxicants in vivo. Toxicology of the kidney, 3rd Ed, Boca Raton: CRC Press; 2005.
19. Lohr JW, Willsky GR, Acara MA. Renal drug metabolism. Pharmacol Rev. 1998; 50: 107-141.
20. Kodner CM, Kudrimoti A. Diagnosis and management of acute interstitial nephritis. Am Fam Physician. 2003;67(12): 2527-2534.
21. Markowitz GS, Perazella MA. Drug-induced renal failure: a focus on failure in the tubulointerstitial disease. Clinica Chimica Acta. 2005;351: 31-47.
22. Arroyo V, Gines P, Gerbes AL, Dudley FJ, Gentilini P, Laffi G, et al. Definition and diagnostic criteria of refractory ascites and hepatorenal syndrome in cirrhosis. International Ascites Club. Hepatology. 1996;23:164-176.
23. Mathew OW, Blessing CD. Hepatoprotective effects of Garcinia kola seed against hepatotoxicity induced by carbon tetrachloride in rats. Biochemistry. 2007;19 (1): 17-21.
24. Alade A, Ani RE. Protective effects of Garcinia kola seed extract against paracetamol-induced hepatotoxicity in rats. J Ethnopharmacol. 1995; 29(2): 207-211.
25. Farombi EO. Mechanisms for the hepatoprotective action of kolaviron: studies on hepatic enzymes, microsomal lipids and lipid peroxidation in carbon tetrachloride-treated rats. Pharmacol. Res. 2000; 42:75-80.
26. Udenze EC, Braide VB, Okwesilieze CN, Akuodor GC. Pharmacological effects of Garcinia kola seed powder on blood sugar, lipid profile and atherogenic index of alloxan-induced diabetes in rats. Pharmacologia. 2012;3(12):693-699.
27. Onasanwo SA, Singh N, Olaleye SB, Palit G. Anti-ulcerogenic and proton pump (H+, K+ ATPase) inhibitory activity of Kolaviron from Garcinia kola Heckel in rodents. Indian J Exp Biol. 2011;49 (6): 461-468.
28. Sridharan B, Micheal TS, Ramachandran A, Ganesh NR, Pragasam V. Citrus Bioflavonoids Ameliorate Hyperoxaluria Induced Renal Injury and Calcium Oxalate Crystal Deposition in Wistar Rats. Adv Pharm Bull. 2015; 5(3): 419-427.

29. Ademola OA, Bester D, Esterhuyse J, Farombi EO. Kolaviron, a biflavonoid of Garcinia kola seed mitigates ischemic/reperfusion injury by modulation of pro-survival and apoptotic signaling pathways. J Intercult Ethnopharmacol. 2017; 6(1): 42-49
30. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the Folin Phenol reagent. J Biol Chem. 1951;193(1):265-75
31. Singhal PC, Sharma P, Sanwal V, Prassad A, Franki N, Reddy K, et al. Morphine modulates proliferation of kidney fibroblasts. Kidney Int. 1998; 53:350-357.
32. Lee CY, Cooksey BA, Bachrecke EH. Steroid regulation of midgut cell death during Drosophilia development. Development of Biology. 2002; 250(1): 101-111.
33. Karie S, Launay V, Deray G, Isnard C. Drugs renal toxicity. Nephrol Ther. 2010; 6(1): 58-74.
34. Almeida AF. Drug-induced renal disease: Prevention strategies. Med Update: 2005; 637-641.
35. Allen PJ. Creatine metabolism and psychiatric disorders: Does creatine supplementation have therapeutic value? Neurosci Biobehav Rev. 2012; 36 (5): 1442-62.
36. Shemesh O, Golbetz H, Kriss J, Myers B. Limitations of creatinine as a filtration marker in glomerulopathic patients. Kidney Int. 1985; 28(5): 830-8.
37. Edmund L, David J. Kidney function tests. In: Carl AB, Edward R, David E, editors. Tietz Textbook of clinical chemistry and molecular diagnostics. 4th ed. New Delhi: Elsevier Inc; 2006: 797–808.
38. Gamble JL, Mckhann CF, Butler AM, Tuthill E. An economy of water in renal function referable to urea. Am J Physiol. 1934; 109:139-154.
39. David IW, William EM, Jeff MS. Urea and ammonia metabolism and the control of renal nitrogen excretion. Clin J Am Soc Nephro. 2015;10:1444-1458.
40. Santhosh S, Sini T, Anandan R, Mathew P. Hepatoprotective activity of chitosan against isoniazid and rifampicin induced toxicity in experimental rats. Eur. J. Pharmacol. 2007;572: 69–73.
41. Mitchell HR, Kline W. Core curriculum in nephrology, Renal Function Testing. Am J Kidney Dis. 2006 47:174–183.
42. Pagana KD. Mosby's Manual of Diagnostic and Laboratory Tests. St. Louis Mosby, Inc., and Rebecca J.F Gale Encyclopedia of Medicine. 1998
43. Domrongkitchaiporn S, Sritara P, Kitiynkara C. Risk factor for development of decreased kidney function in a Southeastern Asian population: a 12year cohort study. J Am Soc Nephro 2005;16(3):791-799.
44. Metges CC, Barth CA. Metabolic consequences of a high dietary protein intake in adulthood of available evidence. J Nutr. 2000; 130: 886-889.
45. Shihabi ZK, Konen JC, Connor ML. Albuminuria vs Urinary Total Protein for Detecting Chronic Renal Disorders. Clin Chem. 1991; 37(5): 621-624.