HEPATOPROTECTIVE AND NEPHROPORTECTIVE EFFECTS OF THE AQUEOUS EXTRACT OF TURMERIC (CURCUMA LONGA) IN RIFAMPICIN AND ISONIAZID-INDUCED HEPATOTOXICITY AND NEPHROTOXICITY IN RATS

MAHDI M THUAWAINI1*, MAWAHIB B GASIM AL-FARHAAN2, KARIMA F ABBAS3
1Department of Pathological Analysis, College of Health and Medical Sciences/Southern Technical University, Basrah, Iraq.
2,3Department of Basic Sciences, College of Applied Medical Sciences, Karbala University, Karbala, Iraq. Email: Mahdi.Murshd@stu.ed.iq

INTRODUCTION
The metabolic processes of the human body are controlled and managed by two vital organs, the liver and kidney [1,2]. The liver and kidney actively metabolized numerus of drugs, hormones, and xenobiotics and maintain our systems. The liver is a main organ in the body and is responsible for the metabolism of internal and external agents. It plays a vital role in drug metabolism and detoxification. Liver injury may be caused by xenobiotic, alcohol consumption, malnutrition, infection, anemia, and medications [3]. Moreover, the liver is expected to be susceptible, especially to drugs and chemicals. Renal system is essential to maintain homeostasis, regulating water and electrolyte balance, and acid-base maintenance, among other crucial functions and also possessed an endocrine function [4]. However, the kidney is the well-known target of toxicity of therapeutic and environment xenobiotics, due to its high blood flow, tubular transport processes, and complex metabolic activities [5]. The drug-induced nephrotoxicity is manifested functionally by a decline in urine concentration, tubular proteinuria, lysosomal enzyme-urea, and declining glomerular filtration rate [2]; furthermore, drug-induced hepatic and nephritic injury is the most common cause for withdrawal of drugs.

However, antitubercular drugs, especially isoniazid and rifampicin (RIF)-induced hepatotoxicity, range from a non-specific elevation of transaminases to fulminant of liver failure. Furthermore, the incidence of hepatic dysfunction is more, when isoniazid (INH) and RIF are used in combination [9]. They were also induced renal toxicity [6-9].

Many medicinal plants showed hepato- and reno-protective effects [10-12], these included Agrimonia eupatoria [13], Althagi maurorum [14], Allium sativum [15], Anchusa strigosa [16], Arctium lappa [17], Astragalus hamosus [18], Bauhinia variegata [19], Brassica nigra [20], Brassica rapa [21], Byrsonima dioica [22], Cannas indica [22], Bryophyllum calycinum [23], Caesalpinia crista [24], Calendula officinalis [25], Capparis spinosa [26], Capsella bursa-pastoris [27], Carthamus tinctorius [31], Carum carvi [32], Cassia occidentalis [33], Cassia equisetifolia [34], Celosia cristata [35], Chenopodium album [36], Cicer arietinum [37], Cichorium intybus [38], Citrullus colocynthis [39], Citrus species [40], Cleistocalycum natae [41], Convolvulus arvensis [42], Cordia myxa [43], Coriandrum sativum [44], Crocus sativus [45], Crotalaria juncea [46], Cuminum cyminum [47], Cupressus sempervirens [48], Curcuma longa [49], Cymbopogon schoenanthus [50], Cynodon dactylon [51], Cyperus rotundus [52], Daucus carota [53], Digitalis species [54], Dodonaea viscosa [55], Ephedra species [56], Equisetum arvense [57], Eupatorium cannabinum [58], Fumaria parviflora [59], Galium verum [60], Helianthus annuus [62], Hibiscus cannabinus [63], Hypericum triquetrifolium [64], Juniperus communis [65], and Jussiaeae repens [66]. This study was designed to investigate the hepatorenoprotective effects of turmeric (C. longa) in experimentally rifampin- and isoniazid-induced hepatotoxicity and nephrotoxicity in rats.

MATERIALS AND METHODS
Experimental animals
A total of 80 Wistar albino adult male rats (Rattus norvegicus), 150-250 g, were used in this study. They were gained from the laboratory animal house, College of Science, Thi-Qar University. The animals were dwelled individually in well-ventilated polypolyene cage, in conditioned room [at temperature (22±3°C), a 12-h light/dark cycle...
and relative humidity were maintained in the room. Diet and water were allowed ad libitum. At temperature (22±3°C), a 12-h light/dark cycle and relative humidity were maintained in the room.

Chemicals and plant extract
Kidney and liver function tests were evaluated using an enzymatic kit (Biomedicals Pvt. Ltd.). RIF and INH were purchased from Sigma Chemicals, and all other chemicals used for this study were of analytical grade. Fine crude ground turmeric (C. longa) was obtained from the local market of Basrah city, Iraq, and diagnosed by the plant herbarium of the College Of Science, Thi-Qar University.

Experimental design
A total of 48 male albino rats were randomly divided into 6 groups: Control, INH + RIF treated turmeric 100 mg/kg without induction of hepato- and reno-toxicity, turmeric 100 mg/kg + RIF and INH, turmeric 100 mg/kg without induction of hepato- and reno-toxicity, and turmeric 200 mg/kg + RIF and INH. The turmeric aqueous extract and INH + RIF (50 mg/kg bw po, daily) were given for 4 weeks [67,68].

Blood sampling and estimation of biochemical parameters
About 24 h after the last doses of the treatments, the rats were anesthetized lightly by chloroform inhalation and then killed by neck dislocation, and then blood samples were collected by heart puncture; each sample was kept into non-heparinized tubes. The blood of non-heparinized tubes was abandoned at room temperature for 30 min and then was centrifuged at 3000 rpm for 15 min, and the sera were kept in deep freeze (-20 °C) until the biochemical examination [aspartate transaminase (AST), alanine transaminase (ALT), alanine phosphatase (ALP), bilirubin, urea in the blood, creatine in the blood, Total protein] were performed.

Histopathological assays
Samples of liver and kidney were taken from the treated and control rats and were prepared for light microscopic study according to routine histotechnical techniques.

Statistical analysis
Student t-test was used to determine the significance among the treated group in comparison with positive and negative controls [69].

<table>
<thead>
<tr>
<th>Groups description</th>
<th>S. ALT IU/L</th>
<th>S. AST IU/L</th>
<th>S. ALP IU/L</th>
<th>Total bilirubin mg/dl</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1: Normal control</td>
<td>59.9±2.31</td>
<td>98.8±4.34</td>
<td>247.4±1.22</td>
<td>2.01±0.22</td>
</tr>
<tr>
<td>Group 2: INH+RIF treated rats</td>
<td>158.8±26</td>
<td>124.4±11.23</td>
<td>393.3±30.4</td>
<td>4.06±0.51</td>
</tr>
<tr>
<td>Group 3: Turmeric aqueous extract 100 mg/kg treated rats</td>
<td>62.7±3.34</td>
<td>101±3.26</td>
<td>234.4±6.34</td>
<td>1.97±0.05</td>
</tr>
<tr>
<td>Group 4: Turmeric aqueous extract 100 mg/kg + INH and RIF treated rats</td>
<td>13.8±2.98</td>
<td>11.2±2.34</td>
<td>35.5±3.83</td>
<td>2.82±0.04</td>
</tr>
<tr>
<td>Group 5: Turmeric aqueous extract 200 mg/kg treated rats</td>
<td>131±2.26</td>
<td>99.2±2.34</td>
<td>33.3±2.16</td>
<td>2.42±0.28</td>
</tr>
<tr>
<td>Group 6: Turmeric aqueous extract 200 mg/kg+INH and RIF treated rats</td>
<td>65.76±2.98</td>
<td>109.34±8.44</td>
<td>231.33±2.44</td>
<td>3.98±0.08</td>
</tr>
</tbody>
</table>

All values expression by mean±SD, (n=8 in each group). Groups 2, 3, and 5 were compared with Group 1 (normal control) and Groups 4 and 6 were compared with Group 2: Different letters mean significant, NS: Not significant. SD: Standard deviation, AST: Aspartate transaminase, ALT: Alanine transaminase, ALP: Alanine phosphatase, RIF: Rifampicin, INH: Isoniazid

RESULTS

Biochemical investigation
According to biochemical investigation, Group 3 treated by turmeric aqueous extract 100 mg/kg and Group 5 treated by turmeric aqueous extract 200 mg/kg for 28 days showed no significant changes compared with Group 1 (control group) in the serum levels of ALT, AST, ALP, and total bilirubin (Table 1) and serum creatinine, urea, and total protein (Table 2) which clearly indicate that both doses of turmeric aqueous extract are safe. However, in comparison with INH- and RIF-treated group (Group 2), both doses of turmeric aqueous extract significantly decreased the elevated serum levels of ALT, AST, ALP, and total bilirubin (Table 1) and serum creatinine, urea, and total protein (Table 2); however, the level of these biochemical parameters significantly stayed more than normal limits.

Histological investigation
The histopathological study revealed that the sections in the liver of rats from control group showed normal cellular architecture with distinguished hepatic cells, sinusoidal spaces, and central vein showing polyhedral hepatocytes and clear-cut hepatic structural architecture regulated in strands around the central vein (Fig. 1).

On the other hand, sections in livers of rats treated with INH and RIF showed massive, cellular necrosis, vacuolization, and ballooning degeneration displayed hard damage in the hepatic architecture. Most of the hepatocytes fused together forming eosinophilic syncytial masses, part of multiple areas, fine-blush in color indicated calcifications. Furthermore, histological exam showed dilatation of hepatic sinusoid, severe degeneration of hepatocytes which appeared with huge vacuolated cytoplasm and inconspicuous cell outlines and the infiltration of inflammatory cells mostly around the portal vein (Fig. 2).

<table>
<thead>
<tr>
<th>Treatment groups</th>
<th>Serum creatinine mg/dl</th>
<th>Serum urea g/dl</th>
<th>Total protein g/dl</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1: Normal control</td>
<td>1.92±0.06</td>
<td>3.73±0.36</td>
<td>7.75±0.18</td>
</tr>
<tr>
<td>Group 2: INH+RIF treated rats</td>
<td>3.19±0.22</td>
<td>4.63±0.51</td>
<td>6.83±0.12</td>
</tr>
<tr>
<td>Group 3: Turmeric aqueous extract 100 mg/kg treated rats</td>
<td>2.51±0.01</td>
<td>3.90±0.08</td>
<td>7.69±0.10</td>
</tr>
<tr>
<td>Group 5: Turmeric aqueous extract 200 mg/kg treated rats</td>
<td>2.90±0.30</td>
<td>3.49±0.05</td>
<td>7.70±0.12</td>
</tr>
<tr>
<td>Group 6: Turmeric aqueous extract 200 mg/kg+INH+RIF treated rats</td>
<td>2.09±0.14</td>
<td>3.51±1.28</td>
<td>6.72±0.43</td>
</tr>
</tbody>
</table>

All values expression by mean±SD, (n=8 in each group), Groups 2, 3, and 5 were compared with Group 1 (normal control) and Groups 4 and 6 were compared with Group 2: Different letters mean significant, NS: Not significant. SD: Standard deviation, RIF: Rifampicin, INH: Isoniazid
Some of the liver sections of rats treated with turmeric aqueous extract (100 and 200 mg/kg) showed normal appearance of hepatocytes and central vein, hepatocytes appeared intact with slight dilatation of central vein, and the portal area showed moderate infiltration of mononuclear leukocytes inflammatory cells compared to isoniazid and rifampicin treated rat. Normal condition (angiogenesis) as noted in the control group (Fig. 4).

Conversely, examination of histopathological sections of kidneys revealed that the control group showed normal renal architecture characterized by normal glomerular and tubular histology, i.e., there were no observable changes in the kidney tissue morphology (Fig. 5).

Sections in the kidney of rats treated with INH and RIF showed severe changes evidenced by thickening and vacuolations in the wall of renal blood vessels, perivascular polymorphonuclear cells infiltration simultaneously evident renal casts, conspicuous vacuolations of the glomerular tufts joined and huge cellularity of the glomerulus tufts, areas of necrosis in renal tubular tissue and cell nuclei pyknosis, and focal of interstitial nephritis infiltrated areas with inflammatory cells mostly mononucleosis with dilatation, edema, and inflammation of tubules (Fig. 6).

Sections in the kidney of rats treated with INH and RIF with turmeric (C. longa) aqueous extract (100 and 200 mg/kg) significantly showed relieved severity of renal lesions and renal tubular injury, reduced dilatation and inflammations of tubules as well as normal renal parenchyma with minimal congestion of blood vessels (Fig. 7).

Some sections in the kidney of rats treated with turmeric (C. longa) aqueous extract (100 and 200 mg/kg) showed that tubule reveals slight degenerative change, almost normal appearance of glomeruli and tubules as a control group (Fig. 8).
on acetaminophen-induced hepatotoxicity in

The elevation in the enzymatic activities of ALT, AST, and ALP can be matched with previous studies [74]. Activities of enzymes (ALT, AST, and ALP) in the serum. The elevation of INH and RIF drugs to the rat tissues was also noticed by elevated to oxidative stress in the cells [73].

membrane safety is lost as a result of lipid peroxides generation, due oxidative stress in the cells [73]. However, the deleterious effect of INH and RIF drugs to the rat tissues was also noticed by elevated activities of enzymes (ALT, AST, and ALP) in the serum. The elevation of these enzymes in our study was matched with previous studies [74].

DISCUSSION

The present study was carried out to evaluate the effect of turmeric (C. longa) aqueous extracts on the renal and hepatic functions. INH and RIF which were used as anti-tuberculosis drugs (ATD) were not free from side effects [70]; it was associated with hepato- and reno-toxicity in some individuals [71].

Fig. 6: Sections in the kidney of rats treated with isoniazid and rifampicin (induction group) showing histological changes as evidenced by thickening and vacuolations in the wall of renal blood vessels, perivascular polymorphonuclear cells infiltration simultaneously evident renal casts, conspicuous vacuolations of the glomerular tufts joined and huge cellularity of the glomerulus tufts, areas of necrotic renal tubular tissue and cell nuclei pyknosis, and focal of areas of interstitial nephritis infiltrated with inflammatory cells infiltration mostly mononucleosis. Dilatation (Hx and E, ×400)

Fig. 7: Sections in the kidney of rats treated with isoniazid and rifampicin and turmeric (Curcuma longa) aqueous extract (100 and 200 mg/kg) showed significantly relieved severity of renal lesions and renal tubular injury in addition to the reduction in dilatation and inflammations of tubules (Hx and E, ×200)

Fig. 8: Sections in the kidney of rats treated with turmeric (Curcuma longa) aqueous extract (100 and 200 mg/kg) showing tubule with slight degenerative change, almost normal appearance of glomeruli and tubules

Oxidative stress is the main attribution mechanism beyond ATD-induced hepatotoxicity [72]. Where, the hepatocytes membrane safety is lost as a result of lipid peroxides generation, due oxidative stress in the cells [73]. However, the deleterious effect of INH and RIF drugs to the rat tissues was also noticed by elevated activities of enzymes (ALT, AST, and ALP) in the serum. The elevation of these enzymes in our study was matched with previous studies [74]. The elevation in the enzymatic activities of ALT, AST, and ALP can be considered as an important index for the diagnosis of liver diseases because these increases were attributed to increased lipid peroxidation, in such case, marked alterations in the molecular organization of lipids was occurred in the cellular membranes which increased membrane permeability and facilitated leakage of cytoplasmic markers into circulation [75].

In the previous studies, it was found that serum creatinine and serum urea were dramatically enhanced in INH- and RIF-treated animals [76,77]. Similarly, in the present study, the biochemical evaluation showed increased levels of serum creatinine and serum urea in the INH and RIF group compared with the control group. Elevation of serum creatinine and serum urea could be attributed to retention of ATD in nephrons causing severe injury [78].

Due to the involvement of oxidative stress in the mechanisms of renal and hepatic injury, the antioxidant properties of medicinal plants were involved in the mechanism of their hepatoprotective activity. They inhibited hepatic oxidative stress by many mechanisms. Curcumin, the major phenolic compound in turmeric, which shows preventive effects in various diseases, possessed antioxidant effects and inhibited extracellular matrix formation by enhancing matrix metalloproteinase expression through peroxisome proliferator-activated receptor gamma and by suppressing connective tissue growth factor expression [79,80].

Many medicinal plants produced hepatoprotective effects through their anti-inflammatory activity and attenuation of many inflammatory processes [81].

Curcumin also induced downregulation of cyclooxygenase-2 expression which is involved in acute and chronic inflammation, hemodynamics, tumorigenesis, renal function, and hepatic fibrogenesis [82].

With regard to histopathological alterations in the rats treated with INH and RIF drugs (Group 2) or associated with the use of C. tinctorius extract with INH and RIF, were similar to the previous studies [83-86].

We can conclude that the hepatoprotective of the aqueous extract of C. tinctorius could be attributed to its antioxidant and anti-inflammatory effects.

CONCLUSION

The results of the present study indicate that turmeric has hepatoprotective actions against RIF- and INH-induced hepatic and renal injury in rats.

ETHICS APPROVAL

The experimental work and uses of animals were performed according to Local Regulations of Animal House, Science College, Thi-Qar University.

AUTHORS’ CONTRIBUTIONS

Mahdi M. Thuwaini (single author) drafted and approved the manuscript.

CONFLICTS OF INTEREST

The authors confirm that this paper’s content has no conflict of interests.

REFERENCES


4. Ligha AE, Jaja B, Numbere NF. Protective effect of Abrus precatorius

296
27. Al-Snafi AE. The chemical constituents, pharmacological and therapeutic importance of Curcuma longa - A review. IOSR J Pharm 2017;7:31-42.


