Prevention of Liver Injury by Moringa oleifera aqueous leaf extract in rats treated with isoniazid and rifampicin

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INTRODUCTION

Drug-induced liver diseases are diseases of the liver that are caused by physician-prescribed medications, over-the-counter medications, vitamins, hormones, herbs, illicit (recreational) drugs, and environmental toxins.[1] Drug-induced liver injury is now the most common cause of acute liver failure. It is the cause of 25% of hospitalisations for jaundice and accounts for 10% of cases of acute hepatitis.[2]

Tuberculosis, caused by Mycobacterium tuberculosis is one of the most common and deadly infectious diseases with nearly one-third of the global population infected and 1.7 million deaths reported in 2006.[3] The most effective anti-tuberculosis therapy (standard therapy) is a combination of isoniazid (INH), rifampicin (RMP), and pyrazinamide (PZA) for eight weeks, followed by INH and RMP for a further 4-7 months.[4] However, there is much evidence of toxicity of these standard drugs in humans,[5-7] with hepatotoxicity being the most serious effect.[8]

Moringa oleifera commonly known as drumstick tree or moringa is an important foodstuff in human nutrition worldwide. The whole plant has been reported to have medicinal benefits.[9] Previous studies have found moringa to have anticancer,[10] antidiabetic,[11] antipyretic,[12] anthelmentic[13] and abortifacient activities.[14] The leaves have demonstrated antioxidant and free radical scavenging activities.[15] Moringa is also used as anti-ulcer treatment.[16] The root is laxative, expectorant, diuretic and good for obstinate asthma.[17] The bark is useful in heart complaints, eye diseases, dyspepsia and enlargement of spleen.[9] Scientific research confirms that moringa leaves contain seven times the vitamin C in oranges, four times the calcium in milk, four times the vitamin A in carrots, two times the protein in milk and three times the potassium in bananas.[18] In view of the many potentials of moringa in nutrition and medicine, this study was undertaken to investigate the preventive role of Moringa oleifera in hepatotoxicity caused by anti-tubercular drugs.

ABSTRACT: Background: Moringa oleifera has been reported to have a lot of therapeutic potential such as antidiabetic, hypocholesterolemic and hypotensive. Objective: This work was undertaken to study the effects of pretreatment and co-administration of aqueous extract of Moringa oleifera leaves on anti-tubercular drug hepatotoxicity in rats. Materials and Methods: Wistar albino rats were divided into six groups of three rats each. Group A was normal control. Group B was the test control administered with anti-tubercular drugs isoniazid (27 mg/kg bw) and rifampicin (54 mg/kg bw) to induce liver damage. Groups C and D were pretreated with 100 mg/kg bw and 200 mg/kg bw of the extract for four weeks, then administered with isoniazid and rifampicin at doses 27 mg/kg bw and 54 mg/kg bw, respectively for same duration. Groups E and F were administered with the same doses of the extracts and anti-tubercular drugs concurrently for four weeks. Serum levels of alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP) and unconjugated bilirubin (uBIL) were analysed to determine the effect of the extract on hepatotoxicity caused by the drugs. Results: Oral pretreatment and co-administration of aqueous extract of Moringa oleifera leaves significantly (P ≤ 0.05) reduced serum ALT, AST and ALP activities, and uBIL concentration compared to the test control. Conclusion: The results suggest the potential protective effect of aqueous extract of Moringa oleifera on anti-tubercular drug hepatotoxicity in rats.

KEYWORDS: hepatotoxicity, isoniazid, liver damage, Moringa oleifera, rifampicin
MATERIALS AND METHODS

Drugs
Isoniazid tablets BP (300 mg) Microlabs Ltd, India and rifampicin capsules BP (300 mg) Maxheal Pharmaceuticals, India were purchased from a pharmacy shop.

Plant Collection and Extraction
Leaves of *Moringa oleifera* were collected in August, 2010 around Gadani village, in Igabi local government area of Kaduna state. The leaves were washed and dried under the shade, then pulverised into powder. Five grams of the powder was soaked overnight in 1000 ml of distilled water and filtered. The residue was dried and reweighed to determine the mass of dried moringa extract, which was used for the experimental work.

Animals
Wistar albino rats (male and female) weighing 110-140 g were obtained from the Animal House, Department of Biological Sciences, Bayero University, Kano. The males were separated from females and housed in cages in a room where a 12-hour light/dark cycle was maintained. They were maintained on water and feed ad libitum throughout the experimental period. Guidelines of Institutional Animal Ethical Committee (IAEC) were observed in the course of the study.

Study Design
Eighteen male and female wistar albino rats were divided into 6 groups of three rats each. Group A were the normal rats without treatment, while group B served as the test control administered with INH and RMP only for four weeks. Groups C and D were pretreated with 100 mg/kg bw and 200 mg/kg bw of moringa extract respectively for four weeks then administered with INH (27 mg/kg bw) and RMP (54 mg/kg bw) for another four weeks. Groups E and F received the same doses of the extract and drugs concurrently for four weeks.

Twenty four hours after the last administration, blood samples were collected from all the experimental animals. Serum alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP) activities and unconjugated bilirubin (uBIL) concentration were determined.

Biochemical investigations
Serum AST, ALT and ALP activities and uBIL concentration were estimated as per the manufacturer’s instructions, using reagents and kits obtained from Randox Laboratories, UK.

Determination of Serum AST and ALT
To estimate serum AST, a reagent blank was prepared by mixing 0.5 ml of Reagent 1 with 0.1 ml of distilled water. For serum samples, 0.1 ml of each sample was mixed with 0.5 ml of Reagent 1. The mixtures were incubated at 37 °C for 30 minutes, then 0.5 ml of Reagent 2 was added to all test tubes and allowed to stand for 20 minutes at 22 °C. Sodium hydroxide (0.4 mol/l, 5.0 ml) was added. Absorbance of the samples was read at 546 nm against reagent blank after 5 minutes.

For estimation of ALT, serum samples (0.1 ml) and 0.5 ml of Solution R1 were dispensed into labelled test tubes. Reagent blank was prepared by dispensing 0.5 ml of Solution R1 and 0.1 ml of distilled water. The mixtures were incubated for 30 minutes at 37 °C, then 0.5 ml of Solution R2 was added to all test tubes. The mixtures were allowed to stand for 20 minutes at 22 °C, 5.0 ml of 0.4 mol/l sodium hydroxide was added to both samples and reagent blank. After 5 minutes, absorbance of the samples was read against reagent blank at 546 nm.

Determination of Serum ALP
Alkaline phosphatase substrate (0.5 ml) was dispensed in labelled test tubes designated for each sample and equilibrated to 37 °C, for 3 minutes. At timed intervals, 0.05 ml each of standard, serum and deionised water (for reagent blank) were added to the designated test tubes and mixed gently. The mixtures were incubated for 10 minutes at 37 °C and 2.5 ml of alkaline phosphatase color developer was then added to all test tubes and mixed thoroughly. Absorbance of the colored solutions was measured spectrophotometrically at 590 nm.

Determination of Serum uBIL
Total bilirubin was first estimated. For each sample, 200 µl of Reagent 1, 50 µl of Reagent 2, 1000 µl of Reagent 3 and 200 µl of sample were mixed and allowed to stand for 10 minutes at 23 °C. 1000 µl of Reagent 4 was added to the mixture and allowed to stand for 20 minutes at 23 °C. The absorbance of the sample was read against sample blank (A_{TB}) at 578 nm.

Total bilirubin (µmol/l) = 185 \times A_{TB} (578 nm)

Then, direct bilirubin was estimated. For each sample, 200 µl of Reagent 1, 50 µl of reagent 2, 2000 µl of 0.9% NaCl and 200 µl of sample were mixed in a cuvette and incubated for 10 minutes at 23 °C. The absorbance was read at 546 nm against sample blank (A_{DB}).

Direct bilirubin (µmol/l) = 246 \times A_{DB} (546 nm)

Unconjugated (indirect) bilirubin = Total bilirubin – Direct bilirubin

Statistical Analysis
All results were expressed as mean ± SD for each group. Data was analysed with student’s t-test using SPSS. P values ≤ 0.05 were considered significant.
RESULTS AND DISCUSSION

Increase in the activities of serum ALT, AST and ALP, and uBIL concentration were observed in rats administered with anti-tubercular drugs, INH and RMP i.e. Group B compared with normal rats in Group A (Table 1). These elevations indicate liver damage characteristic of the drugs. Administration of Moringa oleifera leaf extract significantly (P ≤ 0.05) decreased these parameters in groups C-F (Table 1), although the levels were still elevated compared to the normal (untreated) control.

Both pretreatment and co-administration of the extracts lowered all the biochemical parameters monitored in the study and there was no significant difference in the effect of pretreatment as compared to co-administration. Co-administration of 100 mg/kg of the extract in Group E reduced serum AST levels better than the other groups, and pretreatment with 200 mg/kg of the extract in Group D had a more positive effect in lowering serum ALT activity. However, differences in mean values in co-administration compared to the pretreated groups and vice versa were not statistically significant e.g. co-administration of 100 mg/kg of ALP had mean ± SD value of 43.33 ± 1.527, while pretreatment with 100 mg/kg of the extract had mean ± SD value of 41.66 ± 1.527 (mean ± SD of the paired difference was 1.666 ± 2.886 and P = 0.423). This trend was observed in all the parameters analysed in groups C-F.

One of the hallmark signs of hepatic injury is an apparent leakage of cellular enzymes into the plasma. In addition, the extent and type of liver injury or damage can be assessed based on the presence or absence of specific enzymes in the bloodstream. In general, measurement of ALT, AST and ALP are commonly used as markers in assessing hepatotoxicity. The elevation of serum liver enzyme activities observed in this study suggests hepatocellular damage caused by the anti-tubercular drugs, as these enzymes are normally cytoplasmic in location. Due to the central role of the liver in the conjugation of bilirubin, estimation of this parameter will serve as an indicator of liver dysfunction. In the present study, hepatoprotective effect of moringa leaf extract is manifested by the decrease in serum ALT, AST, ALP activities and uBIL level compared to the test control.

The significant decrease in these parameters following pretreatment and co-administration of Moringa oleifera aqueous leaf extract suggests that the plant extract has a role in preserving the structural integrity of hepatocellular membrane, thus preventing leakage of the analysed parameters into circulation as shown in groups C-F administered with the extracts. Moringa leaves have been discovered to contain vitamin E, an antioxidant enzyme. Analysis of the leaf composition have revealed them to have significant quantities of vitamins A, B, and C, calcium, iron and protein. Moringa also contains alkaloids, cinnamates, anthocyanins, quercetin and kaempferol and proanthocyanidins. Previous studies have reported antioxidant effects of methanolic extracts of leaves, stem and root bark of Moringa oleifera. The leaf extract has also been reported to prevent isoproterenol-induced myocardial damage and aspirin-induced mucosal damage. The protective effect of moringa could be attributed to any or a synergy of the many chemical components of moringa which are known to have beneficial effects. Numerous studies now point to the elevation of a variety of detoxification and antioxidant enzymes as a result of treatment with moringa or with phytochemicals isolated from moringa.

From the result of this study, it is apparent that aqueous extracts of Moringa oleifera leaves afford protective effects on anti-tubercular drugs induced hepatotoxicity. This result is consistent with a report on the hepatoprotective action of Moringa oleifera Lam. against acetaminophen induced hepatotoxicity in rats.

Future study should focus on the protective effects of other parts of the plant such as roots, stem, bark, flowers and pods which have all been reported to have medicinal properties. The mechanism of action of Moringa oleifera in the prevention of hepatic injury associated with anti-tubercular drugs should also be investigated.

<table>
<thead>
<tr>
<th>Groups</th>
<th>ALP (IU/L)</th>
<th>AST (IU/L)</th>
<th>ALT (IU/L)</th>
<th>uBIL (µmol/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A: Normal control</td>
<td>29.33 ± 3.60</td>
<td>17.00 ± 9.90</td>
<td>6.00 ± 1.40</td>
<td>2.49 ± 0.49</td>
</tr>
<tr>
<td>B: Test control</td>
<td>51.00 ± 4.20</td>
<td>52.66 ± 4.60</td>
<td>25.33 ± 12.60</td>
<td>7.46 ± 1.80</td>
</tr>
<tr>
<td>C: Pre-treatment (100 mg/kg)</td>
<td>41.66 ± 1.52b</td>
<td>37.66 ± 25.49a,b</td>
<td>11.33 ± 3.51a,b</td>
<td>3.31 ± 2.45a,b</td>
</tr>
<tr>
<td>D: Pre-treatment (200 mg/kg)</td>
<td>41.33 ± 0.13a,b</td>
<td>39.00 ± 13.85a,b</td>
<td>6.66 ± 1.52a,b</td>
<td>4.11 ± 0.92a,b</td>
</tr>
<tr>
<td>E: Co-administration (100 mg/kg)</td>
<td>43.33 ± 1.52b</td>
<td>30.00 ± 5.19a</td>
<td>11.00 ± 3.46a,b</td>
<td>3.80 ± 0.96a,b</td>
</tr>
<tr>
<td>F: Co-administration (200 mg/kg)</td>
<td>45.33 ± 2.30b</td>
<td>41.66 ± 30.23a,b</td>
<td>15.00 ± 3.46a,b</td>
<td>3.43 ± 0.95a,b</td>
</tr>
</tbody>
</table>

n= 3
a = significant (P ≤ 0.05) compared with the normal control group.
b = significant (P ≤ 0.05) compared with the test control group.
CONCLUSION

The present study indicated that pretreatment (at doses; 100 mg/kg bw and 200 mg/kg bw) and co-administration (100 mg/kg bw and 200 mg/kg bw) with aqueous extract of M. oleifera leaves prevented liver injury in rats administered with anti-tubercular drugs. Thus, the plant can serve as a hepatoprotective agent in hepatotoxicity associated with isoniazid and rifampicin.

REFERENCES

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