Influence of rifampicin and tetracycline administration on some biochemical and histological parameters in albino rats

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KEYWORDS
Rifampicin; Tetracycline; Albino rats; Physiological parameters; Histopathological changes; Liver; Kidney

Abstract The present investigation was designed to evaluate the effect of two oral administered antibiotics (Rifampicin and Tetracycline) on some physiological parameters in the serum of male albino rats. Histopathological alterations in liver and kidney were also examined. Data showed that treatment of rats with rifampicin caused a significant increase in the total cholesterol, triglycerides and LDL-cholesterol levels, while HDL-cholesterol level showed a significant decrease. Moreover, significant increases in serum AST, ALT, bilirubin and urea were observed. Also, the levels of the total protein, albumin and alpha 1-globulin were significantly decreased. No significant changes were recorded in the rest of the globulin fractions (alpha 2-, beta- and gamma-globulin) and albumin/globulin ratio (A/G) as well as creatinine level.

In the serum of tetracycline-treated rats, the triglycerides, LDL-cholesterol, AST, ALT, bilirubin, urea, creatinine and gamma-globulin levels increased significantly. On the other hand, HDL-cholesterol, total protein, albumin levels and A/G ratio showed significant decreases. No significant changes in the total cholesterol, alpha 2- and beta-globulin levels were detected.

Histological examination of the liver and kidney in the rifampicin-treated rats indicated that the liver pathology includes necrosis of hepatocytes, cytoplasmic vacuolation, and distended sinusoids with some lymphatic aggregations. In the kidney, the glomeruli increased in size, the mesangial matrix was expanded and the renal tubules were degenerated. Histological analysis of liver samples of tetracycline – treated rats revealed high vacuolation of the cytoplasm of hepatic cells, sinusoidal dilation, hepatocellular necrosis and disappearance of the cell membrane in some hepatocytes. The kidney sections of rats treated with tetracycline showed shrinkage of the glomeruli, widening of the Bowman’s space, in addition to necrosis and vacuolation of the renal tubules.

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Introduction

Antibiotics are widely used nowadays to cure many types of diseases (Davey, 2000). Antibiotics have different courses of administration depending upon the seriousness of the infection and the potency of the antibiotic taken. The recovery time of
an individual may range from a week to a whole month (Wiegand et al., 2008). One of the foremost concerns in modern medicine is antibiotic resistance. Over the last decade, almost every kind of bacteria has become stronger and less responsive to antibiotic treatment when it is really required (Pearson, 2007). Although health benefits of antibiotics are often emphasized, the side effects of antibiotics are not commonly known (Koju et al., 2005).

Rifampicin is a bactericidal antibiotic drug of the rifamycin group. It is a semisynthetic compound derived from streptomycyes spp that is used as a first line drug for the treatment of tuberculosis (TB) worldwide (Eminzade et al., 2008). Rifampicin produces many metabolic and morphological aberrations in the liver due to the fact that the liver is the main detoxifying site for these antitubercular drugs (Santhosh et al., 2007). The effect of rifampicin on lipid peroxidation was a significant increase after 4 weeks of treatment with mice with rifampicin (20 mg/kg) intraperitoneally (Upadhyay et al., 2007). Also, Tasdug et al. (2007) demonstrated that, there is a significant increase in triglycerides and cholesterol levels of rats after administration with rifampicin (250 mg/kg/day) for 30 days. Santhosh et al. (2006) detected significant increases in triglycerides, cholesterol and free fatty acids in the serum of rats after receiving anti-TB drugs (200 mg rifampicin + 200 mg isoniazid) for 30 days. Slight declines in the level of HDL-cholesterol with concomitant rise in LDL-cholesterol level were also noted (Pal et al., 2008) and Rana et al., 2010 showed significant increases in serum AST and ALT levels of treatment of rats with anti-TB drugs. Elevated levels of AST and ALT were recorded after treatment of patients (Balamurugan et al., 2009) and mice (Upadhyay et al., 2007) with rifampicin. The pharmacokinetics of rifampicin in healthy volunteers and tuberculosis-patients was studied by Rafiq et al. (2010), they observed a decrease in the level of albumin in the patients, as rifampicin binds only to albumin. Santhosh et al. (2007) and Eminzade et al. (2008) showed a significant decrease in total protein and albumin levels in the serum of rats treated with anti-TB drugs. Balamurugan et al. (2009) recorded a significant decrease in the total protein in the serum of rats after treatment with rifampicin (10 mg/kg) for 21 days. Yanardag et al. (2005) recorded a significant increase in the serum bilirubin, urea and creatinine levels of patients that received antituberculous treatments of isoniazid (300 mg/d), rifampicin (600 mg/d), pyrazinamide (2 g/d), and ethambutol (1 g/d) for 8 months. Santhosh et al. (2007) observed a significant increase in the serum bilirubin and a significant decrease in the serum urea levels of rats treated with isoniazid and rifampicin (200 mg each/kg bw/day) for 30 days. Tasdug et al. (2007) recorded a significant increase in serum bilirubin and urea levels, but there were no significant changes in the levels of creatinine in the serum of rats treated with rifampicin (250 mg/kg) for 30 days.

From a histological point of view, Scheuer et al. (1974) found a wide range of histological changes ranging from isolated steatosis (through varying degrees of portal inflammation with neutrophil or mononuclear infiltrate) to confluent necrosis in some patients who developed abnormal liver function tests within 6 weeks of rifampicin therapy. Kalra et al. (2007) reported diffuse microvesicular fatty infiltration with mild portal triaditis in rats treated with isoniazid (50 mg/kg/day) and rifampicin (100 mg/kg/day) for 7 days. Also, Tasdug et al. (2005) pointed out that treatment of rats with anti-TB drugs (250 mg/kg of rifampicin + 50 mg/kg of isoniazid + 100 mg/kg of pyrazinamide) once daily for 12 weeks caused membrane disintegration and loss of the polyhedral structure in the liver, in addition to the presence of other abnormalities like necrosis, macro vesicular steatosis and inflammation.

Lee (1979) reported that rifampicin can cause hemolysis and subsequently acute renal failure, it can also produce interstitial nephritis (direct toxic effect) which is really part of a pan-nephropathy. Moreover, some renal lesions were observed, these lesions may be due to the formation of immune complexes which were detected on capillary glomerular basement membranes by immunofluorescent and electron microscopic techniques. The deterioration in renal function typically appeared acutely, after the reintroduction of rifampicin (Covic et al., 1998).

Tetracycline antibiotics are bacteriostatic agents with a broad spectrum of antimicrobial activity. Besides their antimicrobial activity, it has been shown that tetracyclines may be useful in the treatment of pathological conditions in which acute or chronic inflammations are involved, such as dermatological, periodontal, rheumatic and neurodegenerative diseases (Bastos et al., 2007). Although the tetracyclines retain important roles in both human and veterinary medicines, the emergence of microbial resistance has limited their effectiveness (Chopra and Roberts, 2001). Large doses of tetracycline have been shown to induce hepatic dysfunction in rats (Yin et al., 2006) and humans (Wruble and Cummins, 1965). This dysfunction of the liver resulted in the disturbance of nitrogen metabolism, jaundice and other signs of hepatocellular damage, e.g., increase of serum transaminases (Böcker et al., 1982). It was found that the levels of the total cholesterol, triglycerides and LDL-cholesterol in the serum of rats were significantly increased after treatment with 10 mg/100 g body weight/day of tetracycline for 7 days, while a significant decrease in HDL-cholesterol levels was noticed (Vijayalekshmi Amma and Leelamma, 1991). Also, Hunt and Washington (1994) found a significant increase in AST and ALT levels in the serum of female patients treated with tetracyclines for 2 months. Balashev and Polosova (1981) studied the effect of tetracycline hydrochloride (25 mg/kg/day for 7 and 20 days) on the levels of the total protein and separate protein fractions such as alpha 1-, alpha 2-, beta- and gamma-globulins in the lymph and blood serum of rabbits. They found a significant decrease in the lymph and serum contents of both the total protein and the protein fractions. Moreover, Zallen (2009) recorded significant increases in the levels of serum bilirubin and urea in mice after treatment with tetracycline. Also, Miller and McGarity (2009) demonstrated that patients treated with tetracycline (250 mg/kg) four times daily for 7 days had renal failure. In addition, they found an increase in urea and creatinine levels. Machado et al. (2003) observed a moderate amount of vacuolation in the convoluted tubules and Henle’s loop and foci of necrosis in the tubular epithelium (that increased according to the dose) in the kidney of the offspring of the tetracycline-treated rats for 10 days.

With this background, the object of this study is to evaluate the effect of rifampicin and tetracycline on liver and kidney function in male albino rats. This was achieved by determination of the lipid profile, total protein and its electrophoretic pattern, albumin, creatinine, and urea in the blood of rats treated with rifampicin and tetracycline, and the histopathological study of the liver and the kidney of the treated animals.
Material and methods

The present study was carried out on white male albino rats having body weight range of 120–160 g. After randomization into various groups, rats were acclimatized for 14 days in the new environment before carrying out the experiment. The drugs were administered orally by means of gastric tubes. Rats were administered with rifampicin (200 mg/kg body weight/day, for 30 days) (Santhosh et al., 2007), or tetracycline (140 mg/kg body weight/day, for 7 days) (Vijayalekshmi Amma and Leelamma, 1991) before feeding.

Rats were divided into four groups

- **Group 1**: Control for rifampicin treated rats. Animals received 2 ml saline solution (the solvent of rifampicin) orally for 30 days.
- **Group 2**: Rats were treated with rifampicin orally in a dose of 200 mg/kg body weight/day (dissolved in 2 ml saline solution) for 30 days.
- **Group 3**: Control for tetracycline treated rats. Animals received 2 ml distilled water (solvent of tetracycline) orally for 7 days.
- **Group 4**: Rats in this group were treated with tetracycline orally in a dose of 140 mg/kg body weight/day (dissolved in 2 ml distilled water) for 7 days.

Blood collection was performed 24 h after the end of the 7th and 30th days. Animals were anesthetized with ether, and the blood was collected from the abdominal aorta. The blood sample was left 30 min at 37°C, and then centrifuged at 5000g for ten minutes. The separated serum was used for the estimation of the following physiological parameters:

1. Total cholesterol level was determined according to Deeg and Ziegemohrm (1982).
2. Triglyceride level was determined according to Stein and Myers (1995).
3. LDL and HDL-cholesterol levels were determined according to Valtere (2002).
4. AST and ALT levels were determined according to Schmidt and Schmidt (1963).
5. Serum total protein level was estimated using the Biuret reaction (Young, 1990).
6. Albumin and creatinine levels were determined according to Newman and Price (1999).
7. Electrophoretic separation of proteins was carried out according to Jeppsson et al. (1979).
8. Urea level was determined according to Patton and Crouch (1977).

Liver and kidney were removed and fixed in Bouin’s fluid for 24 h. After fixation, the organs were then dehydrated through ascending grades of ethyl alcohol, and then they were transferred to xylol. The organs were then placed in a mixture of melted wax and xylol (1:1) for about 10 min and then embedded in paraffin wax (56°C). Sections were made, stained with Ehrlich’s hematoxylin, counter stained with eosin and examined by the light microscope (Culling, 1974).

Data were tabulated and analyzed using SPSS-15 (Statistical Package for Social Science version 15) according to Howell (1995).

Results

**Effect of rifampicin administration on the biochemical parameters studied**

Table 1 shows that administration of rifampicin to rats for 30 days caused a significant increase in the total cholesterol, triglycerides and LDL-cholesterol levels (mg/dl); the mean values increased by 6.0%, 27.3% and 21.0%, respectively, than that of the control, respectively. In contrast, the level of HDL-cholesterol in the serum of the treated animals was significantly decreased by 18.8%, as compared to that of the control group.

As shown in Table 1, the levels of AST and ALT were significantly increased in the serum of the treated rats. The mean values increased by 218.7% and 92.7%, respectively, as compared to that of the control groups.

Data also show that a significant increase was noticed in the levels of bilirubin and urea in the serum of the treated animals as compared to that of the control groups. These increments were 45.3% in bilirubin and 62.9% in urea than that of the control. On the other hand, the mean value of serum creatinine did not show any significant difference in the treated group as compared to that of the control group.

Concerning the total protein and its electrophoretic pattern in the serum of the rifampicin-treated animals, it is clear from the data illustrated in Fig. 1 that the mean values of total protein, albumin and alpha1-globulin were significantly decreased as compared to that of the control group. The rest of the protein fractions (alpha2-, beta-, gamma-globulin and globulin) and A/G ratio in the treated animals recorded non-significant changes due to administration of rifampicin.

**Effect of tetracycline administration on the biochemical parameters studied**

Table 2 shows that administration of tetracycline (140 mg/kg body weight/day) to rats for 7 days caused a significant increase in the levels of triglycerides and LDL-cholesterol by 186.1% and 81.3% respectively, as compared to that of the control group. Meanwhile a significant decrease in the level of HDL-cholesterol was noticed in the serum of the treated rats. The mean value decreased by 44.6% in comparison to that of the control group.

The levels of AST and ALT were significantly increased in the serum of the treated rats. The mean values increased by 322.5% and 333.5%, respectively as compared to that of the control groups. On the other hand, treatment with tetracycline for 7 days had no effect on the level of the total cholesterol in the serum of the rats.

Significant increases were also noticed in the levels of bilirubin, urea and creatinine in the serum of tetracycline-treated animals as compared to that of the control group. The values increased by 45.6%, 47.2% and 49.3% for bilirubin, urea and creatinine, respectively when compared to that of the control group (Table 2).
Regarding the effect of tetracycline administration on the total protein and its electrophoretic pattern in the serum of the treated rats, data in Fig. 2 exhibited that the mean values of the total protein, albumin and A/G ratio were significantly decreased from 6.65 to 5.80, 4.04 to 2.66 and 1.53 to 0.87 g/dl, respectively. On the other hand, treatment with tetracycline caused a significant increase in the level of the gamma-globulin fraction in the serum of the rats. The level increased from 0.18 to 0.77 g/dl. The rest of the protein fractions (alpha 1, alpha 2, beta-globulin and globulin) in the treated rats were not significantly changed (Fig. 2).

Table 1  Mean ± SD of total cholesterol, triglycerides, LDL-cholesterol, HDL-cholesterol, AST, ALT, bilirubin, urea, and creatinine levels in the serum of rats, 30 days after treatment with rifampicin.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control</th>
<th>Treated</th>
<th>Difference (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total cholesterol (mg/dl)</td>
<td>177.60 ± 21.03</td>
<td>188.33* ± 16.78</td>
<td>6.0</td>
</tr>
<tr>
<td>Triglycerides (mg/dl)</td>
<td>205.80 ± 21.32</td>
<td>262.00* ± 73.27</td>
<td>27.3</td>
</tr>
<tr>
<td>LDL-cholesterol (mg/dl)</td>
<td>63.20 ± 12.32</td>
<td>76.50* ± 12.66</td>
<td>21.0</td>
</tr>
<tr>
<td>HDL-cholesterol (mg/dl)</td>
<td>73.24 ± 8.73</td>
<td>59.43* ± 13.72</td>
<td>18.8</td>
</tr>
<tr>
<td>AST (U/L)</td>
<td>69.80 ± 8.17</td>
<td>222.50* ± 56.49</td>
<td>218.7</td>
</tr>
<tr>
<td>ALT (U/L)</td>
<td>68.30 ± 7.89</td>
<td>131.67* ± 39.27</td>
<td>92.7</td>
</tr>
<tr>
<td>Bilirubin (mg/dl)</td>
<td>0.75 ± 0.20</td>
<td>1.09* ± 0.32</td>
<td>45.3</td>
</tr>
<tr>
<td>Urea (mg/dl)</td>
<td>22.60 ± 4.16</td>
<td>36.83* ± 5.78</td>
<td>62.9</td>
</tr>
<tr>
<td>Creatinine (mg/dl)</td>
<td>0.37 ± 0.11</td>
<td>0.43 ± 0.13</td>
<td>16.2</td>
</tr>
</tbody>
</table>

Each tabulated value is the average of six individual samples.

* Significant difference from the control at \( P < 0.05 \).

Table 2  Mean ± SD of total cholesterol, triglycerides, LDL-cholesterol and HDL-cholesterol, AST, ALT, bilirubin, urea, and creatinine levels in serum of the rats, 7 days after treatment with tetracycline as compared to that of the control group.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control</th>
<th>Treated</th>
<th>Difference (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total cholesterol (mg/dl)</td>
<td>113.33 ± 12.32</td>
<td>119.00 ± 13.31</td>
<td>4.76</td>
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<tr>
<td>Triglycerides (mg/dl)</td>
<td>39.67 ± 5.28</td>
<td>63.50* ± 6.06</td>
<td>44.31</td>
</tr>
<tr>
<td>LDL-cholesterol (mg/dl)</td>
<td>44.00 ± 7.62</td>
<td>63.50* ± 6.06</td>
<td>44.31</td>
</tr>
<tr>
<td>HDL-cholesterol (mg/dl)</td>
<td>81.57 ± 12.84</td>
<td>45.17* ± 16.80</td>
<td>-44.62</td>
</tr>
<tr>
<td>AST (U/L)</td>
<td>76.17 ± 18.12</td>
<td>321.83* ± 46.68</td>
<td>322.51</td>
</tr>
<tr>
<td>ALT (U/L)</td>
<td>68.17 ± 13.21</td>
<td>295.50* ± 78.57</td>
<td>333.47</td>
</tr>
<tr>
<td>Bilirubin (mg/dl)</td>
<td>0.57 ± 0.38</td>
<td>0.83* ± 0.31</td>
<td>45.61</td>
</tr>
<tr>
<td>Urea (mg/dl)</td>
<td>20.83 ± 4.96</td>
<td>30.67 ± 7.26</td>
<td>47.23</td>
</tr>
<tr>
<td>Creatinine (mg/dl)</td>
<td>0.69 ± 0.22</td>
<td>1.03* ± 0.28</td>
<td>49.27</td>
</tr>
</tbody>
</table>

Each tabulated value is the average of six individual samples.

* Significant difference from the control at \( P < 0.05 \).
Histological findings

Liver

Control for rifampicin-treated rats. Fig. 3 represents a vertical section of the liver of control for rifampicin treated rats (2 ml saline/day for 30 days). It exhibits a normal architecture of the hepatocytes presenting a homogenous cytoplasm and a large spherical nucleus (N) containing one or more nucleoli and a variable amount of dispersed and peripheral heterochromatin. Hepatocytes were arranged in trabecules running radially from the central vein and were separated by sinusoid (s) containing kupffer cells (K). The lumen of sinusoid contained mainly erythrocytes and white blood cells. Kupffer cells (K) were found on the luminal surface of the sinusoid’s endothelium.

Control for tetracycline-treated rats. The liver of control for tetracycline-treated rats (2 ml distilled water for 7 days) revealed almost the same histological picture as control for rifampicin treated rats; it is composed of hexagonal or pentagonal lobules with central veins and peripheral hepatic tetrads embedded in connective tissue. Hepatocytes (arrows) are arranged and separated by sinusoids containing kupffer cells (K). They are regular and contain a large spheroid nucleus with a distinctly marked nucleolus and peripheral chromatin distribution.

Rifampicin-treated rats. The light microscopic study of the liver of albino rats treated with 200 mg/kg body weight, daily for 30 days, shows several changes. There are dilation and congestion of blood sinusoids (s) and numerous hypertrophied kupffer cells (k) in sinusoids (Fig. 4). The liver cells were degenerated, hypertrophy of hepatocytes which have pyknotic nuclei (pyn) and deformed nuclei (dN) is observed (Fig. 5). Furthermore, there is a disorganization of hepatocytes with lysis of cytoplasm and some cells are completely filled with large vacuoles (head arrows-Fig. 4) presenting a balloon like appearance (Fig. 5).

Tetracycline-treated rats. The light microscopic study of the liver of albino rats treated with 140 mg/kg body weight daily for 7 days (Fig. 6) shows necrotic changes, a small pyknotic Figure 3 Light micrograph of a vertical section in the liver of albino rats (the control group), showing a normal architecture without pathological alterations. Hepatocyte (arrows), Spherical nucleus (N), Sinusoids (S), Blood vessel (BV), and Kupffer cells (K). (HE) stain (X400).

Figure 4 Light micrograph of a vertical section in the liver of albino rats after treatment with rifampicin for 30 days. Arrows point to hypertrophied hepatocytes, vacuolated cytoplasm hepatocytes (arrow heads), pyknotic nuclei (pyn), numerous kupffer cells (K), and dilation of blood sinusoids (S). (HE) stain (X400).

Figure 5 Light micrograph of a vertical section in the liver of albino rats after treatment with rifampicin for 30 days. Arrows point to hypertrophied hepatocytes, degenerated hepatocytes (double arrows), hypertrophied kupffer cells (K), deformed nuclei (dN) and pyknotic nuclei (pyn). (HE) stain (X1000).

Figure 6 Light micrograph of a vertical section in the liver of albino rats 7 days after tetracycline administration demonstrating hypertrophied hepatocytes (arrows), cytoplasmic vacuolation (arrows head), abundance of kupffer cells (K) and massive lymphatic aggregation (LA) inside the hepatic tissue and hepatocellular necrosis. (HE) stain (X400).
nucleus with condensed chromatin, lack of nucleolus and a cytoplasm filled with vacuoles. The trabecular structure of the lobules is slightly or distinctly blurred. Some sinusoids are over filled with erythrocytes and the walls of most sinusoids show numerous kupffer cells (K). Also, aggregations of lymphocytes (LA) inside the hepatic tissue are observed.

Kidney

Control for rifampicin-treated rats. The kidney of the control rat has a number of nephrons. The nephron is made up of renal tubules (Fig. 7 arrows) and a renal corpuscle which consists of a cluster of capillaries (glomerulus: G) surrounded by the Bowman’s capsule (BC). The microscopic picture of the glomerulus shows a capillary space covered by endothelial cells on the inner side, which in their lumen contain nucleated blood cells. On the inner side of the Bowman’s capsule, epithelial cells are found. The convoluted renal tubules [distal renal tubules (Drt) and proximal renal tubules (Prt)] are covered by tall columnar cells with a weak eosinophilic cytoplasm, apical microvilli or brush border toward the lumen as shown in Fig. 7.

Control for tetracycline-treated rats. The kidney of control rat of this group (2 ml. distilled water for 7 days) is composed of normal renal glomeruli and renal tubules [distal renal tubules (Drt) and proximal renal tubules (Prt)]. The renal tubules are lined with typical thick cubic epithelium. The tubules have a relatively regular distinct lumen. Lobular organization of the glomeruli and a flat epithelium lining the glomerular capsule can be seen (Fig. 8).

Rifampicin-treated rats. The light microscopic study of the kidney of albino rats treated with rifampicin (200 mg/kg body weight daily for 30 days) shows several changes (Fig. 9). The degeneration of renal tubules [distal renal tubules (Drt) and proximal renal tubules (Prt)] is observed in the kidney, including lysis of cytoplasm and vacuolation (arrows). Also, the glomerulus shows frequent shrinkage. Moreover, atrophy of glomerular capillaries causing Bowman’s space dilation is noticed. In addition; there are casts in some renal tubules. Cellular proliferation appears in the mesengial area (M).

Figure 7 Light micrograph of a transverse section of the kidney of albino rats receiving 2 ml saline/day for 30 days (control group for rifampicin treated rats). Arrows point to distal renal tubules (Drt) and proximal renal tubules (Prt), glomerulus (G), glomerular capillaries (GC), erythrocytes (Er), Bowman’s capsule (BC), and Bowman’s space (BS). (HE) stain (X400).

Figure 8 Light micrograph of a transverse section of the kidney of albino rats receiving 2 ml distilled water/day for 7 days (control group for tetracycline treated rats). Arrows point to distal renal tubules (Drt) and proximal renal tubules (Prt), glomerulus (G), glomerular capillaries (GC), erythrocytes (Er), Bowman’s capsule (BC), and Bowman’s space (BS). (HE) stain (X400).

Figure 9 Light micrograph of a transverse section of the kidney of albino rats after treatment with rifampicin for 30 days, it shows glomerular swelling (GS), tightly filling the Bowman’s capsule (BC), degeneration of distal (Drt) and proximal (Prt) renal tubules (arrows), and some of the renal tubules contain casts (C). (HE) stain (X 400).

Figure 10 Light micrograph of a transverse section of the kidney of albino rats after treatment with tetracycline for 7 days. It shows the atrophy of glomerular capillaries (GC) with Bowman’s space dilation (BS), degeneration of distal (Drt) and proximal (Prt) renal tubules (arrows), and some of the renal tubules contain casts (C). (HE) stain (X400).
Tetracycline-treated rats. The light microscopic study of the kidney of albino rats treated with tetracycline (140 mg/kg body weight) daily for 7 days shows similar changes in the renal tubules and glomeruli as shown in rifampicin treated rats, but of different intensity. Degeneration and necrosis of renal tubules are evident, some tubules appear empty and have casts; C (Fig. 10, arrows). The glomerulus shows frequent shrinkage and complete degeneration of renal glomeruli (Fig. 11, head of arrows). Also, some tubules show separation of the epithelial cells from their membranes causing a wide space between the renal tubules (Fig. 11, double arrow heads).

Discussion

The major disorder encountered in antitubercular drug-induced hepatitis is fatty accumulation in the liver, which is developed either due to excessive supply of lipids to the liver or interference with lipid deposition indicating the antitubercular drug-induced hypercholesterolemic condition (Santhosh et al., 2006). The increment of cholesterol levels in the serum of rifampicin-administrated rats may be attributed to liver dysfunction that has been observed in the histological study as a result of degeneration and necrosis of the hepatocytes caused by the toxic action of rifampicin. Liver synthesizes many end products of metabolism, and then distributed to the body to be used. So, the necrosis and damage in hepatocytes might cause the release of the cholesterol from the hepatocytes into the blood stream (Santhosh et al., 2006). The present study showed that the level of LDL-cholesterol was significantly increased, whereas the level of HDL-cholesterol was significantly decreased in the serum of rifampicin or tetracycline treated rats. These results are in agreement with those reported by Santhosh et al. (2006). The abnormal cholesterol deposition is favored by the dangerous tendency of cholesterol to passive exchange between the plasma lipoproteins and cell membranes (Brown and Goldstein, 1986). Also, the levels of triglycerides were significantly increased in the serum of animals treated with both drugs. Increased lipolysis of triglyceride depot liberates free fatty acid from adipose tissue stores (Stenberg, 1976) and the free fatty acids liberated by the adipose tissue are also taken up by the liver tissue, leading to the hypertriglyceridemic condition (Santhosh et al., 2006). However, Machado et al. (2003) observed that the organelles which changed in the presence of tetracycline were mainly mitochondria in which, the beta oxidation enzyme is inhibited, resulting in an accumulation of triglycerides inside the cytoplasm. They stated that hypertriglyceridemia may be due to increased release of lipoproteins into the circulation. Vijayalekshmi Amma and Leelamma (1991) reported that the uptake of triglyceride rich lipoprotein from the circulation is also decreased which is evident from the decreased activity of lipoprotein lipase of the extrahepatic tissue. They stated that high density lipoproteins are believed to be involved in the transport of cholesterol from the tissue to the liver for its catabolism.

Amino transferases are important classes of enzymes linking carbohydrate and amino acid metabolism. Alanine transaminase (ALT) and aspartate transaminase (AST) are well known diagnostic indicators of liver diseases. Farrell (1995) reported that in cases of liver damage with hepatocellular lesions and parenchymal cell necrosis, these marker enzymes are released from the damaged tissues into the blood stream. The significant elevation in the levels of ALT and AST in the serum of rifampicin or tetracycline-administrated rats in the present study is in accordance with the results of Kikkawa et al. (2006) and Santhosh et al. (2006). Elevated levels of these enzymes in the serum are presumptive markers of drug-induced necrotic lesions in the hepatocytes (Amr and Alaa, 2005). The enhanced susceptibility of hepatocyte cell membrane to drug induced peroxidative damage might have resulted in an increased release of these diagnostic marker enzymes into the systemic circulation. An increase in the AST and ALT levels indicates a reversible change of the cell membrane permeability (Böcker et al., 1982). Also, the activities of these enzymes were significantly lower in the liver tissue of the anti-TB drugs administrated rats (Santhosh et al., 2007).

The decrease in the total protein and albumin recorded in the present study agrees with the results reported by Santhosh et al., 2007; Eminzade et al. (2008), Balamurugan et al. (2009) and Rafiq et al. (2010). The reduction of total protein and albumin levels indicates that the administration of drugs has caused an impairment of liver function, e.g. its capacity to synthesize albumin from the hepatic parenchyma. Khan et al. (2002) reported that there was a differential binding of tetracyclines with serum albumin, while Shen et al. (2009) observed that albumin secretion of gel entrapped hepatocytes was reduced by tetracycline. The decrement of alpha 1-globulin in the serum of rifampicin-administrated rats could be due to liver dysfunction which affects the synthesis of alpha protein fractions in the liver. The increment of gamma-globulin level in the serum of tetracycline-treated rats may be due to hyperplasia of the reticulo-plasmic tissue of the bone marrow induced by tetracycline administration (Mikaelian, 1975).

The elevation in the serum bilirubin which was observed in rifampicin or tetracycline-treated rats is in agreement with the data reported by Tasduq et al. (2005) and Santhosh et al. (2007). Hepatotoxicity is a common adverse effect of these drugs which induce liver damage as judged by elevated serum bilirubin (Eminzade et al., 2008; Pal et al., 2008 and Marzouk et al., 2009).
Urea and creatinine are waste products eliminated from the blood through the kidney by blood filtration process. The significant increase in the level of urea in the serum of rifampicin and tetracycline-treated rats as well as the increase in creatinine in the tetracycline-treated rats is in harmony with the results of earlier studies (Yanardag et al., 2005; Tasduq et al., 2007 and Miller and McGarity, 2009). The non-significant changes in the level of serum creatinine of rifampicin -treated rats could be interpreted by the fact that the level of serum creatinine does not rise until at least half of the kidney’s nephrons are destroyed or damaged (Bhattacharya et al., 2005). It has been reported that the adverse effects of rifampicin occasionally include nephritis and hepatitis (Plumb, 1999). Several isolated cases of acute renal failure (ARF) following rifampicin therapy have been reported (Yanardag et al., 2005) Tetracycline has been shown to induce various forms of nephrotoxicity (Bihorac et al., 1999 and Zallen, 2009). The increment in urea and creatinine levels may be attributed to the impaired renal function which has been shown in the present histological study as a result of the degeneration and necrosis in the glomeruli and renal tubules due to the toxic action of antibiotics on the kidney. This action may have prevented the filtration of the waste products (urea and creatinine) from the blood stream (Bihorac et al., 1999).

The results of the histopathological examination reflect the toxicity of rifampicin to the liver and show a remarkable damage of the architecture of the liver cells. These results are in agreement with those of Awodele et al. (2010) who showed that rifampicin induces hepatic damage. The present detected necrosis of the hepatic cells of rats treated with rifampicin may be attributed to the formation of free radicals, which act as a stimulator of lipid peroxidation and a source for destruction and damage of the cell membrane (Ravi et al., 2010). Also, the current study indicated portal triaditis and an increase in the number of kupffer cells. This is in accordance with the results of Ravi et al. (2010) who found necrosis with moderate to heavy portal triaditis after treatment with anti-TB drugs (Rifampicin and Isoniazid). Cytoplasmic vacuolation of hepatocytes was observed in the present study. Tasduq et al. (2005) suggested that the vacuolation of hepatocytes accompanied by cytoplasmic rarefaction results in the loss of polyhedral structure in the liver of rats treated with rifampicin.

The present data showed that hepatocytes in the liver of tetracycline treated rats exhibited intense diffuse vacuolization with many small and medium vacuoles. These data agree with the findings of Machado et al. (2003). In the study of Hagel-Lewicka et al. (1980), animals treated for a long period with tetracycline presented a single large fatty vacuole in the hepatocytes and more quantity of collagen fiber in the portal spaces, probably due to the continuity of the treatment. Amacher and Martin (1997) used single doses of tetracycline in their experiments and likewise noted the presence of cytoplasmic changes such as the formation of fatty vesicles in the hepatocytes after a few hours of administration, suggesting metabolic changes with accumulation of triglycerides and lipid inclusions after the use of tetracyclines. Kikkawa et al. (2006) also showed fatty changes and focal inflammatory cell infiltration in the liver after treatment with tetracycline. The overall net effects on the expression of lipid metabolism genes indicated an increase in cholesterol and triglyceride biosynthesis and a decrease in β-oxidation of fatty acids (Yin et al., 2006).

The kidney provides the final common pathway for the excretion of many drugs and their metabolites, and therefore is frequently subjected to high concentrations of potentially toxic substances. Drugs and their metabolites are taken up selectively and concentrated by the renal tubular cells before excretion into the urine. So, high intracellular concentrations are attained, particularly in the renal medulla, which has relatively little vasculature compared with the cortex (Aronson, 2003). The present histological findings showed glomerular distortion or shrinkage. Some tubular cells showed hyaline-droplet formation and tubular dilatation. Warner (1975) found similar results. Also, Boulton-Jones et al. (1974) showed diffuse mesangial proliferative glomerulonephritis in the renal biopsy of patients using rifampicin with subacute infective endocarditis, while Salih et al. (2008) observed interstitial nephritis and/or acute tubular necrosis after treatment with rifampicin.

The mechanism of renal injury is thought to be due to an allergic reaction to rifampicin or one of its metabolites causing allergic interstitial nephritis. However, the renal biopsy does not always show heavy infiltration of mononuclear cells and occasionally the picture is that of severe, diffuse or focal tubular necrosis with mild interstitial changes (Grunfeld et al., 1993). The immune complexes get deposited in the blood vessels or interstitium and cause glomerular endotheliosis leading to tubular injury thereby decreasing renal function (Muthukumar et al., 2002). This may be possibly, the cause of renal failure in our study. Likewise, tetracycline has been shown to induce various forms of nephrotoxicity. The current work showed degeneration of renal glomeruli, tubules and irregular cytoplasmic vacuolization. The same results were observed by Mavromatis (1965). The kidney of rat’s offspring showed slight tubular vacuolation and necrosis, more prominent in the newborn, as well as signs of tubular regeneration on the tenth and twentieth days from treatment with tetracycline (Machado et al., 2003). The results of Chambers (2001) concluded that the main mode of elimination of most tetracycline is via renal glomerular filtration, and it is also eliminated via the biliary route. Approximately 60% of tetracycline administered is found in the urine in an unchanged form.

Recommendations

- Over consumption of antibiotics may nullify their benefits and instead, lead to side effects. So, people do not tend to consume them carelessly and without medical advice.
- All patients receiving antibiotics should have their liver and kidney function tests periodically assessed.

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