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Ameliorative Role of Curcumin on Liver of Rats Treated with Gentamicin, Cefotaxime and Metronidazole

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Abstract The liver is the most vulnerable organ against the side effects of xenobiotics. Aminoglycosides especially gentamicin are the widely used antibiotics against Gram negative bacteria. Cefotaxime is a broad spectrum cephalosporin antibiotic used mainly against Gram positive bacteria. Metronidazole is a chemotherapy used in treatment of protozoal and anaerobic bacterial infections. Combination therapy has complementary and synergistic mechanisms of action. The Present investigation focused on evaluating the effect of curcumin on hepatic alterations of gentamicin, cefotaxime, metronidazole, and their combinations. For this study, eighty eight male rats were classified into eleven groups. (First): Served as control, (Second): Curcumin (200mg/kg.b.wt.,p.o.), (Third): Gentamicin (80 mg/kg.b.wt., i.m), (Fourth): Cefotaxime (540 mg/kg.b.wt., i.m), (Fifth): Metronidazole (135 mg/ kg.b.wt., p.o.), (Sixth): Gentamicin with cefotaxime, (Seventh): Gentamicin with cefotaxime and metronidazole, (Eighth): Curcumin one hour prior gentamicin, (Ninth): Curcumin one hour prior cefotaxime, (Tenth): Curcumin one hour prior gentamicin and cefotaxime, (Eleventh): Curcumin one hour before gentamicin, cefotaxime and metronidazole for 14 successive days. Serum was prepared for measuring of Alkaline phosphate (ALP), Alanine aminotransferase (ALT) and Aspartate aminotransferase (AST), liver was removed for homogenate measurements, histopathological & immunohisto-chemical examination. Administration of curcumin indicated a significant ($p < 0.05$) decrease of ALP, ALT, AST, malondialdehyde (MDA) and tumor necrosis factor–alpha (TNF-α) and elevation in glutathione (GSH), catalase (Cat), superoxide dismutase (SOD), and glutathione peroxidase (GP^x) of liver homogenate. Our data showed that curcumin could protect the liver alterations by blocking oxidative damages.

Keyword: Gentamicin, Cefotaxime, Metronidazole, Curcumin, Liver

Introduction

Aminoglycoside antibiotics belong to one of the oldest classes of antibacterial agents used in curative therapies. Gentamicin is one of aminoglycosides was originated from *Micromonospora purpurea*. It is effective against most of the life threatening microbial pathogens, particularly Gram-negative bacterial infections and is also active against *Staphylococcus* and *Enterococcus*, especially in synergy with β-lactams [1,2,3]. Cefotaxime is a member of third generation cephalosporin broad spectrum β-lactam antibiotic. It is considered to be equivalent to cefotriaxone in terms of safety and efficacy [4]. Metronidazole is a 5-nitroimidazole chemotherapy widely used veterinarians and medicines as anti-protozoal and bactericidal chemotherapy [5].

Curcumin is the effective compound of Curcuma longa. It is used for curing many diseases including renal disorders. Curcumin has antioxidant, anti-inflammatory and cell membrane stabilizing equivalent to vitamins C, E, and beta-carotene [6,7].

The target of the present investigation was to evaluate the possible ameliorative role of curcumin against renal oxidative stress induced by combination of gentamicin, cefotaxime and metronidazole in male rats.

Materials and Methods

Drugs

Gentamicin (80 mg / kg, I.M.) [8], Cefotaxime (540 mg / kg, I.M) [9], were obtained from Egyptian Int. Pharmaceutical Industrial Co. (10^{th} of Ramadan, Egypt), Metronidazole (135 mg / kg, orally; Alex. Co. for pharmaceutical industries (Egypt) [10]. Curcumin (200 mg/kg, orally; purchased from Sigma Chemicals Co. (USA) [11].

Experimental Animals

Eighty eight adult male albino rats (weighing 200±10 gm) were used in the present investigation. They were obtained from the Animal Breading Unite, National Organization for drug control and research (Giza, Egypt), and used after one week of quarantine and acclimation, animal groups were divided in separating cages, in controlled temperature (23-25 ºC), humidity (60%), light and dark cycles of 12 hours each. The animals were fed on standard pelleted diet and water *ad libitum*. The experiment was conducted in accordance with the ethical guidelines for investigations in laboratory animals and were approved by the Ethical Committee of Faculty of Veterinary Medicine, Zagazig University, Egypt and comply with the Guide for the Care and Use of Laboratory Animals [12].

Animal Grouping

At eighth day of acclimatization, eighty eight adult male albino rats were divided equally into eleven groups.

Group 1: (Control)
Group 2: received curcumin.
Group 3: received gentamicin.
Group 4: received cefotaxime**.**
Group 5: received metronidazole.
Group 6: received gentamicin with cefotaxime administration.
Group 7: received gentamicin with cefotaxime and metronidazole.
Group 8: received curcumin and gentamicin.
Group 9: received curcumin and cefotaxime.
Group 10: received curcumin gentamicin and cefotaxime.
Group 11: received curcumin, gentamicin, cefotaxime and metronidazole.

Experiment continued for fourteen days, at twenty-four hours after last dose, the animals were anaesthetized using Phenobarbital, blood samples were collected freshly from retro-orbital plexus in non-heparinized tubes and serum was separated by centrifugation for 20 min at 4000 r.p.m. for measuring albumin, total protein, ALP, ALT and AST. the two livers immediately removed and washed in ice saline, and one kept in formalin for histopathology and histochemistry, the second was homogenized in phosphate buffer [13], for determination of reduced glutathione (GSH) [14], catalase (Cat) [15], glutathione peroxidase (GPx) [16], superoxide dismutase (SOD) [17], Malondialdehyde (MDA) [18] and Tumor necrosis factor alpha (TNF-α) [19]. Liver was examined histopathologically and immunohistochemically [20].

Statistical Analysis

Results were expressed as mean ±standard errors of the means (S.E.M.). Comparison between more than two different groups was carried out using the one-way analysis of variance (ANOVA) followed by Tukey-Kramer's Multiple Comparison Test, where $P<0.05$ was considered significant [21].

Results

Effect of curcumin on liver function tests in male albino rats treated with gentamicin, cefotaxime and / or metronidazole

Table (1) displayed that, on comparison to the control group, curcumin, metronidazole, curcumin with cefotaxime, curcumin with cefotaxime and gentamicin and curcumin with cefotaxime, gentamicin, and metronidazole groups revealed non-significant differences in ALP, ALT and AST. Intramuscular administration of gentamicin and Cefotaxime groups evoked a significant increase in ALP, ALT and AST comparing with that of the normal control group, whereas oral administration of curcumin before gentamicin, cefotaxime, gentamicin with cefotaxime, gentamicin with cefotaxime and metronidazole groups induced a significant decrease in ALP, ALT and AST comparing with gentamicin treated group.

Table 1: Effect of curcumin on liver function tests in male albino rats treated with gentamicin, cefotaxime and / or metronidazole

Groups	ALP (U/L)	ALT (U/L)	AST (U/L)
(1) Control (Cont.)	78.60 ± 1.301^{d}	27.89 ± 0.788^{d}	78.97 ± 1.178^{c}
(2) Curcumin (Cur.)	81.24 ± 1.881^{cd}	30.89 ± 0.647^{cd}	80.87 ± 1.877^{c}
(3) Gentamicin (Gen.)	109.70 ± 2.114^{a}	54.84 ± 1.162^{a}	120.70 ± 1.326^{a}
(4) Cefotaxime (Cef.)	86.91 ± 2.099^{c}	36.86 ± 0.989^{bc}	92.98 ± 3.134^{b}
(5) Metronidazole (Met.)	84.46 ± 0.352^{cd}	31.26 ± 2.991^{cd}	85.39 ± 1.941^{bc}
(6) Gen.+Cef.	101.00 ± 0.715^{b}	44.32 ± 1.695^{b}	103.00 ± 1.921^{b}
(7) Gen.+Cef.+Met.	102.30 ± 1.087^{b}	44.58 ± 1.435^{b}	104.50 ± 1.770^{b}
(8) Cur.+ Gen.	93.61 ± 1.550^{c}	36.47 ± 2.238^{c}	96.13 ± 3.568^{b}
(9) Cur.+ Cef.	78.69 ± 0.822^{d}	28.23 ± 0.492^{d}	77.21 ± 5.775^{c}
(10) Cur.+Gen.+Cef.	84.62 ± 1.800^{cd}	34.89 ± 0.606^{cd}	88.78 ± 1.549^{c}
(11)Cur.+Gen.+Cef.+Met.	84.90 ± 1.509^{cd}	34.21 ± 1.423^{cd}	88.96 ± 1.242^{c}

Means with different superscripts in the column are significant ($p > 0.05$)

Effect of curcumin on albumin and total protein in male albino rats treated with gentamicin, cefotaxime and / or metronidazole

Table (2) showed evoked that, curcumin, cefotaxime, metronidazole, curcumin with cefotaxime, curcumin with cefotaxime and gentamicin and curcumin with cefotaxime, gentamicin, and metronidazole groups revealed non-significant differences in albumin but gentamicin induced a significant increase comparing to the control rats.

In curcumin and metronidazole groups displayed non- significant changes in total albumin but gentamicin and cefotaxime groups induced a significant decrease in total protein comparing to control rats. Administration of curcumin before gentamicin, cefotaxime, gentamicin with cefotaxime, gentamicin with cefotaxime and metronidazole groups showed a significant increase in total protein comparing to gentamicin group

Table 2: Effect of curcumin on albumin and total protein in male albino rats treated with gentamicin, cefotaxime and / or metronidazole

Groups	Albumin	Total protein
(1) Control (Cont.)	4.223 ± 0.095^{a}	6.718 ± 0.110^{a}
(2) Curcumin (Cur.)	4.286 ± 0.088^{a}	6.366 ± 0.135^{ab}
(3) Gentamicin (Gen.)	2.642 ± 0.164^{b}	3.185 ± 0.101^{e}
(4) Cefotaxime (Cef.)	3.845 ± 0.040^{a}	5.971 ± 0.059^{b}
(5) Metronidazole (Met.)	4.038 ± 0.053^{a}	6.395 ± 0.180^{ab}
(6) Gen.+ Cef.	3.865 ± 0.145^{a}	4.051 ± 0.067^{d}
(7) Gen.+Cef.+Met.	3.813 ± 0.208^{a}	3.910 ± 0.031^{d}
(8) Cur.+ Gen.	4.065 ± 0.114^{a}	5.046 ± 0.060^{c}
(9) Cur.+ Cef.	4.244 ± 0.053^{a}	6.736 ± 0.093^{a}
(10) Cur.+Gen.+Cef.	3.897 ± 0.146^{a}	6.169 ± 0.170^{ab}
(11) Cur.+Gen.+Cef.+Met.	3.879 ± 0.121^{a}	6.145 ± 0.174^{ab}

Means with different superscripts in the column are significant ($p < 0.05$)

Effect of curcumin on GSH, Cat, GPx, SOD, MDA, and TNF-α of liver in male albino rats treated with gentamicin, cefotaxime and/or metronidazole

Table (2) showed that, oral metronidazole treated group produced a non-significant difference in liver GSH, Cat, SOD, GPx and MDA, respectively, for the normal control group, but gentamicin or cefotaxime groups evoked a significant inhibition in GSH, Cat, GPx, SOD and increase in MDA and TNF-α versus control group except GPx for cefotaxime group, whereas oral administration of curcumin prior gentamicin, gentamicin with cefotaxime or gentamicin, cefotaxime with metronidazole groups induced a significant increase in GSH, Cat, GPx, SOD and decrease in MDA and TNF-α as compared to gentamicin groups.

Table 3: Effect of curcumin on GSH, Cat, SOD, GPx, MDA and TNF-α of liver in male albino rats treated with gentamicin, cefotaxime and/or metronidazole.

Groups	GSH (mg/g)	Cat. (U/g)	SOD (U / g)	GPx (U / g)	MDA (nmol/g)	TNF-α (Pg/g)
(1) Control(Cont.)	54.31±1.622^{a}	2.190±0.171^{a}	332.9±25.69^{a}	55.94±2.438^{a}	22.24±0.425^{d}	38±2.30^{e}
(5) Gentamicin(Gen)	25.46±1.227^{d}	0.570±0.056^{d}	89.14±4.59^{c}	7.36±0.785^{c}	78.68±5.612^{a}	206±4.04^{a}
(6) Cefotaxime (Cef.)	46.29±1.241^{b}	1.710±0.055^{b}	231.8±17.84^{b}	36.47±2.430^{ab}	31.16±0.344^{c}	71.3±1.85^{d}
(7)Metronidazol(Met.)	50.34±2.077^{ab}	1.895±0.062^{ab}	321.0±6.86^{a}	36.47±3.033^{ab}	25.96±0.926^{d}	50±1.52^{d}
(8) Gen. + Cef.	36.68±0.539^{c}	1.178±0.099^{c}	214.0±21.71^{b}	31.61±2.430^{b}	66.08±1.566^{b}	102.7±3.18^{c}
(9)Gen+Cef+ Met.	36.09±1.464^{c}	1.184±0.053^{c}	206.1±7.92^{b}	31.61±2.433^{b}	67.30±0.631^{b}	101.7±3.71^{c}
(10) Cur.+ Gen.	38.24±2.042^{c}	1.506±0.077^{b}	190.2±13.72^{b}	38.90±0.158^{ab}	37.212±2.007^{c}	144.3±3.48^{b}
(12) Cur.+Cef.	53.99±0.983^{a}	2.258±0.109^{a}	332.9±13.72^{a}	36.47±2.341^{ab}	22.23±0.542^{d}	47.6±1.85^{e}
(14)Cur+Gen+Cef	48.08±1.038^{ab}	1.746±0.102^{ab}	301.2±7.93^{ab}	55.12±3.243^{a}	28.48±2.224^{cd}	43±9.29^{e}
(16)Cur+Gen+Cef+ Met	48.53±1.925^{ab}	1.790±0.071^{ab}	299.3±19.67^{ab}	55.12±3.243^{a}	29.31±2.377^{cd}	44±5.56^{e}

Means with different superscripts in the column are significant ($p < 0.05$)

Effect of curcumin on histopathological picture of liver in male albino rats treated with gentamicin, cefotaxime and/or metronidazole

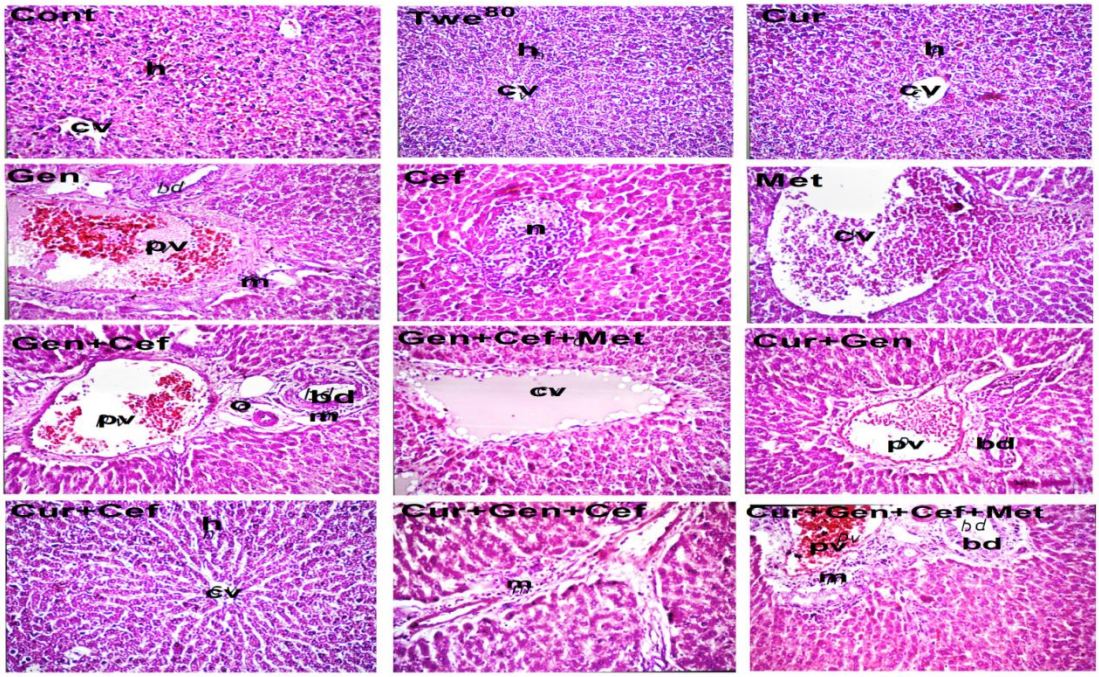

Figure 1: Liver sections of normal control, twe^{80} and curcumin groups, showed no histopath-ological alterations and showed normal histological structure of the central vein (cv) and surrounding hepatocytes (h) but section of liver of

rat treated with gentamicin group, showed severe dilatation and congestion in portal vein (pv) with inflammatory cells infiltration (m) as well as dilatation in the bile ducts (bd) and cefotaxime treated group evoked focal necrosis (n) in hepatic parenchyma, On the other hand, liver section of rat treated with gentamicin and cefotaxime group, elicited severe dilatation and congestion in the portal vein as well as oedema with few inflammatory cells infiltration surrounding the dilated bile ducts. The liver of rats were received gentamicin, cefotaxime and metronidazole displayed sever dilatation in the central vein (cv) by haemolysed blood with granular degeneration (d) in the hepatocytes. Liver section of rats were administered curcumin and gentamicin group, showed congestion and dilatation in portal vein (pv) and bile duct (bd) with moderate inflammatory cells infiltration in portal area while curcumin and cefotaxime group, showed normal histological structure but curcumin, gentamicin and cefotaxime individuals, showed few inflammatory cells infiltration (m) in the portal area and Liver section of rat treated with curcumin, gentamicin, cefotaxime and metronidazole group, showed few inflammatory cells infiltration (m) in the portal area with congestion and dilatation in portal vein (pv) and normal hepatocytes (H&E x40).

Effect of curcumin on the severity of Immunohistopathological reaction using caspase-3 in liver of different experimental groups

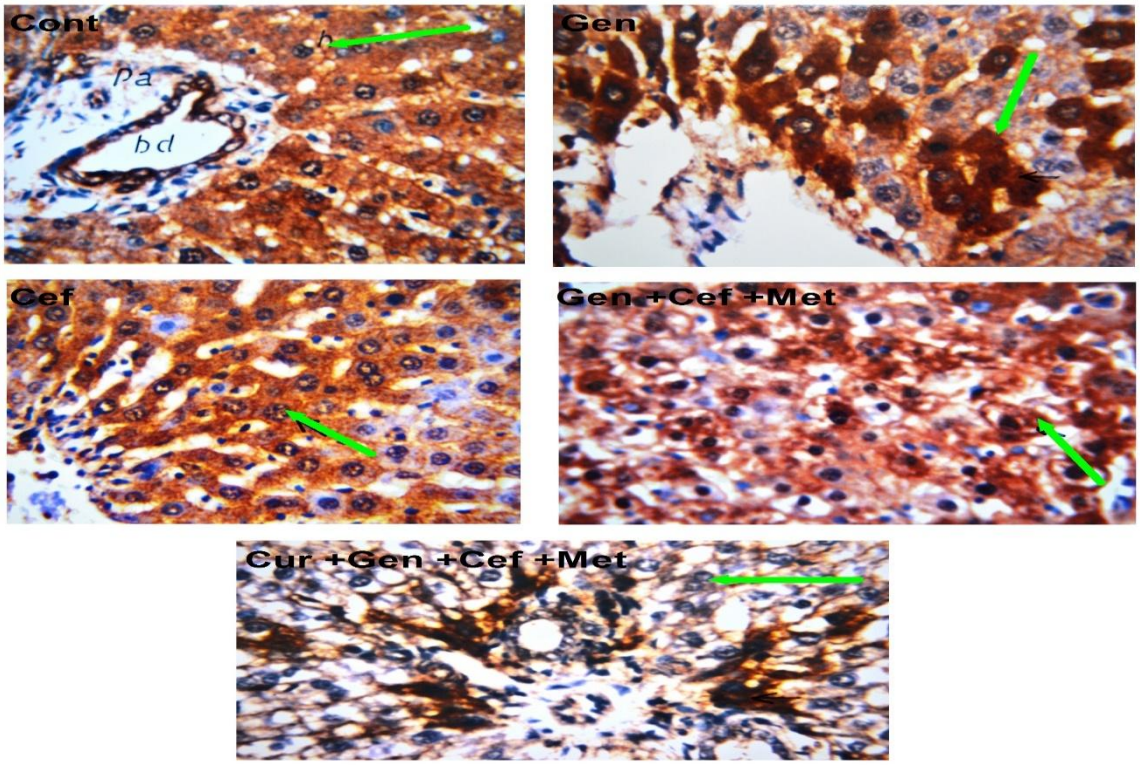

Figure 2: Liver section of control group, showed negative immunohistochemical reaction but Liver section of gentamicin group, displayed severe positive immuno-histochemical while section of rat treated with cefotaxime group, elicited mild positive immunohistochemical reaction in addition to liver section of rat treated with gentamicin, cefotaxime and metronidazole showed positive immunohistochemical reaction and liver section of rat treated with curcumin, gentamicin, cefotaxime and metronidazole evoked mild positive immunohistochemical reaction in the few hepatocytes (arrow) using caspase-3 antibody. (X 80).

Discussion

Aminoglycosides especially gentamicin have long been used in curing of gram negative bacteria resulting from its chemical stability and rapid onset of bactericidal action. Unlike its beneficial effect, gentamicin has hepatotoxic

action. Our biochemical results showed that gentamicin induced hepatic disturbances which were confirmed histopathologically and immunohistochemically.

Biochemically, serum hepatic biomarkers, ALP, ALT and AST were significantly increased while total protein and albumin were significantly decreased in the gentamicin accompanied rats versus curcumin accompanied rats. Our data interpret the increasing of liver biomarkers resulting from hepatocytes injuries and escaping of enzymes in the circulation in agreement with the previous reporters [22-24]. Also, our results indicated a significant increase in MDA and TNF-α and increase in GSH, Cat, SOD and GP^X of liver homogenate of rats treated with gentamicin but these investigations were attenuated by co-administration of curcumin.

In agreement with our data [25] reported that, gentamicin induced an increase of hepatic MDA level and decrease in GSH, Cat.; SOD, GPx, which described by hepatocytes damages and inflammatory cells infiltration. On the same ground [26] described gentamicin toxicity by stimulating synthesis of H_2O_2 and hydroxyl radicals in the mitochondria leading to mesangial cells contraction, altering the glomerular filtration rate. In this way, gentamicin induced a strong accumulation of oxidants (MDA and NO) in the liver while SOD activities and GSH contents were decreased [27].

Organism has a lot of anti-oxidative defense mechanisms for controlling reactive oxygen species preventing cellular damage, including the non-enzymatic (mainly reduced glutathione) and enzymatic defenses (including superoxide dismutase, glutathione reductase, glutathione S-transferase, catalase and glutathione peroxidase) [28]. Also, our results agreed with [29,30] who recorded that the gentamicin group was the most strongest caspase-3-stained tissues. These data showed that, co-administration of gentamicin and cefotaxime resulted in a significant improvement of parameters may be due to cefotaxime decreases the reabsorption of gentamicin in renal tubules in accordance with [31,32] who showed that, cefepime (cephalosporin) induced an improvement in the activities of superoxide dismutase and catalase along with decrease in MDA levels in amikacin (aminoglycoside) group. Our results coordinate with those obtained by [33] reported that, administration of curcumin to gentamicin-treated rats increased liver GSH level and GPx, Cat, and SOD activities. Furthermore, it has been suggested that lipid peroxidation might be a predisposing factor for induction of liver tissue damage. The role of curcumin in reducing the membrane damage is related to its ability to scavenge lipid peroxidation initiating agents. We observed a decrease in MDA and TNF-α levels in the liver tissue of rats treated with curcumin and gentamicin compared with gentamicin alone group. In the present study, the results of histopathological and histochemical examination showed a clear evidence of ameliorative effect of curcumin against gentamicin nephrotoxicity. The protective role of curcumin may be depend on its ability to eliminate the hydroxyl radical [34], superoxide radical [35], singlet oxygen [36], nitrogen dioxide [37], and nitric oxide [38]. Regarding to our results evoked the best effect of curcumin administration one hour before treatment due to its antioxidant, anti-inflammatory and anti-apoptotic effects.

Conclusion

The present study reveals that curcumin has potent protective effect against hepatotoxic alterations of gentamicin in rats.

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