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Protective effects of curcumin against Augmentin-induced hepatotoxicity in rats

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A B S T R A C T

Amoxycillin/clavulanic acid (Augmentin®) is an effective antibiotic which was approved by WHO as a drug for treatment of infection. Its efficacy to inhibit microorganisms had led to their increased use. There is a possibility that the drug may have potential toxic effects following consumption. So, we design this study to investigate if the Amoxycillin/clavulanic acid had toxic effects on liver or not and to explore the role of curcumin in the reduction of this toxic effect.

Forty male albino rats were separated into four groups (10 rats per group); Control group, Augmentin group, curcumin group and curcumin protected group. Amoxycillin/clavulanic acid was administered at 31.83 mg/kg b.wt twice for seven days, curcumin was taken at 300 mg curcumin / kg b.wt for two weeks.

Amoxycillin/clavulanic acid caused a significant elevation in the level of serum malondialdehyde (53.53±0.43nmol/g). Moreover, it increased the serum ALT activity (104.87±1.01 U/L), AST activity (114.62±0.63 U/L) and TNF-α concentration (39.18±0.84 Pg/ml). But, it decreased serum albumin (2.66±0.08g/dl) and total proteins (3.54±0.05g/dl) levels. Furthermore, it reduced hepatic glutathion (GSH) (11.82±0.15nmol/g) and glutathion peroxidase (Gpx) (0.04±0.08nmol/g). It induced a increase in the expression of the pro-inflammatory cytokine (IL-1β) (53.53±0.43) with significant reduction in IL4 (53.53±0.43) in the liver homogenate. Histopathological examination of hepatic tissue revealed ballooning degeneration and hepatic damage. However, curcumin alleviated Augmentin-induced liver damage. These results revealed that Amoxycillin/clavulanic acid is a hepatotoxic drug in rats. Meanwhile, curcumin reverses that harmful hepatotoxic effect.

Keywords: Antibiotics, antioxidants, hepatitis, anti-inflammatory, cytokines.

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1. INTRODUCTION:

The liver has an essential function in managing the homeostasis of the body. It metabolizes fat, carbohydrates and proteins, detoxifies and activates exogenous and

endogenous compounds and it is capable of transforming foreign chemicals. Exogenous substances such as drugs and endogenous compounds such as steroids are metabolized by the liver in presence of various enzymes. Amoxicillin/clavulanic acid is an effective broad-spectrum antibiotic. The β-lactamase-inhibiting characters of clavulanic acid were added to the potent broad-spectrum antimicrobial activity of amoxicillin in tablets**.** Amoxicillin/clavulanic acid is the most frequently recorded antibiotic associated with drug-induced hepatotoxicity in the form of hepatocellular necrosis, cholestasis and other reactions. (Olayinka and Olukowade 2010)**.**

Herbal medicine has attracted the researchers as a result of its efficacy against drug-induced liver toxicity. Curcumin is a widely used herbal substance to protect against drug-induced hepatotoxicity. It is the major constituent of the spice turmeric. Turmeric is a bright yellow spice which is isolated from the rhizomes of curcuma longa (Vetvicka and Vetvickova 2016)**.** The yellow color of turmeric is due to pigments curcumanoids with the curcumin. It is metabolized to curcumin glucuronides, sulfates, tetrahydrocurcumin and hexacurcumin. It is reported to exhibit antioxidant, anti-inflammatory, anti-carcinogenic, antiviral and anti-infectious activities (Zhang etal., 2014; Dalaklioglu etal., 2013 and Gurocak etal., 2013). Curcumin is a potential hepatoprotective substance as it prevents the enzyme cyclooxygenase 2 expression through interference with the NF-Kb activation. In addition, curcumin has many biological activities including antimicrobial, antioxidant, antitumor and anti-inflammatory effects (Singh and Kumar 2017).

Therefore, we attempted to study the possible impact of curcumin supplementation as protective therapy for *in vivo* amoxycillin/clavulanic acid induced hepatic toxicity in rats.

2. MATERIALS AND METHODS

2.1.Experimental animals:

Forty male albino rats were utilized. Rats were taken from the Experimental unit, Faculty of Veterinary Medicine, Benha University, Egypt. Animals were putted in separate clean cages from metal. Fresh and clean drinking water was supplied. Rats were housed at constant environmental and nutritional condition throughout the experimental period.

2.2.Chemicals

Augmentin (GlaxoSmithkline Egypt, Cairo, Egypt) was taken orally by the stomach tube at a dose of 31.83 mg/kg b.wt twice for seven days according to (Olayinka and Olukowade 2010). Curcumin (Curcumin; Sigma Chemical Co., St. Louis, MO, USA) was administrated orally by the stomach tube at a dose of 300 mg/kg b.wt once daily according to (Ukil etal., 2003).

2.3.Experimental design

Forty male rats were separated into four groups randomly (10 animals per group): a control group was taken distilled water orally through a stomach tube; curcumin group was taken curcumin orally using stomach tube at a dose of 300 mg/kg b.wt (Ukil et al. 2003). Augmentin group was received Augmentin orally 31.83 mg/kg b.wt twice daily for 7days according to (Olayinka and Olukowade 2010). Curcumin protected group was taken curcumin orally at a dose of 300 mg/kg b.wt for 2 weeks then given Augmentin orally 31.83 mg/kg b.wt twice a day for seven days. Blood for serum samples was collected from the retroorbital venous plexus and hepatic specimens were taken from all rats at the end

of the experiment for estimation of liver function, the oxidative parameters, gene expression and the histopathological changes.

2.4. Sampling:

Blood specimens were taken from the retro-orbital venous plexus after that hepatic specimens were obtained by scarification. Blood was obtained into two tubes: the first plain centrifuge tube to separate serum for biochemical analysis (ALT, AST, total proteins, albumin and TNF-α) and the second one on EDTA tube for hematological examination.

Liver homogenate (prepared as one gm of liver tissue) was taken from all rats at the end of the experiment. The hepatic tissue was washed and homogenized to yield the homogenate from liver tissue of 10% (W/V; Weight of liver tissue, g per Volume of the buffer, mL).The homogenate was centrifuged at 4000 rpm for 5 min at 4^{o}C for determination of antioxidants.

2.5.Determination of serum ALT, AST, Total protein and Albumin

Serum ALT, AST, total protein and albumin levels were measured using kits supplied by Diamond Diagnostics, Egypt in accordance with the manufacturer instructions.

2.6.Determination of serum TNF-α

Serum TNF-α level was estimated using Quantikine® ELISA Rat TNF-α Immunoassay kit manufactured by R&D systems, Inc, USA.

2.7.Determination of GPx activity

GPx activity in the hepatic homogenate was estimated according to Paglia and Valentine (1967).

2.8.Determination of reduced hepatic GSH activity

GSH activity in the hepatic tissue homogenate was evaluated following to Sedlak and L' Hanus (1982).

2.9.Determination of lipid peroxidation by-products

Lipid peroxidation by-products in hepatic homogenate were done according to (Ohkawa, Ohishi, and Yagi 1979). The level of lipid peroxides was expressed as nmol /mg protein.

2.10.Analysis of mRNA expression of hepatic IL-1β and IL-4 genes using real time-PCR

Expression of various cytokines were analyzed by real time-PCR using sense and anti-sense primers throughout the experiment as previously described (Farid et al. 2010) using the following primers sets: IL-1*β* (GenBank ID: M98820.1), sense (5′-CAC CTC TCA AGC AGA GCA CAG-3′) and anti-sense (5′-GGG TTC CAT GGT GAA GTC AAC-3′); IL-4, sense (5-CAG GGT GCT TCG CAA ATT TTA C-3′) and anti-sense (5-ACCG AGA ACC CCA GAC TTG TT-3′) and 18S rRNA (GenBank ID: NR_046237.1) as a housekeeping gene, sense (5′-GAG GTG AAA TTC TTG GAC CGG-3′) and anti-sense (5′-CGA ACC TCC GAC TTT CGT TCT-3′).

Total cellular RNA was extracted from liver tissue using an RNeasy Mini kit (Qiagen, Valencia, CA, USA) according to the protocol of manufacturer. Real-time PCR was used using a Power SYBR Green RNA-to-C_t 1-step kit (Applied Biosystems, Foster City, CA, USA) according to the protocol of manufacturer.

The real-time-PCR cycling program consisted of reverse transcription at 48°C for 30 min, initial PCR activation at 95°C for 10 min, followed by 40 cycles of 95°C for 15 s, 60°C for 1 min, and a dissociation curve was added to the protocol whenever necessary.

Real-time-PCR assay was done using a 7300 real-time-PCR system (Applied Biosystems).

Thermal cycling and fluorescence detection were performed using a 7300 real-time-PCR system (Applied Biosystems, Foster City, CA, USA).

Changes in gene expression were calculated from the obtained (C_t) values provided by real-time PCR instrumentation using the comparative CT method to a reference (housekeeping) gene (18S rRNA) (Schmittgen and Livak, 2008).

2.11.Histopathological examination:

The hepatic tissue was obtained from all rats and fixed in neutral buffered formalin (10%). For histopathological examination, thin films were prepared according to (Bancroft and Cook 1994).

2.12.Statistical Analysis:

Statistical analysis was done by the usage of the statistical software package SPSS for Windows (Version 16.0; SPSS Inc., Chicago, Ill). The significance of differences between more than two groups was evaluated by ANOVA. If there is a significant difference indicated by one-way ANOVA, then differences between individual groups were measured using LSD test. Results are expressed as the mean ± SEM. A *P*-value of less than 0.05 was considered significant (Kinnear and Gray 2006).

3.RESULTS

3.1.Hematological Results:

There was a significant reduction in RBCs, hemoglobin concentration, hematocrit value with reduction in WBCs and lymphocytes and counts in Augmentin-treated group in comparison with control, curcumin and curcumin-protected groups (Table 1).

3.2.Serum ALT, AST, total proteins, albumin and TNF-α levels

Table 2 showed significant elevations in serum ALT and AST levels with significant reductions in serum total proteins and albumin levels in Augmentin-treated group in comparison with control group. Moreover, non-significant differences of serum ALT, AST, total protein and albumin was recorded in curcumin group in comparison with control group. Moreover, a significant decrease of serum ALT and AST with a significant increase in serum total proteins and albumin levels were found in curcumin protected group in comparison with Augmentin group. Serum ALT and AST in curcumin protected group reviled non-significant difference in comparison with control and curcumin groups.

3.3.Liver malondialdehyde, glutathione (GSH) and glutathione peroxidase (GPx) activity

Table 2 showed significant elevation in liver malondialdehyde levels with significant reductions in liver GSH and GPx levels in Augmentin-treated group in comparison with control group. On the other hand, no significant differences of hepatic malondialdehyde was detected in curcumin group in comparison with control group. Moreover, significant decrease of hepatic malondialdehyde levels with significant increase in hepatic GSH and GPx enzyme activity were found in curcumin protected group in comparison with Augmentin group. Liver malondialdehyde in curcumin protected group showed non-significant differences in comparison with control and curcumin groups. Liver GSH in curcumin protected group reviled a significant decrease in its level in

spite of its release in comparison with control and curcumin groups.

3.4.Liver tissue IL-1β, IL4 mRNA expression and TNFα:

Figure 1, Figure 2 and Table (2) showed the intensity of hepatic IL-1*β* and IL-4 mRNA normalized to 18s rRNA in the curcumin, Augmentin and curcumin protected rats in comparison with control group.

Augmentin administration caused a significant elevation in the expression of hepatic IL-1*β* and TNF-α in comparison to control group, and this up-regulation was ameliorated by curcumin protection. Moreover, there were significant up-regulation for hepatic IL-4 in curcumin group in comparison with control, and this effect became lessen in Augmentin group and curcumin protected group, however; its level still increased than control group. Curcumin group showed non-significant differences in the hepatic IL-1*β* expression and TNF-α in comparison with control group.

3.5.Histopathological examination of hepatic tissue

The examined sections in Augmentin group showed ballooning degeneration in the hepatic parenchyma with a congestion in the portal vein and a few fibrosis in the portal area (Figure C). While, in curcumin protected group, there was a congestion in the central vein (Figure D). No histopathological alterations and the normal histological structure of the central vein and surrounding hepatocytes in the parenchyma were recorded in control (Figure A) and curcumin (Figure B) groups.

4.DISCUSSION

Amoxicillin/clavulanic acid is a broad-spectrum antibacterial drug that is used in many indications for over 20 years and is now primarily used in the respiratory tract infections treatment (Olayinka and Olukowade 2010). It is a combination of amoxicillin, a semisynthetic penicillin, clavulanic acid and a β-lactamase inhibitor. It is one of the most frequent causes of drug-induced hepatitis (Boelsterli and Kashimshetty 2010; Willis 2013)**.** This hepatotoxicity occurs as a result of oxidative stress and lipid peroxidation that are mediated by oxygen free radicals. Hepatocytes are exposed to reactive oxygen species leading to liver damage. The antioxidant molecules provide hepatocytes with resistance to oxidative stress (Li 2003; El-Sherbiny 2009).

In this study, Augmentin administration caused a significant reduction in RBCs count, hemoglobin concentration, and PCV in comparison with control group. It may be attributed to the hepatic injury and hemolytic anemia induced by Augmentin or may be attributed to the destruction of the mature RBCs and the reduction in the rate of erythropoiesis. These effects of Augmentin on RBCs, hemoglobin concentration and hematocrit matched with Mansour H, (2014). Furthermore, Augmentin caused a significant reduction in the WBCs, lymphocytes count with significant elevation in granulocytic count in comparison with control group. The effects of Augmentin in reduction in WBCs count matched with Lee (2009) who reported a significant reduction in WBCs count in Augmentin group which may be attributed to immunosuppressive effect of Augmentin.

Our study reviled that administration of (Augmentin®) in rats in a dose of 31.83 mg/kg b.wt twice daily for 7 days induced liver damage. This is evidenced by significant elevation in serum ALT and AST than the control group. The elevation of the level of ALT and AST is an indicator of hepatocellular

damage (Olayinka and Olukowade 2010). The increase in liver enzymes after Augmentin administration is consistent with results obtained by EL-Sherbiny et al. (2009); De Abajo et al. (2004). Hepatic damage is evidenced also by significant decreases in serum total proteins and albumin in Augmentin group in comparison with control group that matches with Olayinka and Olukowade (2010) and Olayinka et al. (2012). In addition, there is a significant elevation in the level of serum MDA in Augmentin group in comparison with control group which confirms the occurrence of liver injury by lipid peroxidation and failure of antioxidant defense mechanism to scavenge the free radicals. This agrees with El-Sherbiny (2010) and Delemos (2016) who reported that lipid peroxidation has been occurred due to liver injury due to Augmentin administration. Furthermore, there is a significant reduction in hepatic glutathione and glutathione peroxidase in comparison with control group. Glutathione conjugation serves as a protective mechanism where the toxic metabolites are conjugated and removed as glutathione conjugates. The depletion in the glutathione resulted in accumulation of these toxic metabolites which thereafter induced a state of oxidative stress Jordan et al. (2002); Olayinka et al. (2012); EL-Sherbiny et al. (2009).

Moreover, curcumin administration resulted in a significant decrease in Augmentin-induced elevation of serum ALT and AST levels and a significant increase in serum albumin and total protein levels. This revealed that curcumin alleviated the damaging effect of Augmentin on the liver. These effects matched with Vetvicka and Vetvickova (2016). Meanwhile, serum ALT, AST and total protein levels reached to non-significant levels in comparison with control group and curcumin group. This ascertains that curcumin alleviated the liver cell damage, but liver cell function was not returned completely. Furthermore, the result of this study ascertained the powerful anti-oxidant effect of curcumin through a significant reduction in Augmentin-induced up regulation in serum MDA level with a significant elevation in hepatic glutathione and glutathione peroxidase. Similar results of curcumin were reported by Vetvicka and Vetvickova (2016) who reported that curcumin has a powerful anti-oxidant activity in detoxifying free radicals and stabilization of the cell membrane. The liver is the central organ for drug metabolism for reactive metabolites of drugs. These reactive metabolites and their covalent are binding to cellular proteins leading to hepatocellular injury. Reactive metabolites are often produced through oxidation and reduction by cytochrome P450. The balance between production and detoxification are critical in determining the degree of hepatic injury (Yuan 2013).

The mechanism of Augmentin-induced hepatotoxicity in rats is mostly induced by various pro-inflammatory cytokines associated with hepatic toxicity. This observation is supported by the up-regulation of hepatic IL-1β mRNA expression and TNF-α as a result of Augmentin administration. Several laboratories have recorded that inflammatory cytokines are increased in Augmentin toxicity. TNF-α is a major cytokine involved in inflammation that plays a role in liver injury (Clark 2007); Reato and Carlone (2004). Augmentin administration induces TNF-α production by natural killer cells, cytotoxic T lymphocytes and with monocytes (Dufour and Meillet 2005).

Augmentin administration caused significant decrease of IL-4 in comparison with curcumin group. However; its level is still over than control group which may be

attributed to the activated Kupffer cells that released anti-inflammatory cytokines as a compensatory mechanism of the liver cells to Augmentin. IL-4 is an anti-inflammatory immunoregulatory cytokine. It has an important role in regulating antibody production, hematopoiesis and inflammation (Brown and Hural1997). Our results matched with Dufour and Meillet (2005) who concluded that Augmentin administration reduces the anti-inflammatory cytokines production. Moreover, curcumin administration resulted in significant reductions in IL-1β mRNA expression, TNF-α with a significant up-regulation in IL-4 mRNA expression. This indicated that curcumin is a powerful anti-oxidant and anti-inflammatory substance that reverses the inflammatory effects of Augmentin as curcumin reduces the production of proinflammatory cytokines and increases the production of anti-inflammatory cytokines Billerey-Larmonier et al. (2011).

In this study, curcumin administration resulted in significant ameliorations of IL-1β expression and TNF-α in comparison with Augmentin group that associated with a significant elevation in IL-4 mRNA expression. This indicated that curcumin is a powerful anti-oxidant and anti-inflammatory substance that reverses the inflammatory effects of Augmentin as curcumin reduces the production of proinflammatory cytokines and increases the production of anti-inflammatory cytokines. The potent anti-inflammatory effects of curcumin are probably attributed to its ability to modulate various signaling as NF-κB and cyclooxygenase 2 also, this anti-inflammatory effects mediated via inhibition of secreation of pro-inflammatory cytokines (Vetvicka and Vetvickova 2016; Camacho-Barquero et al. 2007; Epstein et al. 2010; Salh et al. 2003; Billerey-Larmonier et al. 2011).

5.CONCLUSION

It was concluded that, Curcumin is a potent anti-inflammatory and anti-oxidant substance that could be used as a remedy which ameliorates the injurious hepatotoxic, inflammatory and oxidative effects induced by Augmentin administration.

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Table 1: RBCs count, Hb concentration, Hct % and WBCs count in Control, Curcumin, Augmentin and Curcumin protected groups (Mean± S.E.M) (*n* = 10).

parameter	Control	Curcumin	Augmentin	Curcumin+Aug.
RBCs (10^6/µL)	09.01 ± 0.50^{b}	08.74 ± 0.25^{b}	06.46 ± 0.30^{a}	08.66 ± 0.30^{b}
Hb (g/dL)	17.73 ± 0.64^{b}	17.88 ± 0.27^{b}	15.17 ± 0.09^{a}	17.49 ± 0.37^{b}
Hct (%)	46.62 ± 0.53^{b}	46.60 ± 0.47^{b}	41.59 ± 0.81^{a}	46.12 ± 0.36^{b}
WBCs (10^3/µL)	07.63 ± 0.20^{b}	07.54 ± 0.35^{b}	06.26 ± 0.18^{a}	07.40 ± 0.30^{b}
Lymphocyte (10^3/µL)	06.21 ± 0.08^{b}	06.43 ± 0.30^{b}	05.07 ± 0.28^{a}	06.29 ± 0.29^{b}
Granulocytes (10^3/µL)	00.62 ± 0.06^{a}	00.53 ± 0.02^{a}	00.49 ± 0.05^{a}	00.50 ± 0.07^{a}
Monocyte (10^3/µL)	00.60 ± 0.06^{a}	00.57 ± 0.07^{a}	00.70 ± 0.09^{a}	00.60 ± 0.04^{a}

Means with different superscripts within the same row were significantly different.

Table 2. ALT, AST, Total Protein, Albumin, TNF-α, MDA, GSH and GSH-PX in Control, Curcumin, Augmentin and Curcumin protected groups (Mean± S.E.M) (*n* = 10).

Parameter	Control	Curcumin	Augmentin	Curcumin+Aug.
ALT (U/L)	22.16 ± 0.57^{a}	23.25 ± 0.34^{a}	104.87 ± 1.01^{b}	23.71 ± 0.37^{a}
AST(U/L)	47.05 ± 0.20^{a}	46.28 ± 0.30^{a}	114.62 ± 0.63^{b}	47.13 ± 0.30^{a}
TP (g/dl)	07.10 ± 0.09^{b}	07.06 ± 0.06^{b}	003.54 ± 0.05^{a}	06.73 ± 0.26^{b}
Albumin (g/dl)	03.71 ± 0.27^{b}	03.76 ± 0.23^{b}	02.66 ± 0.08^{a}	03.36 ± 0.30^{b}
TNF-α (Pg/ml)	10.51 ± 0.27^{a,b}	10.15 ± 0.15^{a}	39.18 ± 0.84^{c}	12.16 ± 0.52^{a,b}
MDA (nmol/g)	30.14 ± 0.55^{a}	30.08 ± 0.52^{a}	53.53 ± 0.43^{b}	30.06 ± 0.44^{a}
GSH (nmol/g)	36.10 ± 0.11^{c}	44.40 ± 0.25^{d}	11.82 ± 0.15^{a}	35.03 ± 0.56^{b}
GPx (nmol/g)	00.39 ± 0.05^{b}	00.50 ± 0.01^{c}	00.04 ± 0.08^{a}	00.37 ± 0.03^{b}

Means with different superscripts with in the same row were significantly different.

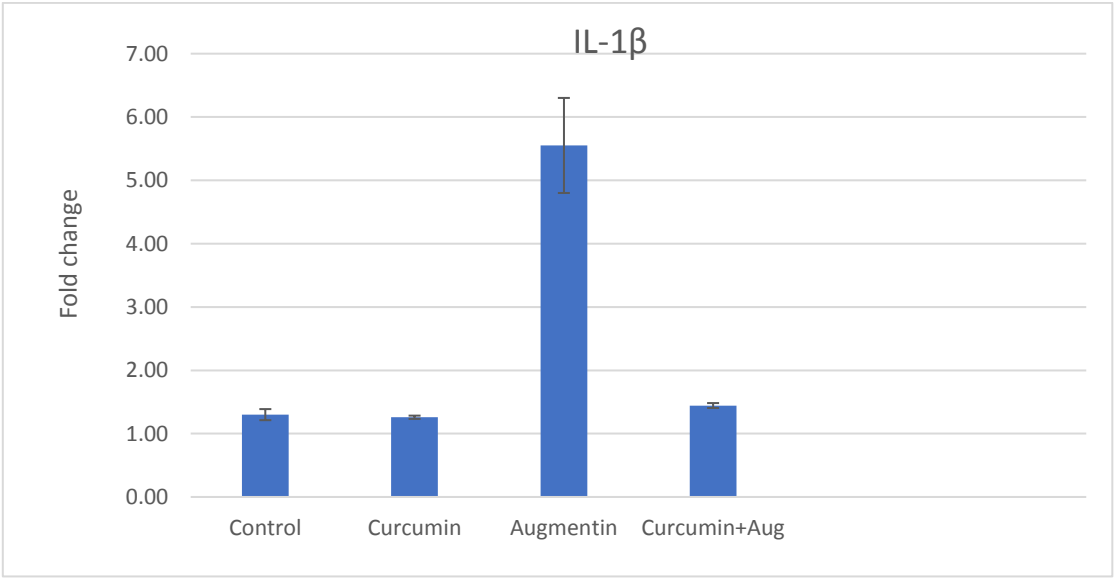


Figure (1)**:** mRNA expression of hepatic IL-1β gene. The expression levels were evaluated by real-time PCR. Means with different superscripts with in the same row were significantly different. Bars represent means ± S.E.M. (*n* = 10).

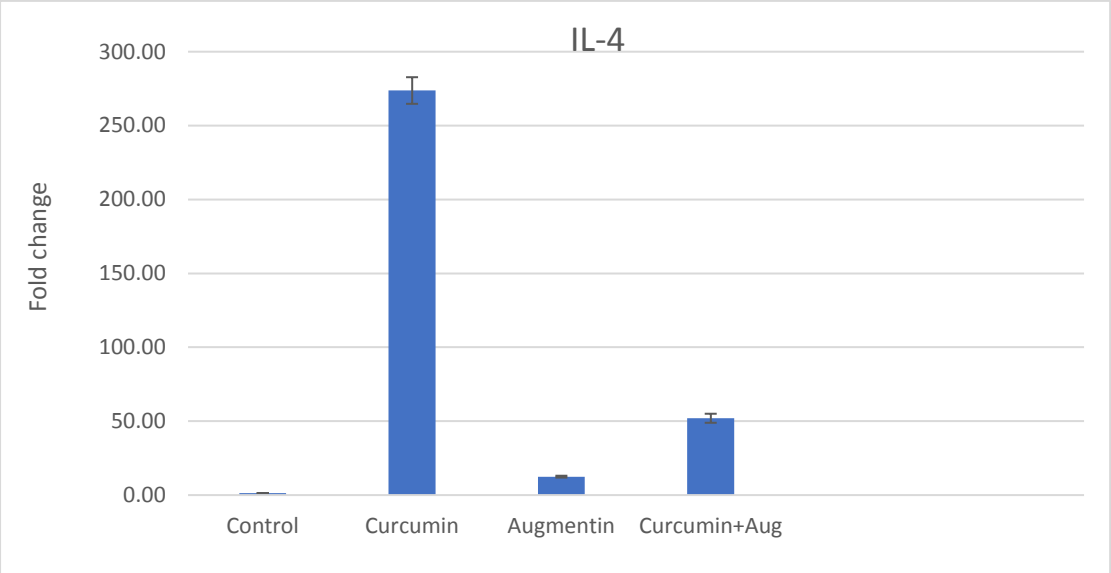
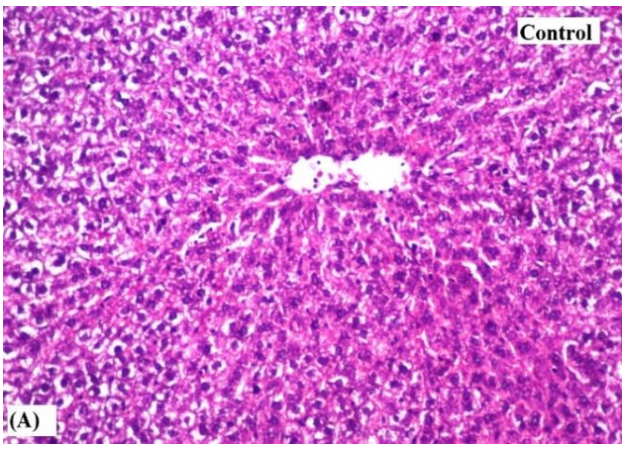
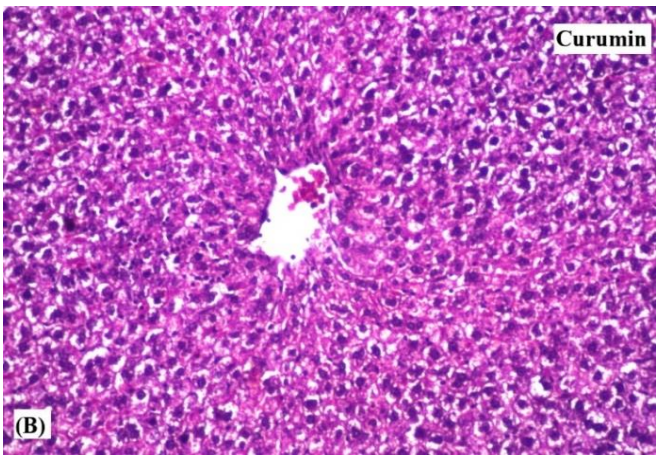
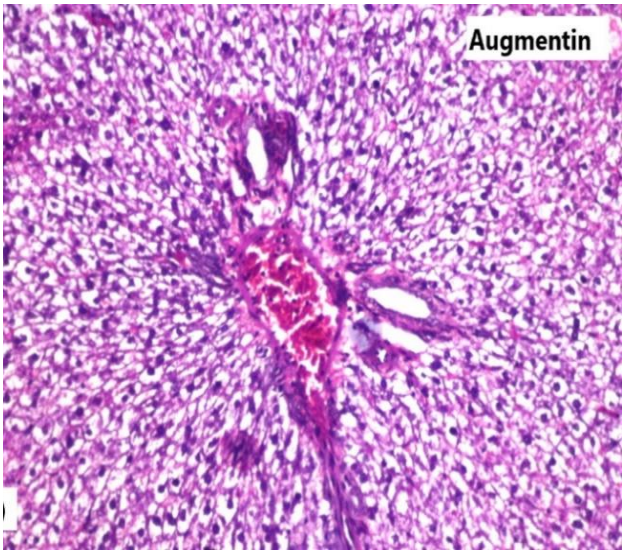


Figure 2. mRNA expression of hepatic IL-4 gene. The expression levels were evaluated by real-time PCR. Means with different superscripts with in the same row were significantly different. Bars represent means ± S.E.M. (*n* = 10).







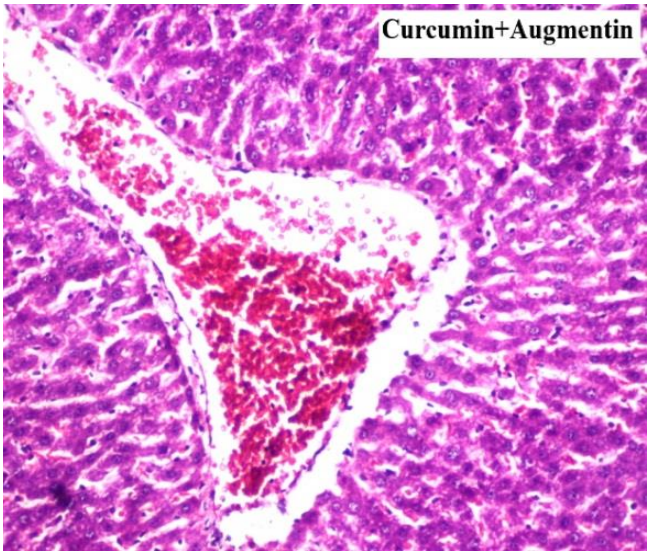


Figure 3. Histopathological examination of rat liver sections of different experimental groups. 40 X magnification.

(A): Control group
(B): Curcumin group
(C): Augmentin group
(D): Curcumin+Augmentin group