

Modulatory Role of Gallic Acid and Vitamin C on Amoxicillin/Clavulanic Acid Combination Induced Hepatotoxicity in Adult Albino Rats

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ABSTRACT

KEYWORDS

Amoxicillin,
Clavulanate, Gallic acid,
Vitamin C,
Hemoxygenase gene.

Amoxicillin/ Clavulanate (AC) combination has become one of the antibiotics most widely prescribed used in the treatment of several bacterial infections, associated with liver injury. This study aimed to investigate amoxicillin/clavulanic acid induced hepatotoxicity and the modulatory effect of gallic acid (GA)/ vitamin C (VTC) individually and in combination on oxidative stress-related liver damage. Sixty-four male albino rats were randomly separated into eight groups; negative control; GA group; VTC group; GA +VTC group; AC- treated group; AC + GA- treated group; AC + VTC treated group and AC+ GA + VTC treated group. A twice daily dose of AC (31.83 mg/kg) and a single daily dose of both GA (60 mg/kg) and VTC (200 mg/kg/day) were introduced to rats orally for 7 consecutive days. After sacrificed, blood was collected for biochemical analysis of aspartate aminotransferase (AST), alanine aminotransferase (ALT), serum tumor necrosis factor alpha (TNF- α), caspase-3, RNA of heme oxygenase-1 gene (HMOX-1) and liver sample for lipid peroxidation and histopathological study. The serum levels of AST, ALT and TNF- α were significantly increased and upregulation of caspase-3 protein in rats treated with AC. Hepatic contents of malondialdehyde (MDA) were markedly increased following AC administration but produced a significant decrease in the levels of reduced glutathione (GSH) and glutathione-S-transferase (GST) along with down regulated the expression of HMOX-1. These findings were in accordance with the histopathological findings. Co-administration of GA and/or VTC along with AC to rats reduced liver injury, oxidative stress, apoptosis and histopathological alterations. So, we concluded that GA and VTC pose a positive modulatory effect against AC induced hepatotoxicity.

Introduction

Drug-induced liver injury is becoming popular around the world. Antibiotics are known as one of the causes of liver injury, due to its high exposure rate (Leitner et al., 2010; Devarbhavi and Andrade, 2014).

Amoxicillin/clavulanic acid (AC) is an oral Broad-spectrum antibacterial compound composite of an antibiotic semi-synthetic penicillin (amoxicillin) and an inhibitor of β -lactamase (potassium clavulanate) (Fig. 1). It has been effectively used for over 20 years in the treatment of various bacterial infections (White et al., 2004; Olayinka and Olukowade, 2010).

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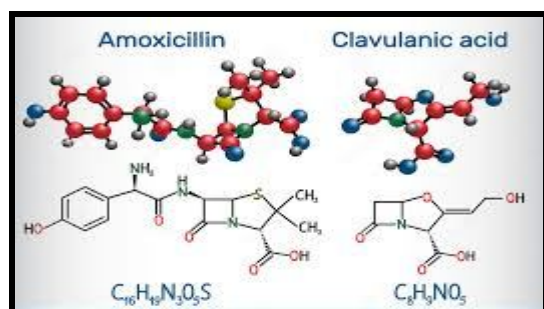


Fig. (1): Chemical structure of amoxicillin/clavulanic acid (Olayinka et al., 2012).

Although AC has become one of the antibiotics most commonly prescribed, the administration of the drug might be correlated with cholestatic and hepatocellular liver injury, which appeared to be primarily due to the clavulanate component (Stine and Chalasani, 2015; Yu et al., 2017a).

Hemeoxygenase (HO) in the heme degradation pathway is a rate-limiting enzyme. Many compounds such as metal ions and heme compounds are produced in animal tissues, especially the liver. Oxidant species have a vital role in the production of heme oxygenase gene by direct action or by consumption of GSH. Increased activity of heme oxygenase will enhance unconjugated bilirubin formation which known a biological buffer against oxidative stress in the liver and a reactive oxygen species (ROS) effective scavenger (Guillermo et al., 2000).

Gallic acid (3,4,5-trihydroxybenzoic acid, GA) is a polyphenolic substance extracted naturally from plants in particular green tea, grapes, various berries and popularly used in food, drugs and cosmetic products. It has been received great attention because of it has been proven to possess powerful antioxidant properties of the diminution reactive oxygen species (ROS), such as superoxide anions, hydrogen peroxide, hydroxyl radicals and hypochlorous acid (Priscilla and Prince, 2009; Chaphalkar et al., 2017).

Vitamin C (ascorbic acid, VTC) is an antioxidant vitamin soluble in water present in the diet which representing essential cofactor for many enzymes involved in different biochemical functions and acts as a reducing agent or an electron donor. The adverse effect of reactive oxygen and nitrogen species is significantly reduced, which can induce oxidative damage to macromolecules like those of lipids, proteins and DNA. It is also capable of scavenging/neutralizing singlet oxygen, hydroxyl, superoxide and water soluble peroxy radical (Jacob and Sotoudeh, 2002; Seghrouchni et al., 2002).

The current work was planned to assess the scavenging antioxidative bioactivities of gallic acid /vitamin C and synergistic modulatory effect of both on oxidative stress-related injury of liver in experimental rats poisoned with amoxicillin/clavulanic acid.

Material and Methods

Chemicals:

Augmentin 625mg (a combination of Amoxicillin/clavulanic acid) was purchased from a local pharmacy in the form of film-coated tablets manufactured by GlaxoSmithKline Egypt). Gallic acid white powder of purity $\geq 98.5\%$ was purchased from Sigma-Aldrich Chemical Co. (USA). Vitamin C powder (100%) purity was purchased from El-Gomhureia pharmaceutical and chemical company, Cairo, Egypt. All other chemical reagents used were analytical grades and were prepared freshly prior to each experiment.

Dosage regimen and vehicle:

All drugs were taken for seven consecutive days, orally via appropriate gastric tube. Amoxicillin/ Clavulanate (Augmentin 625mg) tablets were ground into a powder, dissolved in distilled water resulting in a suspension and it was administered at a dose

represented the human therapeutic dose of (31.83 mg/kg/ twice daily) according to (Olayinka and Olukowade, 2010).

Gallic acid was dissolved in distilled water at a dose of (60 mg/Kg /day) according to (Omobowale et al., 2017).

Vitamin C was dissolved in distilled water at a dose of (200 mg/kg/day) according to (Farombi and Onyema, 2006).

Experimental animals:

Sixty-four (64) adult healthy male albino rats weighing 200-220 g were received from Helwan farm (animal house of the Biological Products and Vaccines; VASERA, Giza, Egypt). Rats were left for one week for acclimatization prior to starting the experiment. All care and procedures adopted for the current study were according to the approval of the animal ethics committee of Scientific Research, Faculty of Medicine, Banha University, Egypt.

Experimental design and treatments:

Rats were separated randomly into eight equal groups of (eight per each) as follows: *Group a* (negative control): Only treated with distilled water till the end of the experiment to measure the basic parameters. *Group b* (GA-treated): treated with GA. *Group c* (VTC-treated): treated with VTC. *Group d* (GA + VTC- treated). *Group e* (AC- treated): was received AC. *Group f* (AC + GA- treated). *Group g* (AC + VTC- treated). *Group h* (AC + GA + VTC- treated).

Sampling:

a. Serum samples:

The rats after 24 hours of last treatment were anaesthetized and sacrificed. Blood was collected from abdominal aorta in two tubes;

one to separate serum for biochemical analysis of (aspartate aminotransferase (AST), alanine aminotransferase (ALT), serum tumor necrosis factor alpha (TNF- α) and caspase-3). The other tube was used to determine RNA of heme oxygenase-1 gene (HMOX-1).

b. Tissue samples:

The liver was quickly removed after scarification, washed with ice cold physiological saline and sectioned into two parts; the first one was stored at -20 °C. Later, the homogenate was centrifuged at 4000 rpm for 5 min at 40 °C to separate the supernatant which was utilized for estimation of malondialdehyde (MDA), reduced glutathione (GSH) and glutathione -S- transferase (GST). The other part from hepatic tissue was used for histopathological samples.

Methods:

a. Determination of Liver function parameters (serum AST and ALT):

They were estimated spectrophotometrically using commercial test kits of (AST and ALT) supplied by Diamond Diagnostics. The level of both (AST and ALT) was expressed as (U/L).

b. Determination of serum tumor necrosis factor alpha (TNF- α) and caspase 3:

They were estimated by enzyme-linked immunosorbent assay (ELISA Rat TNF- α and caspase-3 Immunoassay kits) manufactured by Orgenium, Helsinki, USA. Their levels were expressed as (pg/ml).

c. Determination of liver lipid peroxidation / antioxidant parameters:

Malondialdehyde (MDA), reduced glutathione (GSH) and glutathione -S- transferase (GST) were measured using a colorimetric method (Bio diagnostics, Dokki,

Giza, Egypt) and were expressed as (nmol MDA/g tissue, mg/g and U/g respectively).

d. Analysis of RNA for expression of heme oxygenase-1 gene (HMOX-1):

Expression of HMOX-1 was analyzed by real time-PCR. Total RNA was extracted from serum by using RNA Mini Kit (Qiagen, USA) where the quantity and quality of RNA were evaluated by nano drop (USA) ratio of readings at 260 and 280 nm (A260/280) to detect the purity of RNA. RNA had a 280/260 ratio of 1.9-2.3 (OD260/OD280).

Reverse transcription - quantitative polymerase chain reaction (RT-qPCR)

RNA (5 µg) was then reversed by using the first cDNA synthesis kit of the revert aid TM (Ferments life science, USA) then cDNA was subsequently amplified with the Syber Green PCR Master Kit (Applied Biosystems, USA) in a 48-well plate, PCR was conducted in a 30µ reaction volume containing 5 µl lcDNA, 2.5 µl forward primers, 2.5 µl reverse primers, the following sequences have been designed Hmox-1, 5-ATA GAG CGA AAC AAG CAG A-3 and 5-TAG AGC TGT TTG AAC TTG G-3GAPDH was used as an internal control and sequence of primers was: forward CAGTGCCAGCCTCGTCTCAT and reverse AGGGGCCATCCACAGTCT TC. 5 µl of DNA polymerase, 7.5 µ syber green and 7.5 µ buffer. By using one step real time PCR instrument (Applied Bio systems, USA), PCR amplification were done as follows: initial denaturation at 94 °C for 5 min, then by at 94 °C 30 s, 35 s at 54 °C, and 2 min at 72 °C for a total of 35 cycles and terminal extension at 72 °C for 10 minutes.

Relative quantification of Hmox-1 was done with the 2-ddCt method, and analyzed by ABI prism 7500 the Boxplot software and values were normalized to the quantity of GAPDH.

e. Histopathological evaluation of the liver:

The liver samples were preserved in neutral buffered formalin (10%) solution for fixation and included in bars of paraffin, then sliced at 4-6 microns' thickness, then stained by using Hematoxylin and Eosin (H&E) according to (Bancroft and Layton, 2019).

Statistical analysis

The data was analyzed using SPSS statistical software (20.0; SPSS Inc., Chicago, IL). Kruskal- Wallis was used for multiple comparisons of quantitative variables and Man-Whitney test was applied for the comparison of quantitative variables after establishing their non-normality by K-S test of normality. ANOVA test was used for comparing means of the parametric values by using post hoc test (LSD) for multiple comparisons. Significant differences are found at $p \leq .05$.

Results

All the studied biochemical parameters of negative control, GA-treated, VTC-treated and "GA + VTC"-treated groups showed non statistically significant differences ($p > 0.05$) between them as shown in (Table 1). So the negative control group was chosen as a representative group to be compared with the biochemical results of the other treated groups.

In the current study, the administration of AC orally alone caused a highly significant increase in the mean values of blood (AST, ALT, TNF- α and caspase 3) and hepatic tissue (MDA) along with a significant reduction of hepatic GSH and GST. Furthermore, there was significant down regulation in HMOX-1 gene as compared with control groups. The highest elevated and reduced values of previously measured parameters were recorded in AC-treated group as shown in (Tables 1 and 2).

However, treatment with GA and/or VTC along with AC for the same period in “AC+GA-treated”, “AC + VTC”-treated and “AC + GA + VTC”-treated groups led to significant reduction in the elevated serum levels of measured liver injury biomarkers (AST, ALT, TNF- α , caspase 3) and hepatic tissue (MDA), as well as significant improvement in hepatoprotector indices (GSH and GST) in addition to upregulation of (HMOX-1 gene) as

compared to AC-treated groups alone as shown in (Tables 1 and 2).

Moreover, the previously measured parameters illustrate significant improvement when GA and VTC used together in (“AC + GA + VTC”-treated group) as compared to (AC-treated, “AC + GA-treated” and “AC + VTC”-treated) groups with exception of (AST and caspase-3) where the improvement was insignificant as shown in (Tables 1 and 2).

Table (1): Statistical comparison among the studied groups as regard AST, ALT, caspase-3 and TNF- α .

	AST(U/L) Median (IQR)	ALT(U/L) Median (IQR)	TNF(pg/ml) Median (IQR)	Caspase-3(pg/ml) Median (IQR)
Control group	160 (159-160.75)	47 (44.5-48.75)	4 (2.25-5)	0.7 (0.53-0.9)
GA group	159.5 (157.25-161.75)	46.5 (45-48.75)	3.5 (2.25-4.75)	0.75 (0.55-0.98)
VTC group	159.5 (155.75-161.5)	45.5 (44.25-50.5)	4 (2.25-4.75)	0.75 (0.63-0.9)
GA + VTC group	159.5 (158.25-160)	47 (45.5-49.75)	4 (3.25-5)	0.65 (0.43-1.03)
AC group	411 ^{abcd} (408-414.75)	81.5 ^{abcd} (78.5-84.5)	164 ^{abcd} (152.5-173.25)	10 ^{abcd} (8-11.75)
AC+ GA group	315.5 ^{abcde} (289.75-318.75)	54 ^{abcde} (50.25-65.75)	57.5 ^{abcde} (37.25-66.5)	2.5 ^{abcde} (2-4)
VTC+AC group	314 ^{abcde} (220.5-320.25)	58 ^{abcde} (55.25-62.5)	58 ^{abcde} (37.25-64.75)	3 ^{abcde} (1.05-5.5)
GA +Vit C+ AC group	307 ^{abcde} (234.75-315.75)	48 ^{efg} (45.5-52.5)	17.5 ^{abcdefg} (10-23.5)	2 ^{abcde} (1.13-3.75)
KW Test	43.05	41.89	54.44	48.73
p value	<0.001**	<0.001**	<0.001**	<0.001**

(**) = Highly significant; IQR= Inter Quartile Range; KW: Kruskal-Wallis test; GA= gallic acid ; VTC= vitamin C ; AC= Amoxicillin/clavulanic acid ; AST = Aspartate aminotransferase; ALT= Alanine aminotransferase; TNF- α =tumour necrosis factor alpha; Different letter within the same row indicates significant differences ($p < 0.05$) between experimental groups for the same blood and tissue chemical parameter; ^a→difference versus control group; ^b→ difference versus GA group; ^c→ difference versus VTC group; ^d→ difference versus GA + VTC group; ^e→ difference versus AC group; ^f→ difference versus AC+ GA group; ^g→ difference versus AC.

Table (2): Statistical comparison among the studied groups as regard MDA, GSH, GST, and Heme-oxygenase gene expression:

	Tissue MDA Median (IQR)	Tissue GSH (mean ± SD)	Tissue GST (mean ± SD)	Heme-oxygenase Median (IQR)
Control group	38 (33.75-39.75)	50.67±3.16	9.99±1.74	14.11 (12.78-14.64)
GA group	37 (35.25-39.5)	50.42±3.04	9.81±1.51	13.43 (12.64-14.34)
VTC group	36.5 (32.25-42)	50.12±3.73	9.95±1.99	13.6 (12.71-14.64)
GA + VTC group	37 (33.5-39.75)	49.1±4.61	9.6±1.76	14.09 (12.86-14.57)
AC group	95 ^{abcd} (90-103.5)	17.79 ^{abcd} ±3.83	2.64 ^{abcd} ±1.03	0.075 ^{abcd} (0.06-0.09)
AC+GA group	57.5 ^{abcde} (50.75-61.75)	30.26 ^{abcde} ±4.22	6.49 ^{abcde} ±1.03	4.24 ^{abcde} (3.44-5.52)
VTC+AC group	56.5 ^{abcde} (50.25-63.75)	29.47 ^{abcde} ±2.47	6.03 ^{abcde} ±1.0	4.24 ^{abcde} (3.74-5.21)
GA +Vit C+ AC group	46.5 ^{abcdefg} (40.25- 50.5)	41.69 ^{abcdefg} ±3.52	7.73 ^{abcdefg} ±1.47	8.25 ^{abcdefg} (7.21-9.43)
Test	KW = 49.78	F test = 95.26	F test = 25.0	KW = 53.72
P value	<0.001**	<0.001**	<0.001**	<0.001**

(**) = Highly significant; IQR= Inter Quartile Range; KW: Kruskal-Wallis test; F test: ANOVA; GA= gallic acid; VTC= vitamin C; AC= Amoxicillin/clavulanic acid; MDA =malondialdehyde; GSH = reduced glutathione; GST= glutathione S transferase.

Different letter within the same row indicates significant differences (p<0.05) between experimental groups for the same blood and tissue chemical parameter; ^a→difference versus control group; ^b→ difference versus GA group; ^c→ difference versus VTC group; ^d→ difference versus GA + VTC group; ^e→ difference versus AC group; ^f→ difference versus AC + GA group; ^g→ difference versus AC.

Additionally, the present study documented a significant negative correlation between HMOX-1 gene and MDA values and a

significant positive correlation with (GSH and GST) among the studied groups (Table 3).

Table (3): Correlation between the Heme-oxygenase gene expression and each of MDA, GSH and GST among the studied groups.

	Tissue MDA		Tissue GSH		Tissue GST	
	rho	p	rho	P vau	rho	p
Heme-oxygenase gene expression	-0.974	< 0.001**	0.843	< 0.001**	0.808	< 0.001**

MDA =malondialdehyde; GSH = reduced glutathione; GST= glutathione S transferase; rho: Spearman's rank correlation coefficient

Liver sections in negative control, GA-treated, VTC-treated and “GA + VTC”-treated groups (groups a, b, c and d respectively) illustrate normal lobular architecture with normal central vein and radiating cords of well-arranged polyhedral hepatocytes with rounded vesicular nuclei, separated by blood sinusoids as shown in (Figure 2).

However, the histopathological examination of hepatic tissue from rats in AC-treated group (group e) revealed markedly dilated central vein, severe congested dilated sinusoids, cloudy swelling and necrotic

hepatocytes along with pyknotic nuclei (Figure 3-a, b and c).

Co-administration of GA and /or VTC with AC in “AC + GA-treated” and “AC + VTC”-treated and “AC + GA + VTC”-treated group documented a hepatoprotective effect of GA and VTC where there was only dilated congested central vein and cloudy hepatocytes in group (f); and normal central and necro-inflammatory hepatocytes in group (g). On other side sections of “AC + GA + VTC”-treated group (group h) illustrated normal lobular architecture, normal central vein with minimal sinusoidal dilatation and in as shown in (Figures 4, 5 and 6 respectively).

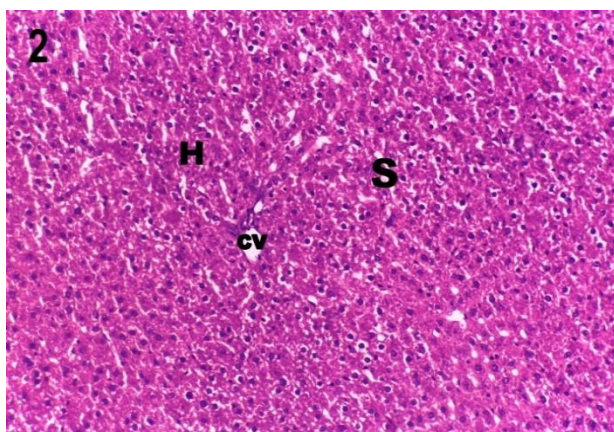


Fig. (2): A photomicrograph of a section in rat's liver prepared from a control rat showing normal architecture with well-arranged polyhedral hepatocytes contained rounded vesicular nuclei (H) normal central vein (CV) and normal sinusoids (S) (H&E x 200).

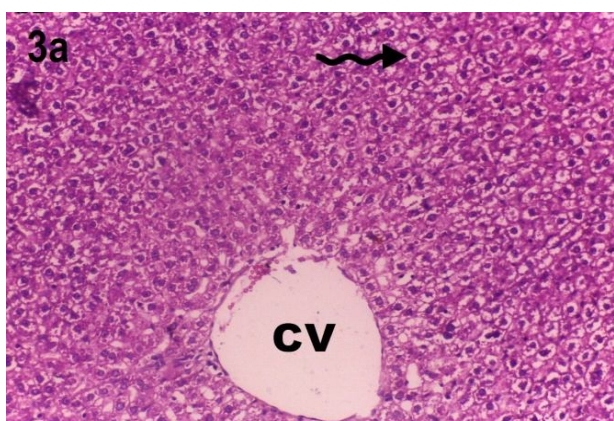


Fig. (3a): A photomicrograph of a section in rat's liver prepared from AC treated group showing marked dilated central vein (CV) and cloudy swelling in hepatocytes (curved arrow) (H&E x 200).

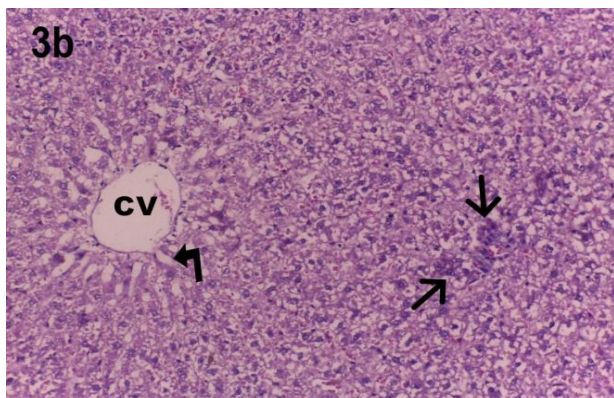


Fig. (3b): A photomicrograph of a section in rat's liver prepared from AC treated group showing dilated central vein (CV), necrotic hepatocytes with pyknotic nuclei (**straight arrows**) and severe dilatation sinusoids (**elbow arrows**) (H&E x 200).

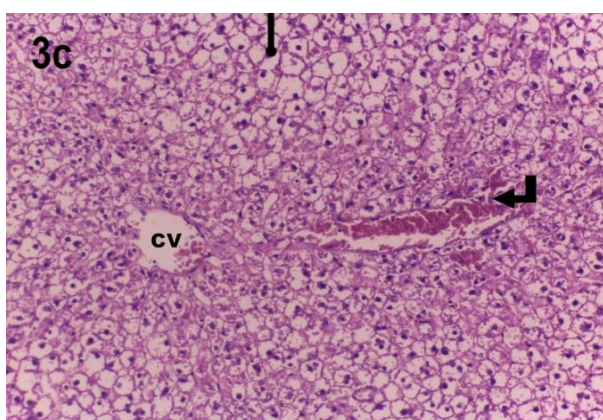


Fig. (3c): A photomicrograph of a section in rat's liver prepared from AC treated group showing dilated congested central vein (CV) and severe congested dilated sinusoids (**elbow arrows**) with hydropic changes in hepatocytes (**drum stick**) (H&E x 200).

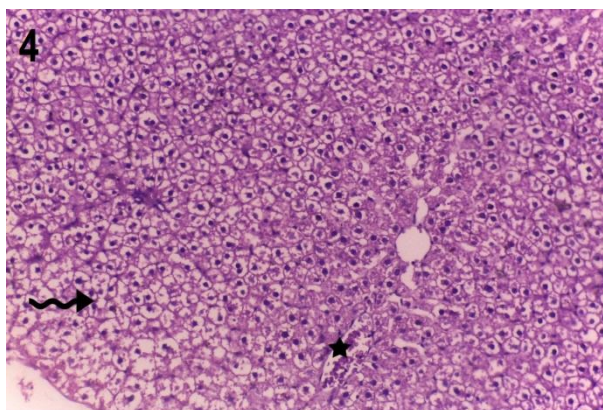


Fig. (4): photomicrograph of a section in rat's liver prepared from AC + GA treated group showing dilated congested central vein (**black star**) and cloudy hepatocytes (**curved arrow**) (H&E x 200).

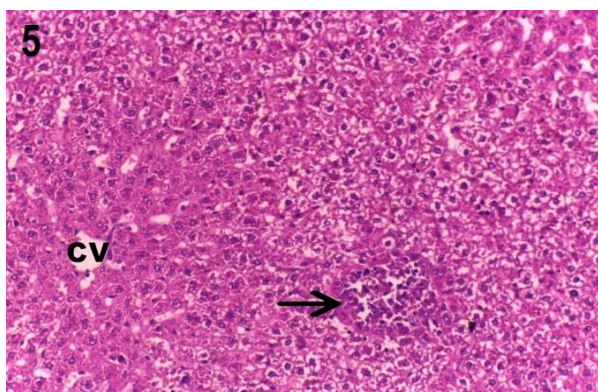


Fig. (5): A photomicrograph of a section in rat's liver prepared from AC + VTC treated group showing normal central vein (CV) and necro-inflammatory hepatocytes (**straight arrows**) (H&E x 200).

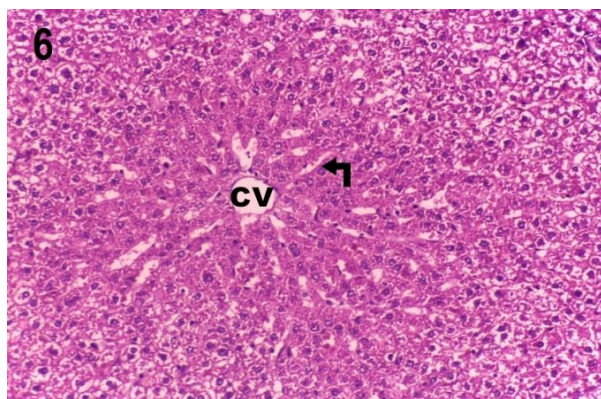


Fig. (6): A photomicrograph of a section in rat's liver prepared from AC + GA+ VTC treated group showing normal central vein (CV) and moderate dilatation of sinusoids (elbow arrows) (H&E x 200).

Discussion

The liver is an imperative central organ that plays a crucial role in the metabolism of many drugs and their reactive metabolites which render it not only the most important organ for detoxification, but also a major target for their toxicity as well (Ghabril et al., 2010; El-Hosseiny et al., 2016).

Amoxicillin, especially in combination with potassium clavulanate (AC), is a widely prescribed antibiotic to prevent and treat Gram-negative bacterial infections. However, many evidences documented that it is the most common drug in clinical medicine strongly associated with drug-induced hepatocellular damage and cholestatic liver injury (Stine and Chalasani, 2015; Yu et al., 2017 b).

The treatment of rats with gallic acid (GA) or vitamin C (VTC) alone or in combination did not illustrate any significant variation in the whole evaluated parameters when compared to non-treated control rats, so negative control group was chosen as a representative group to be compared with the biochemical and histopathological results of the rest of other treated groups.

Regarding liver function biochemical parameters, the findings of the current study documented a marked significant increase in Aspartate aminotransferase (AST) and Alanine aminotransferase (ALT) levels in serum of AC-

treated group when compared with control and other studied groups. EL-Sherbiny et al. (2009) reported an excess activity of plasma AST and ALT on administration of combined AC+ Clavulanate (50 mg/kg, 10mg/kg, respectively, orally for 21 days). Also, Olayinka et al. (2012) and El Shemy (2018) who treated rats with AC at a dose of 31.83 mg/kg body weight twice for seven days reported the same results.

The increased serum level of previously mentioned cytoplasmic hepatocyte enzymes might be due to excessive release of these enzymes into blood stream as a result of toxic effect of AC on liver cells. This cause disruption and damage of hepatocytes cell membranes, destruction and necrosis of hepatocytes. Additionally, the hepatic enzymes activity are probably raised due to significant increase in malondialdehyde (MDA) and reduction in reduced glutathione (GSH) and glutathione -S- transferase (GST) concentration at the AC treated group. These are considered the first line defense in scavenging the free radicals (Gumieniczek, 2005; Sahib et al., 2016).

Tumor necrosis factor alpha (TNF- α) is a well characterized major inflammatory cytokine developed by macrophages/ monocytes during acute inflammation and is responsible for inflammation that plays a role in liver injury (Santos et al., 2015). It is the

first multifunctional cytokine generated from monocytes and macrophages during trauma or infection which can do the inflammatory cascade and share in exacerbating liver injury. It has been noticed that drug exposure during periods of inflammation can increase susceptibility of human's toxicity (Maruf and O'Brien, 2014; Kim et al., 2015; Yao et al., 2016).

In the current study, tumor necrosis factor alpha (TNF- α) activity was significantly increased in AC treatment group when compared to other groups that agreed with the previous results obtained by (Yu et al., 2017 b; El Shemy, 2018). Furthermore, Dufour and Meillet (2005) stated that Augmentin administration induces TNF- α release by cytotoxic T-lymphocytes, natural killer cells and monocytes.

Apoptosis is a state of programmed cell death that occurs under both physiological and pathological situations. However, it was found that free radicals-produced oxidative stress can attack the membrane phospholipids in mitochondria, this leads to loss of mitochondrial membrane potential causing the release of intermembrane proteins, such as cytochrome c, to the cytoplasm which can trigger caspase-3 activation leading to cell apoptosis (Li et al., 2006).

In the present study, oral intake of AC produced marked significant increase in caspase -3 level as compared to the control and other treated groups. Current finding of induction of apoptosis by AC is consistent with previously published studies by El-Kholy et al. (2019) who documented marked increases in the apoptotic markers caspase-3 in the liver of treated rats with oral administration of AC (30 and 60 mg/kg) and this apoptotic effect of the drug was found to be dose-dependent. Amoxicillin/ Clavulanic acid triggered apoptosis is related to the overproduction oxidative reactive molecules along with a

reduction in antioxidant GSH and GST (Yu et al., 2017a).

Lipid peroxidation is an accurate indicative parameter for reactive oxygen species (ROS) created systemic injury (Georgieva et al., 2006). Malondialdehyde is recognized as one of the last products of lipid peroxidation. Its level is generally known as the marker of antioxidant status and oxidative stress (Tsikas et al., 2016; Yu et al., 2017 b). Glutathione S-transferase is a highly diverse family of enzymes which protect against the breakdown products of macromolecules during oxidative stress. GSH supply the cell with multitude of defenses against reactive oxygen species and toxic products. In fact, GSH a major reductant in cells, can reduce a wide variety of disulfides by trans-hydrogenation, and is an important ROS scavenger (Lee et al., 2010).

The current study documented a highly significant increase in MDA concentrations in AC group and a significant reduction of reduced GSH and GST at the same group as compared with other studied groups. The increased production of free radicals MDA accompanied with reduction of the detoxifying capability of the liver (reduction of GSH and GST) in the present study could be attributed to oxidative damage to vital molecules in the cells which leads to the destruction of cell membranes with subsequent release of biomarkers of liver injury. Several previous studies have linked disturbance in the balance between lipid peroxidation by-products; MDA and antioxidant parameter; in AC administration to deleterious hepatotoxic effects. El-Sherbiny et al. (2009) announced that AC or clavulanic acid administration and their combination resulted in a pronounced elevation of the oxidative stress marker as demonstrated by increased lipid peroxidation MDA and depletion of antioxidant molecules such as GSH. Additionally, El-Kholy et al.

(2019) revealed that AC in a drug dose-dependent reduced the hepatic levels of antioxidant molecules including GSH. Also, findings by Olayinka et al. (2012); Delemos et al. (2016); Sahib et al. (2016); Yu et al. (2017a) and El Shemy (2018) were in line with the current work.

Inducible heme oxygenase gene (HO) subtype inducible heme oxygenase gene -1 (HMOX-1) is a key protective molecule in mammalian cells. It is able to relieve acute and chronic liver injury due to its characteristic anti-inflammatory and anti-apoptotic characters, and may be useful as a new strategy of alleviating this type of damage (Li et al., 2019). In cases of inflammation and apoptosis, HO-1 overexpression is one of the protective mechanisms for activation in tissues and organs (Myloie et al., 2015; Wei et al., 2018). It is also involved in maintaining the balance of antioxidants and oxidants in the process of cell damage (An and Shang, 2018; Deng et al., 2018). The results of our study illustrated that acute AC exposure leading to a significant inhibition of HMOX1 gene expression compared to other studied rat's groups. In contrast, concomitant administration of GA and VTC either individually or in combination with AC caused a highly significant upregulation in HMOX1 gene expression with best results obtained when GA and VTC used in combination which probably is due to synergistic effect.

Additionally, we observed the presence of a highly significant negative correlation between HMOX1 gene expression and MDA. So, there was a highly significant positive correlation between HMOX1 gene expression and both antioxidant parameter (GSH and GST) which could be explained by the fact that the induction of HMOX1 is well-formed as a cytoprotective response to liver injury by suppressing oxidative stress, inflammation and reducing apoptotic hepatic damage (Gao et al.,

2017). Yao et al. (2007) documented inhibition in HMOX1 gene expression due to chronic ethanol administration. Also, Kyokane et al. (2001); Dorman et al. (2004) reported that over-expression of HO-1 has been illustrated to be protective against endotoxin mediated hepatic dysfunction. Additionally, stimulation of HO-1 has recently been demonstrated as a defense of human hepatocytes against hypoxia, suggesting that HO-1 induction may have a potential therapeutic effect against inflammatory attacks (Tüzüner et al., 2004).

In this line, Omobowale et al., (2017) found that rats' treatment with GA at a dose of (60 mg/kg body weight orally for 7 days) produced hepatoprotective effect against hepatic damage in Doxorubicin cancer chemotherapy. Furthermore, Oyagbemi et al. (2016) concluded that GA treatment reverses the oxidative stress by enhancing the antioxidant defense system and preventing lipid peroxidation (LPO) and therefore, speculate that the inherent antioxidant property of this compound is responsible for its hepatoprotective effect against cyclophosphamide-induced toxicity. Also, results of the study by Safaei et al. (2018) proposed that oral GA may protect liver tissue from oxidative damages induced by methotrexate by altering the activity of antioxidant enzymes and antioxidant levels. Direct or indirect antioxidant properties of GA can decrease oxidative stress-induced injuries in liver tissue exposed to methotrexate.

Ascorbic acid (vitamin C) represents the first line of antioxidant defense, and this vitamin is likely to be most susceptible to free radical oxidation. It is a strong free radical scavenger as a result of its chemical characters (Olayinka and Olukowade, 2010). In accordance with our study, Abdulkhaleq et al. (2018) reported that pretreatment with vitamin C has been found to be beneficial in hepatic oxidative stress prevention induced by

paracetamol overdose. Moreover, Farombi and Onyema, (2006) documented that VTC ameliorates oxidative stress induced by monosodium glutamate.

The results of our study indicated that GA and VTC possess a powerful anti-inflammatory substance that reverses the inflammatory effects of AC as they reduce the production of pro-inflammatory cytokines. Also, their effects on oxidant markers can lead to a clear improvement in oxidative stress.

In line with the biochemical results, the histopathological evaluation of hepatic sections of rats treated with AC showed marked dilatation and congestion in central vein and sinusoids along with hydropic changes, cloudy swelling and necrotic hepatocytes with pyknotic nuclei. These findings are in agreement with many studies; Sahib et al. (2016) revealed that AC administered orally (0.2232 mg/kg, three times/day for 7 days) in adult albino rats resulted in hepatocyte necrosis, central vein enlargement with subsequent hemorrhage and congestion, in addition to the accumulation of many inflammatory cells. El-Hosseiny et al. (2016) demonstrated that a rat model exposed to Co-amoxiclav (30 mg/kg body weight, orally for 7 days) exhibited provoked histoarchitectural changes manifested by hydropic changes, central vein necrosis and congestion, as well as, mild inflammatory and cholestatic changes. Also, El Shemy (2018) concluded that male rats treated daily with AC at a dose of 31.83 mg/kg b. wt twice for seven days orally resulted in ballooning degeneration and hepatic damage. Moreover, the study by Khan et al. (2018) documented acute cellular swelling and vacuolar degeneration with mild coagulative necrosis of hepatocytes.

The histopathological changes in AC treated group occurred due to lipid peroxidation which inducing disruption in membrane lipids of hepatocyte, with

continuous rise in free radicals production. In contrast, the addition of GA and VTC both individually or with each other in conjunction with AC leads to improvement and reverse these findings to some extent.

Conclusions

The current study provided additional evidence that combined Amoxicillin / clavulanate (AC) could be associated with a potential risk of hepatotoxicity. Moreover, synchronous administration of gallic acid (GA) and vitamin C (VTC) either alone or in combination along with AC exerted beneficial effects in the protection against AC-induced inflammation, oxidative stress and hepatic damage in albino rats. These could be largely attributed to their potent anti-inflammatory effects and their ability to improve and activate antioxidant defense mechanisms.

Recommendations

Based on the results of the current study, it's recommended that:

- 1- The used of Amoxicillin/Clavulanic acid should be under the observation of physicians along with monitoring of diagnostic markers of liver injury in the blood. Furthermore, decrease of its use even in the therapeutic doses especially in high risk and vulnerable groups.
- 2- Administration of vitamin C and gallic acid with Amoxicillin/Clavulanic acid have considerable protective effects against AC-induced hepatic toxicity.
- 3- Additional studies on the cytoprotective role of HMOX-1 gene to be used as a new therapeutic line against drugs induced hepatic toxicity.

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الدور المعدل لحمض الجالريك وفيتامين سي لمجموعة الاموكسيسيلين / حمض الكالفولنيك المسبب لتسمم الكبد في الجرذان البيضاء البالغة

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أصبح الاموكسيسيلين/ حمض الكالفولنيك واحدا من المضادات الحيوية الأكثر وصفا على نطاق واسع حيث يستخدم في علاج الكثير من العدوى البكتيرية ويرتبط ايضا بالاصابة بالكبد. هدفت هذه الدراسة إلى البحث في تسمم الكبد الناجم عن الاموكسيسيلين / حمض الكالفولنيك و التأثير التعديلي لحمض الجالريك / فيتامين سي بشكل فردي ومجتمعين على تلف الكبد الناتج عن الإكسدة. تم فصل أربعة وستين ذكور من الجرذان البيضاء بشكل عشوائي إلى ثماني مجموعات: مجموعة سلبية ضابطة، مجموعة حمض الجالريك، مجموعة فيتامين سي، مجموعة حمض الجالريك و فيتامين سي، مجموعة الاموكسيسيلين/ حمض الكالفولنيك، مجموعة الاموكسيسيلين/ حمض الكالفولنيك و حمض الجالريك، مجموعة الاموكسيسيلين/ حمض الكالفولنيك و فيتامين سي، مجموعة الاموكسيسيلين/ حمض الكالفولنيك و حمض الجالريك و فيتامين سي. تم إعطاء الجرذان جرعة مرتين يوميا من الاموكسيسيلين/ حمض الكالفولنيك (٣١,٨٣ ملجم / كجم) وجرعة يومية واحدة لكل من حمض الجالريك (٦٠ ملجم / كجم) و فيتامين سي (٢٠٠ ملجم / كجم / يوم) عن طريق الفم لمدة ٧ أيام متتالية. وبعد الذبح تم أخذ عينات دم لتحليل أمينوترفرافاز (AST)، ألانين أمينوترفرافاز (ALT) وعامل نخر الورم ألفا (TNF- α)، كاسباس ٣- و الهيمواوكسيجيناز الجيني (HMOX-1) وعينات من الكبد للدراسة بيروكسيد الدهون والهستوباثولوجية. يتضح وجود زيادة ذو دلالة احصائية عالية في مستويات ALT، AST، TNF- α بشكل ملحوظ وزيادة تنظيم بروتين كاسباس ٣- في الجرذان التي تم اعطاءها الاموكسيسيلين/ حمض الكالفولنيك. تمت زيادة المحتويات الكبدية للمالونديالديهيد (MDA) بشكل ملحوظ بعد تناول الاموكسيسيلين/ حمض الكالفولنيك، ولكنها أنتجت انخفاضا كبيرا في المستويات الكبدية من الجلوتاثيون (GSH) و الجلوتاثيون اس ترانسفيريز (GST) وكذلك جنبا إلى جنب مع التعبير المنتظم عن (HMOX-1) وكانت هذه النتائج وفقا لنتائج الهستوباثولوجي. ولذلك فان تناول المشترك لحمض الجالريك و فيتامين سي مع الاموكسيسيلين/ حمض الكالفولنيك للجرذان يؤدي إلى تقليل إصابة الكبد والإجهاد التأكسدي وموت الخلايا المبرمج والتغيرات الهستوباثولوجية.