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THE ROLE OF FOLIC ACID AS A PROTECTIVE DRUG ON SOME OF AUGMENTIN-INDUCED TOXIC EFFECTS IN MALE ALBINO RATS

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ABSTRACT

The current study aimed to investigate the protective role of folic acid against the harmful toxic effects in rats induced by long term treatment by augmentin. In the current experiment, twenty-four adult male albino rats (170-200 g) were divided into four groups. The 1st group was the control group, it received saline solution (1 ml/kg). The 2nd group received folic acid (70 µg/kg). The 3rd group received augmentin (31.83 mg/kg). The 4th group was given augmentin and folic acid simultaneously. All doses were given orally and once a day for three weeks. At the day 22, rats were decapitated, blood samples were collected for hematological examination, whereas others were centrifuged, and serum was obtained and preserved for further biochemical examinations. The results showed that augmentin induced significant decreases in the levels of hemoglobin, hematocrit, RBCs, platelets, WBCs and superoxide dismutases (SOD), and significant increases in serum cholesterol, urea, creatinine, aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), triglycerides, total protein, albumin and malondialdehyde (MAD). Simultaneous administration of folic acid with augmentin caused partial or complete enhancement in the hematological parameters such as hematocrit, WBCs count and hemoglobin. Also, biochemical parameters were partially improved by the folic acid administration such as SOD or completely improved such as MAD by rendering them to values close to the normal saline control groups. According to the present study, it was suggested that co-administration of folic acid with augmentin modulates some of the hematological and oxidative stress parameters induced by augmentin.

Keywords: Augmentin, Folic acid, hematology, nephrotoxicity, hepatotoxicity, oxidative stress and, Albino rats

INTRODUCTION

Drugs are a double-edged weapon benefit, and hazard. Although the drugs provide a benefit by modifying processes in the body, it's not surprising that they, also, have side effects and may cause toxicity [1]. Therapeutic dose from the drug produces the desired beneficial effect without unbearable side effects, but a high dose of drug leads to adverse effects on the body. Also, interactions with drugs are common and they can cause side effects or reduce the beneficial effect of the drugs [2].

Antibiotics fight bacteria by killing them or preventing their reproduction. In general, antibiotics act by inhibiting or regulating the enzymes involved in biosynthesis of cell wall, nucleic acids metabolism and repair, protein synthesis and/or disruption of membrane structure [3]. Augmentin is a penicillin drug class that has broad spectrum of activities against the most commonly bacteria [4]; it is composed of amoxicillin, a β-lactam antibiotic, and potassium clavulanate as β-lactamase inhibitor [5]. Specifically, it is used for otitis media, strep throat, pneumonia, cellulitis, urinary tract infections, animal bites, and tuberculosis [6, 7]. Data from several studies suggest that it is safe in the therapeutic dose but increasing the dose can lead to side effects such as hepatitis, and renal damage [8, 9]. Prolonged use of amoxicillin clavulanate could lead to breakdown of the mitochondrial membrane, moreover, opening of mitochondrial permeability transition pores (mPT pore) leads to expulsion of cytochrome C, lipid peroxidation and reduced energy in liver cells. Also, high dose of augmentin concentrating in the kidney medulla can induce kidney injury through direct toxicity or damage by reduced prostaglandin and increased thromboxane production [10].

Folic acid or vitamin B9, is a water-soluble vitamin that stimulates the hematopoiesis, needed for normal cellular metabolic activities and also helps to prevent changes to DNA that may cause initiation of cancer. It is a powerful antioxidant and used as nutritional supplement and in the treatment of specific types of anemia [11]. Folic acid declines oxidative stress and lipid peroxidation, thereby keeping cells healthy by preventing various harmful processes in cells. Recent studies suggest that folic acid has a protective effect against toxicity [11, 12].

Previous reports showed that of folic acid dietary supplementations have protective effects against several diseases and

injuries. Thus, the current work aimed to investigate the protective and modulatory role of folic acid administration on the damaged hematological parameters, hepatotoxicity, nephrotoxicity and oxidative stress induced by augmentin in male albilno rats.

MATERIALS AND METHODS

Experimental animals

Twenty-four adult male Wistar rats weighting from 170-200g were used in this study; they were kept in cages under hygienic condition and supplied with commercial rodent diet and water ad libitum; they were allowed to acclimate for two weeks in photoperiod (dark light cycle 12h: 12h) and the temperature was adjusted at 20-25 °C with constant humidity before beginning the experiment.

Drugs

Augmentin and folic acid were purchased from pharmacy of Minia university hospital, dissolved in sterile physiological saline solution.

Experimental design

Animals were divided into four equal groups (six rats each), the 1st group was considered as the control group that received saline solution (1ml/kg). The 2nd group received a dose of folic acid (70 µg/kg) [13]. To ensure obtaining augmentin-induced hepatotoxicity and nephrotoxicity, the 3rd group received a dose of augmentin (31.83 mg/kg) [9]. To examine the effects of folic acid on the augmentin-induced oxidative stress, hepatotoxicity and nephrotoxicity, the 4th group was given augmentin and folic acid simultaneously with the same doses mentioned above. All doses were given orally and once a day for three weeks.

Hematological and biochemical Analysis

At the end of the experiment, rats were fasted for 12h; at the day 22, animals were anesthetized by diethyl ether, decapitated. Blood samples were collected immediately and divided into two tubes. The 1st tube contained EDTA as anticoagulant for hematological examination and the 2nd tube for biochemical examination, left at room temperature for 15-30 minutes, to coagulate, freez-centrifuged at 3000 x g for 10 minutes and the serum was transferred into a sterilized tube and preserved for further analysis.

Erythrocyte count, packed cell volume (hematocrit), hemoglobin, platelet count and leukocyte count were determined by using the method of Louderback and Fontana (1976) [14]. Aspartate and alanines aminotransferase (AST/ALT) were determined by the kinetic method of Henry et al. (1974) [15]. Alkaline phosphatase (ALP)

was determined by the kinetic method of Tietz and Ash (1995) [16]. Albumin and total protein were estimated according to the colorimetric method of Young (1995) [17]. To examine the renal toxicity and hepatotoxicity caused by augmentin and their modulation by folic acid, the following parameters were analyzed: the serum total cholesterol was determined by the colorimetric method of Watson (1960) [18], and triglycerides was determined by the colorimetric method of Fossati and Prencipe (1982) [19]. Creatinine was determined according to colorimetric – kinetic method of Burtis (1995) [20]. The method of analysis for blood urea depended on the enzymatic colorimetric method of Sethi and Moorthy (1986) [21]. The serum activity of superoxide dismutase (SOD) was determined by the colorimetric method of Marklund and Marklund (1974) [22]. The serum activity of lipid peroxide (malondialdehyde or MDA) was determined by the colorimetric method of Kei (1978) [23]. The optical densities of all chemical analysis were determined by spectrophotometer (Humalyzer 3000, Germany).

Statistical analysis

The present data were analyzed by Analysis of Variance (one-way ANOVA test) using SPSS version 21. Data were expressed as mean ± S.E.M; (n= 6) and significant values were determined at p value < 0.05 (Tukey post hoc test).

RESULTS

The results of the current work are explained in figures 1-5 and tables 1-5. Figure 1 (A-E) and table 1 summarize the results of administering folic acid on change in the level of hemoglobin (HB), hematocrit (HCT), red blood corpuscles (RBCs) and white blood cells (WBCs) respectively, after oral administration of augmentin. The level of HB is significantly elevated by oral administration of folic acid in comparison with control group; on the other hand, its level is significantly reduced by oral administration of augmentin. After co-administration of folic acid with augmentin, it was clear that it was significantly elevated to a level lower than control group (Fig. 1A).

The value of HCT is not affected by folic acid. A significant decrease in HCT was obtained after oral administration of augmentin, whereas a significant increase in the HCT value was obtained after co-administration of folic acid with augmentin (Fig. 1B).

The number of RBCs is significantly reduced by oral administration of augmentin. However, no improvement was obtained with co-administration of folic acid (Fig. 1C).

An unexpected significant decrease in the platelets number was observed in folic acid group; such decrease was continued in augmentin group. Co-administration of folic acid did not change the lowered number of platelets resulted from augmentin administration (Fig. 1D).

Folic acid administration did not affect the number of WBCs when compared with control group, on the other hand augmentin could decrease the number of WBCs; after administration of folic acid with augmentin, the number of WBCs was significantly increased to a level significantly lower than the control group (Fig. 1E).

Figure 2 (A-E) and table 2 represent the results of administering folic acid on the change in the concentration of AST, ALT, ALP, serum albumin and total protein after oral administration of augmentin. Folic acid did not affect the levels of the above-mentioned liver parameters in comparison with control group; however, a significant elevation in the concentration of those parameters was observed after augmentin treatment, such elevation was also continued even after administration of folic acid with augmentin (Fig. 2A-E).

Figure 3. (A and B) and table 3 explain the effects of folic acid on the augmentin-induced total cholesterol and triglycerides. Administration of folic acid did not affect the level of both cholesterol and triglycerides; administration with augmentin caused and elevation in their concentration compared to control group (A and B). Administration of folic acid had no effect on the augmentin-induced elevation of total cholesterol (A); however, the concentration of triglycerides was increased compared to both augmentin and control groups after administration of folic acid with augmentin (B).

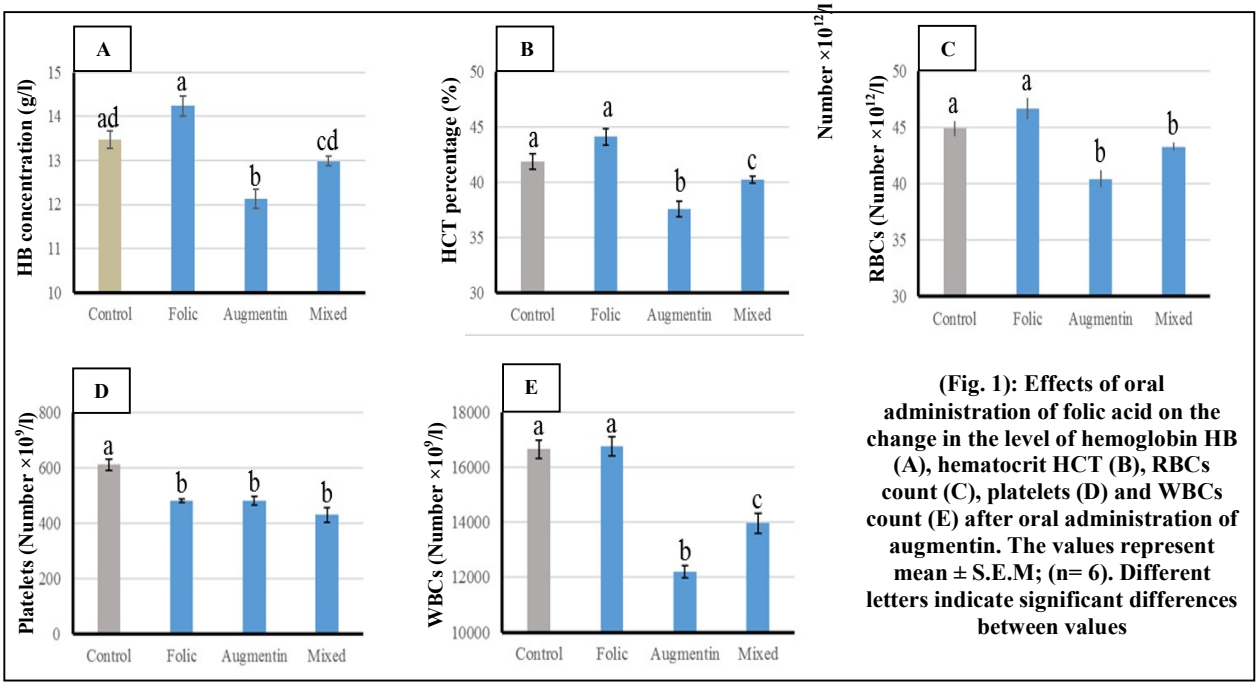
Figure 4 (A and B) and table 4 provide an overview of the effects of administering folic acid on the change in creatinine (A) and blood urea (B) concentrations after oral administration of augmentin. Folic acid group showed a significant upregulation in the creatinine concentration compared to control group, whereas the creatinine was significantly increased compared to both control and folic acid group, such increase persisted even after administering both augmentin and folic acid simultaneously, which means that folic acid did not affect the increased creatinine which was induced by augmentin (Fig. 4A).

The concentration of blood urea did not change after folic acid administration; however, augmentin significantly induced its concentration. After administration of folic

acid with augmentin, the blood urea concentration was kept at a level almost same as augmentin group but significantly higher compared to control group (Fig. 4B).

Figure 5 (A and B) and table 5 summarize the effects of folic acid on the change in SOD and MAD after oral administration of augmentin. Administration of folic acid did not significantly change the level of SOD compared to the control group; the level of SOD is significantly decreased by oral administration of augmentin. After co-administration of folic acid with augmentin, the level of SOD was significantly elevated; however, such elevation did not reach the level of the normal control group (Fig. 5A).

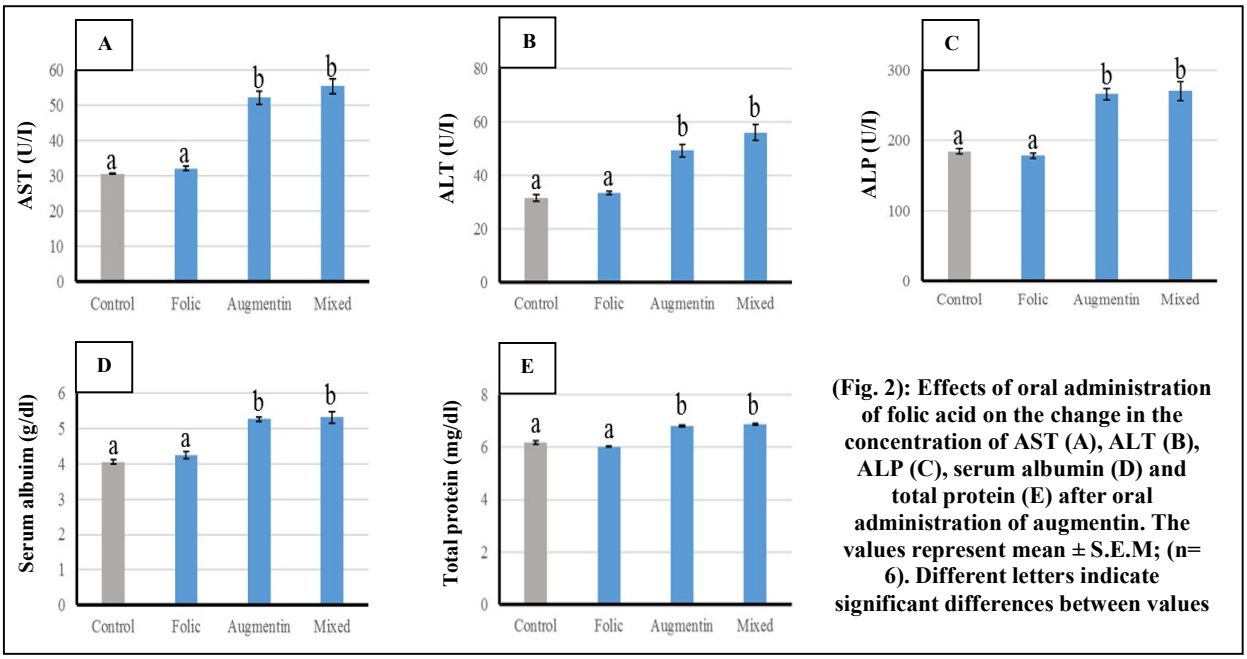
Oral treatment of folic acid did not affect the serum level of MAD which was significantly elevated by augmentin, whereas co-administration of folic acid with augmentin showed significant decrease to a level statistically same as control group (Fig. 5B).



(Fig. 1): Effects of oral administration of folic acid on the change in the level of hemoglobin HB (A), hematocrit HCT (B), RBCs count (C), platelets (D) and WBCs count (E) after oral administration of augmentin. The values represent mean ± S.E.M; (n= 6). Different letters indicate significant differences between values

Table 1: Effects of oral administration of folic acid on the hematological parameters (hemoglobin, hematocrit, RBCs, platelets and WBCs) after oral administration of augmentin. The values represent mean ± S.E.M; (n= 6). Different letters indicate significant differences between values

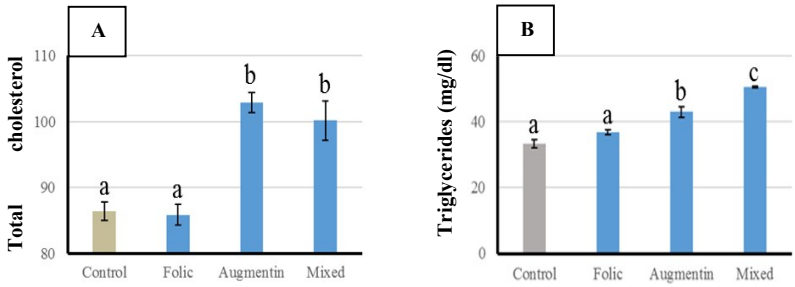
Groups	Hemoglobin (g/l)	Hematocrit (%)	RBCs (number $\times10^{12}$/l)	Platelets (number $\times10^{9}$/l)	WBCs (number $\times10^{9}$/l)
Cont.	13.47±0.20 ^{ad}	41.89±0.69 ^{a}	44.88±0.67 ^{a}	612.33±19.64 ^{a}	16 650±324.04 ^{a}
Folic	14.24±0.23 ^{a}	44.13±0.71 ^{a}	46.65±0.97 ^{a}	482.60±6.69 ^{b}	16 750±352.85 ^{a}
Aug.	12.13±0.22 ^{b}	37.61±0.68 ^{b}	40.42±0.74 ^{b}	482.00±15.22 ^{b}	12 200±227.30 ^{b}
Aug. + Folic.	12.98±0.11 ^{cd}	40.28±0.32^{c}	43.26±0.36 ^{b}	431.00±25.86 ^{b}	13 960±369.59 ^{c}



(Fig. 2): Effects of oral administration of folic acid on the change in the concentration of AST (A), ALT (B), ALP (C), serum albumin (D) and total protein (E) after oral administration of augmentin. The values represent mean ± S.E.M; (n= 6). Different letters indicate significant differences between values

Table 2: Effects of oral administration of folic acid on the parameters of liver functions (AST, ALT, ALP, albumin and total protein) after oral administration of augmentin. The values represent mean ± S.E.M; (n= 6). Different letters indicate significant differences between values

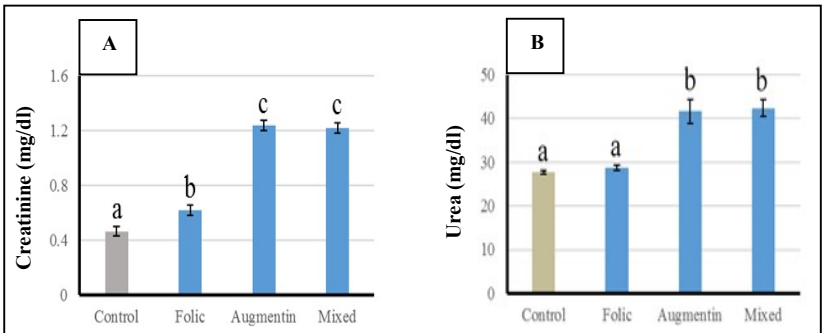
Groups	AST (U/l)	ALT (U/l)	ALP (U/l)	Albumin (g/dl)	Total protein (mg/dl)
Cont.	30.663±0.235^{a}	31.685±1.235^{a}	184.237±3.792 ^{a}	4.058±0.066 ^{a}	6.168±0.064 ^{a}
Folic	32.12±0.64^{a}	33.356±0.654^{a}	178.586±3.581 ^{a}	4.246±0.104 ^{a}	6.026±0.019 ^{a}
Aug.	52.206±1.88^{b}	49.356±2.336^{b}	266.444±8.074 ^{b}	5.27±0.072 ^{b}	6.814±0.033 ^{b}
Aug. + Folic	55.4±2.08^{b}	55.986±2.967^{b}	270.62±13.447 ^{b}	5.322±0.159 ^{b}	6.888±0.039 ^{b}



(Fig. 3) Effects of oral administration of folic acid on the change in the concentration of total cholesterol (A) and triglycerides (B) after oral administration of augmentin. The values represent mean ± S.E.M; (n= 6). Different letters indicate significant differences between values

Table 3: Effects of oral administration of folic acid on level of total cholesterol and triglycerides after oral administration of augmentin. The values represent mean ± S.E.M; (n= 6). Different letters indicate significant differences between values

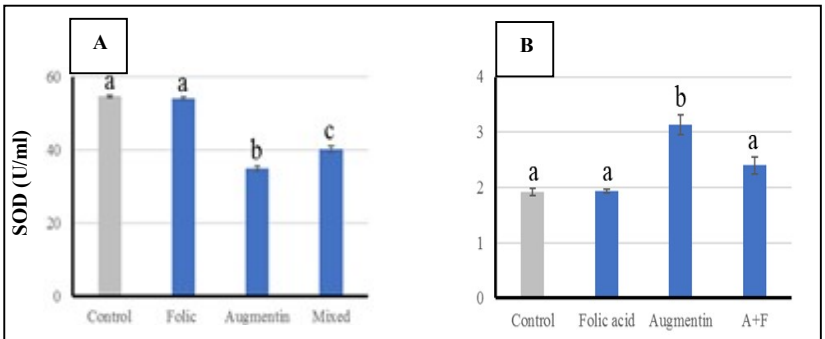
Groups	Total cholesterol (mg/dl)	Triglycerides (mg/dl)
Cont.	86.412±1.42 [a]	33.312±1.35 [a]
Folic	85.836±1.59 [a]	36.798±0.83 [a]
Aug.	102.896±1.53 [b]	42.97±1.56 [b]
Aug. + Folic	100.168±2.95 [b]	50.572±0.21 [c]



(Fig. 4) Effects of oral administration of folic acid on the change in the concentration of creatinine (A) and blood urea (B) after oral administration of augmentin. The values represent mean ± S.E.M; (n= 6). Different letters indicate significant differences between values

Table 4: Effects of oral administration of folic acid on the parameters of kidney functions (creatinine and blood urea) after oral administration of augmentin. The values represent mean ± S.E.M; (n= 6). Different letters indicate significant differences between values

Groups	Creatinine (mg/dl)	Blood urea (mg/dl)
Cont.	0.47±0.03 [a]	27.73±0.38 [a]
Folic	0.62±0.03 [b]	28.69±0.65 [a]
Aug.	1.24±0.04 [c]	41.63±2.75 [b]
Aug. + Folic	1.22±0.03 [c]	42.41±1.94 [b]



(Fig. 5) Effects of oral administration of folic acid on the change in the concentration of SOD (A) and MAD (B) after oral administration of augmentin. The values represent mean ± S.E.M; (n= 6). Different letters indicate significant differences between values

Table 5: Effects of oral administration of folic acid on the oxidative stress parameters (SOD & MAD) after oral administration of augmentin. The values represent mean ± S.E.M; (n= 6). Different letters indicate significant differences between values

Groups	SOD (U/ml)	MAD (nmol/ml)
Cont.	54.63±0.41 [a]	1.92±0.06 [a]
Folic	54.00±0.47 [a]	1.93±0.03 [a]
Aug.	34.93±0.67 [b]	3.13±0.18 [b]
Aug. + Folic	40.23±0.95 [c]	2.40±0.15 [a]

DISCUSSION

The current study aimed to address the changes in the hematological parameters, liver functions, kidney functions and oxidative stress induced by oral treatment with augmentin due to the preventive effects of folic acid as dietary supplement against adverse drug reactions. Interestingly, the most important findings were partial or complete enhancement in the hematological parameters such as hematocrit, WBCs count and hemoglobin; also, partial or complete improvement in the biochemical oxidative stress parameters such as SOD and MAD.

Hematological parameters are important indicators of the health condition of an organism. In this work, augmentin induced hematological disturbances by decreasing HB, HCT%, RBCs number, platelets and WBCs number, such results are consistent with the results of Mintzer et al., (2009) [25]. Moreover, wide spectrum of drug-induced hematologic disturbances (e.g., Pencillins, beta-lactam antibiotics, aspirin, NSAIDs) are mediated by a variety of mechanisms, including immune effects, interactions with enzymatic pathways, and direct inhibition of hematopoiesis [25, 26].

Hematopoietic tissues and leukocytes are two dynamic systems which maintain the homeostasis of an organism by responding quickly to any chemical changes or poisoning in the body. The observed effects of augmentin which were obtained in our study are generally in consistent with many results of various investigations on the animals treated with different chemical factors [26, 27]. It was reported that oral treatment with augmentin, paracetamol, aspirin or lornoxicam resulted in depletion of the RBCs count, hemoglobin concentration, PCV, and WBCs. Such depletion might be attributed to hepatic injury and hemolytic anemia or may be due to the destruction of the mature RBCs which lead to reduction in the rate of erythropoiesis [29-32].

Blood cells provide enough oxygen for tissues, protect the body against infectious diseases and promote coagulation. Because bone marrow is the center of manufacture of blood cells, suppression of bone marrow activity results in depletion of all types of

circulating blood cells. Accordingly, the body will be more susceptible of bacterial and viral infections as a result of WBCs insufficiency [33]; also the number of erythrocytes decreases which lead to decreasing amount of HB and reduced HCT%, thus blood cannot supply tissues with adequate amount of oxygen and therefore causes the symptoms of anemia [34]; whereas the depletion of platelets production causes spontaneous bleeding [35]. Antibiotics and non-steroidal anti-inflammatory drugs are the most common medication may cause bone marrow suppression [25].

In the current study, we observed that some of the undesirable side effects of augmentin were partially or completely improved by administration folic acid simultaneously with it. For example, the augmentin HB, HCT% and WBCs depletions were significantly upregulated by folic acid. We do think that this improving may be attributed to ameliorating the bone marrow activity leading to enhancing the immune functions and production of sufficient RBCs and platelets [34, 36, 37].

Recently, great attention has been focused on the assessment of liver damage by xenobiotics and drugs, which are passing and metabolized into toxic intermediates in hepatic cells. The serum parameters related to liver functions which have been studied in the present work revealed that acute doses of augmentin induced significant increase in the level of AST, ALT, ALP, albumin and total protein. Similar findings were observed that amoxicillin-clavulanate administration induced liver injury [38, 39].

AST, ALT and ALP are normally located in mitochondria, cytoplasm or microsomes of hepatic cells; their increased serum levels may indicate a damage in the hepatic cells and consequently liver damage [40]. The increased serum level of such enzymes may also be attributed to changes in the cell membrane permeability and increased/ decreased catabolism of aminotransferases [41] and it was reported in conditions involving necrosis of hepatocytes [42]. Moreover, the increase in albumin and total protein observed in the present study are not in consent with other findings that showed reduced levels of albumin and total protein induced by augmentin poisoning [43]. On the other hand, our data agreed with those obtained by Agbafor et al. (2015) [44] when their experimental animals were given antibiotics such as ofloxacin and ciprofloxacin. They attributed the unchanged or elevated albumin and total protein level to the liver which keeps its normal functions

within the given doses of the antibiotics. We agree with the above-mentioned authors that the experimental doses of antibiotic used were not strong enough to cause extensive hepatocytes damage to down regulate the synthesis of proteins, thus keeping the protein levels as high as normal in healthy liver.

It was reported that prolonged use of Ciprofloxacin and Amoxicillin Clavulanate could breakdown mitochondrial membrane via induction of opening of mitochondrial membrane permeability transition pore which leads to expulsion of cytochrome C, lipid peroxidation and decrease in energy in the healthy liver cells [10].

Several reports explained that folic acid controlled the activities of the liver enzymes; which is the primary evidence for hepatoprotective activity [44], this is consistent with our results that showed an improvement in the level of liver enzymes when folic acid was given with augmentin. In the current study, we observed that after folic acid administration, no improvement was obtained in the levels of liver enzymes which were affected by augmentin; the differences between our results and the other reports may be attributed to the dose and the duration of using folic acid after stopping administering augmentin.

Our data revealed a significant upregulation in the level of total cholesterol and triglycerides in augmentin group which are consistent with the study of Olayinka and Olukowade (2010) and AL-Harbi (2015) [9, 46]. The elevated levels may indicate a disturbance in fat metabolism as a result of the peroxidation of membranes and modulation of the cellular structure due to toxicity dose of augmentin as well as overproduction of free radicals [47, 48]. The results of our study are in harmony with the previous reports that showed a high level of total cholesterol and triglycerides after amoxicillin clavulanate treatment. Such change in the levels of total cholesterol and triglycerides led to alternation in the membrane permeability [9].

In the current study, it was observed that folic acid did not affect the change in the level of total cholesterol or triglycerides induced by augmentin suggesting that more treatment duration and high dose of folic acid may be required to get such modulation in lipid concentrations obtained by augmentin.

The concentrations of creatinine and blood urea were significantly increased in augmentin, group compared to control group. Blood urea and serum creatinine have been reported to increase in acute and chronic intrinsic renal diseases [49, 50]. The increase

in renal parameters in blood might be observed as a result of lower urinary tract obstruction caused by the drugs and decrease in glomerular filtration which lead to decreased renal perfusion of the kidney, or intrinsic renal lesions [51, 52]. Recent studies showed that almost 70% of acute interstitial nephritis (AIN) were attributed to medications such as NSAIDs, including topical preparations and the selective COX-2 inhibitor celecoxib, penicillin and cephalosporins can cause AIN accompanied with increased serum level of creatinine [53, 54].

The antioxidant enzyme superoxide dismutase (SOD) represents one of the primary intracellular antioxidant defense mechanism against oxidative stress. In the current study, it was observed that augmentin affects the level of SOD by decreasing it significantly when compared with the control group; such observation has been noticed earlier in several studies [55, 56]. When the level of oxidative stresses (OS) is very high, the proteins damage became profound and the activity of SOD decreased either via direct oxidative damage of the SOD molecules, or by alternation in the SOD gene expression, or both [57]. In this study, the observed decrease in the levels of WBCs, was associated with decrease in SOD activity, it was explained that the white blood cells used enzymes such as NADPH oxidase to produce SOD and other reactive oxygen species (ROS) to kill bacteria, also, administration of augmentin decreased antioxidant enzymes in the erythrocytes [58]. Hence, SOD protects the cells from superoxide toxicity. In the present work, when folic acid was co-administered with augmentin, it was clear that the level of SOD was partially enhanced suggesting that folic acid has a potential protective mechanism against oxidative stress and free radical-mediated tissue damage and supporting the observations of different studies [59, 60].

It was observed that augmentin is responsible for MDA level elevation through their effects to increase the oxidative stress factors such as reactive oxygen species (ROS) and hence increasing lipid peroxidation process [61-66]. The current data showed results comparable with the above-mentioned observations of increasing MDA levels. The elevated serum levels MDA as diagnostic biomarker of liver and kidney damage caused by amoxicillin indicated the presence of cellular membrane damage, thus suggested the implication of lipid peroxidation and oxidative stress in the pathogenesis related to amoxicillin treatment [64, 67].

It is of great importance to reduce or minimize the damage occurred by drugs to get the maximum benefits in fighting diseases; one of the most important way to get maximum benefits is to reduce the drugs-induced oxidative stress. Many natural products as well as synthetic products can perform such important role. It was found that *Cucumis sativus*, quercetin, vitamin C have a potential antioxidant action by reducing MDA level and increasing the level of SOD [66, 68, 69].

CONCLUSION

The current results suggested that folic acid at the used dose and duration could improve some of the augmentin-induced hematological disorders as well as the induced oxidative stress factors, but it did not improve the augmentin-induced hepatotoxicity and nephrotoxicity, and as its well known that the augmentin-induced oxidative stress is a key factor of the hepatotoxicity and nephrotoxicity, and from the current results that showed an improvement in the oxidative stress factors, we do think that the improvement in the augmentin-induced hepatotoxicity and nephrotoxicity may require high dose and longer time of folic acid treatment after finishing the treatment with augmentin. Hence, more considerations about the effective dose and duration of folic acid should be taken in mind when using it as protective drug with augmentin.

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