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Full Length Research Paper

Amoxycillin/clavulanic acid combinations (Augmentin® 375 and 625 tablets) induce - oxidative stress, and renal and hepatic damage in rats

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Amoxicillin/clavulanic acid has become one of the most widely prescribed antibiotics being advocated by WHO for treatment of infection. Its efficacy and potential to inhibit microorganisms had led to their increased use. There is the possibility that the drug may have potential toxic effects on humans following consumption. The present investigation was therefore undertaken to study the status of antioxidant defence system, oxidative stress and some biochemical indices following treatment with different combinations of amoxicillin/clavulanic acid (Augmentin® 375 and 625 tablets) treatments in rats. Thirty rats were divided into three groups: Group 1 (control) received no drug; Group II received Augmentin® 375 (14.25 mg/kg body weight), while, Group III received Augmentin® 625 (21.83 mg/kg body weight) twice for seven days. The result indicated that Augmentin® 375 and 625 induced marked renal and liver failure characterized by a significant increase ($p<0.05$) in plasma creatinine, urea, and bilirubin. Similarly, Augmentin® 375 and 625 significantly increased plasma total cholesterol by 29.4 and 38.1%, HDL- cholesterol by 53 and 83.5%, LDL- cholesterol by 36 and 96% and triglycerides by 18 and 26%, respectively when compared with control. Furthermore, plasma AST and ALT was significantly increased ($p<0.05$) by 27.7 and 42.6%, and 33 and 44.4%, respectively in the two treated groups when compared with control. Administration of Augmentin® 375 and 625 also caused significant decrease in hepatic reduced glutathione (GSH) and vitamin C by 35 and 61%, and 38 and 44%, respectively. Similarly, hepatic superoxide dismutase (SOD) and catalase activities decreased significantly by 33 and 56%, and 31 and 48.7%, respectively in the treated group. In addition, Augmentin® 375 and 625 significantly decreased the hepatic levels of glutathioneS-transferase (GST) by 44.7 and 53.4%, respectively. In conclusion, the result of the present investigation show that two different combinations of amoxicillin/clavulanic acid (Augmentin® 375and 625) altered enzymatic and non-enzymatic antioxidant defence system and induced oxidative stress in rats.

Key words: Amoxicillin/clavulanic acid, respiratory tract infection, antioxidant indices, oxidative stress, reactive oxygen species.

INTRODUCTION

Amoxicillin/clavulanic acid is an oral antibacterial combination consisting of the semisynthetic antibiotic amoxicillin and the β-lactamase inhibitor, clavulanate potassium. Amoxicillin is an analogue of ampicillin, derived from the basic penicillin nucleus, 6-aminopenicillanic acid. Chemically, amoxicillin is (2*S*,5*R*,6*R*)-6-[(*R*) (-)-2-Amino-2-(*p*-hydroxyphenyl)acetamido]-3,3-dimethyl-7-oxo-4-thia-1 azabicyclo[3.2.0]heptane-2-carboxylic acid trihydrate (Ball et al., 1980) and may be represented structurally as:

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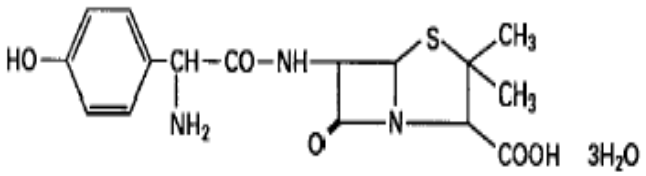
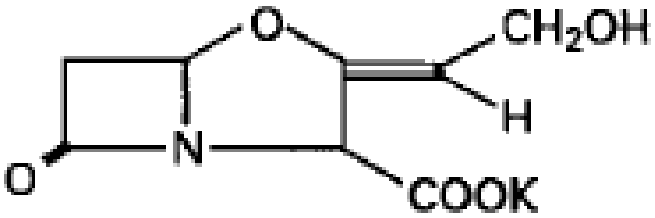


Clavulanicacids, a β-lactam structurally related to the penicillins possess the ability to inactivate a wide variety of β-lactamases by blocking the active sites of these enzymes. Clavulanic acid is particularly active against the clinically important plasmid-mediated β-lactamases frequently responsible for transferred drug resistance to penicillins and cephalosporins.

Chemically, clavulanate potassium is potassium (*Z*) (2*R*,5*R*)-3-(2-hydroxyethylidene)-7-oxo-4-oxa-1-azabicyclo[3.2.0]-heptane-2-carboxylate (Ball et al., 1980), and may be represented structurally as:



Amoxicillin/clavulanic acid (Augmentin®) is a broad-spectrum antibacterial that has been available for clinical use in a wide range of indications for over 20 years and is now used primarily in the treatment of community acquired respiratory tract infections. (White et al., 2004). Amoxicillin/clavulanic acid was developed to provide a potent broad spectrum of antibacterial activity, coverage of β-lactamase-producing pathogens and a favourable pharmacokinetic/pharmacodynamic (PK/PD) profile. The β-lactamase-inhibiting properties of clavulanic acid (Hunter et al., 1978) have been combined with the good oral absorption and potent broad-spectrum antimicrobial activity of amoxicillin (Rolinson, 1979; Comber et al, 1980) in tablets containing amoxicillin trihydrate and potassiumclavulanate.

Augmentin® 375 contains amoxycillintrihydrate equivalent to 250 mg amoxycillin and potassium clavulanate equivalent to 125 mg clavulanic acid. Where as, Augmentin® 625 contains amoxycillintrihydrate equivalent to 500 mg amoxycillin and potassium clavulanate equivalent to 125 mg clavulanic acid. There are different combinations of amoxicillin to clavulanate now in the market. Initially, the adult formulation of amoxicillin/clavulanate was introduced as Augmentin at a three times daily dose of 250 mg of amoxicillin (as amoxicillin trihydrate) plus 125 mg of clavulanic acid (as potassium clavulanate) (Comber et al.,1980). Over the years, the ratio of amoxicillin to clavulanate has been varied to reflect prescribing needs, to improve convenience and as a response to recommendations for the treatment of more severe infections or those caused by resistant organisms.

Liver injury caused by drugs and other chemicals accounts for approximately 5% of all cases of jaundice and encompasses a wide spectrum of diseases ranging from acute and chronic hepatitis to bile duct abnormalities (Friis and Andreasen, 1992; Zaidi, 2003). The antibiotic combination amoxicillin/clavulanic acid has become one of the most widely prescribed antibiotics (Garcia-López et al., 2001). Several reports incriminate amoxicillin/clavulanic acid in the development of cholestatic hepatitis (O'Donohue et al., 2000; Jordan et al., 2002). This results in accumulation of toxic hydrophobic bile acids A recent study has shown that amoxicillin/clavulanic acid is the most common drug involved in drug-induced liver injury and is the most frequently prescribed drug leading to hospitalization for drug-induced liver disease (Andrade et al., 2005). It is noteworthy that oxidative stress and lipid peroxidation that are mediated by oxygen free radicals has been implicated as a common link between chronic liver damage and hepatic fibrosis (Di Sario et al., 2007). Reactive oxygen metabolites are shown to mediate microvascular disturbances by various chemical substances (Parks and Granger, 1988). Hepatocytes are well recognized as being continuously exposed to reactive oxygen species (ROS) in various liver diseases including cholestasis.

To prevent injury from reactive oxygen species (ROS), cells have developed defense systems. Besides scavenger molecules such as glutathione, or α-tocopherol, specific enzymes, the antioxidants enzymes (AOE) fulfil this task. The expression of AOE can be regulated by oxidative stress itself (Shull et al., 1991; Tate et al., 1995). In this study, we investigate the antibiotics amoxycillin/clavulanic acid (Augmentin® 375 and 625 tablets) using status of antioxidants defence-mechanism, oxidative stress and some biochemical indices to identify possible mode of action.

MATERIALS AND METHODS

Chemicals

Augmentin® 375 and 625 tablets were obtained from Danax pharmacy in Ibadan. Glutathione,1-chloro-2,4-dinitrobenzene (CDNB), 5,5′-Dithiobis (2-nitrobenzoic acid) (DTNB), epinephrine and hydrogen peroxide were purchased from Sigma Chemical Company (London,UK). Aspartate aminotransferase (AST), alanine aminotransferase (ALT), urea, creatinine, bilirubin, total cholesterol, HDL-cholesterol, LDL-cholesterol and triglycerides Kit were obtained from Randox laboratories ltd. (Antrim, UK). All other reagents were of analytical grade and were obtained from British Drug House, Dorset, UK.

Animals and treatments

Animals weighing about 130 to 200 g were bred and maintained in the animal house of the department of Chemical Science, Ajayi Crowther University, Oyo, Nigeria. They were kept in wire meshed

cages and fed with commercial rat chow (Bendel feeds Nigeria ltd) and supplied water *ad libitum*.

Thirty rats were divided into three groups of 10 rats per group as follows:

Group A; control group. They were administered with no drug for a period of seven days.

Group B; Animals administered with doses of Augmentin® 375 (mg/kg body weight) twice for seven days.

Group C; Animals administered with doses of Augmentin® 625 (31.83 mg/kg body weight) twice for seven days. All the doses represent the human therapeutic doses. The animals were sacrificed 24 h after the last treatment.

Collection of blood samples for plasma preparation

The rats were sacrificed by cervical dislocation. Blood samples were collected by ocular punctures into heparinized tubes. Plasma was prepared by centrifugation for 10 min at 3000 x g in an MSC (Essex, UK) bench centrifuge. The clear supernatant was used for the estimation of plasma electrolytes, lipid profiles and enzymes. The liver, was immediately removed and rinsed in ice-cold 1.15% KCl, blotted and weighed.

Preparation of cytosolic fractions

The liver, excised from rat, blotted of blood stains, rinsed in 1.15% KClwas homogenized in 4 volumes of ice-cold 0.01 M potassium phosphate buffer, (pH 7.4). The homogenates were centrifuged at 12,500 g for 15 min at 4°C and the supernatants, termed the post-mitochondrial fractions (PMF) were aliquoted and used for enzyme assays.

Renal and liver functions test

Plasma creatinine, urea and bilirubin determination was done using Sigma diagnostic kits. Methods for creatinine assays are based on colorimetric alkaline picrate methods (Jaffe, 1972), with creatinine-picrate complex measured at 492 nm. The urea determination method was based on the fearon reaction (Tietz et al., 1994), with the Diazinechromogen formed absorbing strongly at 540 nm. The dimethysulphoxide method by Tietz et al. (1994) was used for bilirubin determination. The dimethyl sulphoxide form a coloured compound with maximum absorption at 550 nm.

Determination of plasma AST and ALT activities

Plasma AST and ALT activities were determined using Randox diagnostic kits. Determination of AST and ALT activities were based on the principle described by Reltman and Frankel (1957). AST was measured by monitoring the concentration of oxaloacetate hydrazone formed with 2,4-dinitrophenylhydrazine at 546 nm and ALT was measured by monitoring the concentration of pyruvate hydrazone formed with 2,4-dinitrophenylhydrazine at 546 nm.

Determination of plasma lipid profiles

The plasma total cholesterol, HDL- cholesterol, LDL- cholesterol and triglycerides were determined using Randox diagnostic kits and the determination were based on CHOD-PAD enzymatic colorimetric method of Trinder (1969). Cholesterol in the presence of cholesterol oxidase and peroxidase enzymes produced 4-(p)-benzoquinone-monoimino phenazone whose absorbance is read at 546 nm. Low-density lipoprotein (LDL and VLDL) and chylomicron fractions are precipitated quantitatively by the addition of phosphotungstic acid in the presence of magnesium ions. After centrifugation, the cholesterol concentration in the HDL (High density lipoprotein) fraction which remains in the supernatant is determined enzymatically. The triglycerides are determined after enzymatic hydrolysis with lipases. The indicator is a quinoneimine formed from hydrogen peroxide, 4-aminophenazone and 4-chlorophenol under the catalytic influence.

Assay of non-enzymatic antioxidants

Hepatic vitamin C was determined chemically according to the method of Erel et al. (1997) using dinitro phenyl hydrazine (DNPH), while hepatic glutathione was determined according to the method of Jollow et al. (1974).The chromophoric product resulting from the reaction of Ellman's reagent with the reduced glutathione, 2-nitro-5-thiobenzoic acid possesses a molar absorption at 412 nm which was in a spectrophotometer. Reduced GSH is proportional to the absorbance at 412 nm.

Determination of antioxidant enzymes

The procedure of Misra and Fridovich (1972) as described by Magwere et al (1997) was used for the determination of hepatic superoxide dismutase (SOD) activity by measuring the inhibition of auto-oxidation of epinephrine at pH 10.2 and 30°C. Hepatic catalase activity was determined according to the method of Asru (1972) by measuring the reduction of dichromate in acetic acid to chromic acetate at 570 nm. Hepatic glutathione S-transferase (GST) activity was determined by the method described by Habig et al. (1974) using 1-chloro-2,4-dinitrobenzene (CDNB) as substrate.

Protein determination

Protein determination of plasma and all fractions was estimated by the method of Lowry et al. (1951) using bovine serum albumin as standard.

Statistical analysis

The data were analyzed using one way ANOVA followed by Duncan multivariable post-hoc test for comparison between control and treated rats in all groups. P values less than 0.05 were considered statistically significant.

RESULTS

Table 1 shows the effects of different combination of amoxycillin-clavulanic acid (Augmentin® 375 and 625) treatments on plasma creatinine, urea and bilirubin levels.

Augmentin® 375 and Augmentin® 625 treatment significantly increased the plasma creatinine, urea and bilirubin level in the rats by 44 and 76% when compared with control ($p<0.05$), while the plasma urea level was significantly increased by 21 and 45% when compared with control ($p<0.05$). Similarly, plasma bilirubin was increased significantly following Augmentin® 375 and Augmentin® 625 administration by 50 and 100% when compared with control ($p<0.05$).

The plasma lipid profiles are shown in Table 2,

Table 1. Effects of different combination of amoxycillin-clavulanic acid (Augmentin® 375 and 625) treatments on plasma creatinine, urea and bilirubin levels in rats.

Treatment	Creatinine (mg/dl)	Urea (mg/dl)	Bilirubin (mg/dl)
Control	0.25±0.06	34.75±0.5	0.14±0.04
Augmentin® 375	0.36±0.05 (44%)*	42.2±1.48 (21%)*	0.21±0.01 (50%)*
Augmentin® 625	0.44±0.03 (76%)*	50.4±3.05(45%)*	0.28±0.01 (100%)*

The values are the Means ± SD (range) for five rats in each group. * Significantly different from the control p<0.05 Values in parenthesis represent percentage (%) increase.

Table 2. Effects of different combination of amoxycillin-clavulanic acid (Augmentin® 375 and 625) treatments on plasma lipid profiles.

Treatment	total cholesterol (mg/dl)	HDL-cholesterol (mg/dl)	LDL-cholesterol (mg/dl)	Triglycerides (mg/dl)
Control	43.75±4.11	17.0±2.0	15.0±1.15	56.5±1.29
Augmentin®375	56.6±4.3(29.4%)*	26±3.7 (53%)*	23.4±3.2 (36%)*	66.6±2 (18%)*
Augmentin®625	60.4±1.5(38.1%)*	31.2±0.8(83.5%)*	29.4±3.05(96%)*	71.2±0.84(26%) *

The values are the means ± SD (range) for five rats in each group; * Significantly different from the control (p<0.05). Values in parenthesis represent percentage (%) increase.

Table 3. Effects of different combination of amoxycillin-clavulanic acid (Augmentin® 375and 625) treatments on plasma aspartate amino transferase (AST) and alanine amino transferase (ALT) activities in rats.

Treatment	Enzyme activity (U/L)	
	ALT	AST
Control	73.25±2.99	124±1.63
Augmentin® 375	97.6±7.5 (33%)*	158.4±2.2 (27.7%)*
Augmentin® 625	105.8±2.49 (44.4%) *	176.8±3.03 (42.6%) *

The values are the means ± SD (range) for five rats in each group. * Significantly different from the control (p<0.05); Values in parenthesis represent percentage (%) increase.

following treatments with Augmentin®375and 625. The plasma total cholesterol level, HDL- cholesterol, and LDL-cholesterol level were significantly increased by 29.4 and 38.1%, 53 and 83.5%, and 36 and 96%, respectively when compared with control (p<0.05). Similarly, plasma triglyceride was increased significantly following Augmentin® 375 and Augmentin® 625 administration by 18 and 26% when compared with control (p<0.05).

The effect of different combination of amoxycillin-clavulanic acid (Augmentin® 375 and 625) treatments on the hepatic functions of rats are presented in Table 3. Augmentin® 375 and 625 administration significantly increased (p<0.05) plasma aspartate amino transferase (AST) by 27.7% and 42.6% and alanine aminotransferase (ALT) by 33 and 44.4% respectively when compared with control (p<0.05).

The hepatic vitamin C concentration is shown in Figure 1 following treatment with Augmentin® 375 and 625. The vitamin C level was significantly decreased by 38 and 44%, respectively in the treated groups when compared with the control (p<0.05).

The hepatic glutathione concentration is shown in Figure 2, following treatment with Augmentin® 375 and 625. The GSH level was significantly decreased by 35 and 61% respectively in the treated groups when compared with the control (p<0.05).

The hepatic superoxide dismutase (SOD) activity is shown in Figure 3, following treatment with Augmentin® 375 and 625. The activity of the superoxide dismutase (SOD) was significantly decreased in the treated groups by 33%, and 56% when compared with the control (p<0.05).

The hepatic glutathione-S-transferase (GST) activity is shown in Figure 4, following treatment with Augmentin®

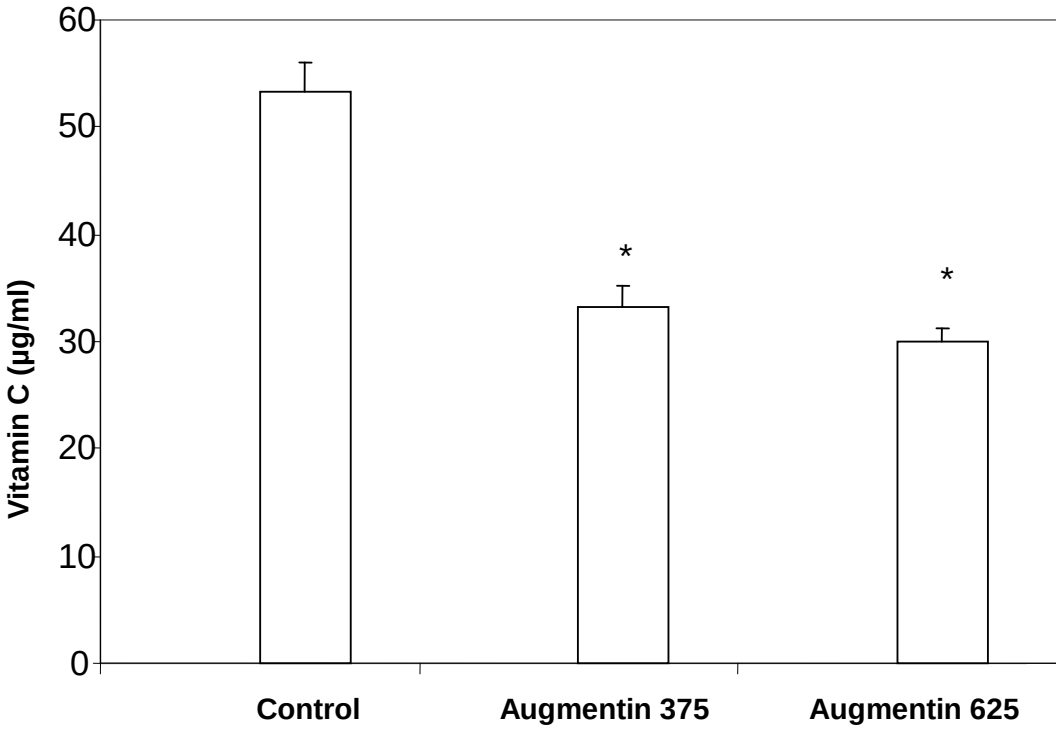


Figure 1. Influence of different combination of amoxycillin-clavulanic acid (Augmentin® 375 and 625) treatment on hepatic vitamin C concentration in rats the values are the means ± SD (range) for five rats in each group. * Significantly different from the control, $p<0.05$ (Duncan's multiple comparison test).

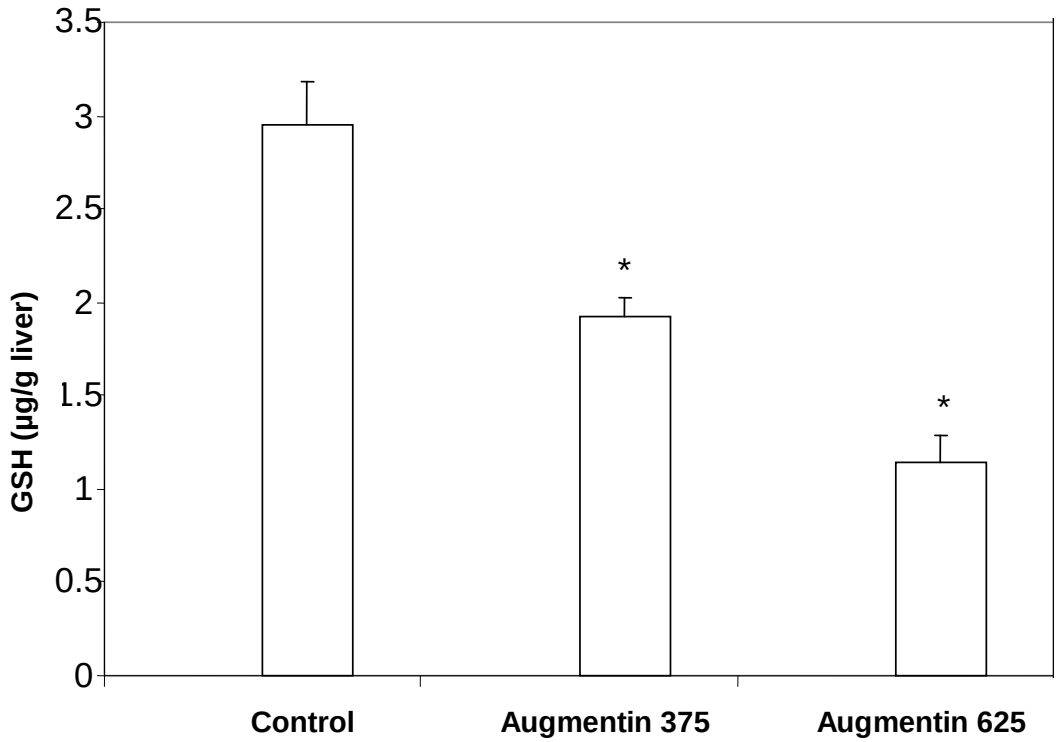


Figure 2. Influence of different combination of amoxycillin-clavulanic acid (Augmentin® 375 and 625) treatment on hepatic GSH concentration in rats. The values are the means ± SD (range) for five rats in each group. * Significantly different from the control, $p<0.05$ (Duncan's multiple comparison test).

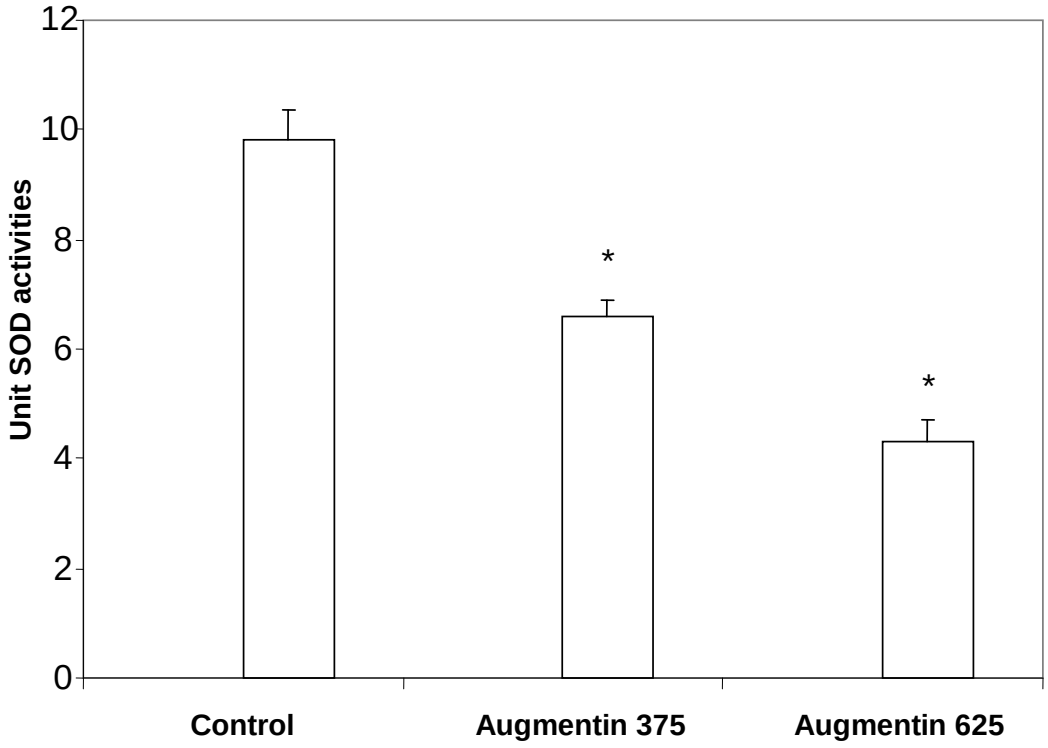


Figure 3. Influence of different combination of amoxycillin-clavulanic acid (Augmentin® 375 and 625) treatment on superoxide dismutase (SOD) activity in rats. The values are the means ± SD (range) for five rats in each group. * Significantly different from the control, $p<0.05$ (Duncan's multiple comparison test).

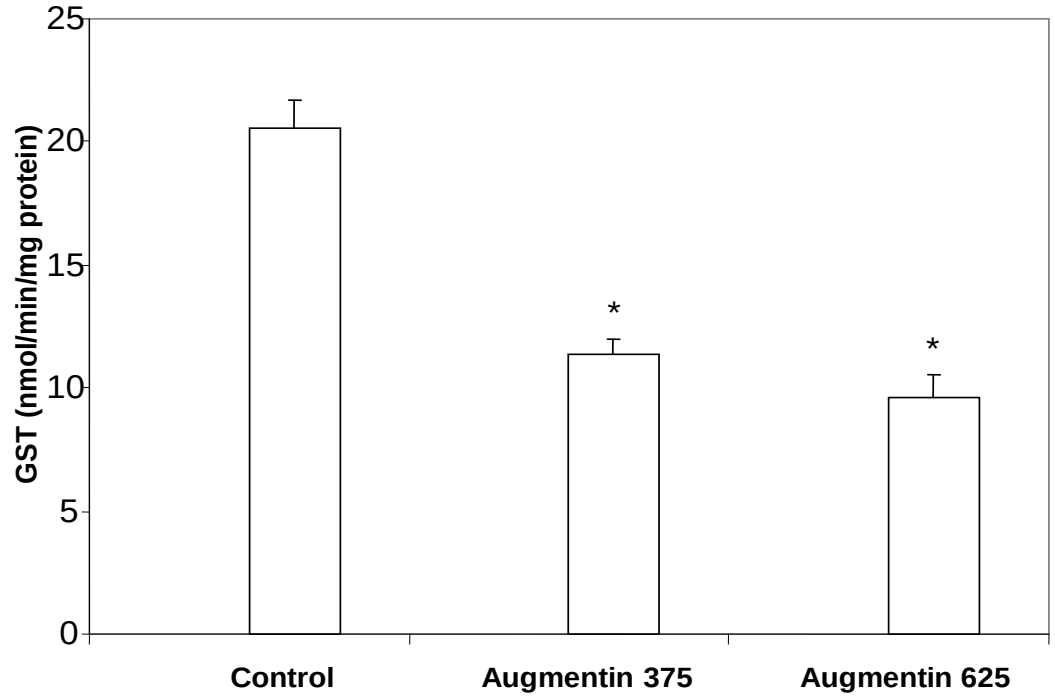


Figure 4. Influence of different combination of amoxycillin-clavulanic acid (Augmentin® 375 and 625) treatment on hepatic glutathione S-transferase(GST) activity in rats. The values are the means ± SD (range) for five rats in each group.* Significantly different from the control, $p<0.05$ (Duncan's multiple comparison test).

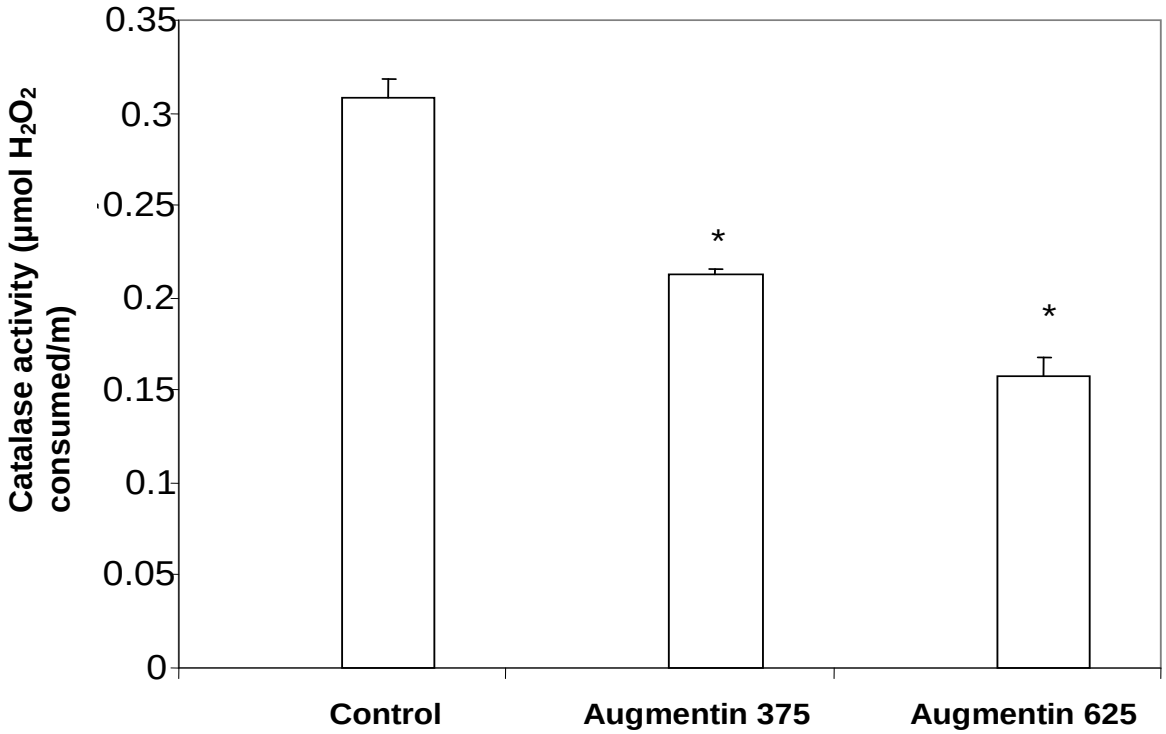


Figure 5. Influence of different combination of amoxycillin-clavulanic acid (Augmentin® 375 and 625) treatment on hepatic catalase activities in rats. The values are the means ± SD (range) for five rats in each group. * Significantly different from the control, p<0.05 (Duncan's multiple comparison test).

375 and 625. The activity of the glutathione-S-transferase (GST) was significantly decreased in all the treated groups by 44.7 and 53.4%, respectively when compared with the control (p<0.05).

The Hepatic catalase activity is shown in Figure 5, following treatment withAugmentin® 375 and 625. The catalase activity level was significantly decreased by 31 and 48.7% in the treated groups when compared with the control (p<0.05).

DISCUSSION

Amoxycillin/clavulanic acid (Augmentin® 375 and 625) is an antibiotic which is being advocated by WHO (2001) as a drug for treatment of infection. Its efficacy and potential to inhibit microorganisms had led to their increased use.

It is well-known that amoxycillin/clavulanic are widely used as broad spectrum antibiotics. However, an increasing number of evidence indicates that they have risk of hepatotoxicity as adverse effect (Zaidi, 2003; Andrade et al., 2005) and the mechanism of their hepatotoxicity remains uncertain.

It is noteworthy that oxidative stress mediated by oxygen free radicals has been implicated as a common link between chronic liver damage and hepatic fibrosis (Di Sario et al., 2007). Reactive oxygen metabolites are shown to mediate microvascular disturbances by various chemical substances (Parks and Granger, 1988). Hepatocytes are well recognized as being continuously exposed to reactive oxygen species (ROS) in various liver diseases including cholestasis. Antioxidant molecules such as glutathione (GSH), vitamin C and antioxidative enzymes such as superoxide dismutase (SOD), and catalase, ordinarily provide hepatocytes with resistance to oxidative stresses (Li et al., 2003).

In the present investigation, we observed that the two combination of amoxycillin/clavulanic acid (Augmentin® 375 and 625) induce renal and liver failure in the rats. This was evident from the renal and liver function test as plasma concentration of creatinine, urea and bilirubin significantly increased in the treated groups, suggesting impairment of renal and liver function. Plasma urea has been reported to increase in acute and chronic intrinsic renal disease (Cameron and Greger, 1998; Orth and Ritz, 1998). The observed significant Increase in plasma creatinine might result due to intrinsic renal lesions, decreased perfusion of the kidney, or obstruction of lower urinary tract by the drugs (Cameron and Greger, 1998). Elevated plasma bilirubin has been associated with hepatocellular damage, intra and extra-hepatic biliary tract obstruction (Renner, 1995; Tredger and Sherwood, 1997).

The two drugs also significantly ($P < 0.05$) increased the

concentration of plasma ALT and AST compared to control. These enzymes elevation in the plasma by the drug might be as a result of release of the enzymes from some tissues indicating tissue damage. Increase in plasma ALT and AST has been reported in conditions involving necrosis of hepatocytes (Macfarlane et al., 2000).

In our study, lipid profiles remained in consonance with the previous reports. The levels of lipids profiles, that is, plasma total cholesterol, HDL- cholesterol, LDL-cholesterol and triglycerides were high in all the treated groups. Investigations on membrane lipids indicated that cholesterol/phospholipids molar ratio coupled with other parameters are the most important determinants of membrane fluidity. The result may suggest a decrease in membrane fluidity and could result in altered membrane function (Schinitzky and Inbar, 1976).

Our data also indicate that the two amoxycillin/clavulanic acid combinations (Augmentin® 375 and 625) affects both enzymatic and non-enzymatic antioxidants profiles. Following Augmentin® 375 and 625 treatment, we observed a decrease in the activities of liver SOD and catalase as already observed in several studies. The antioxidants enzymes catalase, and SOD represent some of the primary intracellular antioxidants defense mechanism against oxidative stress (Erel et al., 1997). Catalase is a tetramerichemoprotein present in the liver cells and erythrocytes at high concentration (Kono and Frodovich, 1982). Its reactivity is important when H_2O_2 concentrations are raised. In previous studies, catalase is known to be inhibited by ROS such as superoxides anion which converts it to ferroxy and ferryl states that are inactive forms of enzymes (Areeku and Boomme, 1986). It is generally accepted that H_2O_2 can be detoxified by catalase which removes it when present at high concentration Therefore, accumulating H_2O_2 arisen from the decreased activities of catalase and in the treated animals will increase H_2O_2 concentration which will inactivate SOD activity and this may render the liver more susceptible to H_2O_2 and hydroxyl-radical induced oxidative stress. Treatment with amoxycillin/clavulanic acid combinations (Augmentin® 375 and 625) induced a significant decrease in hepatic glutathione-S-transferase (GST) activity, reduced glutathione (GSH) and vitamin C. Glutathione S-transferase is a family of enzymes that utilize glutathione in reactions contributing to the transformation of a wide range of compounds, including carcinogens, therapeutic drugs, and products of oxidative stress. Glutathione conjugation serves as protective mechanism whereby potentially toxic electrophilic metabolites are “mopped up” as glutathione conjugates. The decrease in GST activity observed correlates with GSH depletion in the liver. This confirms that the drugs toxicity is associated with cellular depletion of GSH. The level of reduced glutathione is a measure of the cellular redox status (Chance et al., 1979). The alteration produced by the drugs on GSH and GST suggests that the cellular redox status of the treated animals is affected. Aniya and Naito (1993) had reported that severe oxidative stress might result in decrease in GST with concomitant depletion of glutathione.

In addition to vitamin A and β-carotene, vitamin C (ascorbic acid) is known to represent the first line of antioxidant defense (Frei et al., 1988, 1989), and this vitamin is likely to be most susceptible to free radical oxidation. Ascorbate is a good free radical scavenger due to its chemical properties (Buettner and Moseley, 1993; Bielski et al., 1975). Studies have shown that the redox state of intracellular vitamin C is controlled by the intracellular level of GSH (Stocker et al., 1986).

Several reports incriminate amoxicillin-clavulanic acid therapy as a cause of intrahepatic cholestasis (O’Donohue et al., 2000; Jordan et al., 2002) and the possible role of oxidative stress in hepatotoxicity induced by the drug have been established in rats (Gamal et al., 2009). In conclusion therefore, our results suggest that oral administration of amoxicillin/clavulanic acid combinations (Augmentin® 375 and 625) induced oxidative stress, renal and kidney failure, and altered the profiles of enzymatic and non- enzymatic antioxidants defence in the host.

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