Journal of Toxicology and Environmental Health Sciences Vol. 2(6) pp. 85-92, November 2010
Available online at http://www.academicjournals.org/JTEHS
ISSN 2006-9820 ©2010 Academic Journals

Full Length Research Paper

Effect of amoxycillin/clavulanic acid (Augmentin 625®) on antioxidant indices and markers of renal and hepatic damage in rats

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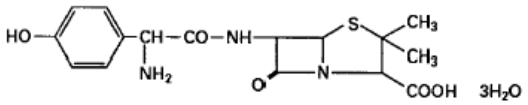
Accepted 16 September, 2010

Amoxycillin/clavulanic acid (Augmentin 625®) is an antibiotic which is being advocated by WHO as a drug 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 defense system, oxidative stress and some biochemical indices following Augmentin 625® treatment in rats. 20 rats were divided into two groups: Group 1 (control) received no drug, while group 2 received Augmentin 625 (21.83 mg/kg body weight) twice for seven days. The result indicated that the drug induced marked renal and liver failure characterized by a significant increase ($p < 0.05$) in plasma creatinine, urea, and bilirubin, by 76, 45, and 100% respectively. Similarly, the drug also significantly increased total cholesterol, HDL cholesterol, LDL cholesterol and Triglycerides by 38.1, 83.5, 96 and 26% respectively compared to control. Furthermore, the administration of the drug significantly increased plasma AST and ALT by 42.6 and 44.4% respectively compared with the control. The drug also caused a significant decrease in hepatic Glutathione (GSH) and Vitamin C by 51 and 44% respectively. Similarly hepatic SOD, Catalase and GST activities were significantly decreased by 56, 33 and 53% respectively. Overall, the result of the present investigation shows that acute dose of amoxycillin/clavulanic acid (Augmentin 625®) altered enzymatic and non-enzymatic antioxidant defense system and induced oxidative stress in rats.

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

INTRODUCTION

An antibiotic is a substance or compound that kills, or inhibits the growth of, microorganism (Davey, 2000). Amoxicillin/clavulanate potassium is an oral antibacterial combination consisting of the semisynthetic antibiotic amoxicillin and the β-lactamase inhibitor, clavulanate potassium (the potassium salt of clavulanic acid). Amoxicillin is an analog of ampicillin, derived from the basic penicillin nucleus, 6-aminopenicillanic acid. Chemically, amoxicillin is (2S,5R,6R)-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 and may be represented structurally as:



Clavulanic acid is produced by the fermentation of streptomyces clavuligerus. It is a β-lactam structurally related to the penicillins and possesses 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)¬ (2R,5R)-3-(2-hydroxyethylidene)-7-oxo-4-oxa-1-azabicyclo[3.2.0]-heptane-2-carboxylate, and may be represented structurally as:

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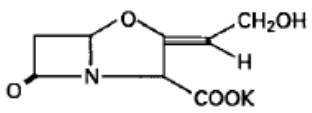

Amoxicillin/clavulanate (Augmentin®) is a broad-spectrum antibacterial that has been available for clinical use in a wide range of indications for over 20 years and is now used primarily in the treatment of community acquired respiratory tract infections. Amoxicillin/clavulanate was developed to provide a potent broad spectrum of antibacterial activity, coverage of β--lactamase-producing pathogens and a favourable pharmacokinetic/pharmacodynamic (PK/PD) profile. These factors have contributed to the high bacteriological and clinical efficacy of amoxicillin/clavulanate in respiratory tract infection over more than 20 years. This is against a background of increasing prevalence of antimicrobial resistance, notably the continued spread of β-lactamase-mediated resistance in *Haemophilus influenzae* and *Moraxella catarrhalis,* and penicillin, macrolide and quinolone resistance in *Streptococcus pneumoniae.* The β--lactamase-inhibiting properties of clavulanic acid (Hunter et al., 1978) were 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 potassium clavulanate. Early studies demonstrated the efficacy of amoxicillin/clavulanate in infections caused by β-lactamase producing pathogens in urinary, respiratory and soft tissue sites (Goldstein et al., 1979; Ball et al., 1980; Martinelli et al., 1981; Leigh et al., 1981) and in diseases such as gonorrhoea and chancroid (de Koning et al., 1981 and Fast et al., 1982). Amoxicillin/clavulanate is now most commonly used in the empirical treatment of bacterial respiratory tract infections, such as community-acquired pneumonia (CAP), acute exacerbations of chronic bronchitis (AECB), acute bacterial rhinosinusitis (ABS) and acute otitis media (AOM).

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) fulfill 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 effects of the antibiotic Amoxycillin/clavulanic acid (Augmentin 625®) using status of antioxidants defense mechanism, oxidative stress and some biochemical indices.

MATERIALS AND METHODS

Chemicals

Augmentin 625 was obtained from Danax pharmacy in Ibadan. Glutathione, 1Chloro,2.4dinitrobenzene (CDNB) ,5,5-dithio bis-2-nitrobenzoic acid (DTNB), epinephrine and hydrogen peroxide were purchased from sigma chemical company (London, UK). ALT, AST, 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, Poole, UK..

Animals and treatments

Male albino rats (Wistar strain) weighing about 130-200 g were bred and housed 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 supply water *ad libitum*.

Twenty rats were divided into two groups of 10 rats per group as follows:

Group A: Animals administered with doses of Augmentin 625 (31.83 mg/kg body weight) twice for seven days.

Group B (control group): They were administered with no drug for a period of 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 occular 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%KCl was 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 Randox 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 diazine chromogen formed absorbing strongly at 540 nm. The dimethy sulphoxide method by Tietz et al. (1994) was used for bilirubin determination. The dimethyl sulphoxide form a coloured compound with maximum absorption at 550 nm.

Plasma electrolytes determination

Plasma Na^+ and K^+ were determined by the use of Jenway clinical PFP7 flame photometer. Plasma bicarbonate ion ($HCO3^-$) determination was done using back titration method described by Tietz et al. (1994).

Determination of plasma AST and ALT activities

Plasma AST and ALT activities were determined using Randox

Table 1. Effects of Amoxycillin-clavulanic acid (Augmentin 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
Amoxycillin-Clavulanic acid (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 amoxycillin-clavulanic acid (augmentin 625®) treatments on plasma electrolytes.

Treatment	Na^+ (mmol/L)	K^+ (mmol/L)	HCO_3^- (mmol/L)
Control	109.5±2.38	60.08±4.43	18.75±1.5
Amoxycillin-Clavulanic acid (Augmentin 625)	105.6±1.95 (3.6%)	64.22±3.27 (6.9%)	23.2±1.1 (23.7%)*

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.

diagnostic kits. Determination of AST and ALT activities were based on the principle described by Reltman and Frankel (1957).

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).

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).

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 1,2-dichloro 4-nitrobenzene (CDNB) as substrate.

Protein determination

Protein determination of the plasma and liver post mitochondrial 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 effect of amoxycillin-clavulanic acid (Augmentin 625®) treatments on plasma creatinine, urea and bilirubin level**.**

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

Table 2 shows the effect of amoxycillin-clavulanic acid (Augmentin 625®) treatments on rat's plasma sodium ion (Na^+)**,** potassium ion (K^+), and bicarbonate ion (HCO_3^-). Augmentin 625 treatment did not significantly increase the plasma sodium ion and potassium ion level when compared with control ($p<0.05$). However, plasma Bicarbonate ion was increased significantly by 23.7% following Augmentin 625 administration when compared with control

The plasma lipid profiles are shown in Table 3 following treatments with amoxycillin-clavulanic acid (Augmentin 625®). The plasma total cholesterol level, high density lipoprotein, low density lipoprotein cholesterol level were

Table 3. Effects of amoxycillin-clavulanic acid (Augmentin 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
Amoxycillin-clavulanic acid (Augmentin625®)	60.4±1.52 (38.1%) *	31.2±0.84 (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 4. Effects of amoxycillin-clavulanic acid (Augmentin 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
Amoxycillin-clavulanic acid (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.

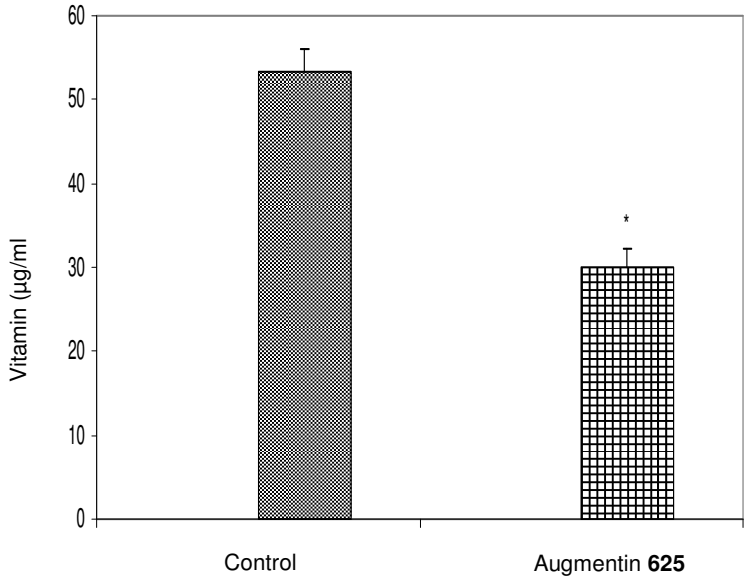

Figure 1. Influence of amoxicillin/clavulanic acid (Augmentin 625®) treatment on hepatic vitamin C concentration in rats. The values are the Means ± SD (range) for five rats in each group. * Significantly different from the control, $p<0.05$ (Duncan's multiple comparison test).

significantly increased by 38.1, 83.5 and 96% respectively when compared with control ($p<0.05$). Similarly, plasma triglyceride was increased significantly following Augmentin 625 administration by 26% when compared with control ($p<0.05$).

The effect of amoxycillin-clavulanic acid (Augmentin 625®) treatments on the hepatic functions of rats are presented in Table 4. The plasma AST activity was increased significantly by 42.6%, while the plasma ALT activity was increased significantly by 44.4% when compared with the control ($p<0.05$).

The hepatic vitamin C concentration is shown in Figure 1 following treatment with amoxicillin/clavulanic acid (Augmentin 625®). The vitamin C level was significantly decreased by 44% in the treated group when compared with the control ($p<0.05$).

The hepatic glutathione concentration is shown in Figure 2 following treatment with amoxicillin/clavulanic

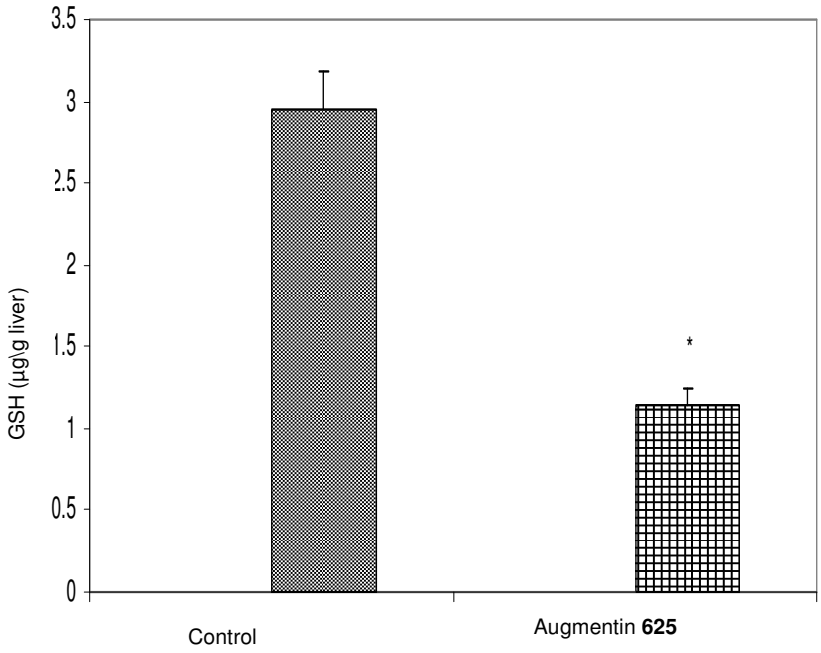

Figure 2. Influence of amoxicillin/clavulanic acid (Augmentin 625®) treatment on hepatic GSH concentration in rats. The values are the Means ± SD (range) for five rats in each group. * Significantly different from the control, $p<0.05$ (Duncan's multiple comparison test).

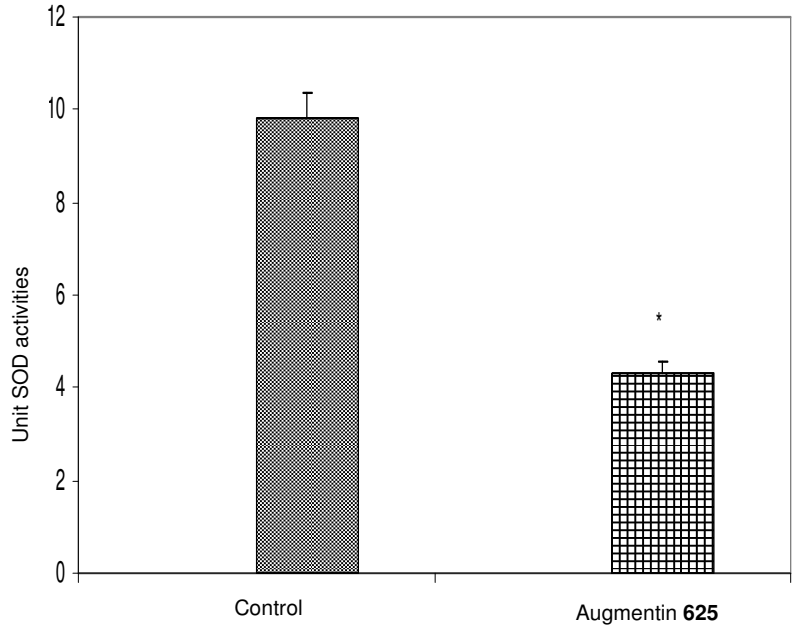

Figure 3. Influence of amoxicillin/clavulanic acid (Augmentin 625®) treatment on superoxide dismutase (SOD) activity in rats. The values are the Means ± SD (range) for five rats in each group. * Significantly different from the control, $p<0.05$ (Duncan's multiple comparison test).

acid (Augmentin 625®). The GSH level was significantly decreased by 51% in the treated group when compared with the control ($p<0.05$).

The hepatic superoxide dismutase (SOD) activity is shown in Figure 3 following treatment with amoxicillin/ clavulanic acid (Augmentin 625®). The activity of the

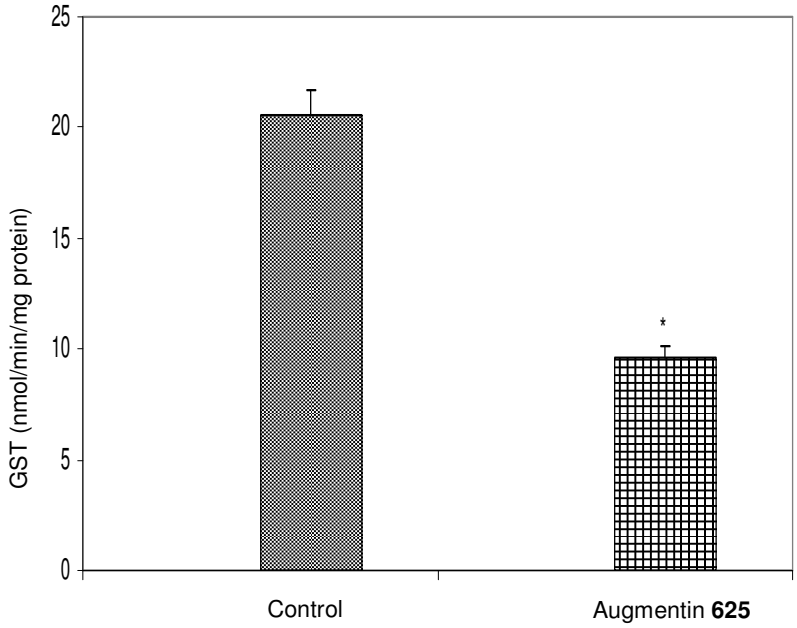

Figure 4. Influence of amoxicillin/clavulanic acid (Augmentin 625®) treatment on hepatic glutathione-s-transferase (GST) activity in rats. The values are the Means ± SD (range) for five rats in each group. * Significantly different from the control, $p<0.05$ (Duncan's multiple comparison test).

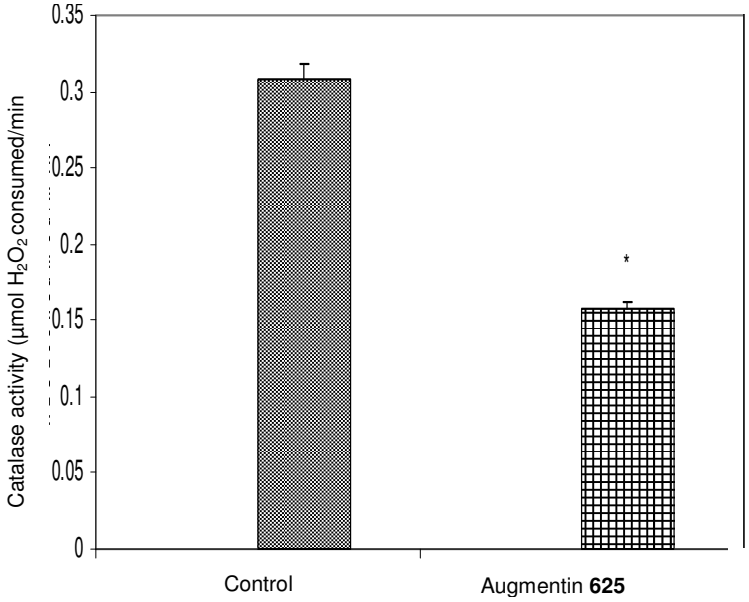

Figure 5. Influence of amoxicillin/clavulanic acid (Augmentin 625®) treatment on hepatic catalase activities in rats. The values are the Means ± SD (range) for five rats in each group. * Significantly different from the control, $p<0.05$ (Duncan's multiple comparison test).

superoxide dismutase (SOD) was significantly decreased in the treated group by 56% when compared with the control ($p<0.05$).

The hepatic glutathione-s-transferase (GST) activity is shown in Figure 4 following treatment with amoxicillin/clavulanic acid (Augmentin 625®). The activity of the glutathione-S-transferase (GST) was significantly decreased in all the treated group by 53% when compared with the control ($p<0.05$).

The Hepatic catalase activity is shown in Figure 5

following treatment with amoxicillin/clavulanic acid (Augmentin 625®). The catalase activity level was significantly decreased by 33% in the treated group when compared with the control ($p<0.05$).

DISCUSSION

In the present investigation, we observed that amoxycillin/clavulanic acid (Augmentin 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 group, 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 drug 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 (Farombi, 2000). The levels of lipids profiles, that is, plasma total cholesterol, HDL-cholesterol, LDL-cholesterol and triglycerides were high in the treated group. 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 amoxycillin/clavulanic acid (Augmentin 625) affects both enzymatic and non-enzymatic anti oxidants profiles. Following Augmentin 625 treatment, we observed a decrease in the activities of liver SOD and catalase as already observed in several studies (Erel et al., 1997; Farombi et al., 2000). 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 tetrameric hemoprotein 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 earlier studies, catalase is known to be inhibited by ROS such as super-oxides 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 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 (Augmentin 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), reported that severe oxidative stress might result in decrease in glutathione-s-transferase with concomitant depletion of glutathione.

In addition to vitamin A and β-carotene, ascorbic acid (vitamin C) 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 et al., 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).

In conclusion, our results suggest that oral administration of amoxycillin/clavulanic acid (Augmentin 625) induced renal and kidney failure, oxidative stress and altered the profiles of enzymatic and non- enzymatic antioxidants defense in the host.

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