

## Administration of Clarithromycin (Claricin®) Induces Changes in Antioxidant Status and Biochemical Indices in Rats

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**Abstract:** Clarithromycin is a well established, semi synthetic, second generation macrolide antibiotic for respiratory, disseminated mycobacterial, skin and *Helicobacter pylori* infections. Although, this drug has proven to be relatively safe, hepatic dysfunction including increased hepatocellular and/or cholestatic hepatitis with or without jaundice has been infrequently reported with its use. This study was therefore designed to investigate the toxic potentials of two different doses of clarithromycin (Clarithicin®) in rats. About 30 rats (Wistar strain) weighing between 180-220 g were completely randomised into 3 treatment groups. Group 1 (Control) received physiological saline while group 2 and 3 were administered 8.8 and 17.6 mg kg<sup>-1</sup> of clarithromycin (Clarithicin®) respectively twice daily for 7 days. Total bilirubin, creatinine and urea levels were significantly ( $p < 0.05$ ) elevated in the plasma of the rats that received the two doses of Claricin® by 62 and 94.6, 60 and 122% and 22 and 31.8%, respectively when compared to the control. Activities of ALP, ALT, AST and GGT were also significantly ( $p < 0.05$ ) higher in the plasma of animals treated with the two doses of the drug by 35 and 44.1%, 17 and 30.9%, 22 and 35.3% and 58 and 133.9%, respectively. Plasma lipid profiles revealed a significant increase ( $p < 0.05$ ) in total cholesterol, HDL cholesterol, LDL cholesterol and triglycerides in the two treated groups by 14 and 27.9%, 21 and 43.42%, 19 and 33.64% 15 and 46.2%, respectively. A significant ( $p < 0.05$ ) increase in the MDA level by 36 and 55%, respectively in the two treated groups was also observed. Furthermore, the two doses of clarithromycin significantly ( $p < 0.05$ ) reduced the hepatic levels of ascorbic acid and reduced glutathione (GSH) by 26.2 and 40% and 55 and 73%, respectively with a concomitant reduction in activities of hepatic GST by 23 and 36%, respectively. Similarly, there was a significant reduction in the activities of hepatic catalase and SOD by 46 and 51.9% and 34 and 52%, respectively in the clarithromycin treated groups. Liver histopathology revealed a mild periportal cellular infiltration by mononuclear cells and severe central venous congestion respectively in the two dose groups while foci of haemorrhages in the renal cortex were observed in the 17.6 mg kg<sup>-1</sup> Claricin® group. These data indicate that oral administration of clarithromycin has adverse effects on both enzymic and non-enzymic antioxidant status and induces oxidative stress as well as renal and liver damages in rats.

**Key words:** Clarithromycin, liver and renal damage, oxidative stress, antioxidants, catalase, SOD, Nigeria

### INTRODUCTION

Clarithromycin (6-O-methyl-erythromycin A) is a well established, semi synthetic, second generation macrolide antibiotic (Piscitelli *et al.*, 1992). It is obtained by substitution of a methoxy group for the hydroxyl group in position 6 of the erythromycin lactonic ring (Fig. 1). This structural change results in improved bioavailability and tolerability and an expanded spectrum of activity over erythromycin (Sturgill and Rapp, 1992). The presence of a methyl group at this position significantly decreases acid catalysed degradation of clarithromycin to inactive products (Sturgill and Rapp, 1992). Clarithromycin is

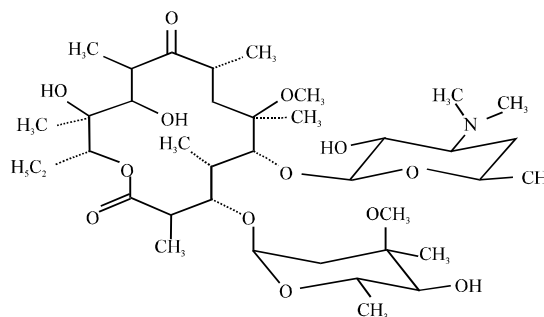


Fig. 1: Structure of clarithromycin

indicated for treatment of infections caused by susceptible organisms including lower respiratory tract

infections such as acute and chronic bronchitis and pneumonia; upper respiratory tract infections such as sinusitis and pharyngitis; skin and soft tissue infections of mild to moderate severity such as folliculitis, cellulitis and erysipelas; helicobacter pylori infection (Hardy *et al.*, 2003; Albataineh and Siddiqui, 2007). It is effective against a wide range of microorganisms including gram-positive cocci, *Haemophilus influenzae*, *Moraxella catarrhalis*, mycoplasma, chlamydia, selected mycobacteria, *Legionella* sp. and protozoan organisms (Klein 1993; Ping-Ing *et al.*, 2008).

Clarithromycin exerts its antibacterial action by inhibiting the intracellular protein synthesis of susceptible bacteria. It selectively binds to the 50S subunit of bacterial ribosomes and thus prevents the translocation of activated amino acids. After oral administration, clarithromycin is absorbed from the gastrointestinal tract with absolute bioavailability of about 50% and is oxidised into 14-(R) hydroxylclarithromycin and N-demethylclarithromycin through hydroxylation at 14-position and N-demethylation, respectively (Suzuki *et al.*, 2003). Clarithromycin and its metabolites are readily distributed into body tissues and fluids.

The body requires oxygen to maintain life and metabolic processes including drug metabolism but certain destructive oxygen derivatives named Reactive Oxygen Species (ROS) are generated during oxygen use (Gupta *et al.*, 2007). Some of these ROS include the superoxide anion radical, hydroxyl radical and hydrogen peroxide. However, the body contains certain antioxidants which protect against the damages caused by ROS and maintains redox homeostasis. These includes both enzymatic (Superoxide Dismutase (SOD), glutathione peroxidase, catalase, etc.) and non-enzymatic (glutathione and vitamins A, E and C) antioxidants. Oxidative stress sets in when the redox balance is disrupted by extreme generation of ROS or when the antioxidant capacity is insufficient (Golden *et al.*, 2002; Thomas, 2000) thereby shifting the balance towards the pro-oxidants side. Oxidative stress has been implicated in lipid peroxidation, protein and DNA damage and in the pathogenesis of certain diseases (Aruoma, 1999; Berlett and Stadtman, 1997; Wallace, 2002).

Although, clarithromycin appears to be safe and generally well tolerated, however elevated liver enzymes in plasma, liver failure, hepatocellular injuries and cholestasis has been reported with its use (Sousa *et al.*, 1997; Shaheen and Grimm, 1996; Fox *et al.*, 2002; Tietz *et al.*, 2003; Albataineh and Siddiqui, 2007). In some cases, this hepatic dysfunction may be severe and may be reversible (Albataineh and Siddiqui, 2007).

Despite years of clinical use, there are limited non-clinical toxicity data on clarithromycin. It has been

reported that the metabolism of some macrolide antibiotics may lead to generation of ROS or free radical intermediates and can cause depletion of antioxidant reserve (Yazar *et al.*, 2004, 2010; Er *et al.*, 2011) where the measurement of oxidative stress indices may provide additional toxicity information. Therefore, the present study was designed to investigate the effect of administration of two doses of clarithromycin on the antioxidant status and some biochemical indices in experimental rats.

## MATERIALS AND METHODS

**Chemicals and reagents:** Claricin® (Clarithromycin 250 and 500 mg) tablets were products of Waves-Biotech Pvt., Ltd. New Delhi, India. Glutathione (GSH), 1-chloro-2, 4-dinitrobenzene (CDNB), 5,5'-dithio-bis-2-nitrobenzoic acid (DTNB), Thiobarbituric Acid (TBA), epinephrine and hydrogen peroxide were purchased from Sigma Chemical Company (London, UK). Alkaline Phosphatase (ALP), Alanine amino Transferase (ALT), Aspartate amino Yransferase (AST), Gamma Glutamyl Transferase (GGT) urea, creatinine, bilirubin, total cholesterol, HDL cholesterol, LDL cholesterol and triglycerides assay kits were products of Randox Laboratories Ltd. (Antrim UK). All other chemicals and reagents were of analytical grade and of highest purity.

### Animals and treatments

**Animals:** Male rats (Wistar strain) weighing between 180-220 g were used in this study. The rats were bred and housed in the animal house of the Department of Chemical Sciences, Ajayi Crowther University, Oyo, Nigeria. They were kept in wire meshed cages at room temperature and under controlled light cycle (12 h light: dark). They were fed with commercial rat chow (Ladokun feeds, Ibadan, Nigeria) and supplied water *ad libitum*. All experiments were conducted without anaesthesia and protocol conforms to the guidelines of the National Institute of Health for laboratory animal care and use.

**Experimental design:** Thirty healthy male albino rats (Wistar strain) were randomly divided into 3 groups of 10 rats each. Group 1 (Control) received physiological saline while group 2 and 3 were administered 8.8 and 17.6 mg kg<sup>-1</sup> body weight clarithromycin (Clarin®), respectively (Table 1). The treatments were administered in divided doses, twice daily for 7 days. The rats were sacrificed 24 h after the last treatment.

**Collection of blood samples for plasma preparation:** Blood was collected from the animals by ocular puncture

Table 1: Experimental design

Experimental groups	Treatments
Group 1 (n = 10)	Control (Normal saline)
Group 2 (n = 10)	Claricin 250® (8.8 mg kg <sup>-1</sup> bw)
Group 3 (n = 10)	Clarinin 500® (17.6 mg kg <sup>-1</sup> bw)

into heparinized tubes and the rats were sacrificed by cervical dislocation. Plasma was prepared by centrifuging blood samples for 10 min at 3000 rpm in an Eppendorf (UK) bench centrifuge. The clear supernatant was used for the estimation of plasma lipid profiles and enzymes.

**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 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-picric acid complex measured at 492 nm. The urea determination method was based on the Feigl reaction (Tietz *et al.*, 1994) with the Diazine chromogen formed absorbing strongly at 540 nm. The dimethyl 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 concentration of urea was determined by the method of Tietz *et al.* (1994).

**Determination of plasma AST, ALT, ALP and GGT activities:** Plasma AST, ALT, ALP and GGT activities were determined using Randox diagnostic kits. Determination of AST and ALT activities were based on the principle described by Reitman 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. ALP was determined in accordance with the principles of Tietz (1995). The p-nitrophenol formed by the hydrolysis of p-Nitrophenyl phosphate confers yellowish colour on the reaction mixture and its intensity can be monitored at 405 nm to give a measure of enzyme activity. GGT activity was measured based on a modification of the method described by Theodorsen *et al.* (1979) using Abbott diagnostic kit.

**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.

**Assay of non-enzymatic antioxidants and lipid peroxidation:** 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 read in a spectrophotometer. Reduced GSH is proportional to the absorbance at 412 nm. The extent of Lipid Peroxidation (LPO) was estimated by the method of Varshney and Kale (1990), the method involved the reaction between Malondialdehyde (MDA) and thiobarbituric acid to yield a stable pink chromophore with maximum absorption at 532 nm.

**Determination of antioxidant enzymes:** The procedure of Misra and Fridovich 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 Sinha (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.

**Histopathological studies:** The method of Baker and Silverton (1985) was employed for the processing of liver for histopathological studies.

**Statistical analysis:** Results were expressed as mean of 10 replicates±SD. Data obtained were subjected to one-way Analysis of Variance (ANOVA) and complemented with Student's t-test using Stat Pac® Statistical Software. Statement of statistical significance was based on p<0.05.

## RESULTS AND DISCUSSION

**Effect of clarithromycin on the levels of plasma creatinine, urea and bilirubin in rat:** The effect of clarithromycin at 8.8 and 17.6 mg kg<sup>-1</sup> on rat plasma total bilirubin, creatinine and urea levels are shown in Table 2. The plasma concentration of bilirubin, creatinine and urea were significantly ( $p<0.05$ ) increased by 62 and 94.6%, 60 and 122% and 22 and 31.8%, respectively in the treated rats when compared with the control ( $p<0.05$ ).

**Effect of clarithromycin on plasma levels of liver enzymes in rat:** Table 3 shows the effect of clarithromycin on the plasma level of the liver enzymes, Alkaline Phosphatase (ALP), Alanine aminotransferase (ALT), Aspartate aminotransferase (AST) and  $\gamma$ -Glutamyl Transferase (GGT). Following the administration of 8.8 and 17.6 mg kg<sup>-1</sup> clarithromycin, the activities of ALP, ALT, AST and GGT in the plasma of rats were significantly ( $p<0.05$ ) increased by 35 and 44.1%, 17 and 30.9%, 22 and 35.3% and 58% and 133.9%, respectively compared with those of animals in the control group.

**Effect of clarithromycin on plasma lipid profiles of rat:** Table 4 shows the plasma lipid profiles of rats following the administration of clarithromycin to rats. The plasma levels of total cholesterol, HDL-cholesterol, LDL-cholesterol and triglycerides significantly increased by 14 and 27.9%, 21 and 43.42%, 19 and 33.64%, 15 and 46.2%, respectively in treated groups when compared with the control group ( $p<0.05$ ).

**Effect of clarithromycin on the activities of hepatic antioxidant enzymes in rat:** The effect of clarithromycin on rat hepatic antioxidant enzymes: Catalase (CAT) and Superoxide dismutase (SOD) is shown in Table 5.

Administration of 8.8 and 17.6 mg kg<sup>-1</sup> clarithromycin caused a significant ( $p<0.05$ ) reduction in the activities of CAT and SOD in the liver of rats by 46 and 51.9% and 34 and 52%, respectively compare to the control. Hepatic Glutathione-S-Transferase (GST) activity was also significantly reduced in the treated group by 23 and 36%, respectively (Fig. 2).

**Effect of Clarithromycin on the levels of hepatic non-enzymatic antioxidant and lipid peroxidation in rat:** The hepatic level of non-enzymic antioxidants: ascorbic acid (vit. C) and Glutathione (GSH) were significantly ( $p<0.05$ ) reduced following administration of 8.8 and 17.6 mg kg<sup>-1</sup> Clarithromycin when compared to the control. Specifically, vit. C level was reduced by 26.2 and 40% (Fig. 3) and GSH by 55 and 73% (Fig. 4) in the treated groups, respectively while MDA level was significantly ( $p<0.05$ ) elevated by 36 and 55% in the treated groups, respectively compared to the control (Fig. 5).

**Effect of clarithromycin on the histopathology of kidney and liver of rats:** Photomicrograph of the liver and kidney following administration of clarithromycin to rats are in Fig. 6 and 7, respectively. In the liver at 8.8 mg kg<sup>-1</sup> of the drug, there was mild periportal cellular infiltration by mononuclear cells (Fig. 6b) while 17.6 mg kg<sup>-1</sup>. Clarithromycin showed a severe central venous congestion (Fig. 6c). In the kidney, there were no visible lesions seen for animals treated with 8.8 mg kg<sup>-1</sup> while there were foci of haemorrhages in the renal cortex of animals treated with 17.6 mg kg<sup>-1</sup> of the drug (Fig. 7c). The potential hepatic and renal toxicity as well as effect on antioxidant status of two doses of clarithromycin (Claricin®) was investigated in rats in this study. Clarithromycin is a macrolide antibiotic commonly

Table 2: Effect of clarithromycin (Clarithicin®) treatment on plasma bilirubin, creatinine and urea levels in rats

Treatment	Bilirubin (mg mL <sup>-1</sup> )	Creatinine (mg dL <sup>-1</sup> )	Urea (mg mL <sup>-1</sup> )
Control	0.45±0.03	0.10±0.02	42.8±3.03
Clarithicin® (8.8 mg kg <sup>-1</sup> )	0.73±0.02 (62%)*	0.16±0.01 (60%)*	52.0±1.6 (22%)*
Clarithicin® (17.6 mg kg <sup>-1</sup> )	0.88±0.03 (94.6%)*	0.22±0.02 (122%)*	56.4±1.1 (31.8%)*

Table 3: Effect of clarithromycin (claricin®) treatment on plasma ALP, ALT, AST and GGT activities in rats

Treatment	ALP (UL <sup>-1</sup> )	ALT (UL <sup>-1</sup> )	AST (UL <sup>-1</sup> )	GGT (UL <sup>-1</sup> )
Control	79.4±2.3	53.6±3.6	61.8±1.6	7.2±0.8
Clarithicin® (8.8 mg kg <sup>-1</sup> )	107.2±1.9 (35%)*	62.8±2.2 (17%)*	75.6±2.4 (22%)*	11.4±1.1 (58%)*
Clarithicin® (17.6 mg kg <sup>-1</sup> )	114.4±2.3 (44%)*	70.2±1.9 (31%)*	83.6±1.1 (35%)*	15.4±1.1 (134%)*

Table 4: Effects of clarithromycin (claricin®) treatments on plasma lipid profiles in rats

Treatment	Total cholesterol (mg dL <sup>-1</sup> )	HDL-cholesterol (mg dL <sup>-1</sup> )	LDL-cholesterol (mg dL <sup>-1</sup> )	Triglycerides (mg dL <sup>-1</sup> )
Control	43.8±1.3	15.2±0.8	22±1.6	31.6±1.5
Clarithicin® (8.8 mg kg <sup>-1</sup> )	50.0±1.6 (14%)*	18.4±1.1 (21%)*	26.2±1.3 (19%)*	36.2±1.9 (15%)*
Clarithicin® (17.6 mg kg <sup>-1</sup> )	56±1.6 (28%)*	21.8±1.3 (43%)*	29.4±1.1 (34%)*	46.2±2.3 (46%)*

Values are the mean±SD (range) for 10 rats. Values in parenthesis represent percentage (%) increase. \*Significantly different from the control ( $p<0.05$ )

Table 5: Effects of Clarithromycin (Clarithin®) treatments on Hepatic Superoxide Dismutase (SOD) activity and catalase activity in rats

Treatment	SOD (units)	Catalase ( $\mu\text{mole H}_2\text{O}_2$ consumed $\text{min}^{-1}$ )
Control	15.4 $\pm$ 1.7	0.13 $\pm$ 0.02
Clarithin® (8.8 mg $\text{kg}^{-1}$ )	10.20 $\pm$ 1.3 (34%)*	0.07 $\pm$ 0.004 (46%)*
Clarithin® (17.6 mg $\text{kg}^{-1}$ )	07.4 $\pm$ 1.1 (52%)*	0.06 $\pm$ 0.01 (52%)*

Values are the mean $\pm$ SD (range) for ten rats. Values in parenthesis represent percentage (%) increase. \*Significantly different from the control ( $p < 0.05$ ). About 1 unit of SOD activity was given as the amount of SOD necessary to cause 50% inhibition of the auto-oxidation of adrenaline to adrenochrome

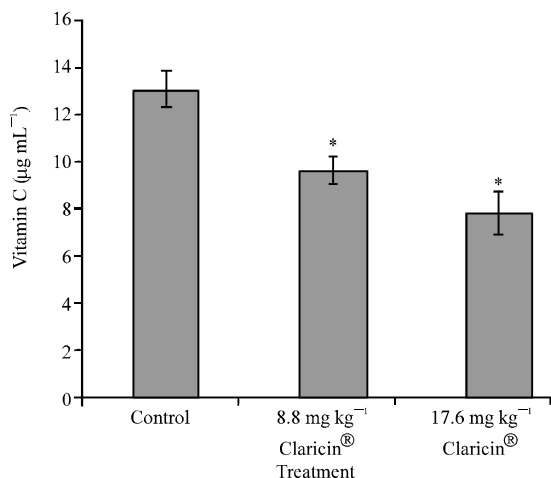


Fig. 2: Influence of clarithromycin (Clarithin®) treatments on hepatic vitamin C concentration in rat. Values are the means $\pm$ SD (range) for 10 rats in each group. \*Significantly different from the control,  $p < 0.05$  (Duncan's multiple comparison test)

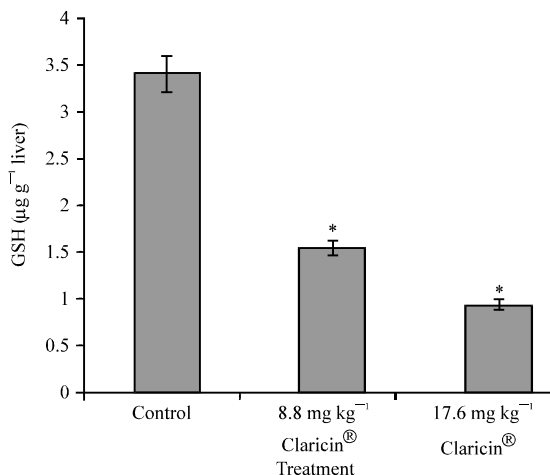


Fig. 3: Influence of clarithromycin (Clarithin®) treatments on hepatic GSH concentration in rat. Values are the means $\pm$ SD (range) for 10 rats in each group. \*Significantly different from the control,  $p < 0.05$  (Duncan's multiple comparison test)

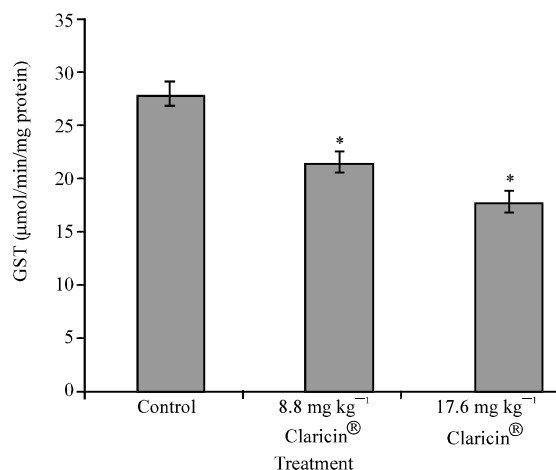


Fig. 4: Influence of clarithromycin (Clarithin®) treatments on hepatic GST concentration in rat. Values are the means $\pm$ SD (range) for 10 rats in each group. \*Significantly different from the control,  $p < 0.05$  (Duncan's multiple comparison test)

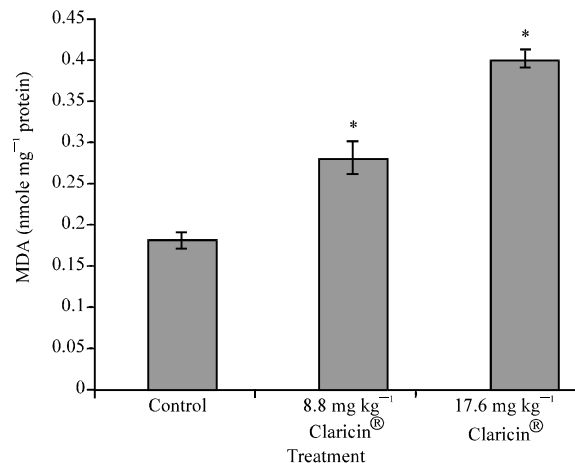


Fig. 5: Influence of clarithromycin (Clarithin®) treatments on hepatic MDA concentration in rat. Values are the Means $\pm$ SD (range) for ten rats in each group. \*significantly different from the control,  $p < 0.05$  (Duncan's multiple comparison test)

indicated for use in upper and lower respiratory infections and has a well-established safety and efficacy profile. Mild and sometimes symptomatic hepatitis is common especially in elderly population taking the antibiotic in high dose (Brown *et al.*, 1995). However, serious side effects are rare and include progressive cholestatic hepatitis (Giannattasio *et al.*, 2006; Fox *et al.*, 2002) and fulminant hepatic failure (Tietz *et al.*, 2003; Shaheen and Grimm, 1996). Cholestasis, defined as impairment of bile flow or production is a well recognized presentation of

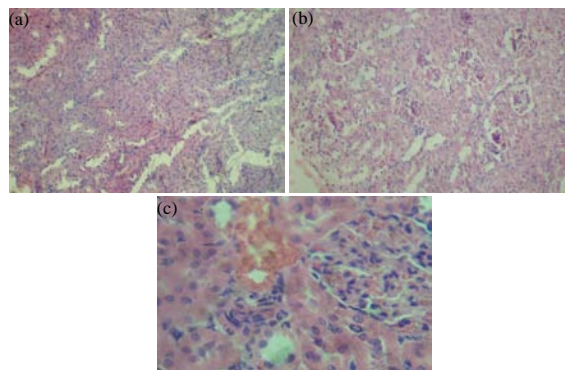


Fig. 6: Effect of Clarithromycin (Clarithin®) on histology of the liver cell. a) Control: No visible lesions seen; b) 8.8 mg kg<sup>-1</sup> (Clarithin®): There is mild periportal cellular infiltration by mononuclear cells; c) 17.6 mg kg<sup>-1</sup> (Clarithin®): There is severe central venous congestion

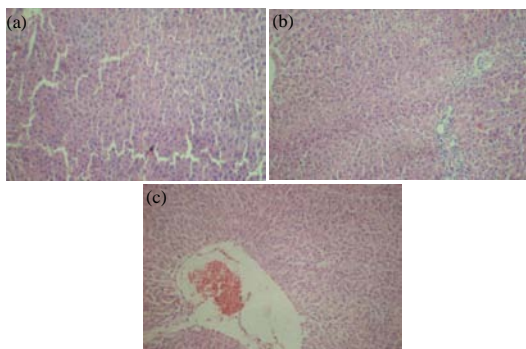


Fig. 7: Effect of Clarithromycin (Clarithin®) on histology of the kidney cell. a) Control: No visible lesions seen; b) 8.8 mg kg<sup>-1</sup> (Clarithin®): No visible lesions seen. c) 17.6 mg kg<sup>-1</sup> (Clarithin®): There are foci of haemorrhages in the renal cortex

drug induced liver disease and drugs account for 17-20% of the cases of cholestatic hepatitis (Friis and Andreassen, 1992).

The toxicity induced by clarithromycin may appear to involve generation of Reactive Oxygen Species (ROS) or reduction in the levels of enzymic and non-enzymic antioxidants. The human body contains non-enzymic antioxidants such as vitamins E and C and reduced Glutathione (GSH) and enzymic antioxidants like Superoxide Dismutase (SOD), Catalase (CAT) and Glutathione Peroxidase (GSHPx) which remove harmful ROS as soon as they are formed and to prevent their deleterious effects (Gutteridge, 1995). This study revealed that clarithromycin caused marked kidney and liver failure in rats. This was evident in the significantly ( $p < 0.05$ )

elevated levels of urea, creatinine and bilirubin in the plasma of rats following the administration of clarithromycin, suggesting an impairment of kidney and liver function.

Urea and creatinine are metabolic waste products that are freely filtered by the glomeruli of the kidneys (Gaspari *et al.*, 1998) and their serum/plasma concentrations are commonly used to screen for renal or cardiovascular diseases (Nankivell, 2001; Traynor *et al.*, 2006). Plasma creatinine is mainly produced by the metabolism of creatine or creatine phosphate in skeletal muscle (Nankivell, 2001) while urea is a metabolic product of protein metabolism. Furthermore, the biochemical processes involved in the biosynthesis of these metabolic wastes are mediated via oxidation-reduction (redox) processes (Mollica *et al.*, 1998; Wyss and Kaddurah-Daouk, 2000). Elevation of the plasma levels of creatinine and urea is an indication of abnormal renal function (Perrone *et al.*, 1992; Mouton and Holder, 2006). Elevated plasma bilirubin has been associated with hepatocellular damage, intra and extra-hepatic biliary tract obstruction, intravascular and extra cellular haemolysis and neonatal jaundice (Renner, 1995; Tredger and Sherwood, 1997).

Phosphatase enzymes are produced by specific cells/tissues and organs. Alkaline and total acid phosphatases are produced by several parts of the body especially in the liver. The serum/plasma levels of these enzymes are used as surrogate markers for toxicities of the appropriate tissues/organs (Wang *et al.*, 1981; Chu and Lin, 1998). The two doses of clarithromycin (Clarithin®) significantly ( $p < 0.05$ ) increased the concentration of plasma ALP, ALT, AST and GGT compared to control. The elevation in the plasma level/activity of these enzymes by the drugs might be as a result of their release from some tissues indicating tissue damage. Plasma ALP elevation has been attributed to increased osteoblastic activity such as in hyperparathyroidism, osteomalacia, neoplasm and also in hepatobiliary diseases (Moss and Rosalki, 1996). Both ALT and AST are marker enzymes for liver function and integrity (Jens and Hanne, 2002). Liver enzymes are usually raised in acute hepatotoxicity or mild hepatocellular injury, regenerative or reparative activity (Ishak, 2004) but tend to decrease with prolonged intoxication due to damage to the liver (Jens and Hanne, 2002; Cornelius, 1979). They are also present in red blood cells, heart cells, muscle tissue, pancreas and kidneys. When body tissue or an organ such as the heart or liver is diseased or damaged, additional AST and ALT are released into the bloodstream. Both ALT and AST levels are reliable indicators of liver damage. The elevated level

of aminotransferases and phosphatases observed in the 8.8 and 17.6 mg kg<sup>-1</sup> dose of Claricin® treated animals is highly indicative of hepatic toxicity (Vahdati-Mashhadian *et al.*, 2005; Ewaraiah and Satyanarayana, 2010).

The investigation further revealed that the two doses of clarithromycin (Clarithcin®) alter the antioxidant status of rat. This was obvious by the significant reduction in the activities of SOD, CAT and GST in the liver of clarithromycin (Clarithcin®) treated animals. The significantly decreased activity of the ROS scavenging enzyme, SOD and CAT by exposure to clarithromycin, conforms to previous study on macrolide antibiotics (Yazar *et al.*, 2002). This may be due to the damaging effects of free radicals possibly generated by the action of the drug.

The antioxidant enzymes CAT and SOD represent the primary intracellular antioxidant defence 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 Fridovich, 1982). It is generally accepted that H<sub>2</sub>O<sub>2</sub> can be detoxified by catalase which removes it when present at high concentration. Catalase is known to be inhibited by ROS such as superoxide anion which converts it to ferroxyl and ferryl states that are inactive forms of enzymes (Areeku and Boonme, 1986). SOD catalyzes the dismutation of superoxide radicals (O<sub>2</sub><sup>-</sup>) to hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) and molecular oxygen (O<sub>2</sub>) and therefore protects catalase against inhibition by superoxide anion. Thus, the balance of this enzyme system may be essential to get rid of superoxide anion and peroxides generated in subcellular compartments of the liver. The antioxidant enzymes, superoxide dismutase and catalase constitute a mutually supportive team of defence against reactive oxygen species.

Glutathione-S-Transferase (GST) is a family of multifunctional isozymes found in all eukaryotes, catalysing both glutathione dependent conjugation and reduction reactions. One main function of GST is to catalyse the biotransformation of xenobiotics including drugs detoxification in the mercapturic acid pathway, leading to the elimination of toxic compounds (Hayes and Pulford, 1995) and also acting as an antioxidant enzyme (Adaramoye and Adeyemi, 2006). In this study, GST activities were inhibited in animals treated with the two doses of clarithromycin. This observation therefore suggests that these drugs may alter the expression and activities of antioxidant enzymes as a result of toxic metabolites generated during their biotransformation.

The level of reduced GSH is a measure of non-enzymic antioxidant and cellular redox status of cells

in higher animals (Chance *et al.*, 1979). Ascorbic acid (vitamin C) in addition to vitamin A and E is known to represent the first line of antioxidant defence (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). Studies have shown that the redox state of intracellular vitamin C is controlled by the intracellular level of GSH (Stocker *et al.*, 1986). The results revealed that clarithromycin treatment decreased the overall redox status in the liver as indicated by a significant decrease in the level of hepatic GSH and vitamin C. The observed decrease in GSH and vitamin C levels further confirmed the formation of reactive oxygen species or toxic metabolites by the two dose of clarithromycin.

Oxidative damage to unsaturated lipids is a well-established general mechanism for oxidant mediated cellular injury (Yagi, 1994). In addition to extensive experimental studies, increased lipid peroxidation has been reported in a wide variety of clinical and toxicological conditions (Pryor, 1997). Free radical mediated lipid peroxidation has been associated with the pathogenesis of many diseases and clinical conditions (Frankel and Neff, 1983).

Malondialdehyde (MDA) an end product of lipid peroxidation in plasma may come from three different sources: circulating endogenous lipid peroxides, MDA produced in platelets during prostaglandin H<sub>2</sub> and Thromboxane (TXA<sub>2</sub>) synthesis and from other sources (Hamberg *et al.*, 1974; Bourgan *et al.*, 1982; Warso and Lands, 1984). The data from this study indicated an elevation in hepatic lipid peroxidation and plasma lipid profiles following treatment with the two doses of clarithromycin (Clarithcin®). The remarkable elevation of malondialdehyde in the liver of all the treated groups is an evidence of intensification of lipid peroxidation processes and an indication of toxicity. MDA which is one of the products of lipid peroxidation has been the most extensively studied marker. The increased lipid peroxidation in this study may be related to the altered lipid profiles. This may result in alteration in membrane integrity. The apparent increase in MDA formation indicates that clarithromycin (Clarithcin®) is capable of inducing oxidative stress which may overload the cell's endogenous detoxification mechanism. This observation agrees with previous studies on certain macrolide antibiotics (Salvemini and Cuzzocrea, 2002; Berger and Chioloro, 2007).

The histological alterations observed in the liver of clarithromycin-treated rats characterized by severe central venous congestion and mild periportal infiltration by

mononuclear cells coupled with the presence of foci of haemorrhages in the renal cortex is an indication of disruption of cellular architecture. All these abnormalities might have resulted due to the formation of highly reactive radicals because of oxidative threat caused by the drug which disrupted normal cellular functioning of the liver and the kidney.

## CONCLUSION

The results of this study show that clarithromycin induced renal and hepatic damage in rats and its mechanism of toxicity appears to proceed through the generation of free radicals or depletion of the antioxidant systems.

## REFERENCES

- Adaramoye, O.A. and E.O. Adeyemi, 2006. Hepatoprotection of d-galactosamine-induced toxicity in mice by purified fractions from *Garcinia kola* seeds. Basic Clin. Pharmacol. Toxicol., 98: 135-141.
- Albataineh, H. and F. Siddiqui, 2007. Acute liver failure secondary to clarithromycin. A case report and a literature review. Pract. Gastroenterol, 31: 87-89.
- Areeku, S. and Y. Boonme, 1986. Catalase activity in red cell and liver of mice infected with plasmodium bergeri. Southeast Asia. J. Trop. Med. Public Health, 17: 48-52.
- Aruoma, O.I., 1999. Free radicals, antioxidants and international nutrition. Asia Pacific J. Clin. Nutr., 8: 53-63.
- Baker, F.J. and R.E. Silverton, 1985. Introduction to Medical Laboratory Technology. 6th Edn., Butterworths, London, Pages: 480.
- Berger, M.M. and R.L. Chioloro, 2007. Antioxidant supplementation in sepsis and systemic inflammatory response syndrome. Crit. Care Med., 35: 584-590.
- Berlett, B.S. and E.R. Stadtman, 1997. Protein oxidation in aging, disease and oxidative stress. J. Biol. Chem., 272: 20313-20316.
- Bourgan, R.H., C. Deby, G. Deby-Dupont and R. Andries, 1982. Enhancement of arterial thromboformation by uric acid, a free radical scavenger. Biochem. Pharmacol., 31: 3011-3013.
- Brown, B.A., R.J. Jr. Wallace, D.E. Griffith and W. Girard, 1995. Clarithromycin-induced hepatotoxicity. Clin. Infect Dis., 20: 1073-1074.
- Buettner, G.R. and P.L. Moseley, 1993. EPR spin trapping of free radicals produced by bleomycin and ascorbate. Free Radic Res. Commun., 19: S89-S93.
- Chance, B., H. Sies and A. Boveris, 1979. Hydroperoxide metabolism in mammalian organs. Physiol. Rev., 59: 527-605.
- Chu, T.M. and M.F. Lin, 1998. PSA and acid phosphatase in the diagnosis of prostate cancer. J. Clin. Ligand Assay., 21: 24-34.
- Cornelius, C.E., 1979. Biochemical evaluation of hepatic function in dogs. J. Am. Anim. Hosp. Assoc., 15: 25-29.
- Er, A., E. Ulutas, F. Altan, G. Cetin, A. Bulbul, M. Elmas and E. Yazar, 2011. Tulathromycin disturbs blood oxidative and coagulation status. Afr. J. Biotechnol., 10: 3243-3247.
- Erel, O., A. Kocyigit, S. Avci, N. Aktepe and V. Bulut, 1997. Oxidative stress and antioxidative status of plasma and erythrocytes in patients with vivax malaria. Clin. Biochem., 30: 631-639.
- Ewaraiah, M.C. and T. Satyanarayana, 2010. Hepatoprotective activity of extracts from stem of *Mussaenda erythrophylla* Lam. Against carbon tetrachloride-induced toxicity in rats. JPRHC, 2: 23-31.
- Fox, J.C., R.S. Szyjowski and S.O. Sanderson, 2002. Progressive cholestatic liver disease associated with clarithromycin treatment. J. Clin. Pharmacol., 42: 676-680.
- Frankel, E.N. and W.E. Neff, 1983. Formation of malonaldehyde from lipid oxidation products. Biochem. Biophys. Acta, 754: 264-270.
- Frei, B., R. Stocker and B.N. Ames, 1988. Antioxidants defenses and lipid peroxidation in human blood plasma. Proc. Nat. Acad. Sci. USA., 85: 9748-9752.
- Frei, B., L. England and B.N. Ames, 1989. Ascorbate is an outstanding antioxidant in human blood plasma. Proc. Natl. Acad. Sci. USA., 86: 6377-6381.
- Friis, H. and P.B. Andreasen, 1992. Drug-induced hepatic injury: An analysis of 1100 cases reported to the Danish Committee on Adverse Drug Reactions between 1978 and 1987. J. Int. Med., 232: 133-138.
- Gaspari, F., N. Perico, M. Matalone, O. Signorini, N. Azzollini, M. Mister and G. Remuzzi, 1998. Precision of plasma clearance of iothexol for estimation of GFR in patients with renal disease. J. Am. Soc. Nephrol., 9: 310-313.
- Giannattasio, A., M. D'Ambrosi, M. Volpicelli and R. Iorio, 2006. Steroid therapy for a case of severe drug-induced cholestasis. Ann. Pharmacother., 40: 1196-1199.
- Golden, T.R., D.A. Hinerfeld and S. Melov, 2002. Oxidative stress and aging: Beyond correlation. Aging Cell, 1: 117-123.
- Gupta, S., A. Agarwal, J. Banerjee and J.G. Alvarez, 2007. The role of oxidative stress in spontaneous abortion and recurrent pregnancy loss: A systemic review. Obstet. Gynecol. Surv., 62: 335-347.



- Gutteridge, J.M., 1995. Lipid peroxidation and antioxidants as biomarkers of tissue damage. Clin. Chem., 41: 1819-1828.
- Habig, W.H., M.J. Pabst and W.B. Jakoby, 1974. Glutathione-S-transferases. The first enzymatic step in mercapturic acid formation. J. Biol. Chem., 249: 7130-7139.
- Hamberg, M., J. Svensson and B. Samuelsson, 1974. Prostaglandin endoperoxides. A new concept concerning the mode of action and release of prostaglandins. Proc. Natl. Acad. Sci., 71: 3824-3828.
- Hardy, R.D., A.M. Rios, S. Chavez-Bueno, H.S. Jafri and J. Hatfield *et al.*, 2003. Antimicrobial and immunologic activities of clarithromycin in a murine model of *Mycoplasma pneumonia*-induced pneumonia. Antimicrob. Agents Chemother., 47: 1614-1620.
- Hayes, J.D. and D.J. Pulford, 1995. The glutathione s-transferase supergene family: Regulation of *gst* and the contribution of the isoenzymes to cancer chemoprotection and drug resistance. Crit. Rev. Biochem. Mol. Biol., 30: 445-600.
- Ishak, K., 2004. Hepatobiliary and Skeletal Muscle Enzymes and Liver Function Tests. In: Veterinary Laboratory Medicine Interpretation and Diagnosis, Meyer, D.J. and J.W. Harvey (Eds.). 3rd Edn., W.B. Saunders Co., Saint Louis, Missouri, ISBN: 9780721689265, pp: 169-192.
- Jaffe, B., 1972. What made the radical break? N. Engl. J. Med., 286: 156-157.
- Jens, J.J. and H. Hanne, 2002. A review on liver function test. [http://home3.inet.tele.dk/omni/hemo chromatosis\\_iron.htm](http://home3.inet.tele.dk/omni/hemo chromatosis_iron.htm).
- Jollow, D.J., J.R. Mitchell, N. Zampaglione and J.R. Gillette, 1974. Bromobenzene induced liver necrosis: Protective role of glutathione and evidence for 3,4-bromobenzeneoxide as the hepatotoxic metabolite. Pharmacology, 11: 151-169.
- Klein, J.O., 1993. Clarithromycin: Where do we go from here? Pediatr Infect Dis. J., 12: S148-151.
- Kono, Y. and I. Fridovich, 1982. Superoxide radicals inhibit catalase. J. Biol. Chem., 257: 5751-5754.
- Lowry, O.H., N.J. Rosebrough, A.L. Farr and R.J. Randall, 1951. Protein measurement with folin phenol reagent. J. Biol. Chem., 193: 265-275.
- Magwere, T., Y.S. Naik and J.A. Hasler, 1997. Effect of choloquine treatment on antioxidant enzymes in rats liver and kidney. Free Radic. Biol. Med., 22: 321-327.
- Mollica, M.P., S. Iossa, G. Liverini and S. Soboll, 1998. Oxygen consumption and biosynthetic function in perfused liver from rats at different stages of development. Cell. Mol. Life Sci., 54: 1277-1282.
- Moss, D.W. and S.B. Rosalki, 1996. Enzyme Tests in Diagnosis. Hodder Arnold, London, ISBN: 034055245X, Pages: 304.
- Mouton, R. and K. Holder, 2006. Laboratory tests of renal function. Anaesthesia Intensive Care Med., 7: 240-243.
- Nankivell, B.J., 2001. Creatinine clearance and the assessment of renal function. Aust. Prescr., 24: 15-17.
- Perrone, R.D., N.E. Madias and A.S. Levey, 1992. Serum creatinine as an index of renal function: New insights into old concepts. Clin. Chem., 38: 1933-1953.
- Ping-Ing, L., W. Mei-Hwan, H. Li-Min, C. Jong-Min and L. Chin-Yun, 2008. An open, randomized, comparative study of clarithromycin and erythromycin in the treatment of children with community-acquired pneumonia. J. Microbiol. Immunol. Infect., 41: 54-61.
- Piscitelli, S.C., L.H. Danziger and K.A. Rodvold, 1992. Clarithromycin and azithromycin: New macrolide antibiotics. Clin. Pharm., 11: 137-152.
- Pryor, W.A., 1997. Cigarette smoke radicals and the role of free radicals in chemical carcinogenicity. Environ. Health Perspect., 105: 875-882.
- Reitman, S. and S. Frankel, 1957. A colorimetric method for the determination of serum glutamic oxalacetic and glutamic pyruvic transaminases. Am. J. Clin. Pathol., 28: 56-63.
- Renner, E.L., 1995. Liver function tests. Ballieres Clin. Gastroenterol., 9: 661-677.
- Salvemini, D. and S. Cuzzocrea, 2002. Oxidative stress in septic shock and disseminated intravascular coagulation. Free Radic. Biol. Med., 33: 1173-1185.
- Shaheen, N. and I.S. Grimm, 1996. Fulminant hepatic failure associated with clarithromycin. Am. J. Gastroenterol., 91: 394-395.
- Sinha, A.K., 1972. Colorimetric assay of catalase. Anal. Biochem., 47: 389-394.
- Sousa, C., J. Correia, J. Santos, F. Silvestre and A. Bernardo, 1997. Cholestatic hepatitis probably induced by clarithromycin. Gastroenterol. Clin. Biol., 21: 632-633.
- Stocker, R., M. Weidemann and N. Hunt, 1986. Possible mechanisms responsible for the increased ascorbic acid content of *P. vinckei-infected* mouse erythrocytes. Biochim. Biophys. Acta, 881: 391-397.
- Sturgill, M.G. and R.P. Rapp, 1992. Clarithromycin: Review of a new macrolide antibiotic with improved microbiologic spectrum and favorable pharmacokinetic and adverse effect profiles. Ann. Pharmacother., 26: 1099-1108.
- Suzuki, A., I. Iida, M. Hirota, M. Akimoto and S. Higuchi *et al.*, 2003. CYP isoforms involved in the metabolism of clarithromycin *in vitro*: Comparism between the identification from disappearance rate and that from formation rate of metabolites. Drug Metab. Pharmacokin., 18: 104-113.

- Theodorsen, L., J.H. Stromme, M. Horder, W. Gerhardt and J. Waldenstrom *et al.*, 1979. Recommended method for the determination of creatine kinase in blood modified by the inclusion of EDTA. The committee on enzymes of the scandinavian society for clinical chemistry and clinical physiology (SCE). Scand J. Clin. Lab. Invest., 39: 1-5.
- Thomas, M.J., 2000. The role of free radicals and antioxidants. Nutrition, 16: 716-718.
- Tietz, A., M.H. Heim, U. Eriksson, S. Marsch, L. Terracciano and S. Krahenbuhl, 2003. Fulminant liver failure associated with clarithromycin. Ann. Pharmacother., 37: 57-60.
- Tietz, N.W., 1995. Clinical Guide to Laboratory Tests. 3rd Edn., W.B. Saunders, Philadelphia.
- Tietz, N.W., E.L. Prude and O. Sirgard-Anderson, 1994. Tietz Textbook of Clinical Chemistry. 2nd Edn., W.B. Saunders Company, London, ISBN: 0721656102, pp: 1354-1374.
- Traynor, J., R. Mactier, C.C. Geddes and J.G. Fox, 2006. How to measure renal function in clinical practice? Clinical review. BMJ., 333: 733-737.
- Tredger, J.M. and R.A. Sherwood, 1997. The liver: New functional, prognostic and diagnostic tests. Ann. Clin. Biochem., 34: 121-141.
- Vahdati-Mashhadian, N., H. Rakhshandeh and A. Omid, 2005. An investigation on LD50 and subacute hepatic toxicity of *Nigella sativa* seed extracts in mice. Pharmazie, 60: 544-547.
- Varshney, R. and R.K. Kale, 1990. Effects of calmodulin antagonist on radiation induced lipid peroxidation in microsomes. Int. J. Radiat. Biol., 58: 733-743.
- Wallace, S.S., 2002. Biological consequences of free radical-damaged DNA bases. Free Radic. Biol. Med., 33: 1-14.
- Wang, M.C., L.D. Papsidero, M. Kuriyama, L.A. Valenzuela, G.P. Murphy and T.M. Chu, 1981. Prostate antigen: A new potential marker for prostatic cancer. Prostate, 2: 89-96.
- Warso, M.A. and W.E.M. Lands, 1984. Pathophysiological modulation of arachidonate metabolism. Clin. Physiol. Biochem., 20: 70-77.
- Wyss, M. and R. Kaddurah-Daouk, 2000. Creatine and creatinine metabolism. Physiol. Rev., 80: 1107-1213.
- Yagi, K., 1994. Lipid peroxides and related radicals in clinical medicine. Adv. Exp. Med. Biol., 366: 1-15.
- Yazar, E., V. Altunok, M. Elmas, B. Tras, A.L. Bas and V. Ozdemir, 2002. The effect of tilmicosin on cardiac superoxide dismutase and glutathione peroxidase activities. J. Vet. Med. B, 49: 209-210.
- Yazar, E., E. Oztekin, A. Sivrikaya, R. Col, M. Elmas and A.L. Bas, 2004. Effects of different doses of timicosin on malondialdehyde and glutathione concentrations in mice. Acta Vet. Brno, 73: 69-72.
- Yazar, E., A. Er, K. Uney, A. Bulbul, G.E. Avci, M. Elmas and B. Tras, 2010. Effects of drugs used in endotoxic shock on oxidative stress and organ damage markers. Free Radic Res., 44: 397-402.