

ASSESSMENT OF ARTESUNATE TOXICITY ON RENAL TISSUE OF MALE ALBINO MICE

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ABSTRACT

About 500 million people suffer from malaria leading to death in 2 to 3 million cases. Acute renal failure occurs as a complication of *P. falciparum* malaria in less than 1% of cases, but the mortality in these cases may be upto 45%. Artesunate is an antimalarial drug used for the treatment of falciparum malaria in regions of malaria endemicity. The effect of varying doses of artesunate on functional indices of mice kidney was investigated. Oral doses of 150 mg/kg.b.wt and 300 mg/kg.b.wt of artesunate were administered for a period of 21 and 45 days. Effects were observed on some biochemical parameters like protein, cholesterol, LPO (TBARS), SOD, GSH, G-Px, G-Rx, GST and histology of concerned tissue using standard methods. Gravimetric indices were also recorded. The results of investigation revealed lowered level of protein and elevated cholesterol content in the test tissue after 21 and 45 days of administration of both low and high dose of Artesunate. Similarly, oxidative parameters SOD, GSH, G-Px, G-Rx were found to be decreased and LPO (TBARS) and GST were found to be increased as compared to control animals after the termination of experiment. The histological findings after H&E staining showed interstitial lymphoplasmacytic nephritis and necrosis in the epithelial cells of the renal tubules at 21 days post treatment. Cytology of renal cells was also altered compared to control slides after 45 days post treatment. Treatment with high dose of Artesunate showed hemorrhage within interstitial tissue and proliferation of epithelial cell lining of renal tubules followed by necrotic glomerular tufts. Thus, obtained results clearly indicate the adverse effects of artesunate administration on functional capacities of the kidney.

INTRODUCTION

Malaria affects about 500 million people annually and it is a leading cause of mortality and morbidity in the developing world. It remains a major public health problem in endemic regions (Bremar *et al.*, 2004). The treatment of malaria has posed great challenge to medicine and development of efficient antimalarial drugs. This is due to the development of resistance of parasite to mainly antimalarial agents (Wongsrichanalai *et al.*, 2002; Sharma, 2005) resulting in huge impact on the socio-economy of man (Sachs and Malaney, 2002). Discovery of artemisinin and its derivatives (artesunate, artemether, arteether, and dihydroartemisinin) have given renewed hope for combating resistant malaria. Artesunate is an antimalarial drug used for the treatment of falciparum malaria in regions of malaria endemicity. It has been used in combination with other antimalarial drugs due to evidence of enhancement of efficacy and delay in development of resistance (Taylor *et al.*, 2003). However it is still used as monotherapy in many countries including some regions in Asia for the treatment of uncomplicated malaria (Saunders *et al.*, 2012). In addition to its anti malarial action, artesunate is also being considered an anti-tumour drug due to evidence of its effect against several tumor cell lines including pancreatic (Du *et al.*, 2010), breast and colon cancers (Liu *et al.*, 2011). This increases its propensity for use with existing drugs in co morbidities. However its current use along with lamivudine, an antiretroviral agent used in the treatment of Hepatitis B infection

(HBV) in Africa due to the rising prevalence of the infection, requires important safety assessment.

Serious concern has been raised about uncontrolled use of artemisinin derivatives in endemic areas of the disease. Moreover, self-medication is quite common and purchase of antimalarials in the open market is rampant (Akanbi *et al.*, 2005). The possibility of overdose and misappropriation in the usage of antimalarial agents are very common, all of which could lead to toxic effects of the drugs (Agboruche, 2009 and Izunya *et al.*, 2010).

Renal pathology associated with malaria, ranging from electrolyte imbalance to more serious incidences with acute renal failure is part of complications that occur with Plasmodium falciparum infection (Naqvi *et al.*, 2003). The kidney performs several excretory and regulatory functions including blood pressure control and maintenance of extracellular environment (Ferguson and Waikar, 2012). The high extent of delivery of materials including xenobiotics, via their passage through the tubular lumen contributes to renal injury resulting from several clinically used drugs, and experimental toxins alike (Hagos and Wolff, 2010), with the metabolic and excretory roles of the kidney making it vulnerable to various forms of renal injury. Consequently, alteration in structural components of the kidney, which may result from disease or toxicological effects of drug, may result in deleterious effects that often affect its optimal function. Alterations in renal function leading to increase or decrease

renal clearance may result in changes in blood concentrations and pharmacological responses (Doogue and Polasek, 2011).

Artesunate has been established to partly owe its antimalarial action to the formation of free radicals. Much of the data in the literature shows that reactive oxygen species (ROS) and oxidative stress are implicated in nephropathy (Manucha, 2007; Ghosh *et al.*, 2010). With the geographic overlap of malaria, concurrent therapy with this life saving drugs is often useful. We thus hypothesize that the simultaneous use of artesunate may result in nephrotoxic consequences that may lead to structural and functional changes in the kidney.

Hence this study investigated the renal consequences of artesunate administration in healthy adult Swiss albino mice.

MATERIALS AND METHODS

Animals

Healthy, adult male albino mice (*Mus musculus*) of Swiss strain weighing about 30-35 gram (gm) were used for the experiment. The animals were housed in an air conditioned animal house at a temperature of $26 \pm 2^{\circ}\text{C}$ and exposed to 12h light: 12h dark cycle and were maintained on a standard Amrut mice feed (Pranav Agro Industries) and water *ad libitum*. Animals were caged separately in accordance with the experimental protocol into different groups (Groups A to E) as per the guidelines set by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), India and experimental protocols were approved by the institutional animals' ethics committee (167/1999/CPCSEA).

Experimental Design

Artesunate was prepared in double distilled water and orally given to mice via feeding canula with a hypodermic syringe. The two doses selected for the present study were based on therapeutic dose of artesunate administered for the treatment of uncomplicated malaria (Angus *et al.*, 2002). Adult Swiss albino male mice weighing between 30-35 gm were kept in six groups of ten animals each.

Experimental Protocol

Group A1 (control) received only feed and water *ad libitum* for 21 days.

Group A2 (control) received only feed and water *ad libitum* for 45 days.

Group B received Artesunate (150mg/kg.b.wt) for 21 days.

Group C received Artesunate (300mg/kg.b.wt) for 21 days.

Group D received Artesunate (150mg/kg/b.wt) for 45 days.

Group E received Artesunate (300mg/kg.b.wt) for 45 days.

Artesunate was administered as per the experimental protocol. At the end of each treatment, animals were euthanized, dissected and kidney was carefully dissected out, blotted free of blood and weighed. Tissue was than processed for biochemical and histological evaluation.

Protein estimation

Protein levels in the kidney of control and all treated groups of animals were estimated by the method of Lowry *et al.* (1951). Protein containing preparation when treated with phenol reagent of Folin-Ciocalteu, a deep blue colouration develops,

which is measured colorimetrically at 540nm.

Cholesterol estimation

The levels of cholesterol in the kidney of control and all treated groups of mice were estimated by the method of Zlatkis *et al.* (1953). In the presence of concentrated sulphuric acid and glacial acetic acid, cholesterol forms a colored complex with ferric chloride (FeCl_3) which was measured on Systronics Digital Spectrophotometer 167 against blank at 540nm.

Lipid peroxidation (LPO)-Thiobarbituric acid reactive species (TBARS)

The Thiobarbituric acid reactive species (TBARS) levels in kidney of control and all treated animals was determined by the method of Ohkawa *et al.* (1979). The method is based on the formation of a red chromophore that absorbs at 532nm following the reaction of thiobarbituric acid (TBA) with malonyl dialdehyde (MDA) and other breakdown products of peroxidised lipids collectively called as thiobarbituric acid reactive substances (TBARS).

Superoxide dismutase (SOD)

The activity of superoxide dismutase (SOD) in kidney of control and all treated animals was assayed by the modified spectrophotometric method of Kakkar *et al.* (1984). In this method, the formazan formed at the end of the reaction indicates presence of the enzyme. One unit of the enzyme activity is defined as the enzyme concentration required to inhibit 50% of the optical density of chromogen formed in one minute at 560 nm under the assay condition.

Glutathione (GSH)

The concentration of glutathione in kidney of control and all treated groups of mice was assayed by the method of Ellman (1959). Glutathione (GSH) present in the tissue oxidizes 5, 5'-dithiobis-(2-nitrobenzoic acid), (DTNB) to form yellow colored complex which can be read at 412nm. The absorbance is proportional to amount of GSH.

Glutathione peroxidase (G-Px)

Activity of GPx was estimated in kidney of control and treated animals by the method of Rotruck *et al.* (1973). Glutathione peroxidase acts on hydrogen peroxide and splits it in to two water molecules, while doing so it consumes two hydrogen atoms from two molecule of GSH. As byproduct one molecule of reduced glutathione (GS-SG) is obtained. Thus, the amount of GSH consumed per 10 minutes is related to activity of GPx.

Glutathione reductase (GRx)

The estimation of glutathione reductase was done by the method of Carlberg and Mannervik (1985). Glutathione Reductase can be measured by the measuring the rate of NADPH oxidation. The oxidation of NADPH to NADP^+ is accompanied by a decrease in absorbance at 340 nm. Since GR is present at rate limiting concentrations, the rate of decrease is directly proportional to the GR activity in the sample.

Glutathione-S-transferase (GST)

Glutathione-S-transferase activity was measured in kidney of control and treated group animals by modified method of Habig *et al.* (1974). Glutathione-S-transferase catalyse the conjugation of reduced glutathione - via a sulfhydryl group - to electrophilic centers on a wide variety of substrates. Hence

the quantification of GSH-CDNB conjugate formed by reaction of CDNB and GSH in presence of enzyme source may be used to measure the activity of GST.

Histological study

Histological studies were carried out by using the standard technique of haematoxyline and eosin staining. For light microscopic examination kidney tissues from each group were dissected out, blotted free of blood and fixed in 10% formalin immediately after the autopsy. Fixation was carried out at room temperature for 18 hours, after which they were transferred to 70% alcohol. Several changes of 70% alcohols were given, there after tissues were dehydrated by passing through ascending grades of alcohol, cleared in xylene, embedded in paraffin wax (58 to 60°C M.P) and transverse sections (T.S) were cut at 5 μ m on a rotary microtome. These sections were stained with haematoxyline and eosin, dehydrated, cleared in xylene and mounted in DPX (Distrene Plastisizer Xylene) as permanent slide. The photomicrographs of the relevant stained section slides were taken with the aid of a camera attached to biological light microscope.

Statistics

All the data are presented as MEAN \pm SE. Statistical analysis was performed using the trial version of SPSS software package version 16.0 (USA). Comparison between groups was made by one-way analysis of variance (ANOVA) taking significance at $P < 0.05$ followed by Student's t-test taking significance at *** $P < 0.001$, ** $P < 0.005$ and * $P < 0.01$. Tukey's honestly significance difference (HSD) post hoc test was used for comparison among different treatment groups ($P < 0.05$).

RESULTS

Terminal body weight and tissue weight

Reduction in body weight ($P < 0.01$) was observed in Artesunate 150 mg/kg bwt treated mice for 45 days and artesunate 300 mg/kg bwt treated mice for 21 days compared to control mice. Maximum reduction, in body weight was observed in Artesunate 300 mg/kg body wt treated mice ($P < 0.001$) for duration of 45 days (Table 1).

Weight of kidney declined following Artesunate treatment in a dose dependent manner. The reduction in tissue weight was highly significant ($P < 0.001$) when 300 mg/kg bwt of Artesunate was administered as compared with control mice (Table 1).

Biochemical analysis

Artesunate administration at 300 mg/kg bwt for 21 days ($P < 0.005$) and 45 days ($P < 0.001$) significantly decreased the protein content in kidney, while low dose of 150 mg/kg bwt showed significant ($P < 0.005$) changes in total protein content after 45 days as compared with the control group values (Table 2).

Oral administration of high dose of Artesunate for 21 days ($P < 0.005$) and 45 days ($P < 0.001$) produced a significant increase in cholesterol level in the kidney when compared with the control group (Table 2). On the other hand low dose of (150 mg/kg bw) Artesunate showed significant ($P < 0.005$) escalation in cholesterol content only after 45 days.

Table 1: Body weight and organ weight of control and treated animals 21 days post-treatment

Groups	Body Weight (Gm)	Kidney Wt. (Mg)
Control (A1) (21 Days)	39.03 \pm 0.13	364.6 \pm 0.90
Artesunate (150mg/Kg Bwt.) (B) 21 Days	39.05 \pm 0.03 ^{ns}	355.4 \pm 1.04 ^{ns}
Artesunate (300 Mg/Kg Bwt.) (C) 21 Days	35.36 \pm 0.07*	326.7 \pm 1.14**
Control (A2) (45 Days)	38.41 \pm 0.07	365.2 \pm 1.20
Artesunate (150mg/Kg Bwt.) (D) 45 Days	35.53 \pm 0.14*	327.8 \pm 1.64**
Artesunate (300 Mg/Kg Bwt.) (E) 45 Days	29.30 \pm 0.04***	312.3 \pm 1.77***

Values are mean \pm S.E., * $p < 0.01$, ** $p < 0.005$, *** $p < 0.001$, ^{ns}-Non Significant Analysis of variance at $p < 0.05$ significance level.

Table 2: Protein and cholesterol content in kidney of control and treated animals

Groups	Protein (Mg/100 mg Tissue Wt.)	Cholesterol (Mg/100mg Tissue Wt.)
Control (A1) (21 Days)	16.41 \pm 0.25	0.49 \pm 0.02
Artesunate (150mg/Kg Bwt.) (B) 21 Days	13.02 \pm 0.15 ^{ns}	0.56 \pm 0.04 ^{ns}
Artesunate (300 Mg/Kg Bwt.) (C) 21 Days	10.22 \pm 0.26**	0.61 \pm 0.01**
Control (A2) (45 Days)	16.63 \pm 0.25	0.52 \pm 0.01
Artesunate (150mg/Kg Bwt.) (D) 45 Days	11.30 \pm 0.25*	0.62 \pm 0.01**
Artesunate (300 Mg/Kg Bwt.) (E) 45 Days	9.62 \pm 0.12***	0.65 \pm 0.01***

Values are mean \pm S.E., * $p < 0.01$, ** $p < 0.005$, *** $p < 0.001$, ^{ns}-Non Significant Analysis of variance at $p < 0.05$ significance level.

Table 3: Showing alteration in Lpo (Tbars) and Sod content in kidney of control and treated animals.

Groups	Lpo (Mg)	Sod (Mg)
Control (A1) (21 Days)	71.1 \pm .90	0.45 \pm 0.03
Artesunate (150mg/Kg Bwt.) (B) 21 Days	81.5 \pm 2.74 *	0.33 \pm 0.08 *
Artesunate (300 Mg/Kg Bwt.) (C) 21 Days	84.3 \pm 2.12 **	0.22 \pm 0.01 ***
Control (A2) (45 Days)	72.3 \pm 2.13	0.44 \pm 0.02
Artesunate (150mg/Kg Bwt.) (D) 45 Days	82.2 \pm 1.84 *	0.25 \pm 0.03 **
Artesunate (300 Mg/Kg Bwt.) (E) 45 Days	86.1 \pm 1.12 ***	0.21 \pm 0.02 ***

Values are mean \pm S.E., * $p < 0.01$, ** $p < 0.005$, *** $p < 0.001$; Analysis of variance at $p < 0.05$ significance level.

Table 4: Showing alteration in oxidative parameters Gsh and Gst levels in kidney of control and treated animals

Groups	Gsh (μ g/100 Mg Tissue Wt.)	Gst (Units/Mg Protein)
Control (A1) (21 Days)	64.54 \pm 0.44	0.325 \pm 0.01
Artesunate (150mg/Kg Bwt.) (B) 21 Days	55.99 \pm 0.91 *	0.393 \pm 0.04 *
Artesunate (300 Mg/Kg Bwt.) (C) 21 Days	53.09 \pm 0.24 **	0.389 \pm 0.03 **
Control (A2) (45 Days)	61.99 \pm 0.36	0.343 \pm 0.02
Artesunate (150mg/Kg Bwt.) (D) 45 Days	54.82 \pm 0.45 *	0.395 \pm 0.05 **
Artesunate (300 Mg/Kg Bwt.) (E) 45 Days	51.82 \pm 0.32 ***	0.432 \pm 0.04***

Values are mean \pm S.E., * $p < 0.01$, ** $p < 0.005$, *** $p < 0.001$; Analysis of variance at $p < 0.05$ significance level.

Level of LPO (TBARS) was significantly increased ($P < 0.01$) in animals treated with 150 mg/kg.b.wt of Artesunate for 21 and 45 days. It was also found to be significantly elevated when high dose of artesunate was administered for 21 ($P < 0.005$) and 45 ($P < 0.001$) days (Table 3).

Artesunate administration at high dose (300 mg/kg bwt.) for 21 and 45 days exhibited significantly ($P < 0.001$) decreased superoxide dismutase (SOD) activity in kidney. Similarly, low dose of artesunate at 21 days ($P < 0.01$) and 45 days ($P < 0.005$) showed significant reduction in SOD activity as compared with the control group values (Table 3).

Oxidative parameter GSH recorded a significant decline after administration of Artesunate at high dose for 21 ($P < 0.005$) and 45 ($P < 0.001$) days in the treated animals as compared to control group. Low dosed group too registered a significant ($P < 0.01$) decrease in GSH level of treated mice after 21 and 45 days. (Table 4).

High dose of Artesunate treatment brought about a significant decline in G-Px and G-Rx levels of treated mice after 21 ($P < 0.005$) and 45 ($P < 0.001$) days respectively. With low dose treatment similar significant ($P < 0.005$) reduction in G-Px level were noted in treated animals after both 21 and 45 days administration as compared to control animals. (Table 5).

GST activity was found to be significantly increased in treated animals after 21 ($P < 0.005$) and 45 ($P < 0.001$) days administration of high dose of Artesunate. Low dose treatment of Artesunate also caused a significant enhancement in GST activity in treated mice after 21 ($P < 0.01$) and 45 ($P < 0.005$) days. (Table 4).

Histopathological analysis

Control: The histology of control mice kidney revealed normal structure of Bowman's capsule lined with outer parietal layer and inner visceral layer with intact glomeruli. The renal cortex contained a number of renal corpuscles scattered between the renal tubules. Each renal corpuscle was formed of a glomerulus surrounded by a Bowman's capsule (Plate A, Fig. 1 and 2).

Treated group: Kidney of animals treated with artesunate 150 mg/kg showed interstitial lymphoplasmacytic nephritis and

necrosis in the epithelial cells of the renal tubules (Plate B, Fig. 3) at 21 days post treatment. Cytology of renal cells was also altered compared to control slides (Plate C, Fig. 5) after 45 days post treatment. Treatment with high dose of Artesunate (300 mg/kg) for 21 days showed hemorrhage within interstitial tissue (Plate D, Fig. 7). While at 45 days post treatment proliferation of epithelial cell lining of renal tubules followed by necrotic glomerular tufts were seen. Certain other areas showed hyalinization and necrosis as well (Plate E, Fig. 9).

DISCUSSION

Artesunate, an antimalarial drug was introduced by world health organization (WHO) to combat the problem of multidrug-resistant *P. falciparum* malaria (Philip, 2008; Efferth and Kaina, 2010). In our study, effects of orally administered artesunate on indices of kidney function tests have been investigated. Kidney function tests are required either to demonstrate the presence or absence of active lesion in kidney, or to assess the normal functioning capacity of nephron (Yakubu *et al.*, 2006). Cumming *et al.*, 1996 reported that artesunate undergoes bioactivation in the liver to arteminol, the active antimalarial agent, this results in generation of reactive oxygen species or free radicals (Li *et al.*, 2005). These radicals could damage enzymes and tissues such as the kidney and liver (Nontprasert *et al.*, 2000; Anyasor *et al.*, 2009).

Data obtained from the present work have shown that biochemical and histopathological results were in accordance with each other. Organ weights are an essential part of toxicological and risk assessment of drugs, chemicals and other biologicals (Michael *et al.*, 2007). The present study showed statistically significant differences in relative kidney weights in the treated mice as compared with the control group. It has been reported that a change in relative or absolute weight of an organ after drug administration is an indication of the toxic effects of that drug (Simmons *et al.*, 1995; Maina *et al.*, 2008). These findings are similar to an earlier report on artemether (Raji *et al.*, 2005) and that of Okanlawon and Ashiru (1998) who reported that administration of chloroquine to rats for 7 weeks significantly reduced their body and organ weights.

Reduction in body weight as well as organ weight post antimalarial drug treatment has also been reported by Zahid and Abidi, 2003 and Salman *et al.*, 2010. The observed weight loss could be a result of reduced consumption of food *i.e.* loss of appetite. In the present investigation the total protein content of kidney revealed declining trend which was dose and time dependent. The reduction in protein could be due to the toxicity of artesunate which probably prevents the enzymatic synthesis of DNA and RNA. It is also suggestive of disturbances in protein metabolism and/or altered physiology. Impairment in protein turnover can lead to multiple disturbances at receptor level, enzyme functions or glandular secretions. The observed disturbances in the gravimetric indices in the present work are probably attributed to the disturbances in protein metabolism.

Cholesterol is an essential structural component of cell membranes and the outer layer of plasma lipoproteins. Increased cholesterol levels may be related to the effect of artesunate on lipid metabolism and the permeability of intoxicated cells (Eraslan *et al.*, 2007). Similar result indicating

Table 5: Showing alteration in oxidative parameters G-Px and G-Rx levels in kidney of control and treated animals

Groups	G-Px (Gsh Consumed/Mg Protein)	G-Rx (Moles Nadph Oxidized/Min/Mg Protein)
Control (A1) (21 Days)	12.13 ± 0.38	1.49 ± 0.21
Artesunate (150mg/Kg Bwt.) (B) 21 Days	9.38 ± 0.27 **	1.11 ± 0.20 **
Artesunate (300 Mg/Kg Bwt.) (C) 21 Days	8.60 ± 0.39 **	1.07 ± 0.16 **
Control (A2) (45 Days)	11.98 ± 0.43	1.45 ± 0.24
Artesunate (150mg/Kg Bwt.) (D) 45 Days	8.72 ± 0.43 **	1.08 ± 0.17 **
Artesunate (300 Mg/Kg Bwt.) (E) 45 Days	7.90 ± 0.26 ***	0.95 ± 0.15 ***

Values are mean ± S.E., ** $p < 0.005$, *** $p < 0.001$; Analysis of variance at $p < 0.05$ significance level.

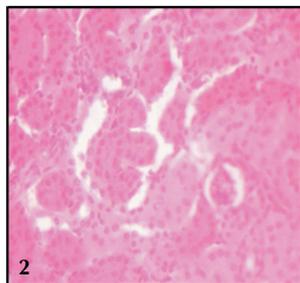
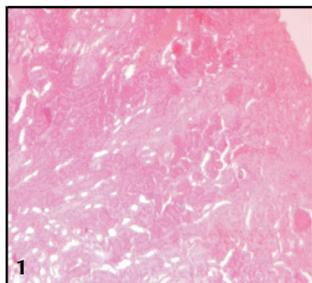


Plate A: Transverse sections (T.S) of Kidney of control mice (*Mus musculus*). (Haematoxylin-Eosin stain, 5 μ section)

Figure 1: T. S of control mice Kidney showing normal structure of Bowman's capsule lined with outer parietal layer and inner visceral layer with intact glomeruli X 250.

Figure 2: Magnified views of figure-33 X 1000

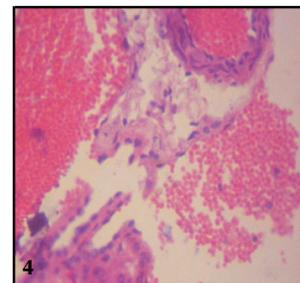
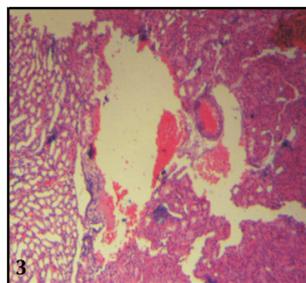


Plate B: Transverse sections (T.S) of Kidney of Artesunate (150 mg/kg body wt) treated mice for 21 days. (Haematoxylin-Eosin stain, 5 μ section)

Figure 3: T.S of Kidney 21 days Artesunate (150 mg/kg body wt) treated mice shows interstitial lymphoplasmacytic nephritis and necrosis in the epithelial cells of the renal tubules 250 X.

Figure 4: Magnified views of figure-37 X 1000

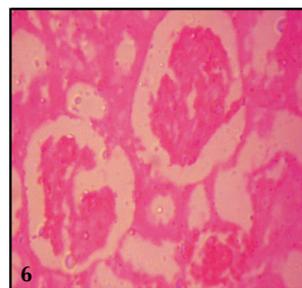
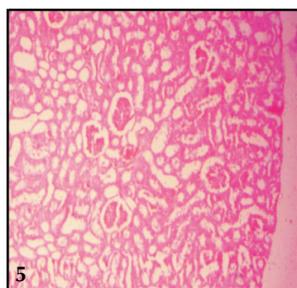


Plate C: Transverse sections (T.S) of Kidney of Artesunate (150 mg/kg body wt) treated mice for 45 days. (Haematoxylin-Eosin stain, 4.5 μ section)

Figure 5: T. S. of Kidney 45 days Artesunate (150 mg/kg body wt) treated mice showing Cytology of renal cells was also not altered X 250

Figure 6: Magnified views of figure-39 X 1000

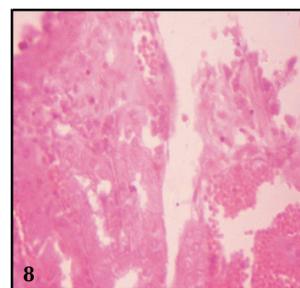
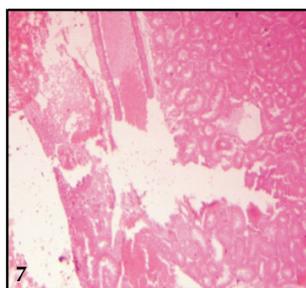


Plate D: Transverse sections (T.S) of Kidney of Artesunate (300 mg/kg body wt) treated mice for 21 days. (Haematoxylin-Eosin stain, 5 μ section)

Figure 7: T.S of Kidney 21 days Artesunate (300 mg/kg body wt) treated mice showing the showed hemorrhage within interstitial tissue X 250

Figure 8: Magnified views of figure-41 X 1000

an increase in the cholesterol level was reported by Desai *et al.* (2011). The elevation of the tissue cholesterol may be attributed to enhance cholesterol synthesis and / or reduced cholesterol catabolism. High cholesterol content is also considered as an indicator of heart dysfunction. This increase in cholesterol content could be due to the interference of artesunate with cholesterol metabolism.

The intracellular antioxidant system includes different kinds of free radicals scavenging antioxidant enzymes along with non enzyme antioxidants like GSH and other thiols. Further, SOD, GST, G-Rx and G-Px constitute the first line of cellular antioxidant defense enzyme. In the presence of excess free radicals due to some toxin load, this equilibrium is lost and results in oxidative insult. The results obtained in the present study indicate implications of artesunate treatment on oxidative parameters clearly.

Elevation of malondialdehyde (MDA) levels is a reflection of increased oxidative stress and provides evidence of lipid peroxidation (Ozturk *et al.*, 2003). Free radicals react with cellular macromolecules and have a deleterious effect on lipids of cell membrane resulting in cellular destruction. The end product of this phenomenon is MDA which is a well known and reliable indicator of lipid peroxidation. Peroxidation of

membrane lipid change the permeability of cell membrane, disrupt its integrity thereby impairing cell integrity. The level of MDA, end product of lipid peroxidation was found to be high in CCl₄ treated group leading to tissue damage and failure of antioxidant defense mechanism against free radicals (Darbar *et al.*, 2011). Results of our investigation revealed a significant increase in the LPO (TBARS) level in kidney of treated mice which suggests an increased peroxidation of lipid with concomitant loss of cellular functions in the body by artesunate treatment. Similar results have been shown with fluoride toxicity in liver and kidney by Vasant and Narasimacharya, 2011. Moreover, Murugavel and Pari in 2004 also showed increased lipid peroxidation and decreased enzymatic as well as non enzymatic antioxidant due to chloroquine toxicity. These findings are also in agreement with our results.

Superoxide radicals are one of the most important reactive oxygen free radicals produced constantly in living cells. These reactive oxygen species (ROS) includes superoxide anion radicals (O₂⁻), H₂O₂ and highly reactive hydroxyl radical (.OH). During cellular respiration ROS are produced collectively via sequential biological reduction of molecular oxygen (Scandalios, 2002) which is rapidly converted into H₂O₂ by superoxide dismutase (SOD). SOD has an antitoxic effect

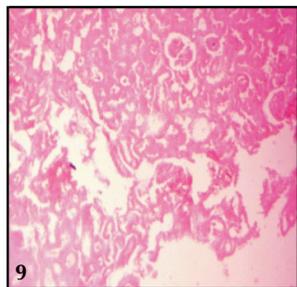


Plate E: Transverse sections (T.S) of Kidney of Artesunate (300 mg/kg body wt) treated mice for 45 days. (Haematoxylin-Eosin stain, 5 μ section)

Figure 9: T.S of Kidney 45 days Artesunate treated mice showing proliferation of epithelial cells lining renal tubules followed by necrotic glomerular tufts, other show hyalinization and necrosis X 250

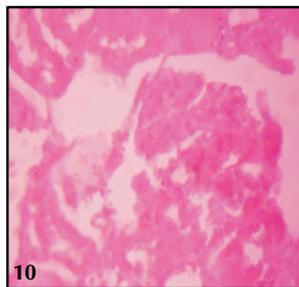


Figure 10: Magnified views of figure-9 X 1000

against the superoxide anion. Thus it serves as a primary defense mechanism against superoxide anion and prevents further free radical generation. Results of our study showed that SOD activity in the kidney of treated mice declined in dose and time dependent manner. This decreased SOD activity is suggestive of accumulation of super oxide anion radical which in turn could be responsible for increased lipid peroxidation. Concomitant decline in GSH, G-Px and G-Rx with an increase in LPO activity supports the susceptibility of tissue to injury. Similar results have been shown with metal toxicity (Chinoy, 2002; Chinoy and Shah, 2004).

GSH is a major non protein thiol in living organisms which plays a central role in coordinating the body's antioxidant defense mechanism. The tissue glutathione concentration reflects its potential for detoxification and is critical in preserving the proper cellular redox balance as well as for its role as a cellular protectant (Mari *et al.*, 2001). The GSH/GST system is a critical factor in protection of cells and organ against toxicity. The thiol containing metabolites reacts with GSH which are then converted into less harmful and more water soluble molecules and the reaction is catalysed by enzyme GST. Artesunate treatment at both low and high dose brought about a significant reduction in GSH activity of kidney of treated mice which clearly indicates the toxic effect of this antimalarial drug on body's defense mechanism.

Glutathione peroxidase (G-Px) plays a significant role in the peroxyl scavenging mechanism by maintaining functional integration of cell membrane. It reduces H₂O₂ to H₂O by oxidizing reduced glutathione. A decrease in G-Px would increase the concentration of H₂O₂ thereby signaling an increase in oxidative stress. Our present finding of the decreased activity of glutathione peroxidase corroborates with that of earlier findings (Chitra *et al.*, 2003).

Glutathione reductase (G-Rx) is concerned with maintenance of cellular level of GSH by catalyzing fast reduction of oxidized glutathione (GSSG) to reduced form. A decrease in the activity of G-Rx would further decrease the concentration of ascorbic acid thereby compromising the defense mechanism of cell, in turn disrupting the normal function and architecture of

concerned tissue.

Glutathione S Transferase (GST) is an important oxidative non enzyme antioxidant playing the key role in cellular detoxification by catalyzing the reaction of glutathione with the toxicant to form an S- substituted glutathione. It plays an essential role in eliminating toxic compounds by conjugation (Townsend and Tew, 2003). In our study the increase in GST activity along with LPO suggests inability of GST to utilize glutathione and protect the tissue against artesunate induced toxicity.

Histological results of kidney revealed that mice treated with low dose artesunate exhibited increased cellularity and matrix deposition leading to expansion of glomerular tuft and obliteration of Bowman's space. High dose artesunate treatment showed proliferation of epithelial cell lining of renal tubules and necrotic glomerular tufts while medullary rays and straight tubules showed hyalinization and necrosis. The disrupted renal parenchyma observed in the present study could be correlated with the observation of David, 2001. Other authors have also demonstrated similar renal histological changes in rats after exposure to chemical compounds containing phenol (Nakazawa *et al.*, 2009).

Hence, the observed changes in the renal histology as well as biochemical parameters could be attributed to artesunate toxicity. Similar results have also been documented with chloroquine toxicity (Dattani *et al.*, 2010). The outcome of the present work helps in evaluating the toxic effects of the antimalarial drug artesunate on the renal tissue and questions the fact that it is a safe drug.

CONCLUSION

In the light of our results and literature knowledge, we can conclude that the use of antimalarial such as artesunate should be monitored especially in patient with history of renal dysfunctions as it is significantly toxic at the recommended dosage.

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