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# Protective Effect of Dietary Vitamin E (α Tocopherol) on Artemisinin Induced Oxidative Liver Tissue Damage in Rats

## Efecto Protector de la Vitamina E en la Dieta (α Tocoferol) sobre el Daño Oxidativo del Tejido Hepático Inducido por Artemisinina en Ratas

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**SUMMARY:** This experiment was designed to study the effects of oral administration of artemether which is the most rapid-acting class of antimalarial drugs and the possible protective effect of vitamin E taken with it on the liver of albino rats. A total of twenty-four adult male albino rats were used in this study and were divided into four groups. Group one served as a control and rats in group two exposed to oral intake of artemether daily for fifteen days. The third and fourth groups treated with artemether plus low and high doses of vitamin E respectively. At the end of the experiment, the rats were sacrificed, and the livers were obtained and processed for histological, biochemical and statistical studies. Histological study of the hepatocytes of rats exposed to artemether showed nearly complete disintegration of most cellular contents except few numbers of mitochondria and rough endoplasmic reticulum. Also, the cytoplasm of these cells had few lysosomes, many vacuoles and irregular nuclei with abnormal distribution of chromatin and were shown. The hepatic sinusoids were dilated and filled with blood and vacuoles and bile ductules were abnormal in its structure. Treatment with low and high doses of vitamin E in concomitant with artemether ameliorated the hepatic histopathological lesions and its parenchyma attained nearly normal structure. As far as biochemical changes, alanine aminotransferase (ALT) and aspartate aminotransferase (AST) in rats treated with artemether were significantly elevated as compared to the control. Superoxide dismutase (SOD) and malondialdehyde (MDA) levels were significantly increased in the liver in rats treated with artemether. However, vitamin E ameliorated the rise in ALT and AST with decreased MDA concentration and levels of SOD as compared to the corresponding artemether group values. Results of the present suggest that artemether has a harmful and stressful effect on hepatic tissue and the treatment with vitamin E may alleviate this toxicity.

**KEY WORDS Artemether, Rats, Vitamin E, light and electron microscopy, Biochemical, Statistical analysis.**

## INTRODUCTION

Malaria plays a critical role in the global infectious disease burden with significant morbidity and mortality. Clinical malaria is characterized by a systemic inflammatory response induced by asexual Plasmodium parasites which is responsible for most malaria-related deaths globally (World Health Organization, 2017). Severity of disease is determined by multiple factors, including parasite species and timing of antimalarial treatment (Gachot & Ringwald, 1998). Most severe falciparum malaria manifestations are most likely related to cytoadherence of parasitized red blood cells to the vascular endothelium (Pain *et al*., 2001).

Hepatic dysfunction and jaundice are common features of severe malaria (Jain *et al.*, 2016). Histopathological changes in the liver range from hepatocyte necrosis, granulomatous lesions, Kupffer cell hyperplasia, malarial pigmentation, cholestasis, monocyte infiltrations to malarial nodules (Srivastava *et al*., 1996; Murthy *et al*., 1998; Anand & Puri, 2005). Pronounced elevations in liver function tests often shortly upon initiation of curative treatment in patients with imported uncomplicated malaria that might arise through a mechanism other than drug induced hepatotoxicity, or the substantial sequestration observed in severe disease (Molyneux *et al.*, 1989; White *et al.*, 2014).

Artemisinin is obtained from the Chinese medicinal herb Artemisia annua, for its potent antimalarial activity (Tu, 2016). Apart from the antimalarial property, the therapeutic

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applications of artemisinin and its derivatives (including artesunate, artemether, artenimol, and artelinic acid) are wide and huge (Whirl-Carrillo *et al.*, 2012). Artemisinin has also been found to be associated with many other activities including anticancer activity, anti-inflammatory activity, and antibacterial and antiviral activity (Efferth *et al.*, 2008; Whirl-Carrillo *et al.*; Alesaeidi & Miraj, 2016).

Artemether (ART) is the methylated derivative of artemisinin. In addition to the amazing antimalarial effect of ART, it showed anti-parasitic properties toward many protozoan parasites such as Leishmania, Toxoplasma gondii and Trypansoma spp (Mishina *et al.*, 2007). One of the great advantages of ART therapy is its prophylactic action. The prophylactic effect of ART is defined by its ability to eradicate the developing stages of schistosomula, so that the egg laying mature female worms are not allowed to develop in the vasculature (Xiao *et al.*, 2000).

Vitamins have indispensable role in almost all biochemical reactions and they are ideal antioxidants able to increase tissue protection from oxidative stress due to their easy, effective and safe dietary administration in a large range of concentrations (Cadenas & Cadenas *et al.*, 2002; Kanter *et al.* 2005).

Vitamin E (α- Tocopherol) is the primary membrane bound, lipid-soluble, chain-breaking antioxidant that protects cell membranes against lipid peroxidation (Bulger & Maier *et al.*, 2003; Soylu *et al.* 2006). Vitamin E pre-treatment has been reported to be beneficial in preventing formaldehyde-induced tissue damage in rats (Gurel *et al.*, 2005; Gulec *et al.*, 2006). The preventive effect of vitamin E on endotoxin-induced oxidative stress in rat tissues is suggestive of its antioxidant activity (Kale *et al.*, 1999; Kheir-Eldin *et al.*, 2001). The antioxidant vitamin E was shown to reduce lysosomal phospholipidosis (Honegger *et al.*, 1995).

This experiment was designed to study the histological and ultrastructural effects of oral administration of normal therapeutic doses of artemether and coadministration of vitamin E may reduce the hepatotoxicity induced by artemether.

## MATERIAL AND METHOD

**Animals:** All ethical points regarding the treatment of laboratory animals were observed in this research. A total of twenty-four male albino rats (*Rattus norvegicus*) of (160-170 g) were purchased from Experimental Animals Production Center, king Khalid University, Saudi Arabia. They were clinically healthy and were acclimatized to the experimental conditions for fifteen days before start of the experiment. During this period, the rats were housed in plastic cages with galvanized iron filter tops and placed in quiet room with natural ventilation and 12:12-h light–dark cycle. Clean food and water were given to rats ad libitum throughout the experimental period. All the experimental procedures were carried out in accordance with international guidelines for care and use of laboratory animals.

**Experimental design and animal grouping:** After acclimatization for two weeks, the rats were randomly divided into four groups, with six animals in each group as follow:

Group A: rats served as a control were orally administered 10 ml/kg of body/day of normal saline.

Group B: rats received artemether orally 0.125 mL, 2.3mg $kg^{-1}$ daily 1 for fifteen days.

Group C: rats received 0.125 mL, 2.3 mg $kg^{-1}$ of artemether orally concomitantly with 50 mg/kg vitamin E (α-tocopherol) dissolved in corn oil by oral gavages daily for fifteen days.

Group D: rats received 0.125 mL, 2.3 mg $kg^{-1}$ of artemether concomitantly with 100 mg/kg vitamin E /100 (α-tocopherol) dissolved in corn oil by oral gavages daily for fifteen days.

**Biochemical study.**

**Assessment of serum levels of liver enzymes.** All serum samples were processed to determine the enzymatic activities of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) with a spectrophotometric technique by the Olympus AU-2700 auto analyzer and presented as IU/L.

**Measurement of some oxidative stress factors.** Liver tissues from all experimental rats were minced and homogenized (10 %, w/v) separately in ice-cold saline. To determine the following oxidative stress factors, homogenates were centrifuged at 18,000 g (148C) for 15 min. The thiobarbituric acid substrate assay was used to measure malondialdehyde (MDA; nmol/g wet tissue) as an indicator of lipid peroxidation, with a spectrophotometer method. In this method, a spectrophotometric measurement of the color produced during the reaction to thiobarbituric acid (TBARS Assay Kit, Item No. 10009055, Cayman Chemical Company, Ann Arbor) with MDA at 535 nm was used according to the method described by Ohkawa *et al.*,

(1979). To estimate superoxide dismutase (SOD, Superoxide Dismutase Assay Kit, Item No. 706002, Cayman Chemical Company, Ann Arbor) activity (an antioxidant) (Units/mg protein) (Khan *et al*., 2012).

**Histopathological examination:** Necropsy of the rats was performed, and liver samples were fixed at 10 % neutral buffered formalin. Paraffin embedded blocks were routinely processed. 5-mm thick sections were stained with hematoxylin-eosin and examined under a microscope. Then random 10 microscopic fields were examined in X40 magnification according to Bancroft & Gamble (2008).

**Transmission electron microscopy (TEM):** For TEM, liver specimens from both control and treated rats were immediately preserved in 2.5 % glutaraldehyde, trimmed and diced into 1 cubic millimeter sizes, fixed in glutaraldehyde solution in 0.1 M sodium cacodylate buffer, pH 7.2, and placed in a thermal box cooled to 4 °C for 2 h. They were post-fixed in 1 % osmium tetroxide in a sodium cacodylate buffer and then dehydrated in ascending series of ethyl alcohol and embedded in Spur's resin. Ultrathin sections stained with uranyl acetate and lead citrate were examined by TEM (JEM-1011, Jeol Co., Japan) operated at 80 KV in the Electron Microscopy Unit, Pathology Department, College of Medicine, King Khalid University (Eid *et al*.., 2017).

**Statistical Analysis.** The results are expressed as mean ± standard error. Statistical significance between the different groups was determined by using a one-way analysis of variance (ANOVA) in the SPSS 21 software package. Post hoc test was performed for between-group comparisons by using the Tukey multiple comparison tests. The level of significance was set at $p < 0.05$.

## RESULTS

### Biochemical Results

**Serum enzymes**. Figure 1, A&B showed that ALT and AST in rats treated with artemether were significantly elevated, respectively, as compared to the corresponding control group. However, vitamin E (Groups III, IV) significantly ameliorated the rise in ALT and AST, as compared to the corresponding artemether group values.

**Tissue lipid peroxidation and antioxidant markers.** Figure 1, C&D Results indicated that SOD and MDA levels were significantly increased in the liver in rats treated with artemether. Vitamin E (Group III, IV) effectively prevented the oxidative damage induced by artemether, which decreased MDA concentration significantly in comparison to artemether treated group. Artemether treatment decreased the levels of SOD in the liver. By contrast, increased levels of SOD were observed in Vitamin E plus artemether treated groups.

**Histopathological assay.** The histological examination of the liver sections of control animals showed its normal architecture. The normal liver consists of several hepatic lobules. Each lobule is formed of cords of hepatocytes radiating from the central vein. The cell cords are separated by narrow blood sinusoids lined by Kupffer cells and endothelial cells. The hepatocytes are large polyhedral with acidophilic cytoplasm and darkly stained nuclei, few of these cells are bi-nucleated (Fig. 2A). Portal tracts contain the hepatic triad, which consists of one or more branches of the portal vein, a branch of the hepatic artery and a small bile duct (Fig. 3A).

Histological assessment of the hepatocytes after administration of artemether showed loss of the normal hepatic architecture. The hepatocytes appeared having cytoplasmic vacuolation and pyknotic nuclei, inflammatory cell infiltration and highly dilated hyperemic central and portal veins. Hemorrhage in hepatic parenchyma especially in the blood sinusoids was observed and Kupffer cells were actively proliferating, markedly increased in size and number (Figs. 2B, 3B).

Treatment with low and high doses of vitamin E in concomitant with artemether greatly ameliorated the hepatic histopathological lesions and the hepatic parenchyma attained nearly normal structure and organization. Preserved normal hepatic architecture and normal blood sinusoids with minimal dilatation and congestion of some portal and central veins and minimal cellular infiltrations were seen (Figs. 2C-D and 3C-D).

**Ultrastructure results.** No pathological changes were observed in the ultrastructure of rat's liver cells in the control group. The cytoplasmic organelles as well as the nuclei of the hepatocytes exhibited normal appearance. The cytoplasm contained numerous mitochondria dispersed all over the cytoplasm. The mitochondria were spherical in shape with well-developed cristae, while the rough endoplasmic reticulum was closely packed parallel with flattened cisternae studded with ribosomes. The nucleus was rounded with a distinct nuclear envelop and the nucleoplasm showed aggregations of euchromatin and heterochromatin. In addition, the hepatic sinusoid, localized between the hepatocytes and lined with endothelial cells, was also shown in (Figs. 4A-D).

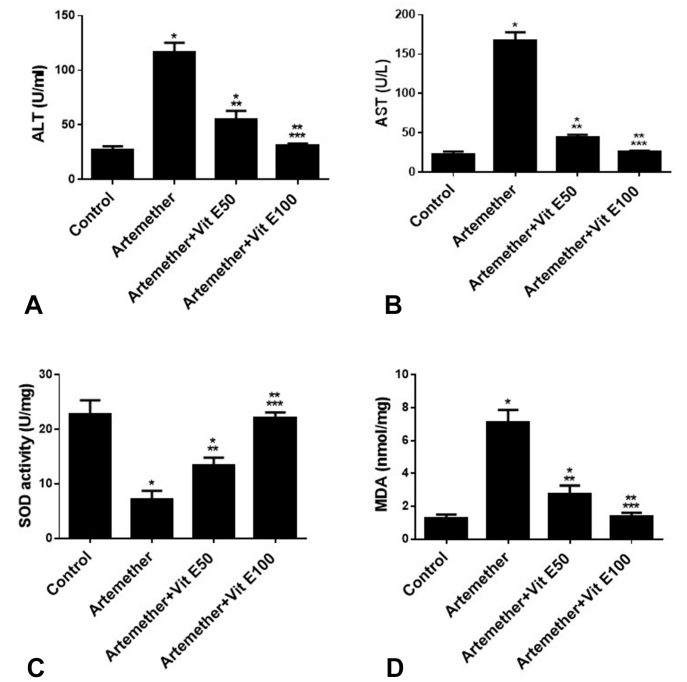


Fig. 1. ALT, AST, MDA and GPx. (A & B) showing that ALT and AST in rats treated with artemether are significantly elevated, respectively, as compared to the corresponding control group. However, vitamin E (Groups III, IV) significantly ameliorate the rise in ALT and AST, as compared to the corresponding artemether group values. (C & D) indicating that SOD and MDA levels are significantly increased in the liver in rats treated with artemether. MDA concentration and the levels of SOD in the liver are significantly decreased in vitamin E plus artemether treated groups in comparison to artemether treated group.

Results represent the mean (±SD); n=6 for each group. Experiments were performed in triplicate. *p<0.05 versus control, **p<0.05 versus Artemether, ***p<0.05 versus Artemether+ vitamin E 50.

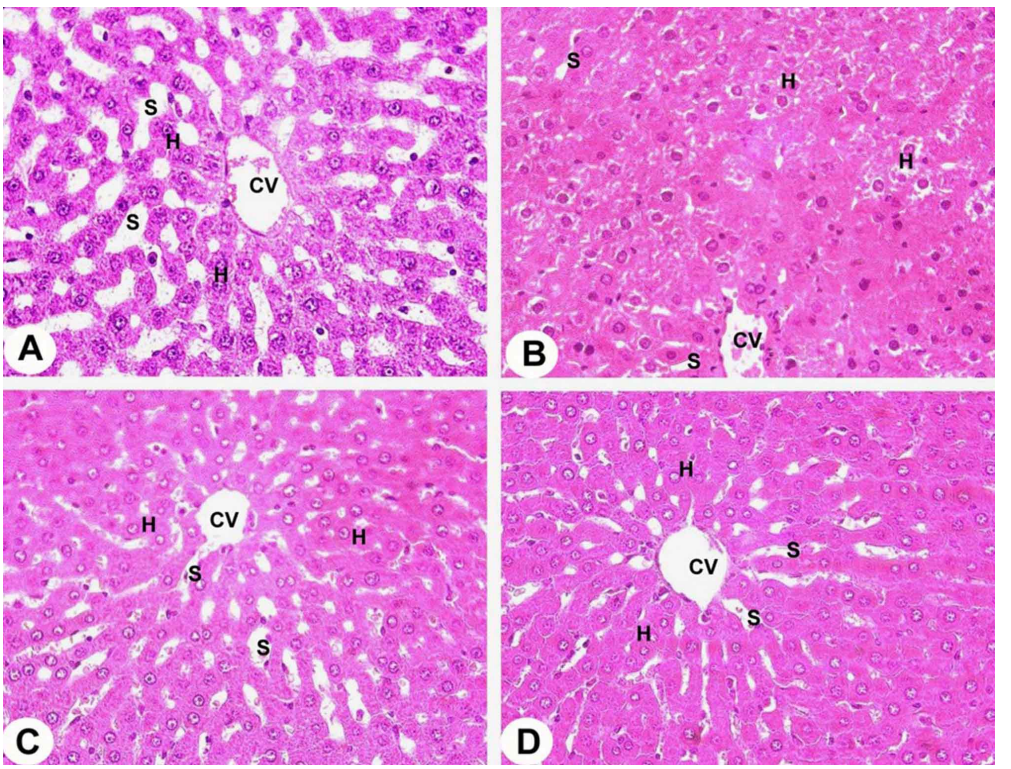


Fig. 2. Histological micrographs obtained from liver tissues around the Central vein. **A.** A photomicrograph of liver section of the control rat showing the characteristic histological structures of hepatocytes (H): central vein (CV), blood sinusoids (S) and kupffer cells. (H & E X400). **B.** A photomicrograph of liver section of rat given artemether showing degenerated ballooned hepatocytes (H) with severely pyknotic nuclei, dilated blood sinusoids (S), swelling in kupffer cells and central vein (CV). (H & E X400). **C.** A photomicrograph of liver section of mice treated with vitamin E low dose in concomitant with artemether showing nearly normal hepatocytes (H), normal central vein (CV) and mild dilatation of blood sinusoids (S). (H & E X400). **D.** A photomicrograph of liver section of mice treated with vitamin E high dose in concomitant with artemether showing nearly normal structure of hepatocytes (H), central vein (CV), blood sinusoids (S) and Kupffer cells. (H & E X400).

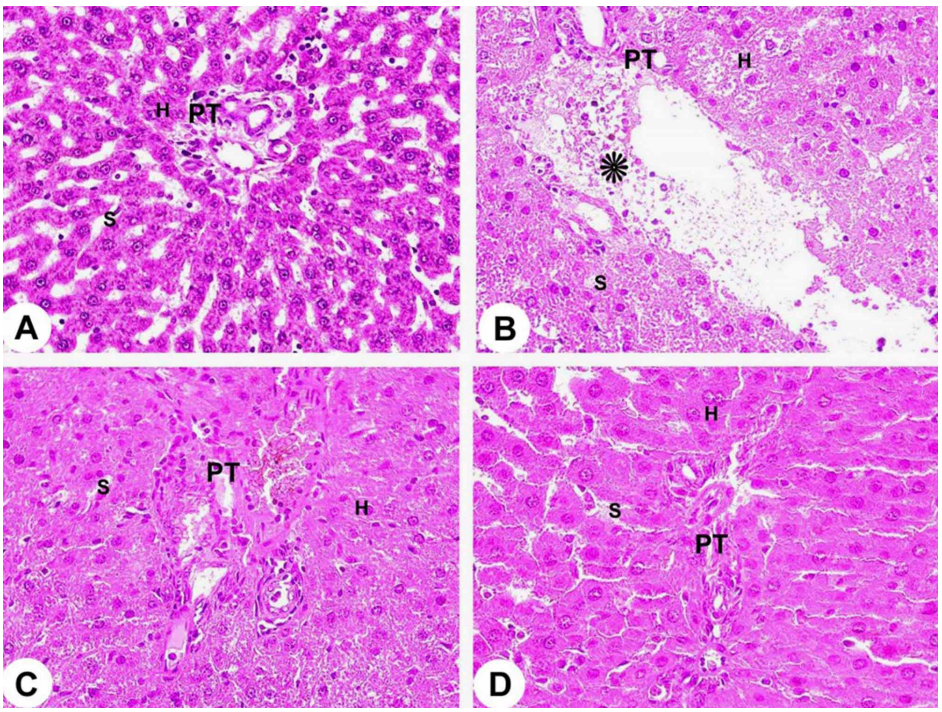


Fig. 3. Histological micrographs obtained from liver tissues around the portal tract. **A.** A photomicrograph of liver section of the control rat showing the characteristic histological structures; portal tract (PT) with its contents, hepatocytes (H), blood sinusoids (S) and kupffer cells. (H & E X400). **B.** A photomicrograph of liver section of rat given artemether showing highly distorted portal tract (PT) with inflammatory cells infiltration (*) in the portal area, degenerated hepatocytes (H), bile ductules, hyperemic portal vein. (H & E X400). **C.** A photomicrograph of liver section of mice treated with vitamin E low dose in concomitant with artemether showing hepatocytes (H) with dilatation of portal tract (PT) and hemorrhage inside, in addition to some inflammatory cells. (H & E X400). **D.** A photomicrograph of liver section of mice treated with vitamin E High dose in concomitant with artemether showing mild nearly normal structure of hepatocytes (H) with its portal tract (PT) and blood sinusoids (S). (H & E X 400).

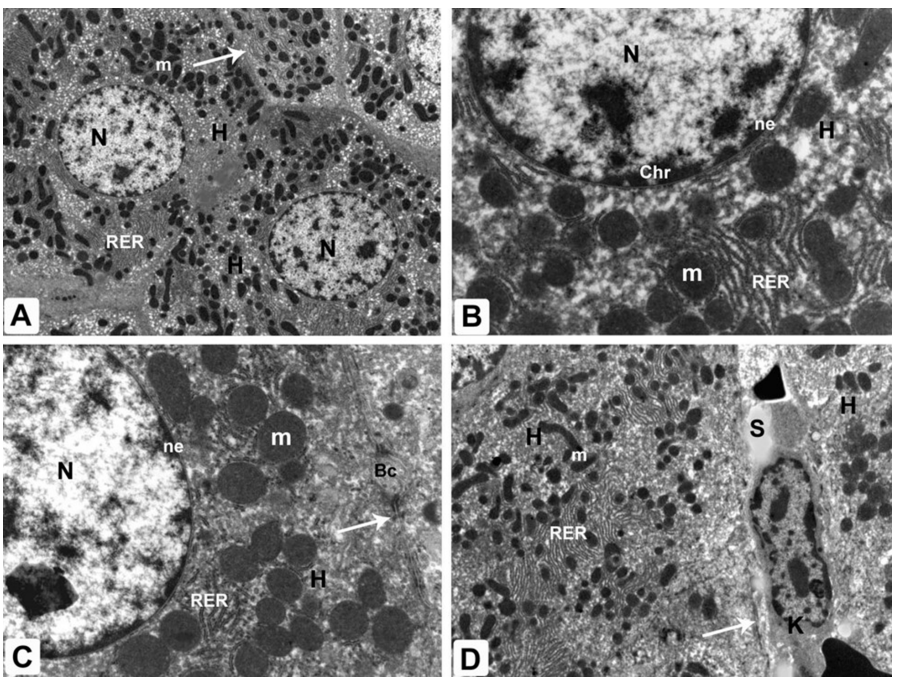


Fig. 4. Transmission electron micrographs (TEM) obtained from the liver tissues of the control group. **A.** Electron micrograph of hepatocytes (H) of the control rat showing hepatocytes with normally distributed rough endoplasmic reticulum (RER), usual pattern of mitochondria (M) and cell junction (arrow) and euchromatic normal nucleus (N). X 5000. **B.** Higher magnification of hepatocytes (H) of the control rat showing hepatocytes with normally distributed rough endoplasmic reticulum (RER), usual pattern of mitochondria (M) and cell junction (arrow) and intact nucleus (N) with normal distribution of its chromatin (Chr) and nuclear envelope (ne). X 15000. **C.** Electron micrograph of hepatocytes (H) of the control rat showing hepatocytes with normally distributed rough endoplasmic reticulum (RER), usual pattern of mitochondria (M) and cell junction (arrow) and euchromatic normal nucleus (N). X 15000. **D.** Electron micrograph of hepatocytes (H) of the control rat administered normal saline showing hepatocytes with normally distributed rough endoplasmic reticulum (RER), usual pattern of mitochondria (M) and cell junction (arrow), euchromatic normal nucleus (N) and intact blood sinusoids (S). X 5000.

On using the electron microscope in this study of the artemether treated rats, nearly complete disintegration of most cellular contents except few numbers of mitochondria and rough endoplasmic reticulum. While swelling in mitochondria and fragmented rough endoplasmic reticulum was observed in. Also, the cytoplasm of these cells had many vacuoles and irregular nucleus with abnormal distribution of chromatin. Some of the cells presented variation in size and shape of mitochondria and few lysosomes which were electron dense in appearance. In addition, hepatic sinusoid, as displayed in was markedly dilated and filled with blood and vacuoles (Figs. 5A-D).

Examination of ultrathin sections of liver from rats treated with artemether and vitamin E with low and high doses showed amelioration of the histological changes induced by artemether as some hepatocytes appeared as the control showed normal structure of mitochondria; nucleus and cell membrane and normal rough endoplasmic reticulum distribution in the cytoplasm but some mitochondria showed loss of cristae and few cytoplasmic vacuoles were still present. Also, the hepatic sinusoid contained little or no cell debris (Figs. 6A-D and 7A-D).

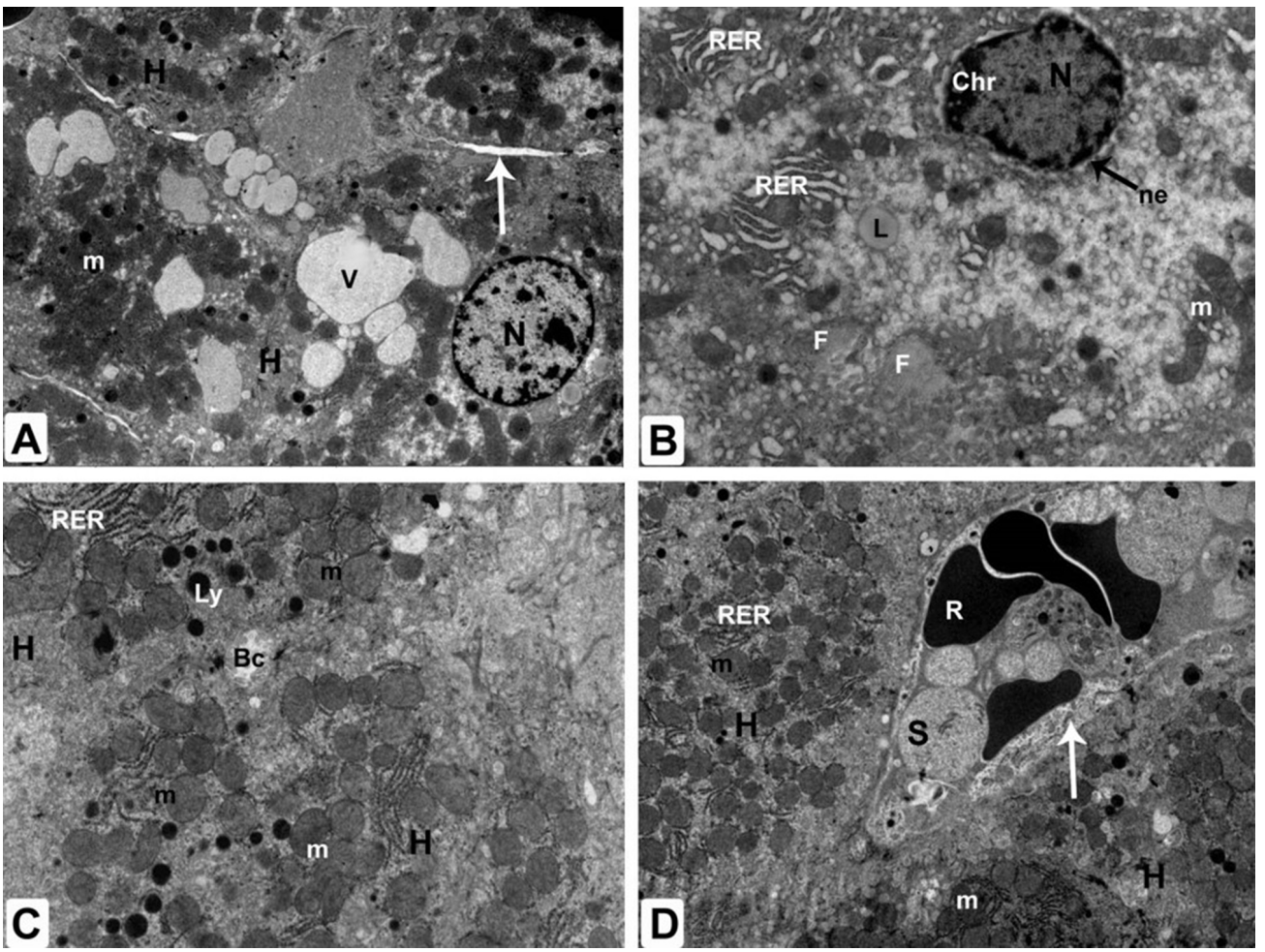


Fig. 5. Transmission electron micrographs (TEM) obtained from the liver tissues of the artemether -treated group. **A.** Electron micrograph of hepatocytes (H) of rats given artemether showing nearly complete disintegration of most cellular contents with abnormally shaped mitochondria (M), nucleus (N) with abnormal distribution of its chromatin, and abnormal cell junction (arrow). Note the breakdown of rough endoplasmic reticulum (RER) and higher content of vacuoles (V). X 5000. **B.** Electron micrograph of hepatocytes (H) of rats given artemether showing swollen mitochondria (M), fragmented rough endoplasmic reticulum (RER), lipid droplets (L), pyknotic nucleus (N) with condensation of heterochromatin (Chr) and irregular outline and nuclear envelop (ne) and higher content of collagen fibrils (F). X 15000. **C.** Electron micrograph of hepatocytes (H) of rats given artemether showing abnormally shaped mitochondria (M), breakdown of rough endoplasmic reticulum (RER), many lysosomes (L) and abnormal bile canaliculi (BC) with disturbed microvilli. X 15000. **D.** Electron micrograph of hepatocytes (H) of rats given artemether showing abnormally shaped mitochondria (M), breakdown of rough endoplasmic reticulum (RER) and blood sinusoids (S) with abnormal microvilli (arrow) and filled with many red blood cells (R) and vacuoles. X 5000.

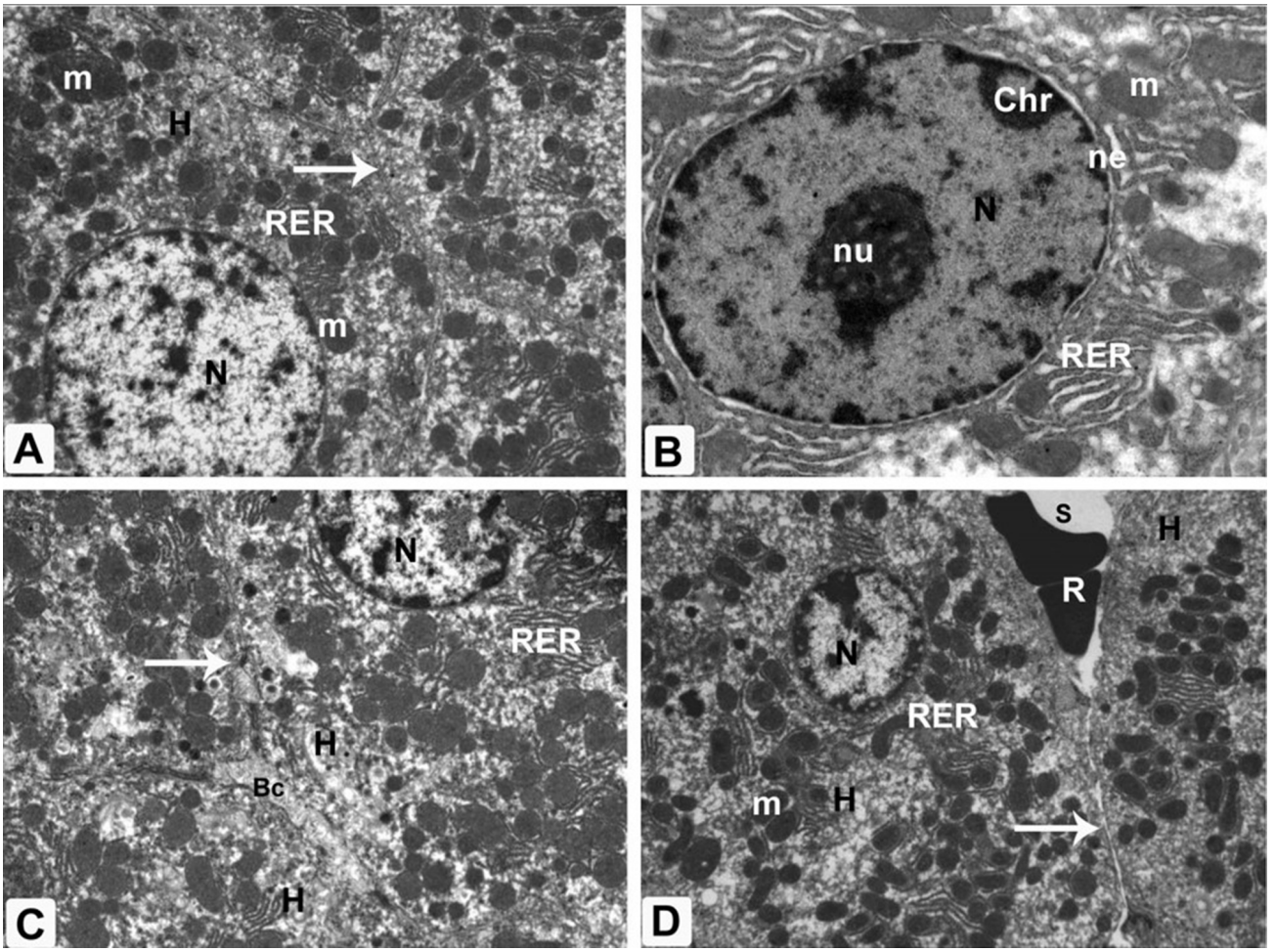


Fig. 6. Transmission electron micrographs (TEM) obtained from the liver tissues of the artemether -treated plus low dose of vitamin E group. **A.** Electron micrograph of rat hepatocytes (H) given artemether with low dose of vitamin E showing normal structure of mitochondria (M) but some mitochondria showed loss of cristae, nucleus (N) still have abnormal distribution of chromatin and little affection of rough endoplasmic reticulum (RER) in the cytoplasm, few cytoplasmic vacuoles still present and abnormal cell junction (arrow). X 5000. **B.** Higher magnification of rat hepatocytes (H) given artemether with low dose of vitamin E showing normal structure of mitochondria (M), nucleus (N) and little affection of rough endoplasmic reticulum (RER) in the cytoplasm, aberrant cell junction (arrow) and few cytoplasmic vacuoles still present. X 15000. **C.** Electron micrograph of rat hepatocytes (H) given artemether with low dose of vitamin E showing normal structure of mitochondria (M), nucleus (N) and little affection of rough endoplasmic reticulum (RER) in the cytoplasm, normal bile canaliculi (BC), few cytoplasmic vacuoles still present and abnormal cell junction (arrow). X 15000. **D.** Electron micrograph of rat hepatocytes (H) given artemether with low dose of vitamin E showing normal structure of mitochondria (M), nucleus (N) and little affection of rough endoplasmic reticulum (RER) in the cytoplasm, blood sinusoids (S) with normal microvilli filled with some red blood cells (R), few cytoplasmic vacuoles still present and abnormal cell junction (arrow). X 5000

## DISCUSSION

The present study was undertaken to evaluate the possible protective effect of vitamin E against artemether-induced histological alterations in liver tissue of male albino rats.

The present investigation showed mice treated with artemether impaired several histopathological alterations in liver tissues. Histological assessment of the liver after administration of artemether showed loss of the normal hepatic architecture and the hepatocytes appeared having cytoplasmic vacuolation and pyknotic nuclei. They also showed inflammatory cell infiltration and highly dilated hyperemic central and portal veins. Hemorrhage in hepatic parenchyma especially in the blood sinusoids was observed and kupffer cells were actively proliferating, markedly increased in size and number.

Disturbed hepatic architecture of the liver which

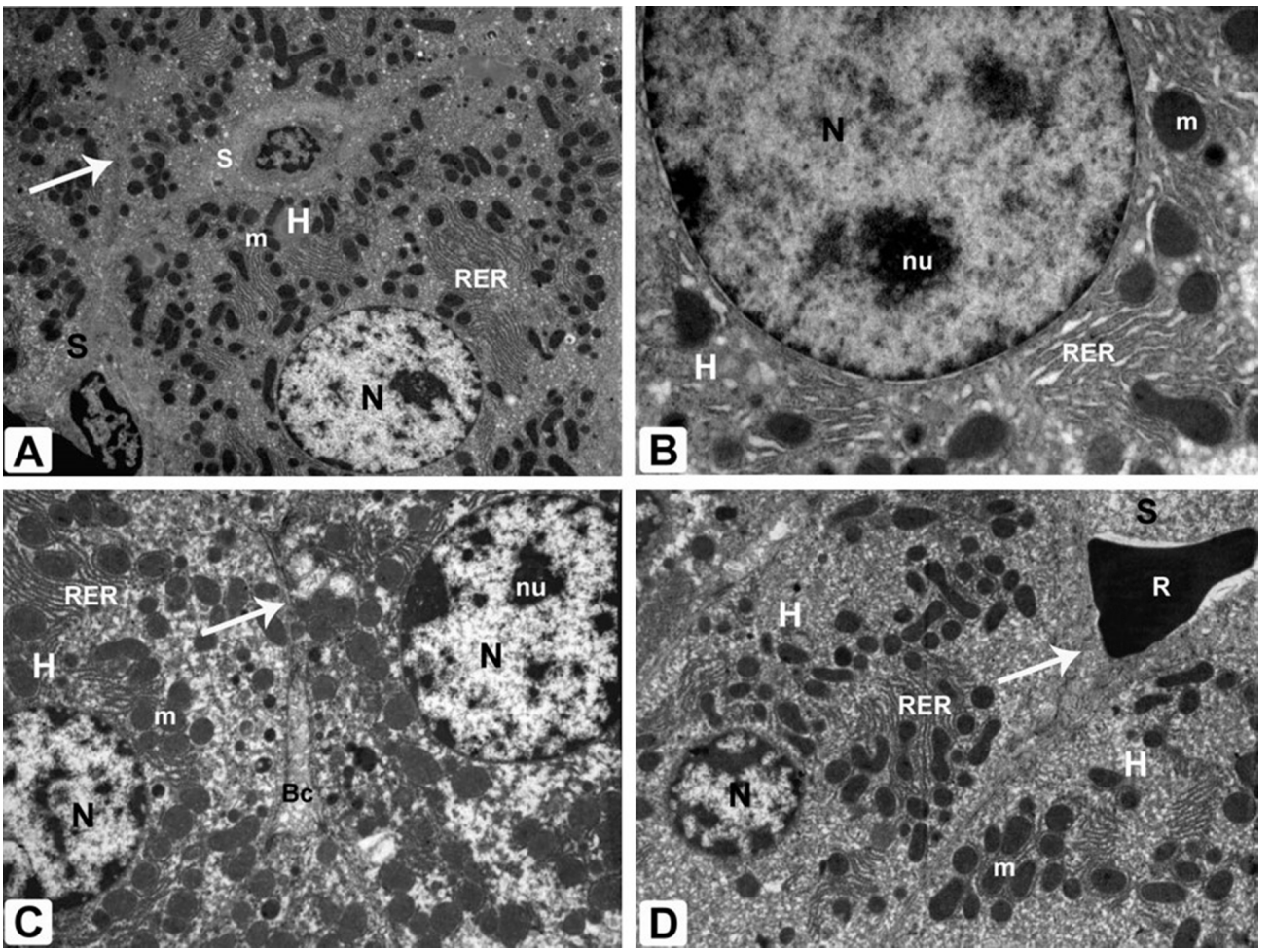


Fig. 7. Transmission electron micrographs (TEM) obtained from the liver tissues of the artemether -treated plus high dose of vitamin E group. A, B, C &D. All electron micrographs of the rat hepatocytes showing partial morphological changes compared to the changes seen in artemether alone.

observed in this study was explained as a result of oxidative damage in hepatocellular proteins or necrotic changes in hepatocytes that lead to irregularity in the orientation of the hepatocyte plates and disturbing hepatic architecture (Abraham *et al.*, 2002). Dilatation of central veins, blood sinusoids, and portal veins were attributed to inflammatory changes or ischemia and hypoxia following high-fat diet (Arvanitidis *et al.*, 2009). The dilatation also might be due to developing hypertension after obesity induced by high-fat diet (Elahi *et al.*, 2009). Furthermore, cytoplasmic vacuolation was attributed to lipid peroxidation because of oxidative stress that damage cell membrane as well as membranes of cell organelles leading to increase in their permeability and disturbance of theion's concentrations in the cytoplasm and cell organelles (Rubin, 2001; López Panqueva, 2014). Extravasation of bile then may be associated with inflammatory reaction.

Ultrastructurally, in this current experiment of the artemether treated rats, nearly complete disintegration of most cellular contents except few numbers of mitochondria and rough endoplasmic reticulum. Also, the cytoplasm of these cells had many vacuoles and irregular nucleus with abnormal distribution of chromatin and few lysosomes which were electron dense in appearance. In addition, hepatic sinusoid was markedly dilated and filled with blood and vacuoles. Ballooned hepatocytes can be attributed to microtubular disruption and severe cell injury (Tiniakos & Kittas, 2005). Moreover, rarefaction of the cytoplasm may be due to proliferation of smooth endoplasmic reticulum and glycogen accumulation (Lotowska *et al.*, 2014).

Mitochondrial abnormalities that were observed in the present study coincided with the previous results and attributed to decreased intramitochondrial protein synthesis,

respiratory chain dysfunction and increase of cytosolic calcium caused by oxidative stress (Lotowska *et al.*). Enlarged mitochondria was represented as an adaptive process to oxidative stress (Le *et al.*, 2004) or suppression of mitochondrial division because of lowered protein synthesis, while dilated rough endoplasmic reticulum may be due to lipid peroxidation (Dai & Chen, 2006).

The nuclear changes were attributed to mitochondrial dysfunction with subsequent decrease in oxidative phosphorylation that leads to decrease in cellular adenosine triphosphate (ATP). With prolonged depletion of ATP, structural disruption of protein synthetic apparatus occurs, and irreversible damage to mitochondrial and lysosomal membranes followed by cell necrosis (Kumar *et al.*, 2008). Nuclear vacuolation of some hepatocytes was also detected and was named by pathologists as glycogenated nuclei which may be due to accumulation of glycogen in the nuclei, and this is a common finding in liver biopsies with Wilson disease and diabetes (Brunt & Tiniakos, 2009).

On examining the liver tissue by light and electron microscopy, on treatment with low and high doses of vitamin E in concomitant with artemether, greatly ameliorated the hepatic histopathological lesions and the hepatic parenchyma attained nearly normal structure and organization was seen. Preserved normal hepatic architecture and normal blood sinusoids with minimal dilatation and congestion of some portal and central veins and minimal cellular infiltrations were shown. Vitamin E represents one of the most fascinating natural resources that have the potential to influence a broad range of mechanisms underlying human health and disease (Catalgol & Ozer, 2012). Vitamin E may effectively minimize oxidative stress, lipid peroxidation and toxic effects of reactive oxygen species in biological systems (Claycombe & Meydani, 2001).

Increased activities of serum AST and ALT in this study were support the histological findings which often used as markers of hepatic injury as they indicate cellular leakage of intracellular enzymes and loss of liver cell membrane stabilization (Sabiu *et al.*, 2014). Oxidative injury to a cell promotes peroxidation of membrane-bound lipids whose harmful products give rise to damage of macromolecules. In this study, MDA was used as an indicator to assess lipid peroxidation, and reduced SOD as indicator of hepatoprotectives for cells. The increased concentration of MDA in the liver tissues of rats and the decreased concentrations of SOD are suggestive of facilitated lipid peroxidation resulting in tissue damage and failure of body's antioxidant defense mechanisms to hinder the formation of excessive free radicals. It has been reported by Khan *et al.*, (2003) and El-Sokkary (2006), that artemether caused a significant increase in lipid peroxidation of liver tissue owing to free radical damage in necrotic and degenerative livers of rats.

Although the mechanism of liver toxicity remains elusive, oxidative stress, as a result of overproduction of reactive oxygen species (ROS) and compromised antioxidant capacity, has been hypothesized to play a role in the etiology of toxicity (Pippenger *et al.*, 1991). In particular ROS and free radicals show genotoxic activity (Pippenger, 2003) and number of studies have investigated the possibility that VPA treatment is associated with oxidative stress in patients (Cengiz *et al.*, 2000) and in animal models (Tong *et al.*, 2005).

In conclusion, results of the present suggest that artemether has a harmful and stressful effect on hepatic tissue. Treatment of vitamin E may alleviate artemether toxicity by the reduction of oxidative damage of artemether in liver tissues and alterations of cardiac energy metabolism.

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**RESUMEN:** Este experimento fue diseñado para estudiar los efectos de la administración oral de arteméter, la clase de medicamentos antipalúdicos de acción rápida, y el posible efecto protector de la vitamina E en el hígado de ratas albinas. Se utilizaron un total de 24 ratas albinas machos adultas y se dividieron en cuatro grupos. El grupo uno sirvió como control y las ratas en el grupo dos recibieron la dosis oral de arteméter diariamente durante 15 días. Los grupos tres y cuatro fueron tratados con arteméter, más dosis bajas y altas de vitamina E, respectivamente. Al final del experimento, se sacrificaron las ratas y se obtuvieron y procesaron los hígados para estudios histológicos, bioquímicos y estadísticos. El estudio histológico de los hepatocitos de ratas expuestas a arteméter mostró una desintegración casi completa de la mayoría de los contenidos celulares, excepto algunos mitocondrias y retículo endoplásmico rugoso. Además, el citoplasma de estas células tenía pocos lisosomas, muchas vacuolas y núcleos irregulares con distribución anormal de cromatina. Los sinusoides hepáticos esta-

ban dilatados y llenos de sangre y vacuolas, y los conductos biliares tenían una estructura anormal. El tratamiento con dosis bajas y altas de vitamina E en forma concomitante con arteméter mejoró las lesiones histopatológicas hepáticas y su parénquima alcanzó una estructura casi normal. En cuanto a los cambios bioquímicos, la alanina aminotransferasa (ALT) y la aspartato aminotransferasa (AST) en ratas tratadas con arteméter se elevaron significativamente en comparación con el control. Los niveles de superóxido dismutasa (SOD) y malondialdehído (MDA) aumentaron significativamente en el hígado en ratas tratadas con arteméter. Sin embargo, la vitamina E mejoró el aumento de ALT y AST con una disminución de la concentración de MDA y los niveles de SOD en comparación con los valores correspondientes del grupo de arteméter. Los resultados del presente estudio sugieren que el arteméter tiene un efecto dañino y estresante sobre el tejido hepático y el tratamiento con vitamina E puede aliviar esta toxicidad.

**PALABRAS CLAVE: Arteméter; Ratas; Vitamina E; Microscopía electrónica; Bioquímica; Análisis estadístico.**

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