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## **Protective effects of vitamin C against oxidative stress induced by diethylene glycol in liver and kidney**

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**Abstract**---This study aims to investigate the vitamin c protective influence against oxidative stress, liver and kidney destructions made by diethylene glycol in rabbit model. Eighteen healthy male rabbits were grouped into 3 classes. Control group: without any treatment. DEG group were treated with 40 mg /kg /day of DEG for 14 days while DEG with vitamin C group with 40 mg /kg /day of DEG with 10mg /kg/day of vitamin C for 14 days. All animals were euthanized at day 14 of the study. Venous blood samples (5ml) were collected from rabbits via jugular vein and serum samples were isolated and kept at -20 °C till time of analysis. Serum analysis was done by colorimetric Assay kit for total antioxidant capacity (TAC). Tissue samples from liver and kidneys were isolated, pigmented by hematoxylin and eosin (H & E) pigments and investigated under a light microscope for histopathological changes. Results: The TAC in the treatment group was significantly reduced at 14<sup>th</sup> day of study when compared to the other groups. While non- significant differences were found between control and treatment with vitamin C groups. Control sections of the liver and kidney have normal histological structure while tissue sections of treatment group appeared to have large zone of necrosis with suppuration surrounded by fibrosis and inflammation. Sections from treatment with vitamin C group appeared to have mild pathological changes. Conclusion: DEG (40 mg / kg /day) for 14 days can result a decrease in TAC with noticeable destructive changes in tissues of liver and kidneys in rabbits. Vitamin C has a useful effect as a good hunter for oxygen free radicals. It keeps the physiological integrity of tissues and decreases toxic effects.

**Keywords**---Diethylene glycol, Vitamin C, Liver, Kidney, Total antioxidant capacity.

## Introduction

Diethylene glycol (DEG) is a hygroscopic, viscous liquid with a sweetish taste that is transparent, colorless, and almost odorless. Besides its wide range utilization in industry, it is connected to several famous mass poisonings dating back to 1937. [1]. IF utilized with a diluent in pharmaceutical preparations, diethylene glycol which is a chemical widely available, is linked to multiple mass poisonings. Diethylene glycol is widely found in a variety of compounds, such as automotive products and wallpaper remover [2].

DEG is a nontoxic substance. The toxicity of this compound's metabolites after hepatic and renal metabolism results in poisoning. The presence of poisoning after DEG exposure is assumed to be dependent on endogenous alcohol dehydrogenase (ADH) activity. The liver, kidneys, and gastrointestinal tract all contain ADH. When ADH activity is strong, more active products of DEG metabolism are produced, increasing the risk of DEG poisoning [3]. Any substance that, if at low amounts in comparison with those of an oxidizable substrate, slows or inhibits the oxidation of the substrate. Removal of O<sub>2</sub>, scavenging reactive oxygen/nitrogen species or limiting ROS/RNS synthesis, binding metal ions required for ROS catalysis, and upregulation of endogenous antioxidant defenses are some of the mechanisms of antioxidant action [4]. Antioxidants are becoming more popular because of their oxidative degradation inhibitory properties in food, pharmaceutical items, and body. This also true against oxidative processes of stress-mediated disease [5].

The rabbit digestive flora is used to manufacture Vitamin C, also known as L-ascorbic acid. It is also called simply ascorbate while a food source of 25–30 mg / rabbit / day could be recommended for digestive problems, for example post weaning. Furthermore, as a natural scavenger of free radicals, its effectiveness in neutralizing reactive oxygen species is enhanced by the oxidation of vitamin C to monodehydroascorbate, reacting to free radicals. Furthermore, ascorbic acid protects against harmful effects [6]. The goal of this study is to see the vitamin c protective influences against oxidative stress, liver and kidney destructions which diethylene glycol in rabbit induces.

## Material and Methods

The investigation was carried out in an animal house as well as in scientific laboratories., in the interval between November,2021 to June,2022. the Research Ethics Committee and Scientific Committee at theDepartment of Dental Basic Science of College of Dentistry / University of Mosul approved this research. And the approval number is UoM. Dent/A.L.15/22 on 10.02.2022. Eighteen local healthy mature male rabbits of body weight of  $1 \pm 0.25$  kg will be included in the study. The rabbits were housed under standard animal housing condition with the room temperature of  $25 \pm 2^\circ\text{C}$ . All animals will have access to food (standard diet) and water. They were under the supervision of a veterinarian for every day

checking of the animal's condition and general health.

Rabbits were divided into 3 groups as follow:

Control group: without any treatment rabbits were maintained on standard diet and water only.

DEG group: treated with 40 mg in every kg body weight daily of DEG by oral gavage for 14 days and were sacrificed on the 14<sup>th</sup> day.

DEG with vitamin C group: treated with 40 mg/kg body weight/day of DEG with 10mg/ kg body weight/day of vitamin C by oral gavage for 14 days and then sacrificed on the 14<sup>th</sup> day.

### **Biochemical estimations:**

After the 14<sup>th</sup> day of cure administration, the rabbits will be sacrificed to take 5ml venous blood samples via jugular vein at the time of animal sacrificing. At room temperature, it was left for clotting for 30 min followed by centrifuging for 10 minutes at 3000 rpm. Serum was extracted and sampled and kept at -20 degrees Celsius until analyzing TAC (Colorimetric Assay Kit (ABTS, Enzyme Method Elabscience)).

### **Histopathological Assessment:**

Every rabbit's liver and kidney tissue was isolated and submerged in 10% formalin for 48 hours embedded in paraffin wax, sectioned longitudinally at (5mm), then Hematoxylin and eosin (H & E) stains were used to stain the tissue, which was then examined under a light microscope by a pathologist.

### **Results**

No mortality was observed in any dosing group during the study period. In this study, ANOVA test was used and indicated a highly significant difference among all groups for TAC as Table (1) shows:

Table (1): ANOVA test comparisons for each TAC between the study groups.

#### **The Comparison Between the Groups:**

| <b>ANOVA</b> |                |    |             |         |         |
|--------------|----------------|----|-------------|---------|---------|
|              | Sum of Squares | Df | Mean Square | F       | Sig.    |
| Cross Groups | 2.156          | 2  | 1.078       | 270.906 | 0.000** |
| in Groups    | 0.060          | 15 | 0.004       |         |         |
| Total        | 2.216          | 17 |             |         |         |

\*\* Highly Significant at  $P \leq 0.01$

The TAC in the treatment group was significantly reduced at 14<sup>th</sup> day of study when compared to the other groups. While non- significant differences were found between control and treatment with vitamin C groups. Figure (1)

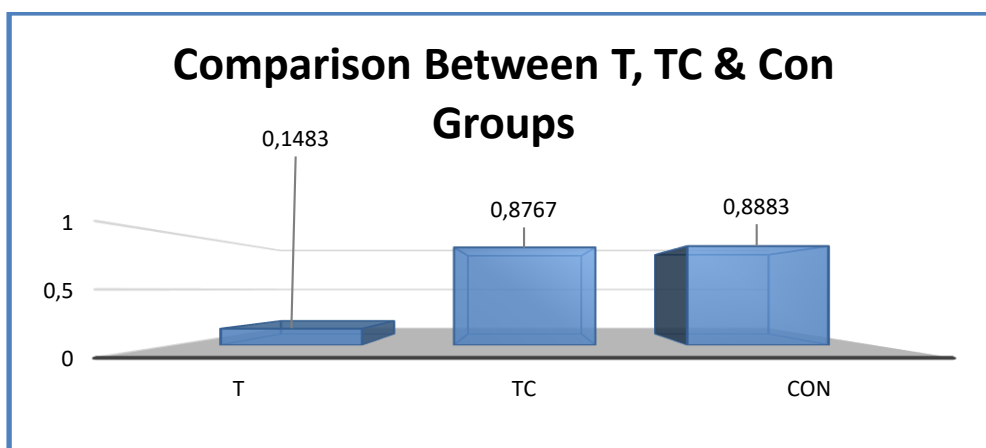


Figure (1): comparisons between the TAC measurements of all study groups.

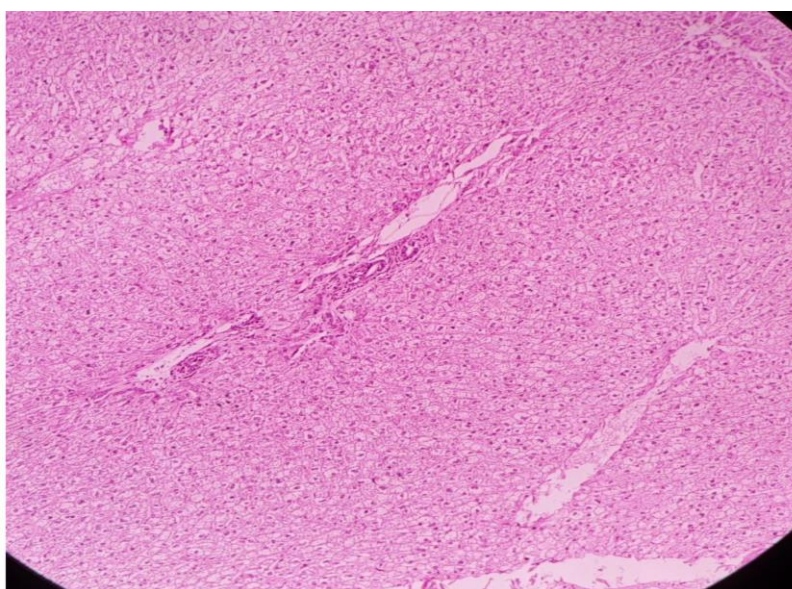


Figure (2): normal liver in control group.

**The histopathological results:**

The histological structure of all liver and kidney sections taken from the control group appeared to be normal.. Figures (2) and (3).

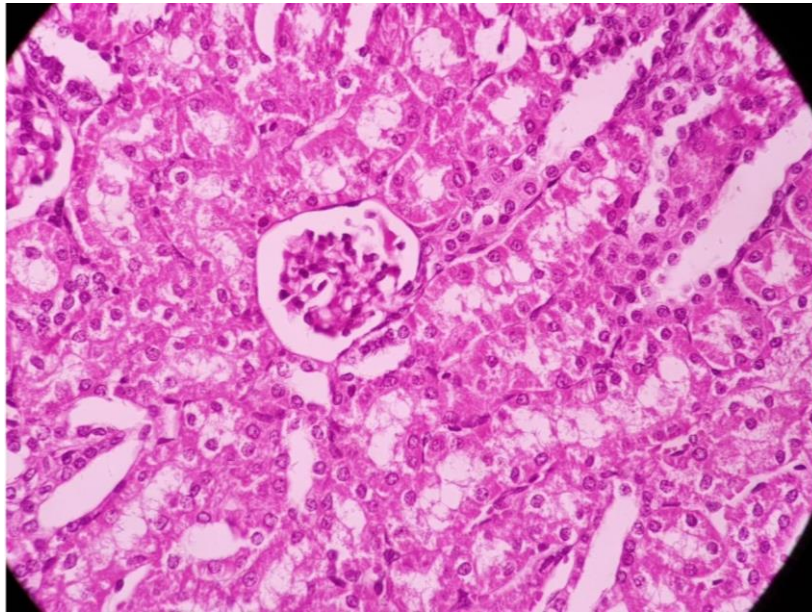
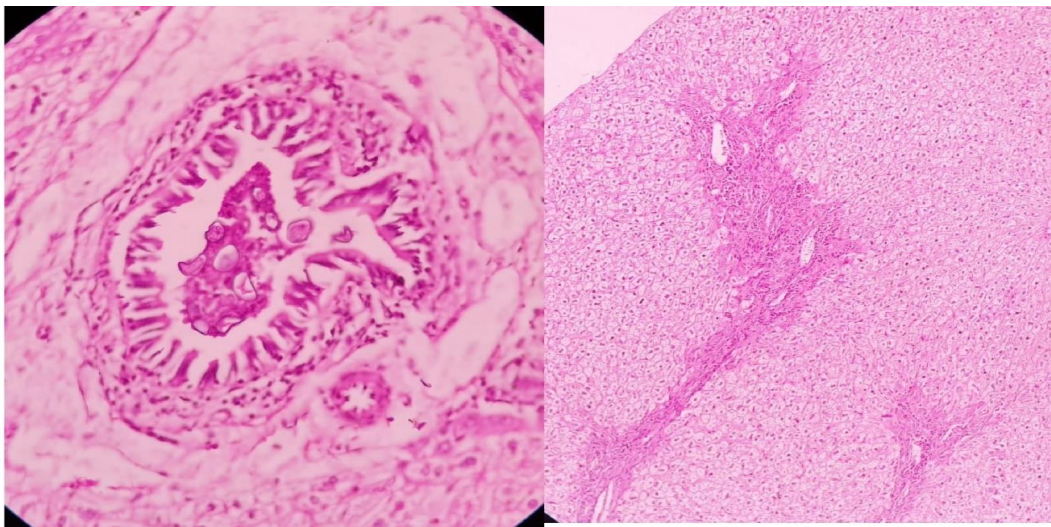


Figure (3): normal kidney control group

Tissue sections of treatment group appeared to have large zone of necrosis with suppuration surrounded by fibrosis and inflammation.

The treatment group's liver sections revealed extensive vacuolar degeneration. Also, it showed oncotic hepatocyte necrosis and a central vein congestion and sinusoidal stenosis as in Figure (4).



B. .A

Figure (4, A and B ): Liver sections of treatment group. H&E 10X H.D

The treatment group's kidney sections exhibit glomeruli atrophy, necrosis of epithelial cells lining renal tubules, Bowman's space dilatation and vacuolar degeneration. After the orally administered DEG, the study reported tubular

necroses, it also showed interstitial inflammatory responses. These necroses and inflammatory responses were in renal tissues. Microscopic observations of tubular vacuolation and an inflammatory reaction in the interstitial space in renal tissue. H&E 10X H.D Figure (5).

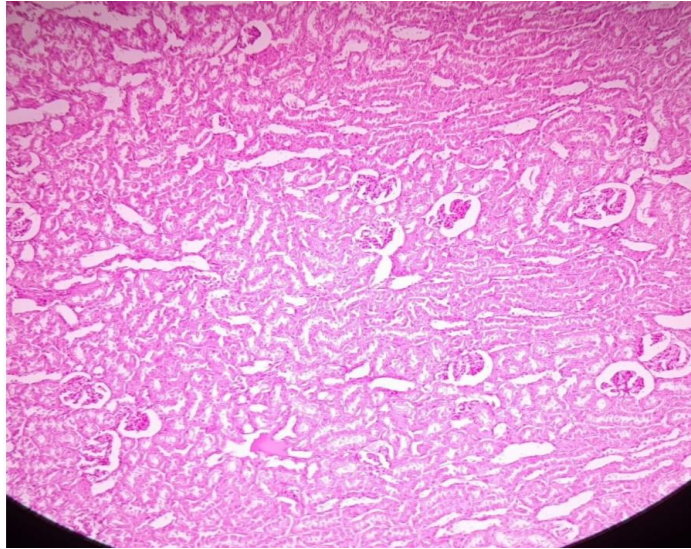


Figure (5): Pathological abnormalities in the DEG group's renal tissue.

Sections of liver taken from treatment with vitamin C group appeared to have mild cell swelling or cloudy degeneration and single cell necrosis of hepatocytes, congestion of central and portal vein. Figure (6).

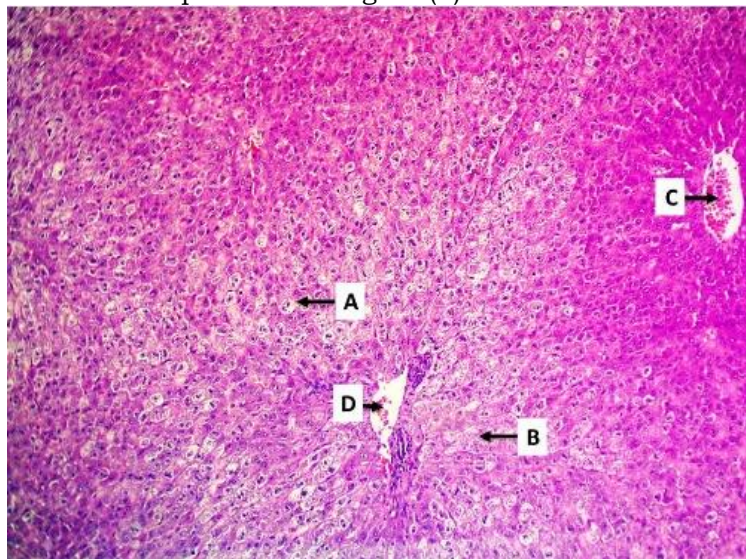


Figure (6): Liver sections of treatment with vitamin C group. mild cell swelling or cloudy degeneration (A) and single cell necrosis (B) of hepatocytes, congestion of central (C) and portal vein (D). H&E 10X H.D

Kidney sections of treatment with vitamin C group appeared to have mild changes including vacuolar degeneration and necrosis of epithelial cells lining renal tubules. Figure (7).

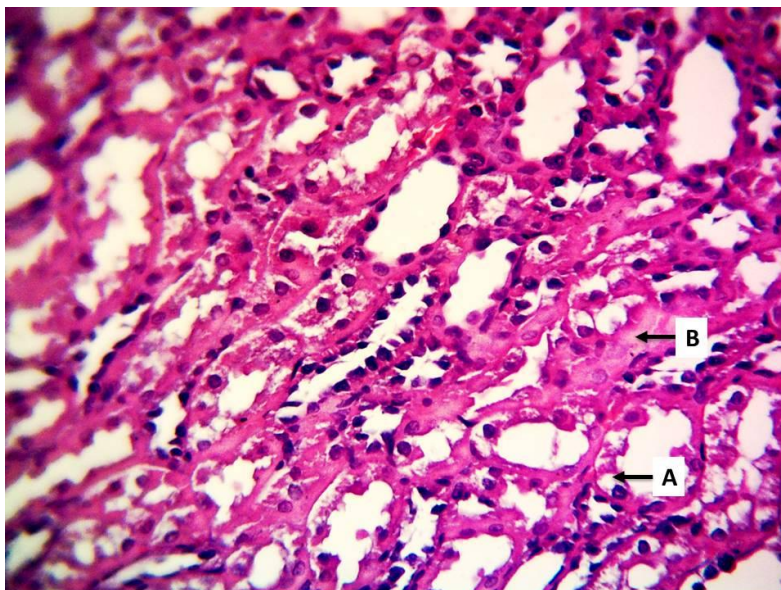


Figure (7): Kidney sections of treatment with vitamin C group show mild vacuolar degeneration (A) and necrosis of epithelial cells lining renal tubules(B). H&E 40X.

## Discussion

Oxidative stress is caused by a miss proportion between building up creating oxygen reactive species (ROS) in tissues and cells besides the biological system's abilities of detoxification of these reactive products. Despite the fact that ROS are normally formed as a by-products of oxygen metabolism with wide ranges of physiological functions (e.g., cell signaling), Environmental factors (e.g., UV, ionizing radiations, pollutants, xenobiotics (e.g., antitubercular medicines) and heavy metals) cause an imbalance that leads to cell and tissue damage (oxidative stress) [7]. In this work, DEG exposure resulted in the production of free radicals and reactive species, resulting in redox imbalance and cytotoxicity. Ascorbic acid is a chain-breaking antioxidant inhibiting peroxidative spread by interacting with membrane- which is bound by oxidized vitamin E and returns it to its original conditions. Several enzyme processes, such as those causing the manufacturing amino acids and peptide hormones, need vitamin C. This means that vitamin C acts as a ROS scavengers and could help in the prevention of the oxidative damages when oxygen concentrations rise and apoptosis occurs [8].

In the current study, after adding vitamin C to treatment with DEG there was amelioration in the histological changes as it is seen in DEG with vitamin C group. Results demonstrate significant decrease in TAC in DEG group compared to control group and no significant difference in TAC between control group and DEG with vitamin C group which confirm vitamin C's anti-oxidant properties against DEG-induced oxidative damage which agree with the fact that

antioxidants, such as vitamin C, may help to reduce oxidative stress and thus contribute in the restoration of the antioxidant defense system [9]. In accordance with previous studies. The beneficial vitamin C outcome is due to it's a very effectiveness as antioxidant, and an efficient hunter for oxygen free radicals and its being active in maintaining the physiological integrity of different tissues [10, 11, 12].

The kidney and liver are key organs for any toxicity in laboratory animals, DEG can cause liver impairment and kidney-toxicity. Lin B et al. studied 64 patients receiving armillarisin-A, 15 had DEG poisoning. There was a less damage Liver in the non-DEG-poisoned group than in the DEG-poisoned group and the poisoned patients had a considerably higher incidence of renal disease [13]. Results of our study demonstrate marked changes in Liver and kidney histology in DEG group can be noticed as large zone of necrosis with suppuration surrounded by fibrosis and inflammation. When compared to the control subjects, the liver of the treatment subjects reveals extensive vacuolar degeneration and hepatocyte oncotic necrosis, central vein congestion, and sinusoids stenosis. The livers of acetaminophen-treated and of starving animals, show significant glycogen depletion as a result of severe glutathione (GSH) depletion [14]. Glycogen depletion started in the centrilobular region and spread outward resembling what our trials with high dose DEG poisoning shows. GSH shortage causes severe glycogen depletion in the livers in the animal with GSH-depleting drugs, in which the GSH deficit causes glycogen breakdown through the change of the GSH/GSSG ratio, which subsequently stimulates glycogenolysis. [15]. The substantial depletion of glycogen in animals given 10 g/kg DEG is most probably due to its notable GSH depletion because of the DGA's capacity to significantly raise the reactive oxygen species, resulting in significant rises in in cellular redox status confirming the results of this study.

Kidney of treatment group shows glomeruli atrophy, Bowman's space dilatation, vacuolar degeneration and necrosis of epithelial cells lining renal which agree with the fact that ethylene glycol damages liver tissue by causing vacuolar degeneration, hepatocyte coagulative necrosis, and mononuclear inflammatory cell infiltration. It damages the renal tissues, causing the kidney to stop functioning normally in terms of endocrine and metabolic activities [16].

The renal is damage from DEG could be due to the metabolism to EG, producing the renal damages [17]. In the literature, the rats administered oral gavages with 10 or 0, 2, 5 g/kg DEG and blood collected tissues of the liver and kidney at 48 h. Rats with 10 g/kg DEG showed a metabolic acidosis, toxicities of the renal (grown BUN and creatinine and cortical necrosis) and liver (centrilobular necrosis, grown serum enzyme, and a significant depletion of glycogen. This confirms the current work.

The literature indicated the DEG nephrotoxic metabolite is DGA depleting the in vitro to ATP, producing the reactive species of oxygen, inhibiting the succinate dehydrogenase, and finally killing necrotic proximal tubule cells [18]. In addition, DGA is known for a remarkable accumulation in the livers and kidneys in comparison to the levels of plasma DGA in rats which are intoxicated by DEG [19]. The DGA significance in the toxicity of DEG has been proved in humans in

the Panama epidemic, in which high DGA concentrations in the urine and serum associate strongly with case statuses (odds ratio > 999) in comparison to DEG and its other likely metabolites (2-HEAA, oxalate, glycolate, EG). Furthermore, DGA total the urine and serum were evidently higher than other different metabolites [20].

The effect of antioxidant and reactive oxygen scavengers is proved to be effective in the protection of the animal liver and kidney [21]. The possible mechanism causing free radical rise in the liver and kidney after treating with ethylene glycol as the systemic circulation bringing the toxic substance into these organs producing free radicals. Later, there is a gradual infiltration of leukocytes and reduced antioxidant enzyme activities could keep the tissues under excessive oxidative stress [22].

The current work proves that the DEG can lead to a decrease in TAC with considerable destructive changes in tissues of liver and kidneys in rabbits. More studies are warranted to observe the impact of DEG exposure on general health in animals and human.

## Conclusion

This study contributed to DEG toxicity. DEG (40 mg/kg/day) for 14 days decreases TAC with big destructive changes in tissues of liver and kidneys in rabbits. Vitamin C has a useful effect as a good hunter for oxygen free radicals. It keeps the physiological integrity of tissues and decreases toxic effects.

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