Evaluation of the Possible Anti-oxidant Effect of Omega-369 Against Cisplatin - Induced Nephrotoxicity in Mice

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Abstract:

Background: Nephrotoxicity is the adverse effect of substances on renal function. one of these substances is cisplatin. cisplatin (CP) treatment has long been linked to Nephrotoxicity due to oxidative stress mechanisms. Triple omega 3-6-9 is a combination of unsaturated fatty acids (UFAs)With omega-3 (n-3) and omega-6 (n-6) being polyunsaturated fatty acids (PUFAs) and omega-9 (n-9) being a monounsaturated fatty acid (MUFA).

Aim: the reason for this study was to discover if omega-3,6,9 fatty acids could protect the kidney from the effects of cisplatin.

Methods: Thirty-five albino male mice were allocated to one of five groups. group 1 received liquid paraffin, group 2 received cisplatin (10 mg/kg) by single intraperitoneal injection, group 3 received omega-3,6,9 (50 mg/kg), group 4 received omega-369 (100 mg/kg) mice of group 5 received vit E(100 mg/kg) The mice were treated with Omega 3,6,9, vit E, and liquid paraffin once daily by oral gavage for 7 days. in day 8 mice in group 3,4and 5 received single intraperitoneal injection of cisplatin .

Results: Group 2 had significantly lower levels of glutathione peroxidase and superoxide dismutase than group1, significantly greater levels of malondialdehyde, serum urea nitrogen and serum creatinine ($p<0.05$) , glutathione peroxidase and superoxide dismutase level significant increase in group3,4 and 5. whereas

malondialdehyde, serum urea nitrogen an creatinine levels were significantly decrease in groups 3, 4, and 5 when compared with group 2 ($p<0.05$).

Conclusion: Omega-3,6,9 fatty acids exhibit anti oxidative effects so reduce the risk of cisplatin-induced kidney injury.

Keywords: Cisplatin, nephrotoxicity, omega 3,6,9, oxidative stress.

Introduction:

The body's major organ for achieving and carrying out a number of vital activities, such as detoxification, extracellular fluid management, equilibrium, and the expulsion of toxic metabolic waste, is the kidney(1). Nephrotoxicity is characterized as a sharp reduction in kidney function brought on by toxic chemicals and medications. Crystal nephropathy, thrombotic microangiopathy, glomerular injury, inflammation, and renal tubular toxicity are just a few of the mechanisms that cause nephrotoxicity. (2). Drugs like cisplatin, which cause oxidative stress and tubular mitochondrial damage, are examples of toxic agents and drugs that can impair the tubular transport system. The development of cisplatin-induced nephrotoxicity is hypothesized to be influenced by a variety of routes. Cellular absorption and accumulation, oxidative stress (3), inflammation (4), vascular injury (5), and necrotic and apoptotic pathways of renal tubular cells are among the molecular mechanisms covered(6) Major routes of Cisplatin-induced nephrotoxicity include the production of reactive oxygen species (ROS), buildup of lipid peroxidation products in the kidneys which disturbed antioxidative systems lead to cell damage(5)and reduction of anti-oxidant enzymes such as Superoxide dismutase (SOD-1), glutathione (GSH), catalase (CAT), and glutathione peroxidase (GPX-1) (7).

The body needs dietary lipids since they are a vital part of all biological systems. All lipids can be produced by humans, with the exception of long-chain fatty acids from the Omega 3, Omega 6, and Omega 9 groups. The two primary long-chain polyunsaturated fatty acids of omega 3 are docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA)(8). Omega6 contains polyunsaturated fatty acids, while the majority of Omega9 is monounsaturated(9). Since mammalian cells are unable to produce alpha-linolenic acid or linoleic acid, which are both members of the Omega-3 and Omega-6 families, respectively, they must be obtained in appropriate amounts from food(10). Oleic acid (OA), a non-essential FA that is a member of the Omega-9 family, can be produced by the body when it has an appropriate supply of Omega-3 and Omega-6(11). In relation to monounsaturated fatty acid omega-9 fatty acids, oleic acids are among them. high percent of olives oil composed of these fatty acid. In addition, oleic acid exerts an impact on the

biochemical and functional attributes of cellular membranes in numerous tissues. This fatty acid is also among the most abundant neuromembrane phospholipids(12).

Omega3 supplementation may increase or decrease the production of pro- and/or anti-inflammatory cell signaling molecules, which have been identified as anti-inflammatory compounds. Numerous studies have supported the positive effects of dietary Omega-3 PUFAs on immune function, lipid peroxidation, and antioxidative properties. It has been demonstrated that omega-3 PUFAs have anti-inflammatory properties(13). this study's objective was to discover if omega-369 fatty acids could protect the kidney of male mice from the effects of cisplatin.

Materials and Methods

Animals

Thirty five albino male mice were used in this studies these mice were placed in animal house of the college of pharmacy at Baghdad University, these mice weighing in an average 25-30 grams. Kept under standard temperature, humidity, and light-dark cycles, The animals were provided with consistent access to pellet food and an unrestricted supply of water from the faucet.

Chemicals and Drugs

Omega-3,6,9 was purchased from Source (Adrien gagnon,canada), liquid paraffin from (Riedel-de Haan GmbH,Germany),normal saline 0.9% was purchased from (Pioneer,Iraq). Cisplatin (1 mg/mL, 50 mL vial) was purchased from (Accord, United Kingdom). SOD,GPX,MDA ELISA kits were bought from(My Biosource ,USA) .

Preparation of omega-369 stock solution

Two stock solutions of omega-369 were prepared by dissolving 1ml of omega-369 in 39ml of liquid paraffin and 1ml of omega 369 dissolve in 79 ml of liquid paraffin then the solutions were mixed by vortex mixer to obtain a final concentration respectively according to omega-369 doses for mice(100mg/kg) and(50mg/kg).

Pilot study

This study was achieved because there was no previous dose used in other studies. It has been used three groups of mice each group with 4 mice, first group receive omega 369 at dose 50 mg/kg orally for 7

consecutive days, second group receive omega 369 at dose 100 mg/kg orally for 7 consecutive days and third group receive omega 369 at dose 200 mg/kg orally for 7 consecutive days. The mice in group three dead before the 7 days finished, so the dose of third group have been canceled.

Experimental Design

Five groups of Thirty-five mice were created at random, each of seven mice, as follows:

Group I (control group): mice were administered 0.5mlof liquid paraffin orally for7 consecutive days.

GroupII. (Model group): mice were administered0.5mlof liquid paraffin orally for7 consecutive days; then on day 8 cisplatin at dose (10mg/kg) was administered intraperitoneally (14).

Group III (omega369+Cisplatin): mice were administered with omega-369(50mg/kg) orally for 7 consecutive days then on day 8 cisplatin at dose (10mg/kg) was administered intraperitoneally.

Group IV (omeg369+Cisplatin): mice were administered with omega-369(100mg/kg) orally for 7 consecutive days then on day 8 cisplatin at dose (10mg/kg) was administered intraperitoneally.

Group V(vitamin E+ cisplatin):mice were administered with alpha tocopherol-(100mg/kg)orally for 7consecutive days then on day 8 cisplatin at dose (10mg/kg) was administered intraperitoneally (15).

On day nine, blood samples were collected under diethyl ether anesthesia. After blood collection, all mice were euthanized by cervical dislocation, and kidney tissues were separated for analysis.

Preparation of Tissue Homogenate Sample

right kidneys were removed, washed in cold normal saline, then weighed using an electrical homogenizer.before being placed in a plain tube with 2.7 ml of PBS. The homogenate was then centrifuged with a cold centrifuge for 20 minutes at 14000 rpm, and the fluid was collected and frozen for later use to quantitatively measure SOD, GPX and MDA.

statistical analysis

The average standard deviation (D) values are presented in the study, to carry out the statistical analysis, the 25th edition of the Statistical Package for Social Sciences (SPSS) was utilized in evaluating group differences ,Statistical significance was established at a P value of 0.05 using an independent T-test

RESULTS

omega 369 administrations impact onGPX-1

as shown in Figure (1) there was a significant reduction in renal GPX-1 of Cisplatin treated mice (group II) as compared to that of the control (group I) $p < 0.05$ .but there was a significant increase of renal GPX-1 levels of omega-369(100mg/kg) + Cisplatin (groupIV) and alpha tocopherol at dose (100 mg/kg) compared to the Cisplatin treated mice (group II) $p<0.05$,the renal GPX-1 levels of omega-369(50mg/kg)+Cisplatin (group III) showed no significant different as compared with mice that were given cisplatin (group II)($p>0.05$) .-in contrast renal GPX-1 mean values of group III showed a significant difference from that of (group IV) ($p< 0.05$).

omega 369 administrations impact on SOD-1

as shown in Figure (2) there was a significant reduction in renal SOD-1 of Cisplatin treated mice (group II) as compared to that of the control (group I) $p <0.05$. however, there was a significant rise. of renal SOD-1 levels of omega-369(100mg/kg) + Cisplatin (group IV), omega-369(50mg/kg) + Cisplatin (groupIII) and alpha tocopherol at dose (100 mg/kg) compared to mice that were given cisplatin (group II) $p<0.05$, Group III renal SOD-1 also showed significant different when compared to renal SOD-1 of (group IV) ($p<0.05$).

omega 369 administrations impact on MDA:

the Figure (3) showed that a significant increase in renal MDA of mice that were given cisplatin (groupII) in comparison to (group I) MDA levels, but there was significant decrease of renal MDA for mice treated with omega 369 at dose(50mg/kg) (groupIII), omega 369 at dose (100 mg/kg) (group IV) and alphatocopherol at dose (100mg/kg)(group V) as compared with cisplatin group. Group III renal MDA also showed significant different when compared to renal MDA of (group IV) ($p<0.05$).

omega 369 administrations impact on kidney function

the Figure (4,5) showed that a significant increase in serum urea nitrogen and serum creatinine of mice that were given cisplatin (groupII) in comparison to(group I) sUN and sCR levels, but there was significant decrease of sUN and sCR for mice treated with omega 369 at dose(50mg/kg) (groupIII), omega 369 at dose (100 mg/kg) (group IV) and alphatocopherol at dose (100mg/kg)(group V) as compared with cisplatin group

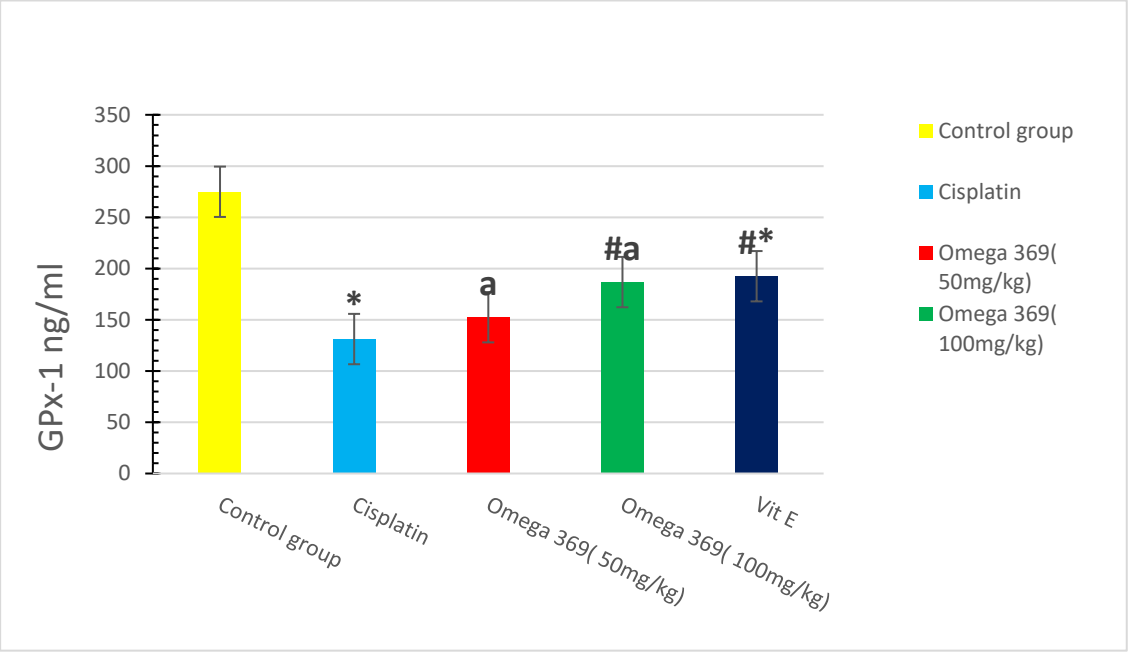

Figure1: Effects of omega-369 on renal GPX-1 levels

- * is used to indicate significant difference compared to liquid paraffin control group.
- # is used to indicate significant difference compared to Cisplatin group.
- a is used to indicate significant difference when comparing combination groups III and IV ; P <0.05 n=7

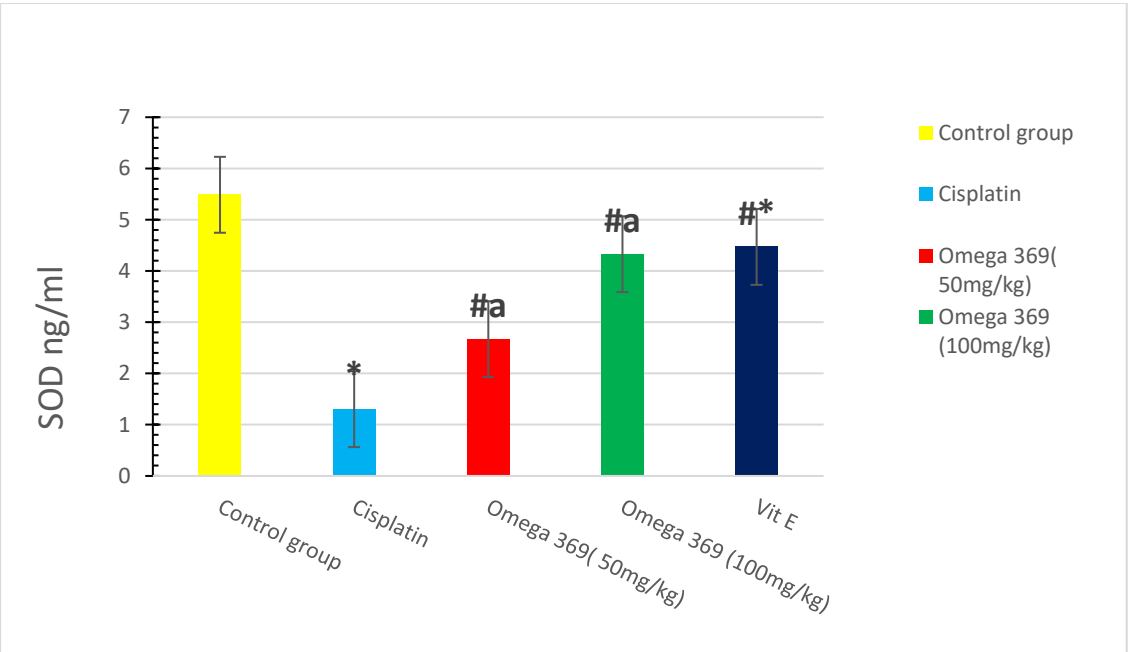

Figure2: Effects of omega-369 on renal SOD levels.

- * is used to indicate significant difference compared to liquid paraffin control group.

- # is used to indicate significant difference compared to Cisplatin group.
- a is used to indicate significant difference when comparing combination groups III and IV ; P <0.05 n=7

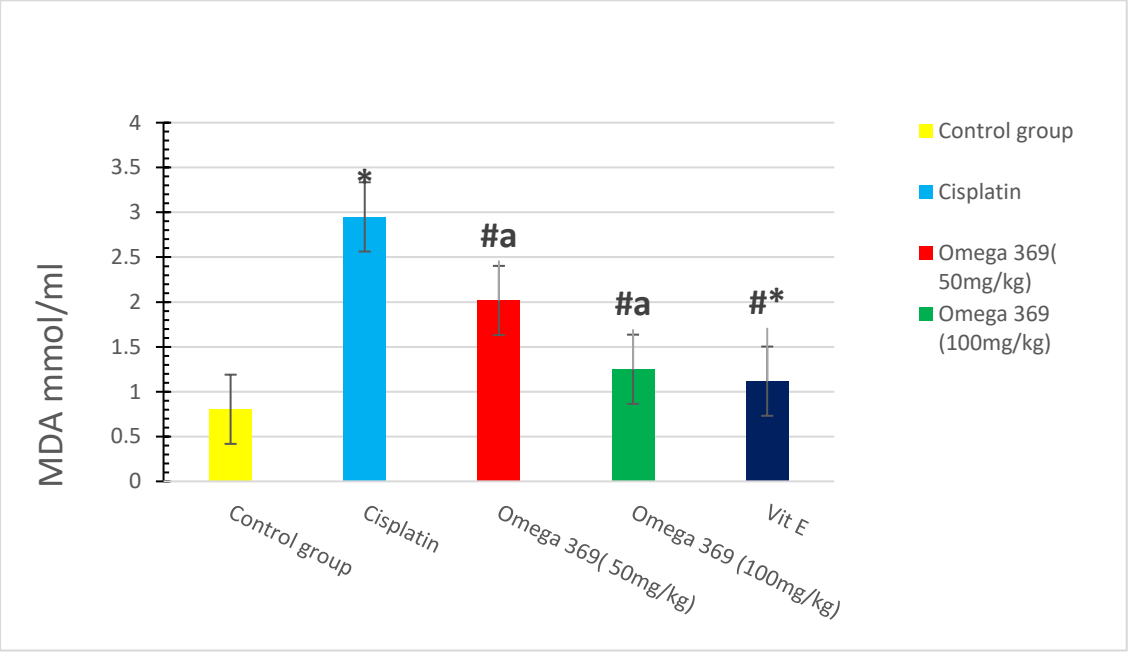

figure3: Effects of omega-369 on renal malondialdehyde (MDA) levels

- * is used to indicate significant difference compared to liquid paraffin control group.
- # is used to indicate a significant difference from the Cisplatin group.
- a is used to indicate significant difference when comparing combination groups III and IV ; P <0.05 n=7

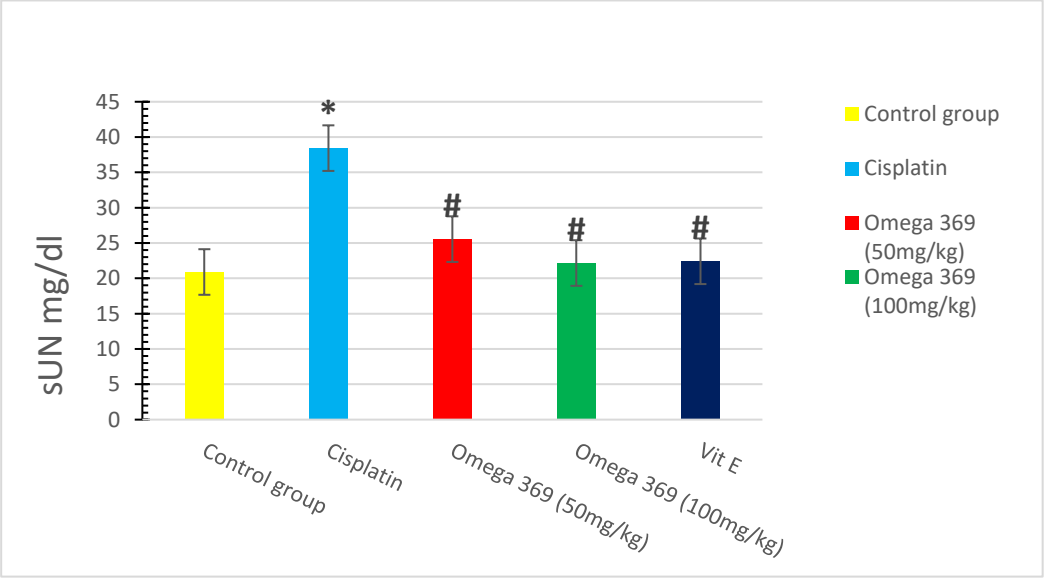

figure4: Effects of omega-369 on serum urea nitrogen(sUN)serum levels

-*significant difference compared to liquid paraffin control group.
- # denotes significant difference compared to Cisplatin group; P <0.05.n=7

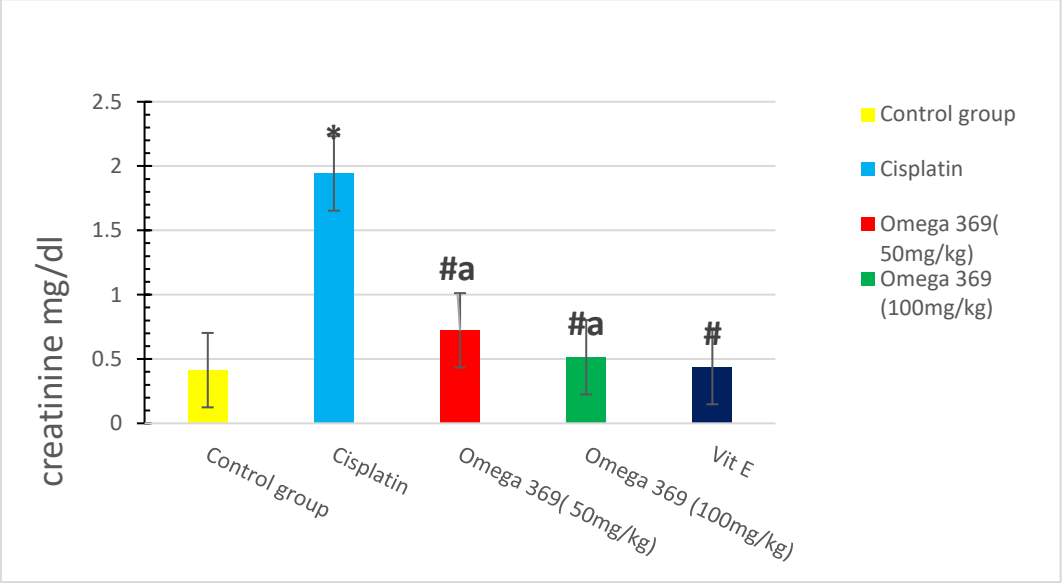

Figure5: Effects of omega-369 on serum Cr levels.

-*significant difference compared to liquid paraffin control group.
- # denotes significant difference compared to Cisplatin group
- a denotes significant difference when comparing combination groups III and IV; P <0.05.n=7

DISCUSSION

The main dose-limiting aspect of cisplatin therapy that hinders its clinical application in cancer chemotherapy is reported as cisplatin-induced nephrotoxicity(16). Among other pathways, oxidative stress and inflammation are linked to the development of nephrotoxicity brought on by cisplatin(17).

As shown in Figure 1, the current study found that (GP-X) significantly decreased in Group 2"positive control" group compared to Group 1 "negative control" group. This result is in line with a prior study that suggested that the injection of Cisplatin lowered the activities of antioxidants such GPX-1(18), Hydrogen peroxide is controlled and eliminated by the crucial antioxidant enzyme GP-X through the conversion of hydrogen peroxide to water. As a result, it prevents the Fenton reaction, a mechanism in which hydrogen peroxide interacts with iron to make the very flammable and dangerous hydroxyl radical(19). The renal GP-X in Group 2 "positive control" in this study significantly decreased as a result of the oxidative stress caused by cisplatin. Free radical production has increased, which was the cause of this. Additionally, according to the results of the current study, Group 2 "positive control" mice's renal SOD levels considerably dropped as compared to Group 1 "negative control" mice. In Figure 2, This result is in line with a prior study that suggested lower antioxidant activity, such as SOD, after Cisplatin treatment(18), While administering mice with

Omega'369 (50 mg/kg), Omega'369 (100 mg/kg), and VIT E (100 mg/kg) through oral gavage for seven days prior to intraperitoneal single injection (IP) of cisplatin (10 mg/kg) on day eight of the test resulted in a significantly higher level of renal SOD compared to Group2 "positive control", This result is in line with past research on the antioxidant advantages of MUFAs, which demonstrated that diets high in oleic acid were less susceptible to oxidative damage and indicated an increase in antioxidant enzyme levels(20). Figure 3 shows that in this study's animals Group 2 "positive control" mice had considerably higher kidney MDA levels than Group 1 "negative control" mice. This outcome is in line with earlier research that found higher MDA levels following Cisplatin treatment(21). This is explained by an increase in oxidative stress attributable to mitochondrial malfunction following Cisplatin treatment, excessive ROS generation causes renal buildup of lipid peroxidation products, and an elevated MDA level(22). Lipid peroxidation is the reaction of free radicals with lipids that have carbon-carbon double bonds. When plasma phospholipid and hydroxyl radicals mix, the outcome of this process is MDA(23). For seven days, mice were given oral gavage treatments of Omega'369 (50 mg/kg), Omega'369 (100 mg/kg), and vitamin E (100 mg/kg) before receiving an intraperitoneal single injection (IP) of 10 mg/kg. cisplatin significantly reduced kidney MDA levels on day eight of the test when compared to Group 2's "positive control" . This outcome is consistent with a previous study that discovered that orally administered oleic acid, a monounsaturated fatty acid, prevented lipid peroxidation(24). Cisplatin induced nephrotoxicity is characterized by impaired renal function leading to increased levels of sUN and sCr(25), which was evident in the present study by significant elevation in the mean values of sUN and sCr levels of Cisplatin group (group II) compared to the control ($p<0.05$) as shown Figures (4,5). These findings are consistent with earlier research that indicated elevated levels of these markers caused by Cisplatin-induced direct nephrotoxicity(22). Recent research has shown that this is partly due to the stimulation of iNOS production (26).The production of ROS and inflammatory responses in Cisplatin-induced nephrotoxicity may be responsible for the deterioration of kidney function(21). In the current study, mice with omega-369(50mg/kg)+ cisplatin(group III) ,and omega (100 mg/kg) + cisplatin (group IV) had significantly lower levels of sUN ,sCr and UA. thus enhancing renal function. No prior research has been done on the effects of omega 369 on kidney function. Unfortunately, prior research have documented a decrease in sUN and sCr after omega3 treatment(27).

in this study vit E (100mg/kg) improve renal function by decrease the sCr , sUN and s UA .These findings are consistent with earlier research that indicated decrease levels of these markers by vit E(28).

CONCLUSION:

The findings of this investigation imply that Omega 369 has an antioxidative effect because it significantly increased (GP-X) and (SOD) levels in mouse kidney tissue homogenate while also significantly reducing (MDA) levels in the same tissue homogenate.

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