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Cardiac and Renal Protective Effect of Omega-3 PUFA via PPAR γ and PTEN Expression in Gold Nanoparticles Intoxicated Rats.

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ABSTRACT

This study aimed to explore the toxic impacts of Gold nanoparticles (GNPs) on oxidative stress and expression of Peroxisome proliferator-activated receptor Gamma (PPAR γ) and Phosphatase and tensin homolog (PTEN) in cardiac and renal tissues of rats. The potential impact of n-3 polyunsaturated fatty acids (n-3 PUFA) in attenuating GNPs cytotoxicity was also studied. The study comprised four groups (10 rats each), G I control; G II injected with a GNPs for six days; GIII injected with GNPs and co -administered orally with n-3 PUFA as GII; G IV: ingested with n-3 PUFA followed by GNPs as GII. The results showed that either pre or co-administration with n-3 PUFA significantly ameliorated the levels of malondialdehyde superoxide dismutase, PPAR γ and PTEN expression in both the heart and kidney tissues nearly to their normal levels. The present data also revealed that pre and co supplementation with n-3 PUFA, markedly ameliorated the alterations in cardiac and kidney .The current biochemical investigations were confirmed by histopathological examination. The present study concluded that n-3PUFA could ameliorate the cardio and renal cytototoxic effect induced by GNPs. The mechanisms underlying these promising effects could be through increasing PPAR γ and PTEN expression which ameliorates antioxidant status.

Keywords: Heart, kidney, PPAR γ , PTEN

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INTRODUCTION

The small sizes of the nanoparticles and large surface to volume ratio put the nanoparticles in a position for tremendous and wide applications essentially in biomedicine [1]. Although metal nanoparticles have received increasing attention due to their widespread medical, consumer, industrial, and military applications, studies have correlated particle size of some metal-based nanoparticles (e.g., Ag, Au, and Cu) with toxicity, even if the same material is relatively inert in its bulk form [2, 3]. Nanoparticles enter the human body through ingestion, inhalation, and skin contact or genitourinary tract and become deposited in vital organs such as brain, heart, liver, or kidneys Studies have shown that nanoparticles interact with biomolecules leading to DNA and protein damage [4, 5].

Goldnanoparticles(GNPs)are widespread scientific achievements over nanotechnology or have much biological applications. They perform as carriers for drug delivery for gene therapy 6. There are different reports about the behavior of toxicity about these nanoparticles so much depends on the modifications changes like the degree concerning absorption performance, form, yet the diameter size about spherical shape [7]. Attempts to discover prophylactic agents which are effective for mitigating cardiac and renal injuries induced by GNPs are considered very urgent in clinical practice. Therefore, it is important to find treatment that can counteract the adverse impacts of GNPs to protect cardiac and renal tissues from damage.

N-3 polyunsaturated fatty acids (n-3 PUFA, α-linolenic acid, eicosapentaenoic acid, and docosahexaenoic acid) are main fatty acids that should be ingested as a part of the diet because they cannot synthesize endogenously 8 n-3 PUFA have a key role in the cellular membrane composition, so affecting cell liquidity and biochemical signaling of the cell membranes. n-3 PUFA has many other effects, including anti-oxidative stress, anti-inflammatory and antioxidants. N-3 PUFA have antioxidant beneficial effect against tumor and cardiovascular illness [9]. N-3 PUFA acts as a natural ligand for certain nuclear receptors known as peroxisome proliferator-activated receptors-γ (PPAR γ) that affect gene expression. N-3 PUFA is one of PPARγ ligands, which have been reported to exhibit beneficial effects through the stimulation of these receptors [10].

Peroxisome proliferator-activated receptor Gamma (PPARγ), a ligand-activated transcription factor, has function among a number of cellular features like glucose homeostasis, lipid metabolism, or control of oxidative stress. The cardio protective effect of PPARγ activation has been studied extensively up on the years making them potential therapeutic targets for cardiovascular disorders, congestive heart failure and myocardial infarction [11]. Recent researches indicate that PPARγ is also concerned in the normal kidney development, renal lipid metabolism, through activation regarding renin-angiotensin system [12].

Phosphatase and tensin homolog (PTEN) is a tumor suppressor inhibiting several key signaling pathways. Its lipid phosphatase activity negatively regulates PDK1-mediated Akt phosphorylation by dephosphorylating PIP3 to PIP2 [13]. Meanwhile, PTEN's protein phosphatase can prevent cell spreading and relocation through dephosphorylation of focal adhesion kinase (FAK) and advance cell differentiation and apoptosis by inhibiting SHC/SOS/ GRB2 and mitogen-activated protein kinase (MAPK) pathways [13]. Past investigations proposed that PPAR-γ can regulate PTEN gene transcription, work collectively along PTEN to incite cell cycle arrest and become one of the most attractive anti-cancer mechanisms [14, 15].

To the best of our knowledge no previous reports have demonstrated the toxic impact of GNPs on the expression of cardiac and renal PPAR γ, and PTEN which have the important roles in protection against oxidative damage. Also, no previous studies have shown the prophylactic effect of n-3 PUFA against toxicity of GNPs induced alterations in these markers.

This study aimed to explore the adverse toxic impacts of GNPs on oxidative stress markers (MDA and SOD) as well as on the expression of PPAR γ and PTEN as protective markers in cardiac and renal tissues of rats. The study also was extended to explore the potential impact of n-3 PUFA in attenuating GNPs cytotoxicity by regulating these markers. Histopathological studies on cardiac and renal tissues were done to confirm the biochemical results.

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MATERIALS AND METHODS

Materials:

Trihydrated tetrachloroauric acid ($HAuCl_4.3H_2O$ 99.9%) and Trisodium citrate ($Na_3C_6H_5O_7.2H_2O$) will be obtained from Aldrich and used as received.

Synthesis of Gold Nanoparticles:

Gold nanoparticles were prepared by reduction of gold salts in the present of suitable stabilizing agent that prevent particle agglomeration. In our experiment, aqueous solution of chloroauric acid ($HAuCl_4$) was heated with different reducing agent [16].

Characterization of Gold Nanoparticles: [17]

1. Transmission electron microscope: for studying shape and morphology of the prepared particles by TEM (Jeol, JSM-6360LA, Japan).
2. The absorption maxima at different wave lengths of visible light and UV was determined using UV-visible spectrophotometer. (Unican UV-Vis spectrometry model, UV5-220).
3. Zeta potential and particle size distribution: Gold nanoparticles (GNPs) will be determined using Nano Zetasizer particle analyzer. by dynamic light scattering (DLS) using Malvern zeta sizer

Experimental design:

Forty adult male albino rats (150-170 g) were utilized in this study. The animals were gotten from Laboratory Animal Production, King Fahd Research Centre, King Abdulaziz University. Animals were housed under controlled conditions (23-25ºC, humidity 50-65%, 12 h dark/light cycles). Animals were provided by standard rat pellet food and water ad libitum. Rat handling was performed in accordance to the roles of the King Abdulaziz University, Faculty of Science The animals were left for 7 days for adaptation and then classified into 4 groups, each of 10 rats as follows:

Group I: served as control. Group II: injected i.p. with a suspension of gold nanoparticles of about 20 nm with a dose of 20 µg/kg body weight for 6 days [18]. Group III: injected as in group II accompanied with oral supplementation of omega-3 fatty acid (Abbott product GmbH, Germany) with a daily dose of 100 mg/kg body weight for a period of 6 days [19]. Group IV: supplemented orally with omega-3 fatty acid with an each day dose of 100 mg/kg body weight for duration of 6 days, prior to injection with Gold nanoparticles as in group II. At the end of the experiment, rats were fasted nightlong (12-14hours). Blood specimens were gathered in tubes for clotting and serum separation. The tubes were centrifuged at 2000g for 15 min and the isolated serum was stored at -20 ºC till use for estimating of some biochemical markers. The animals were then scarified by decapitation and the hearts and kidneys were removed, washed with cold saline and utilized for tissues biochemical investigations. Parts of the tissues had been preserved in 10% neutral buffered formalin solution for histopathological study.

Biochemical investigations:

1-Serum analysis

Urea and creatinine were measured as biomarkers of kidney injury while AST and LDH were measured for detection of heart injury utilizing an automated analyzer.

2- Biochemical investigations in the heart and kidney tissues

The presence of oxidative stress was determined by measuring levels of malondialdehyde and SOD enzyme using commercial assay kits according to the manufacturer's instructions.

Quantitative estimation of PPAR-γ and PTEN levels were done in kidney and heart tissues by ELISA using a commercial kit according to the manufacturer's instructions.

ISSN: 0975-8585

3- Histopathological studies

Kidney and heart tissues could be studied to evaluate the cytotoxic effects of gold nanoparticles and beneficial effect of n-3PUFA

Specimens of liver were immediately fixed in 10%formalin, treated with ascending concentration of ethanol and then cleared in xylol and embedded in paraffin blocks. The blocks of liver Specimen were then sectioned (3-5 µm). The sections were stained with Haematoxylin and Eosin (H&E) [20].

Statistical analysis

The mean values of different markers were analyzed utilizing analysis of variance (ANOVA). Values were considered statistically significant at $p \leq 0.05$.

RESULTS

Characterization of prepared gold nanoparticles:

Transmission Electron Microscope (TEM) of prepared gold nanoparticles showed spherical particles with a size range of 19.1 to 24.4 nm. (fig.1). However, UV-visible spectrophotometer showed the absorption profile of chemically prepared gold nanoparticles (fig.2). The surface plasmon band for the prepared nanoparticles was obtained at 530nm. Particle size analysis and zeta potential measurements showed that the hydrodynamic diameter of chemically prepared gold nanoparticles was 19.620 and the PDI was 0.289 (fig.3). The zeta potential of the prepared gold nanoparticles colloidal solution was performed using Malvern, UK. The zeta potential of prepared gold nanoparticles was -36.5 mv (fig.4).

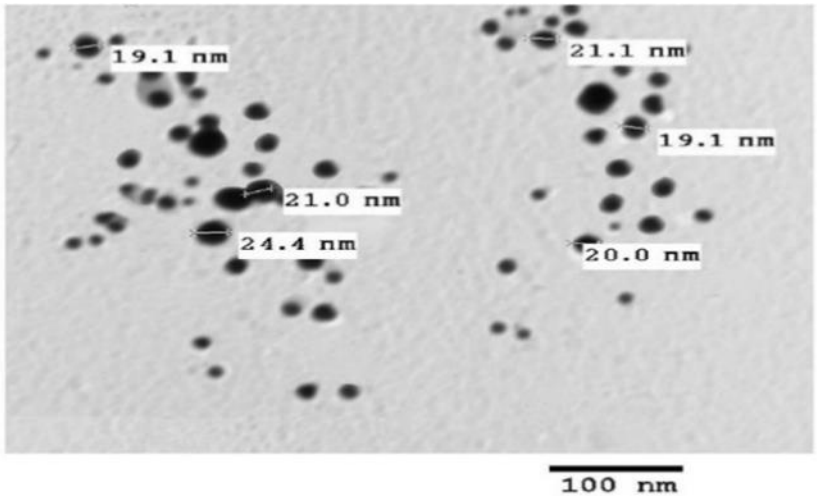

Figure (1): TEM of spherical gold nanoparticles. (Mag.13000x)

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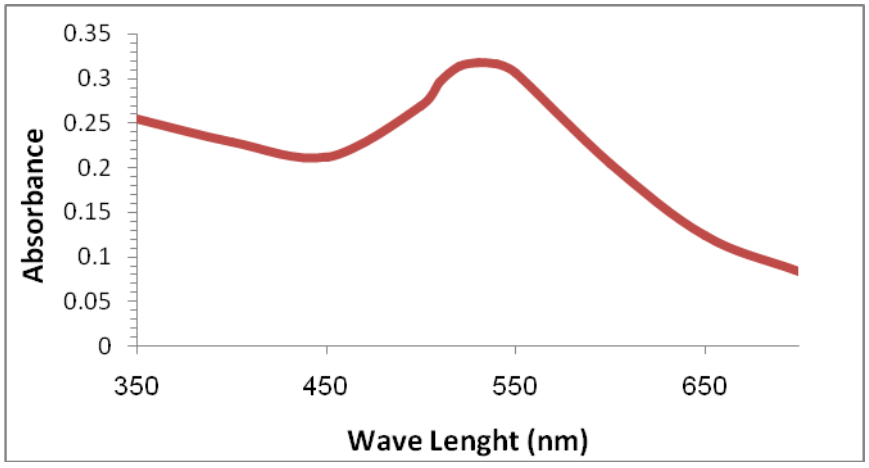

Figure (2): Absorption spectra of prepared gold nanoparticles. (λmax= 530 nm)

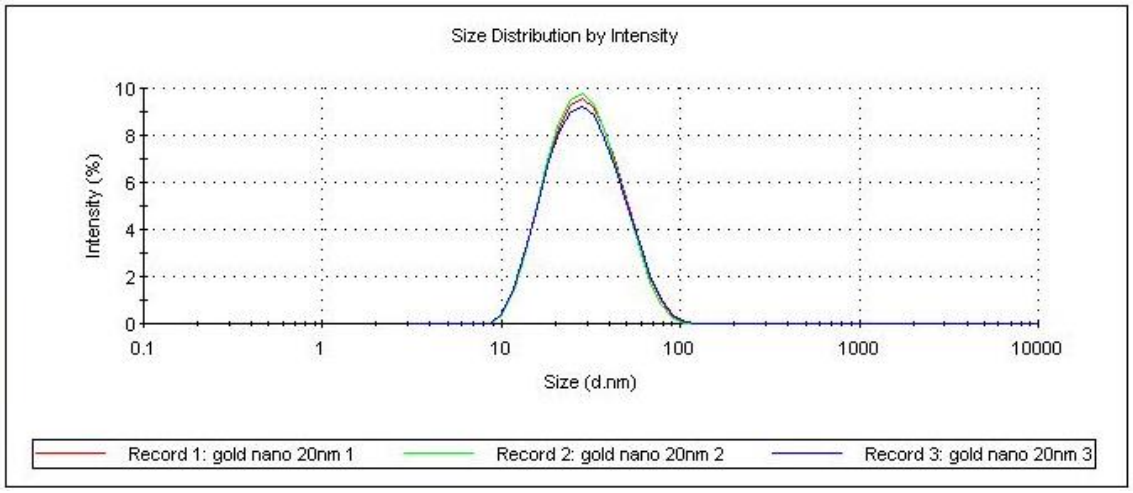

Figure (3): Particle size distribution of prepared gold nanoparticles.

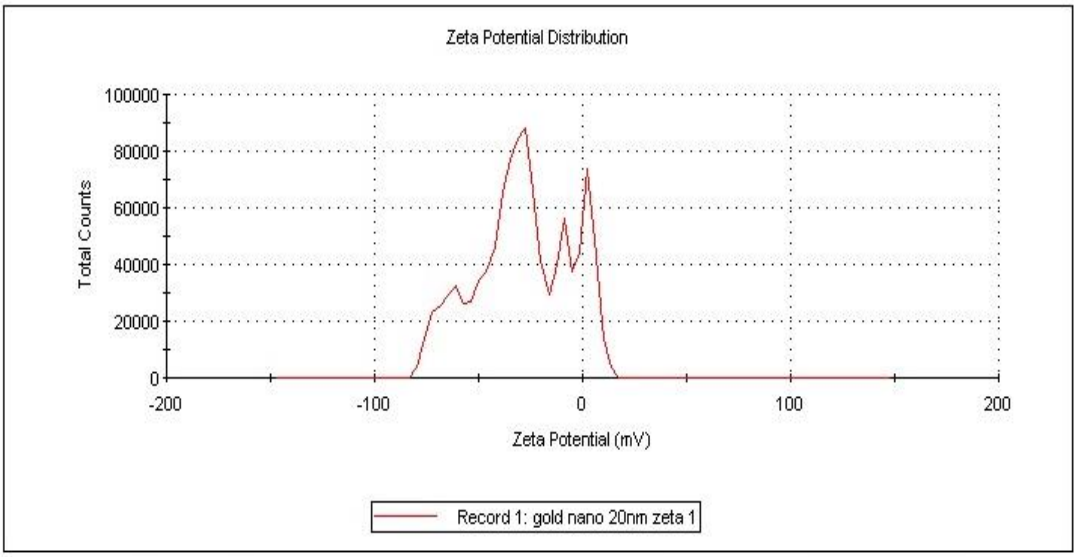

Figure (4): Zeta potential of prepared gold nanoparticle

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Biochemical studies:

The levels of the serum kidney function markers (urea and creatinine) and heart function biomarkers (AST, LDH) were significantly increased in GNPs intoxicated group versus control group [P≤ 0.05, Figures 5 and 6 respectively]. Co-supplementation of omega-3 with GNPS recorded significant reduction in these biomarkers levels versus control and non -supplemented one at p<0.05. However pronounced significant reduction were recorded in animals pre treated with omega-3 before GNPs intoxication versus all other treated groups at p<0.05. Omega-3 FA administration greatly improved these biomarkers close to their normal levels.

Biomarker of oxidative tissue damage malondialdehyde (MDA) and activity of antioxidant enzyme superoxide dismutase (SOD) in kidney and heart tissues of controls and all the studied groups were depicted in figures (7,8) respectively. It was demonstrated that MDA level in animal tissues treated with GNPs was significantly increased versus controls p<0.05. Co-supplementation of omega-3 with GNPS recorded significant reduction in MDA level versus control and non -supplemented one at p<0.05. However pronounced significant reduction in MDA was recorded in animals pre treated with omega-3 before GNPs intoxication versus all other treated groups at p<0.05.

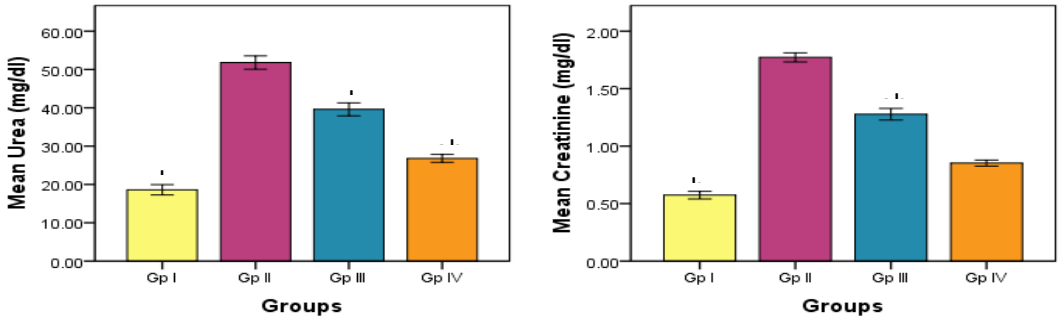

Figure (5): Levels of serum urea and creatinine (biomarkers of kidney injury) in different experimental groups. Data are presented as mean ± S.E. of 10 rats. a significant difference versus control. b significant difference versus GNPs, c significant difference versus GNPs+ omega -3.

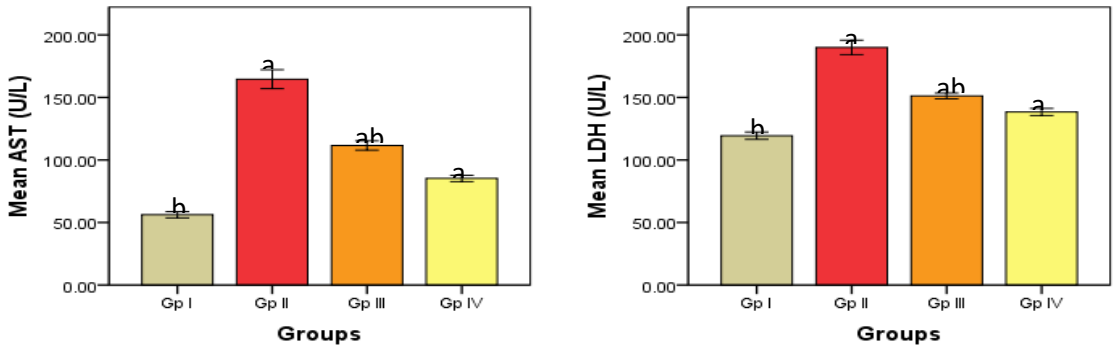

Figure (6): Levels of serum AST and LDH (biomarkers of heart injury) in different experimental groups. Data are presented as mean ± S.E. of 10 rats. a significant difference versus control. b significant difference versus GNPs, c significant difference versus GNPs+ omega -3

The levels of SOD was significantly higher in kidney tissues in animals treated with GNPs versus controls p<0.05. Co-supplementation of omega-3 with GNPS recorded significant reduction in SOD level versus control and non -supplemented one at p<0.05. However significant reduction in SOD was recorded in animals pre treated with omega 3 before GNPs intoxication versus all other treated groups at p<0.05 (fig 7).However, in heart tissues there was reduction in SOD enzyme activity in GNPs intoxicated group which was concomitant with elevation in MDA significantly versus control at p<0.05 (fig. 8). On the other hand, significant elevation in SOD activity was reported in GNPs animals supplemented with omega-3 versus control and GNPs groups, however pre-supplementation markedly ameliorated its level significantly to be nearly to the control level versus other groups at p<0.05.

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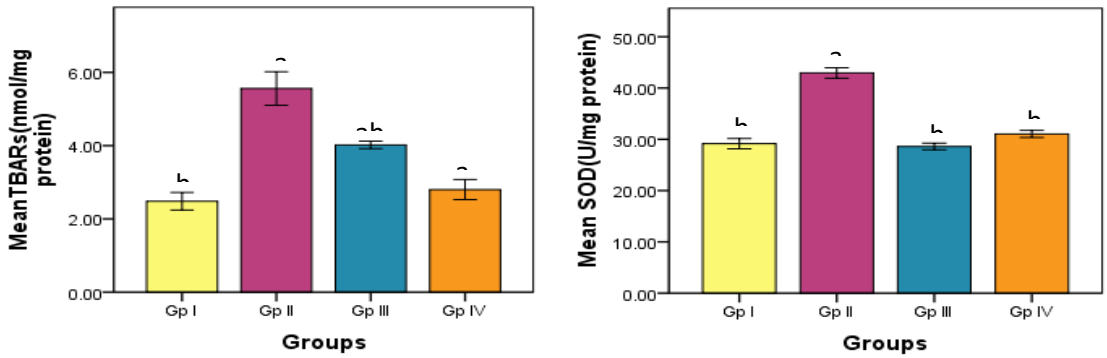

Figure (7): Levels of Malondialdehyde and Superoxide dismutase (biomarkers of oxidative stress) in kidney tissues of different experimental groups). Data are presented as mean ± S.E. of 10 rats. a significant difference versus control. b significant difference versus GNPs, c significant difference versus GNPs+ omega3

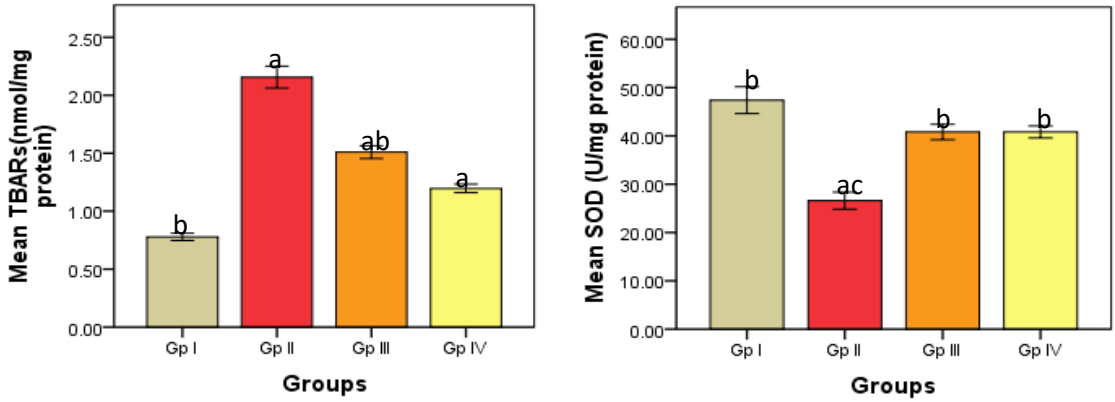

Figure 8: Levels of Malondialdehyde and Superoxide dismutase (biomarkers of oxidative stress) in heart tissues of different experimental groups. Data are presented as mean ± S.E. of 10 rats. a significant difference versus control. b significant difference versus GNPs, c significant difference versus GNPs+ omega3

Meanwhile, PPAR γ and PTEN levels in kidney and heart tissues illustrated in figures (9, 10) showed remarkable significant reduction following administration of GNPs versus control at $p<0.05$. While the levels were ameliorated in pre and co- supplementation groups with omega-3. The pronounced prophylactic effect of omega-3 was more significant in pre supplemented groups than co-supplemented one at $p<0.05$ in the kidney as compared to heart groups. It should be noted here that pre-supplementation with omega-3 induced marked ameliorating effect than co-administration in the kidney wile it had the same effect in heart tissues.

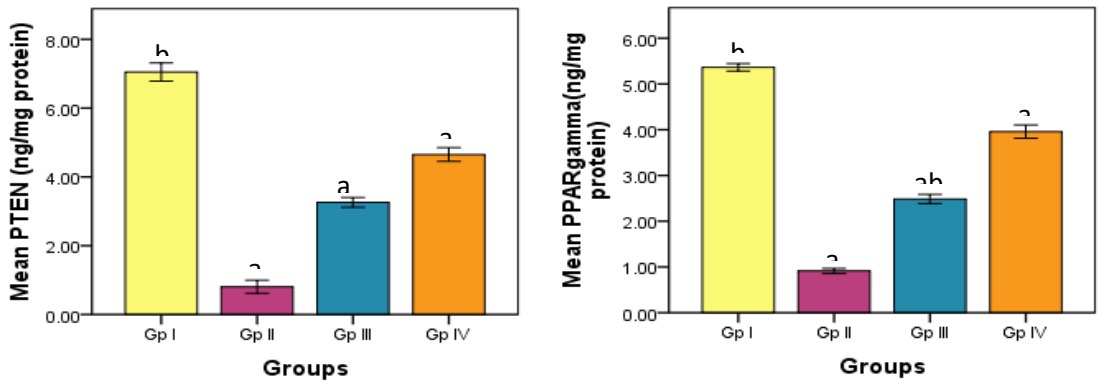

Figure (9): Levels of PTEN and PPAR γ in kidney tissues of different experimental groups. Data are presented as mean ± S.E. of 10 rats. a significant difference versus control. b significant difference versus GNPs, c significant difference versus GNPs+ omega -3

ISSN: 0975-8585

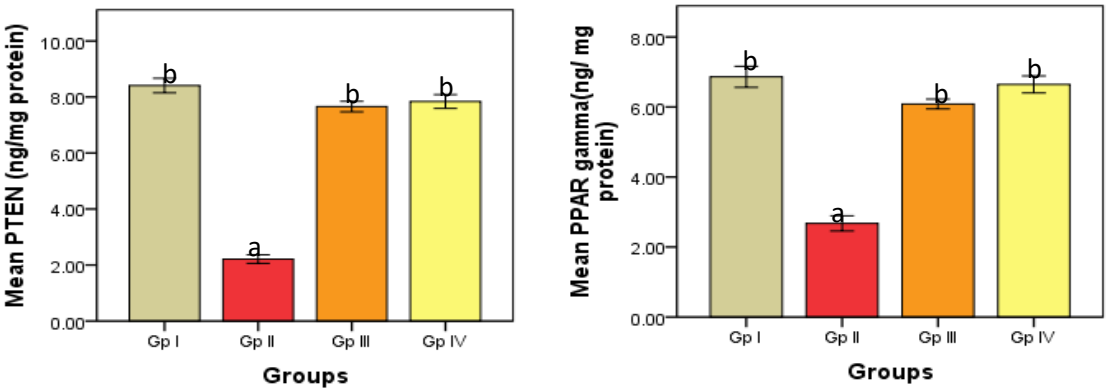

Figure (10): Levels of PTEN and PPAR γ in heart tissues of different experimental groups. Data are presented as mean ± S.E. of 10 rats. a significant difference versus control. b significant difference versus GNPs, c significant difference versus GNPs+ omega-3.

Histological Study of kidney and heart Tissues:

A portion of the kidneys and the hearts from each experimental rat was fixed separately in 10% buffered neutral formalin. These samples were dehydrated by passing successfully in dehydrated in ascending concentration of ethanol, cleaned with xylene. The tissues were then embedded in paraffin wax and sections of about (5-6) µm thickness were cut with a microtom. The sections were mounted on glass slides, then stained with haematoxylin and eosin (H&E) and examined microscopically for histopathological changes using an optical microscope equipped with a camera Figures (11,12) .

The results of the histopathological examination of the kidneys revealed that Normal glomerular and tubular histology was seen in the normal control group 1 rats while group 2 showed marked renal inflammation that was reduced in group 3 and there was no pathological changes in kidneys of group 4 when compared with normal control group 1rats(fig 11).

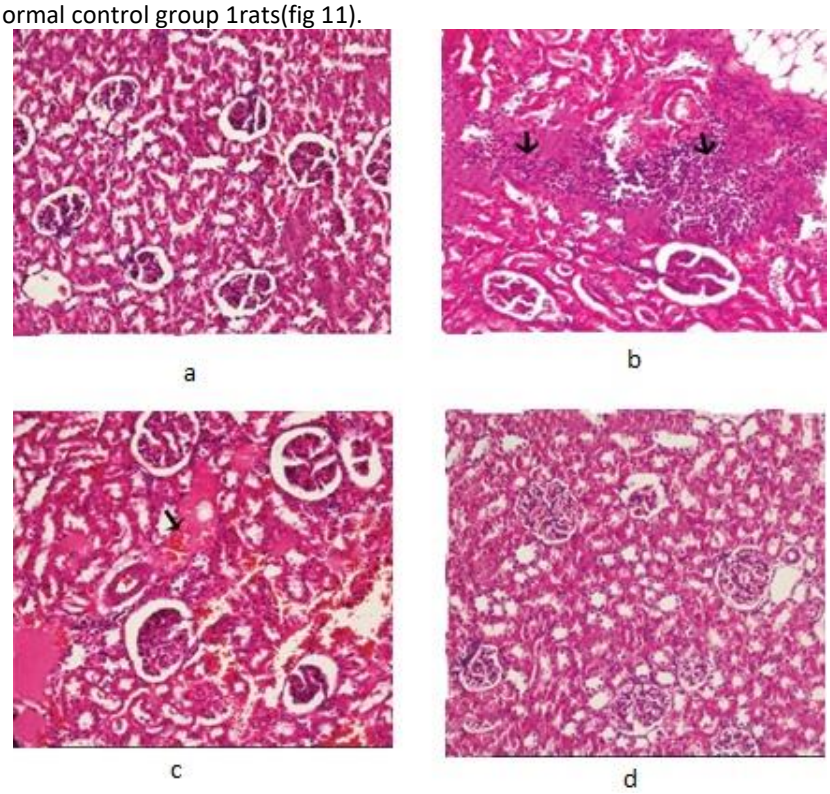

Figure (11): H&E stained sections of kidney tissues of different studied groups.
a- Section of normal rat kidney in control group showing normal glomerular and tubular histology (x100)

b- Section of gold nanoparticles injected group (X100) showing Renal tissues damage. Arrows show areas of inflammation (cellular infilteration).
c- Section of gold nanoparticles+ omega-3 supplemented group (X100) showing Slight regression of normal renal histology with reduced inflammation.
d- Section of pre supplementation of omega-3 + GNP (X100) showing regression of normal renal structure as well as Normal size of glomeruli.

Light microscopy of the heart tissue sections of control rats showed the normal cellular architecture of the heart tissue. GNPs intoxicated rats (Group 2) showed the necrotic changes in myocardial tissue. The tissue sections of both the co and pre supplementation of omega-3 (Groups 3 and 4) showed normal architecture of the heart tissue (fig 12).

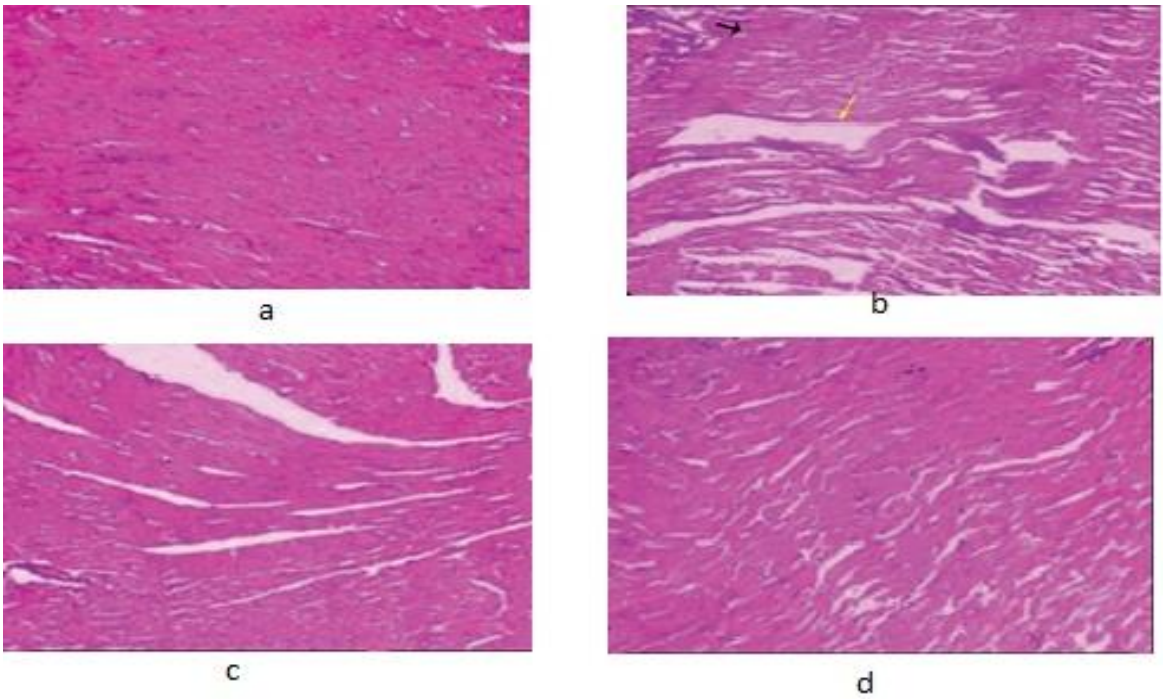

Figure (12): H&E stained sections of heart tissues of different studied groups.

a- Section of normal rat heart in control group showing normal cyto architecture of the heart tissue (regular morphology of the heart) (X100).
b- Section of gold nanoparticles injected group (X100) .Showing Degenerative (yellow arrow) and Necrotic (black arrow) changes in the myocardial tissue.
c- Stained section of gold nanoparticles+ omega-3 supplemented group (X100) showing Normal cyto architecture of the heart tissue.
d- Section of gold nanoparticles+ omega-3 supplemented group (X100) showing Normal cyto architecture of the heart tissue.

DISCUSSION

The benefits of nanomaterials ought to be weighed against their capability toxic effects. Gold nanoparticles are in particular promising for their easy synthesis in diverse shapes and the capability to conjugate them with peptides and proteins to target them to interact with unique molecules [21].

Regarding the present study, morphology and size of prepared gold nanoparticles were determined by transmission electron microscope (TEM), Zeta potential and particle size analysis which revealed the presence of completely spherical gold nanoparticles with smooth surfaces and have sizes of nearly 20nm. The absorption spectra of gold nanoparticles suspension were also determined by UV-visible spectrophotometer and showed that the plasmon absorption was clearly visible and its maximum absorption peak was at 530 nm for the prepared gold nanoparticles. These results are in accordance with a previous study [22].

Nanoparticles are recognized to set off reactive oxygen species (ROS) production, leading to an oxidative strain whilst redox state of the cell is imbalanced. ROS induction by means of nanoparticles is considered the number one motive of nanotoxicity, and has been attributed to the presence of pro-oxidant, useful groups on their reactive surface or because of nanoparticle-cell interactions [23].

In the present study MDA levels in animals tissues treated with GNPs was significantly increased versus controls This indicates the accelerated production of free radicals in these organs as a result of intraperitoneal administration of GNPs among rats, concomitant with the accelerated production over MDA. Khan et al.,[24] found the identical result for MDA in rat liver organ. The smaller particle (average diameter 10-20 nm) has a greater toxicity than the larger ones.

Co-supplementation of omega-3 with GNPS recorded significant reduction in MDA level versus control and non -supplemented one. However pronounced significant reduction in MDA was recorded in animals pre treated with omega-3 before GNPs intoxication versus all other treated groups which replicate the useful antioxidant effect of omega-3 in the tissues which more liable to lipid peroxidation.

The observed decrease in lipid peroxidation by omega-3 fatty acid is in concordance with reports by Sarsilmaz et al., [25] and Iraz et al., [26] .This may be due to the ability of omega-3 fatty acid to up-regulate expression of the antioxidant enzymes and molecules, and also down-regulate the genes associated with or suppression of the production of reactive oxygen species [27].

The antioxidant and anti-inflammatory effects of Omega-3 via scavenging of free radicals and inhibiting lipid peroxidation have been mentioned earlier by Pauwels and Kostkiewicz [28]. This oxidant/antioxidant theory may explain the protective role of Omega-3 fatty acids towards the cardiotoxicity and nephrotoxicity concerning GNPs suggesting that it is a possible beneficial adjunct in the treatment of diseases associated with oxidative stress.

The SOD which catalyzes the dismutation of the superoxide anion into hydrogen peroxide and molecular oxygen is one of the most important antioxidative enzymes .In the current study the level of SOD was significantly higher in kidney tissues in animals treated with GNPs versus controls, these results are matching with the results which were achieved in a previous study [29, 30].

The increase in the SOD activity in kidney tissues, which is indicative of a protective response to oxidative stress [31], is consistent with the elevated MDA concentration in the kidney. We suggest that this dose of GNPs might have a pro-oxidant effect in the kidney.

Kidney and bile can excrete the absorbed nanoparticles in the systemic circulation. Renal clearance of Nps is influenced by particle size and surface charge, the most widespread organ accumulation and distribution are the smaller GNPs (10 - 20 nm) [32].

Co- and pre supplementation of omega-3 with GNP recorded significant reduction in SOD level in the kidney tissues. It was documented that, the mode of action of Omega-3 can be intercepted pharmacologically at different levels with agents that scavenge free reactive oxygen, block their generation, or enhance endogenous antioxidant capabilities [33].

While in heart tissues there was reduction in SOD enzyme activity in GNPs group which was concomitant with elevation in MDA significantly versus control .On the other hand, significant elevation in SOD activity was reported in GNPs animals supplemented with omega-3 versus control and GNPs groups, however pre-supplementation markedly ameliorated its level significantly to be nearly to the control level versus other groups.

Same observation was noticed by Ferchichi *et al.,* [34] who noticed increased malondialdehyde level and decreased CuZn-superoxide dismutase, catalase, and glutathione peroxidase activities in rat as results of oxidative response of GNPs administration.

The GNPs have potent effect on biological system and cause undesirable effects.The disturbance in the natural balance between oxidative stress and antioxidant defense indices is one of these damaging effects which carry out various pathological effects. The changes in antioxidant and oxidative stress markers would possibly be refer the production of ROS [35].

Long-chain n-3 PUFAs has been proven to act both immediately by inhibiting arachidonic acid metabolism and circumlocutory by changing the expression of inflammatory genes via effects on transcription factor activation [36].

The current study also demonstrated that injection of GNPs to rats, markedly down modulated the expression of PPAR-γ and PTEN compared with control animals. The decreased in PPARγ and PTEN expression in the heart and kidney of GNPs intoxicated rats may consider a pathophysiological response to the severity of GNPs cytotoxicity, suggesting an alteration in the expression of these proteins as a results of tissues damage induced by GNPs toxicity. Prophylactic or therapeutic administration of n-3 PUFA to GNPs intoxicated rats, markedly up-regulated the expression of PPAR-γ and PTEN.

These findings was in agreement with Anderson *et al.,* [37], who suggested that PPAR-γ activation is a mechanism by which n-3 PUFAs enhance mitochondrial fatty acid oxidation and antioxidant capacity in human atrial myocardium, and that this preoperative therapeutic regimen may be optimal for mitigating oxidative/inflammatory stress associated with cardiac surgery.

Organ-protective actions such as reduction of cardiovascular events and proteinuria and delaying the progression of diabetic nephropathy can be done by activation of PPAR [38]. PPAR-γ has been related with control of inflamation by inhibiting cytokine incitement of COX2 and inducible nitric oxide synthase (iNOS) expression in various cell writes diminish production of proinflammatory cytokines and restrains vascular smooth cell movement [39],Previous study demonstrate that PPAR-γ can upregulate PTEN at the transcriptional level [40].

Past investigation exhibit that PPAR-γ can upregulate PTEN at the transcriptional level [41]. Current confirmation recommends that PTEN may assume a vital part in the advancement of fibrosis. Kral *et al.,* demonstrated that inhibition of PTEN action initiated lung fibrosis through enactment of proinflammatory and profibrotic pathways [41]. Past investigations demonstrated a connection between the PTEN/Akt pathway and heart fibrosis [43]. Some proof specifically represented that PTEN controlled renal fibrosis during diabetes mellitus [44].

PTEN inactivation causes accumulation of circulating inflammatory cells into the kidney and production of inflammatory substances, which provoke the development of ischemic AKI recommending that PTEN activation could be a potent medicine strategy for ischemic AKI [45].

It has been, exhibited that fish oil applies its helpful impact on breast tumor development by expanding PTEN mRNA and protein expression in breast cancer cells which promote apoptosis [46]. Our result may indicate that the anti-cytototoxic impact of n-3 PUFA is PPARγ and PTEN dependent.

The heart function biomarkers (AST, LDH) and kidney biomarkers (urea and creatinine) were significantly increased in GNPs intoxicated group versus control group. Co-supplementation of omega-3 with GNPS recorded significant reduction in these biomarkers levels versus control and non -supplemented one. However pronounced significant reduction were recorded in animals pre treated with omega-3 before GNPs intoxication versus all other treated groups. Omega-3 FA administration greatly improved these biomarkers close to their normal levels.

The results were in line with earlier reported studies, which have shown that the amount of diagnostic markers present in plasma is directly proportional to the damage of tissue [47.48].

Following pretreatment with fish oil, there was critical diminishment in the levels in regards to these enzymes showing that fish oil repress the lipid oxidation then therby oxidative stress. ROS development require the actuation of the arachidonic acid with the action of the enzyme 5-lipooxygenase (5-LOX) [49]. Fish oil which is rich in n-3 PUFAs i.e. EPA and DHA meddle with arachidonic acid cascade by methods for inhibiting

5-LOX. Fusion of the n-3 PUFAs with cell layer, increased antioxidant status normalizes the oxidative state, controls the physical status of membrane lipids and avoids ascends in intracellular Ca with start of oxidative pressure [48].

It was found that 10 and 50 nm of gold nanoparticles administration in rats increased the creatinine and urea levels compared with controls, due to excessive clearance of gold nanoparticles through kidney or the short period of the treatment. These effect were comparable to the results of the existing study (the difference lied among the size of nanoparticles: 20nm [32].

The measure of creatinine among the blood depends upon the speed of renal glomeruli work, which recommends the renal performance. At the point when creatinine level is higher than the ordinary level it fundamentally disturbs renal capacity. Increments in creatinine level can furthermore reveal chronic kidney disease and renal damage. Regulation of urea by kidneys forms an urgent piece of metabolism. In addition to the role that urea has as a transporter of nitrogenous waste, this combination has a part in common trade that happens in nephrones system. It permits then reabsorption of water and particles that are discharge forexcreation in the urine [50].

The chemoprotective effect regarding Omega-3 on kidney tissue was confirmed by the attenuation of serum urea and creatinine in pre and co- supplemented groups.

These results are consistent with the results of Attaia *et al.,* [33]. The mode of action of Omega-3 can be intercepted pharmacologically at different levels with agents that scavenge free reactive oxygen, block their generation, or enhance endogenous antioxidant capabilities.

In the present study, light microscopic examination of heart tissues sections of GNP intoxicated rats showed degenerative and necrotic changes in myocardial tissue. Sections of both co and pre supplementation of omega-3 showed normal architecture of the heart tissue. The result was matched with the previous results of Abdelhalim *et al*., after 7 days of i.p. administration of GNPs (10 and 20nm) in rats [35] .The histological heart adjustments may propose that GNPs could meddle with the antioxidant defense mechanism and prompting reactive oxygen species generation which thusly may emulate an inflammatory reaction.

In concurrence with mass surface science, metallic NPs have impressive synthetic reactivity, while ionic NPs have been seen to gather protein layers when presented to the cytoplasm or in the lymphatic fluid. This protein layer is perhaps associated with the interaction of the nanoparticle with the cellar system [51].

The results of the histopathological examination of kidney tissues revealed that GNPs injection showed marked renal inflammation that was reduced in co administration of n-3 and there was no pathological changes in kidneys was observed in presupplementation with n-3 which support the prophylactic effect of n-3PUFA.The adverse histocytological alterations in rat kidney in response to GNPs toxicity have been previously documented by Abdelhalim and Jarrar [52] who have suggested that the histological changes may result from interaction of GNPs with cellular components of the kidney, causing oxidative stress and inflammation.

Balasubramanian *et al*., [53]. found that the concentration of gold nanoparticles is negligible among the kidney in the initial period of infusion and increments on time. The reason of this is the serum protein wich cover the GNPs and may change shape, size, charge, or even their hydrodynamic diameter. Every protein together with negative charge that joins the nanoparticles can be discharged by glomerular membrane.

Omega-3 treatment caused a significant decrease in the histopathological changes induced by GNPs in the heart and the kidney and partially restored these changes in GNPs plus Omega-3 treated groups. So, the present results indicated that pre and coadministration of Omega- 3 had protective role against cardio and renal toxicity, induced by GNPs.

CONCUSSION

The present study indicates that administration of n-3PUFA could ameliorate the cardio and renal cytototoxic effect induced by GNPs administration. The mechanisms underlying these promising effects could

be through increasing PPAR γ and PTEN expression which ameliorates antioxidant status, in addition the results support the prophylactic impact of n-3PUFA.The study also provide novel experimental evidence of omega-3 PUFA protective effect against GNPs induced cardiac and renal cytotoxicity

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