Pharmacophore

ISSN-2229-5402

Journal home page: http://www.pharmacophorejournal.com

PROPHYLACTIC EFFECT OF OMEGA-3 FATTY ACID ON GOLD NANOPARTICLES TOXICITY IN RATS' BRAIN

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ARTICLE INFO

Received:
07th Sep 2017
Received in revised form:
18th Dec 2017
Accepted:
25th Jan 2018
Available online:
28th Feb 2018

Keywords: *Gold nanoparticles; Toxicity; omega-3*

ABSTRACT

Objective: This study was undertaken to investigate the toxic effects of gold nanoparticles (GNPs) on rat's brain with and without supplementation of omega-3 fatty acid.
Methods: This study was conducted at King Fahad research center, KAU, Jeddah, on 60 rats divided into four groups,15 rats each. Group I serves as control, Group II injected ip with GNPs for 6 days. Group III: injected as in group II accompanied with oral supplementation of omega-3 Group IV: supplemented orally with omega-3, followed by injection with GNPs. Oxidative stress, DNA damage immuno-inflammatory mediator's biomarkers, and CNS enzymes were determined. Histopathological studies in brain tissues was also done.
Results: The study revealed the presence of oxidative stress in the treated animals, accompanied by an increase in 8-hydroxydeoxyguanosine, caspase-3 and pro inflammatory markers TNF-α and NFkB. Gold nanoparticles also caused a significant decrease in the levels of CNS enzymes AChE and MAO, co and pre-supplementation of omega-3 attenuate all of the parameters to normalcy. However, pronounced attenuation were recorded in animals pre- treated with omega-3.
Conclusion: The results indicated the protected role of omega-3 against GNPs neurotoxic damage in rats brain through its down regulation of the studied parameters. The biochemical result was supported by histopathological findings. Pretreatment with omega-3 was indicating a possible prophylactic impact of omega-3.

To Cite This Article: Iman A. Sharaf, Manal EA El-Halwagy ,Hanan S. Alnahdi, (2018), "Prophylactic effect of omega-3 fatty acid on gold nanoparticles toxicity in rat's brain", ***Pharmacophore, 9(1),*** *56-68.*

Introduction

Nano-biotechnology offers the potential for the improvement of impeccably delicate diagnostics and organ/tumor-focused on remedies. In the direction of latest a long time, there has been great estimated excitement for creating nanoparticles as successful drug transport devices. Gold nanoparticles (GNPs) are relied upon to have an extensive style of makes utilization of later on in mild of the fact that they're easy to synthesize and have correct biocompatibility [1].

15–50 nm GNPs can cross the blood–brain barrier (BBB), bringing about their aggregation in the brain. The biosafety of metallic gold is surely understood, and it has been in vivo use subsequent to the 1950s [2].

Nanoparticle innovation likewise has the ability to deal with Alzheimer's and Parkinson's illnesses. However, a success focusing of medicinal drugs to the brain remains a test in view of the prohibitive houses of the BBB [3].

The healthy BBB shields the mind from blood-borne nanoparticle publicity; in any case, in a few pathological situations have been appeared to increase BBB penetrability to nanoparticle[3] .The usage of nanoparticles afterward is prone to quite affect human health. As a consequence, it's far important to examine the impacts of nanoparticles at the brain [4].

Nanomaterial toxicity can happen via a few distinct mechanisms in the body. The fundamental molecular mechanism of in nanotoxicity is the instigation of oxidative stress which produces free radicals. These reason damage to biological components through lipid peroxidation, and DNA damage [4].oxidative anxiety may also have an element in the enhancement of inflammation through upregulation of transcription elements (e.g. nuclear component-κB) [5].

A growing region of examination is inspecting the neurobehavioral parts of omega-3 unsaturated fats (alpha-linolenic corrosive, docosahexaenoic corrosive (DHA), and eicosapentaenoic corrosive) is examining [6].The neurobehavioral components of omega-three unsaturated fat (alpha-linolenic corrosive, docosahexaenoic corrosive (dha), and eicosapentaenoic

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corrosive) and the fundamental part of those essential fats within the operating of the vital fearful gadget [6].DHA is plentiful in the brain [7] and had a potential to affect cell signaling by using alter the composition of lipid rafts [8].
Incorporation of DHA into cell membranes outcomes in lowering lipid peroxidation and oxidative stress in neurons [9]. The DHA omega-3 additionally reduces proinflammatory mediators and anti-inflammatory compounds in conjunction with materials that protect brain cells called neuroprotectins [10].
To the best of our knowledge no reports demonstrate the neuroprotective effect of omega-3 FA against gold nanoparticles neurotoxicity. The intention of the prevailing work was to evaluate the toxicological impact of gold nanoparticles administration on rats brain. The study also was extended to explore the capacity impact of omega-3 fatty acid as a protective agent with antioxidants and anti-inflammatory properties in a trial to minimize or prevent the cytotoxic effect of gold nanoparticles on nervous system enzymes, oxidative stress, inflammation and DNA damage. Histopathological study of rat brain tissue was examined to confirm the biochemical investigations.

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 (HAuCl4) was heated with different reducing agent [11].

Characterization of Gold Nanoparticles [12]:

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

This study comprised 60 male albino rats (150-200 g body weight) divided into four groups, 15 rats each. The animals were housed in cages. The use of animals and experimental design were approved by unit of biomedical ethics, King Abdulaziz University Medical College, Jeddah, KSA, which are in compliance with the national and international laws and policies (7th edition). All procedures were performed according to the National Institutes of Health Guiding Principles in the Care and Use of Animals. Animals were allowed to acclimatize at the experimental environment for 2 days before dosing initiation. The animals were randomly divided into 4 groups (n=15 each). 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 [4]. 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 [13]. 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, accompanied via injection with Gold nanoparticles as in group II.
Rats were scarified by decapitation after overnight fasting (12-14 hours), and the brains were removed. Parts of the brains had been preserved in 10% neutral buffered formalin solution for histopathological study.

A) **Biochemical investigations in the brain tissues**

The presence of oxidative stress was determined by measuring levels of malondialdehyde and glutathione peroxidase (GPx) were assayed using commercial assay kits according to the manufacturer's instructions. DNA damage and apoptosis were investigated by measuring 8- hydroxydeoxyguanosine (8-OHdG) and caspase -3 using ELISA kits in step with the producer's instructions. The inflammatory-mediators, Nuclear Factor-κB (NF-κB), and TNF-α had been measured using the ELISA assay kits following the instructions furnished by means of the producer. Nervous system enzymes, acetyl choline esterase and monoamine oxidase were assayed using commercial assay kits according to the manufacturer's instructions.

B) **Histopathological studies**

Brain tissues could be studied to evaluate the cytotoxic effects of gold nanoparticles. For histological examination, brain (small pieces of cerebrum) became dissected and right away fixed in 10% neutral buffered formalin solution, processed by way of using a graded ethanol series, after which embedded in paraffin. The paraffin sections have been cut into 5 µm thick slices and

stained with hematoxylin and eosin for light microscopic examination. The sections were then regarded and photographed [14].

Statistical analysis

Data were analyzed by comparing the values for different treatment groups with the values of individual controls. Results were expressed as mean ± SE. Significant differences among the values were analyzed using the one-way analysis of variance (ANOVA) followed by Bonferroni's test post-ANOVA.

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. (Figure.1). However, UV-visible spectrophotometer showed the absorption profile of chemically prepared gold nanoparticles (Figure.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 (Figure.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 (Figure. 4).

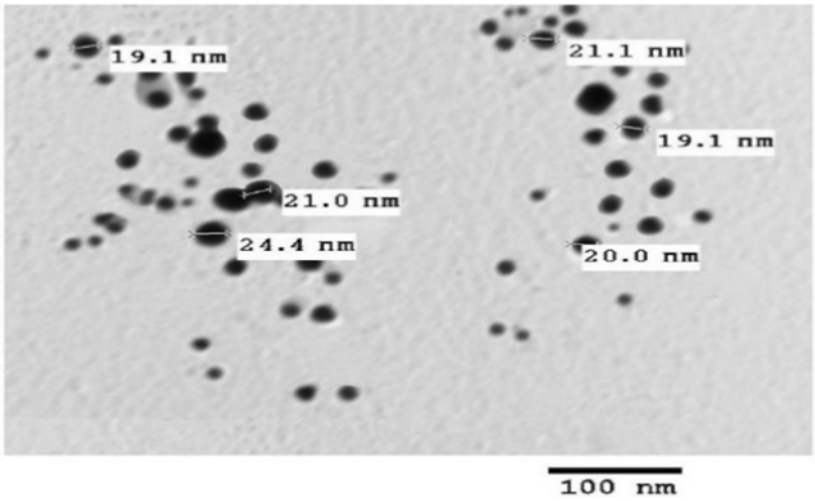

Figure 1: TEM of spherical gold nanoparticles. (Mag.13000x)

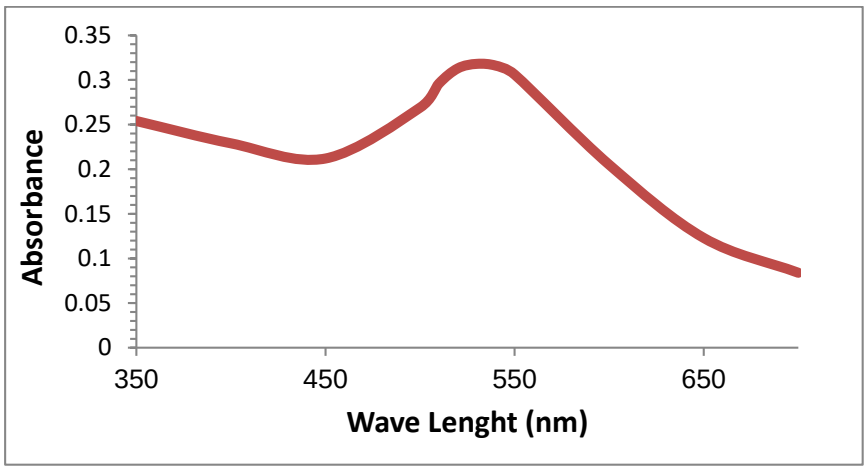

Figure 2: Absorption spectra of prepared gold nanoparticles. (λmax= 530 nm)

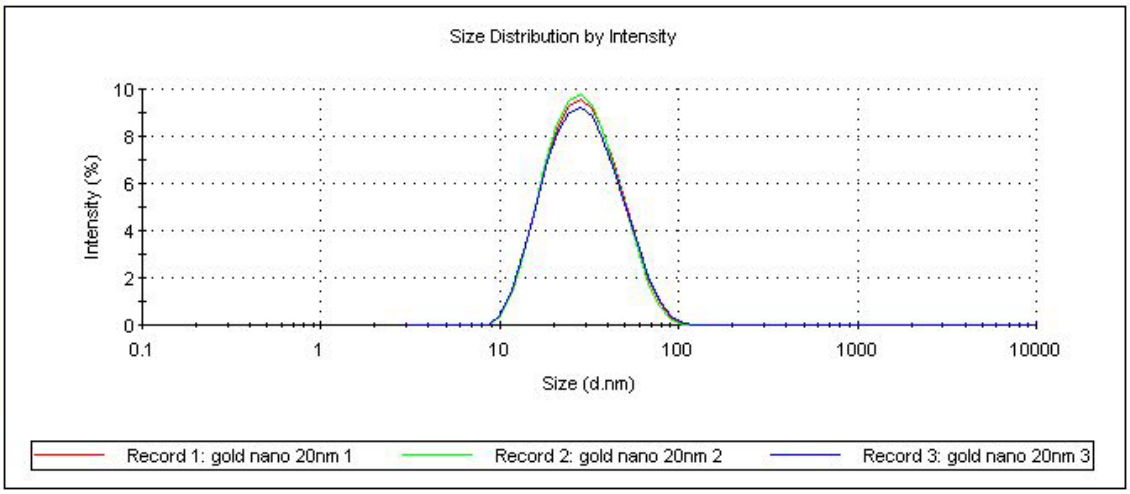

Figure 3: Particle size distribution of prepared gold nanoparticles.

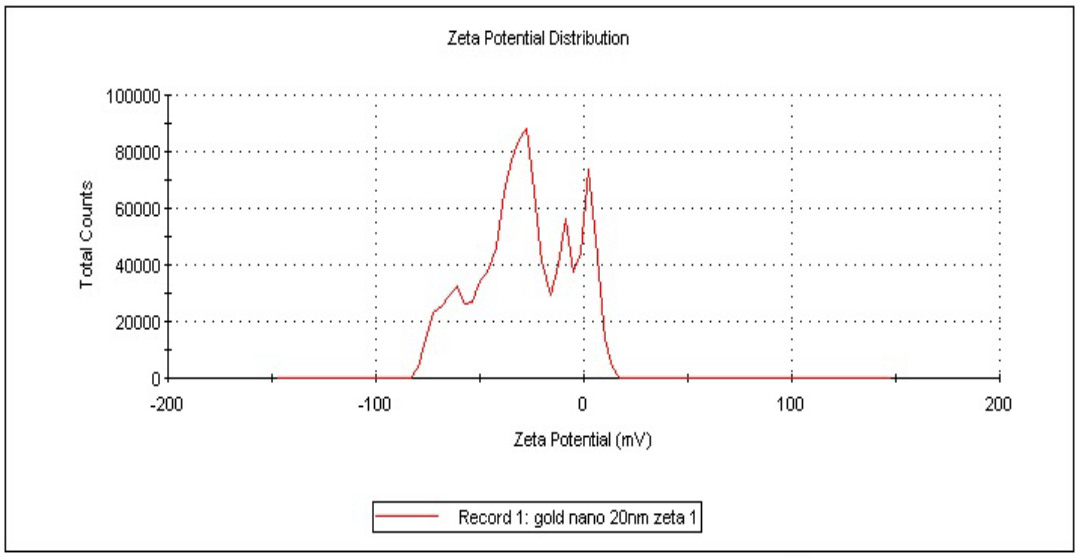

Figure 4: Zeta potential of prepared gold nanoparticle

Biochemical studies

Biomarker of oxidative tissue damage malondialdehyde (MDA) and activity of antioxidant enzyme glutathione peroxidase (GPX) in brain tissues of controls and all the studied groups were depicted in figures (5,6). It was demonstrated that MDA level in brain tissues of animals 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 pretreated with omega-3 before GNPs intoxication versus all other treated groups at $p<0.05$ (Figure. 5). Reduction in GPX enzyme activity in brain tissues in GNPs group was concomitant with elevation in MDA significantly versus control at $p<0.05$ (Figure. 6). On the other hand, significant elevation in GPX 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 $p<0.05$.

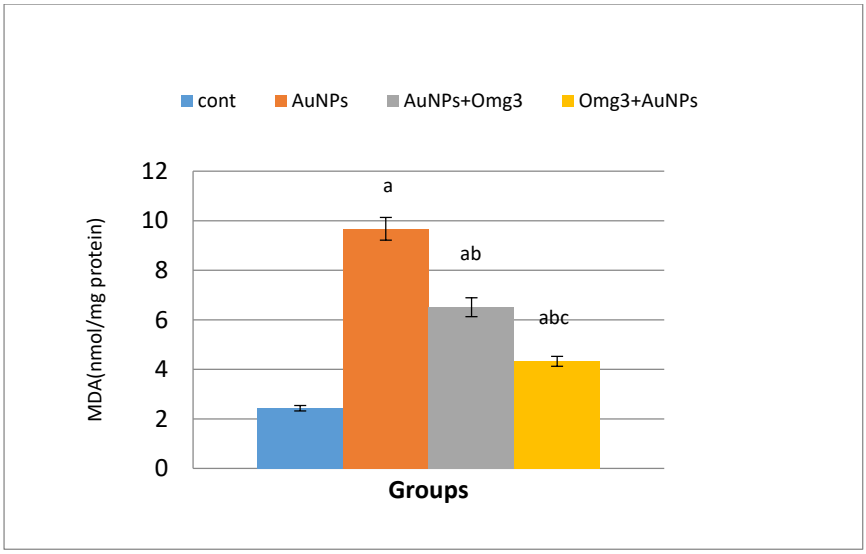

Figure 5: Levels of MDA (oxidative stress biomarker) in Brain of different experimental groups Data are presented as mean ± S.E. of 15 rats
a significant difference versus control
b significant difference versus AuNPS
c significant difference versus AuNPS+ omega -3

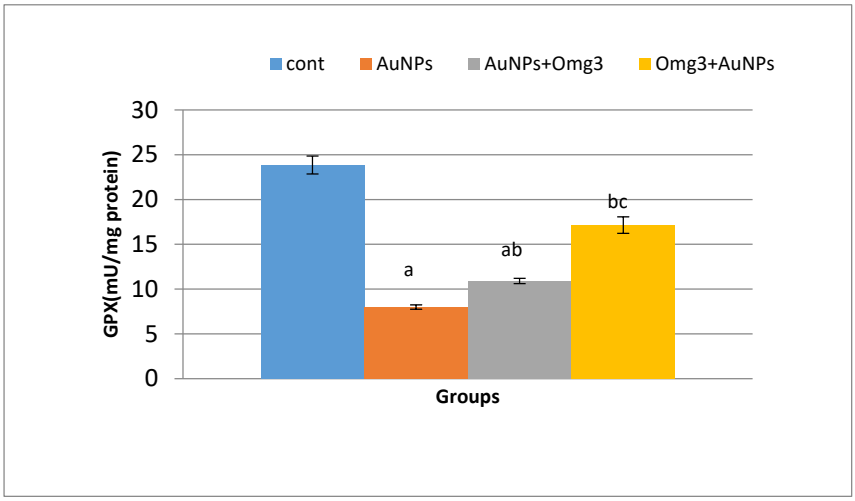

Figure 6: Levels of GPX (antioxidant biomarker) in Brain of different experimental groups
Data are presented as mean ± S.E. of 15 rats
a significant difference versus control.
b significant difference versus AuNPS
c significant difference versus AuNPS+ omega -3

In the present work 8- hydroxyguanosine (8-OHdG) which is a major product of DNA oxidation was elevated significantly in brain tissues treated with GNPs versus control groups at $p<0.05$ (figure 7). Pre and co-supplementation with omega-3 to GNPs treated animals alleviate oxidation of DNA in brain tissues, the pronounced mitigating effect was significant versus control and GNPs groups and versus each other at $p<0.05$. On the other hand, apoptotic marker, caspase 3, was markedly significantly elevated in brain tissue due to GNPs intoxication versus control at $p<0.05$ (figure 8). However, pre-supplementation with omega-3 has pronounced anti – apoptotic effect on caspase 3 to nearly reach the control level than co- supplementation.

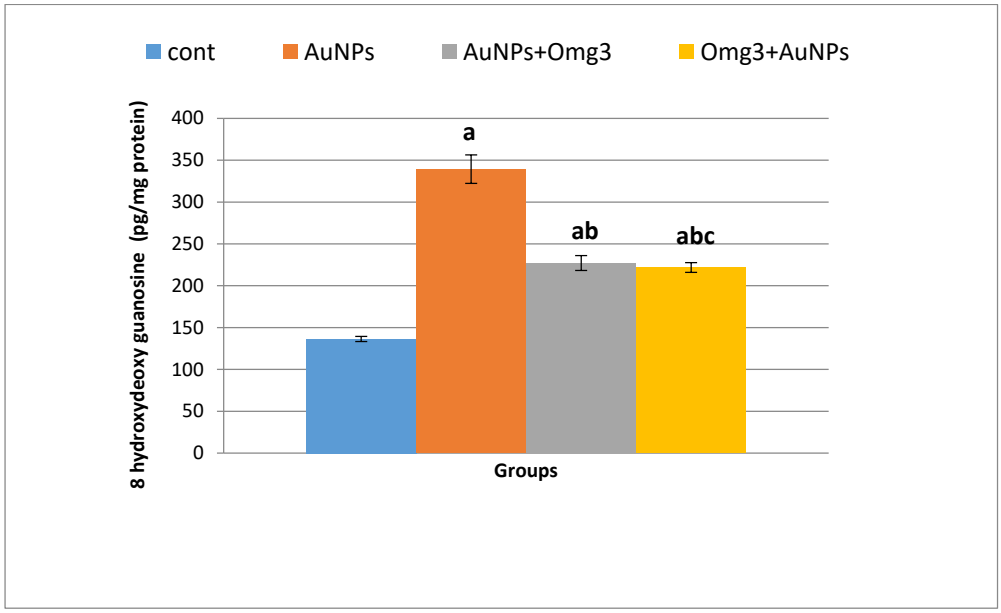

Figure 7: Levels of 8- hydroxydeoxyguanosine (biomarker of DNA damage) in Brain of different experimental groups Data are presented as mean ± S.E. of 15 rats.
a significant difference versus control.
b significant difference versus AuNPS
c significant difference versus AuNPS+ omega -3

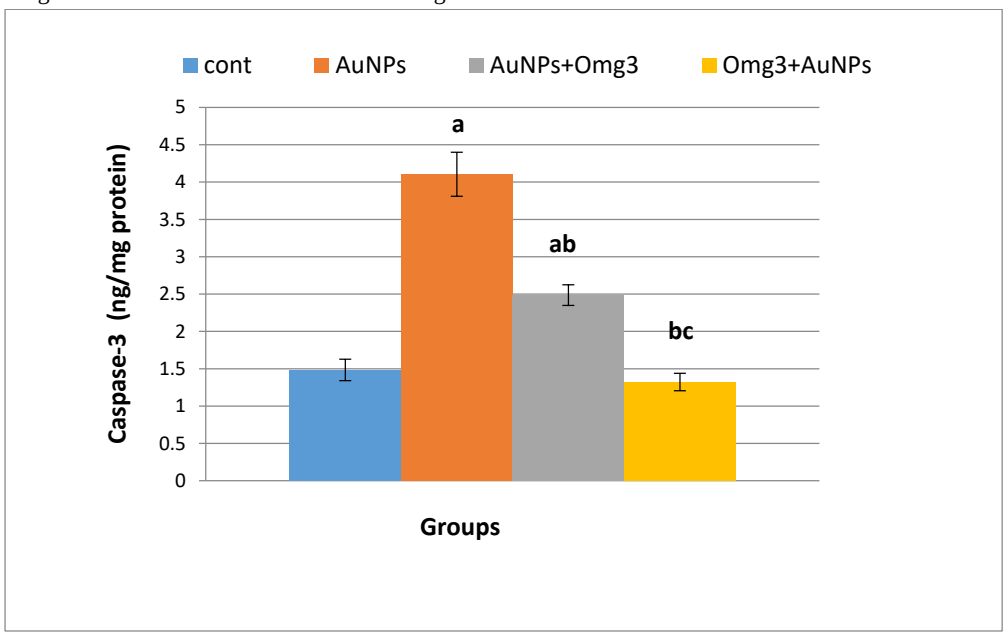

Figure 8: Levels of Caspase-3 (apoptotic biomarker) in Brain of different experimental groups Data are presented as mean ± S.E. of 15 rats
a significant difference versus control
b significant difference versus AuNPS
c significant difference versus AuNPS+ omega -3

Meanwhile, proinflammatory cytokins, Tumor necrosis factor alpha (TNFα) and NF-kB, demonstrated in figures (9,10) showed remarkable significant elevation following treatment with GNPs versus control at $p<0.05$. While the levels were ameliorated in pre and co- supplementation groups with omega-3. The pronounced anti-inflammatory effect of omega-3 was more significant in pre supplemented groups than co-supplemented one at $p<0.05$ as compared to other groups. It should be noted here that pre-supplementation with omega-3 induced marked ameliorating effect than co-administration.

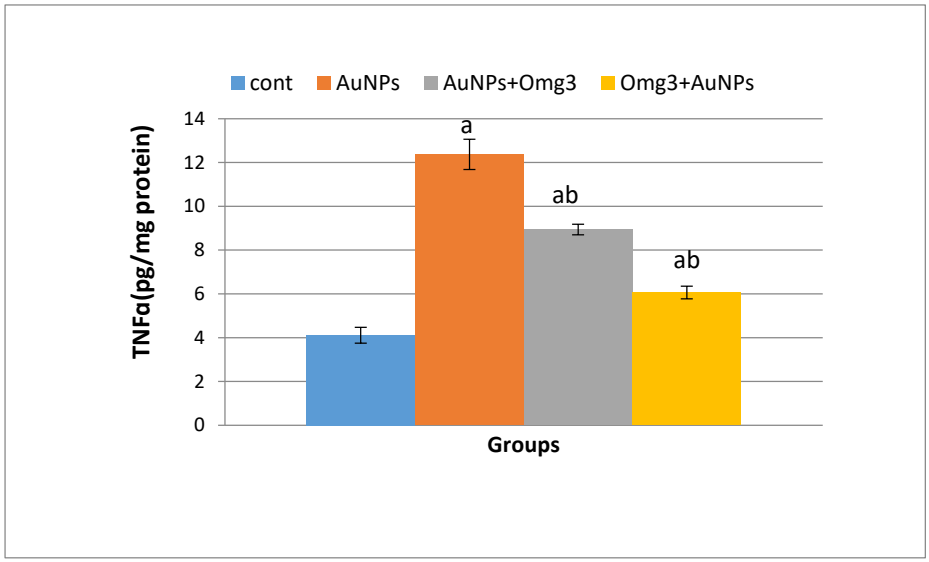

Figure 9: Levels of TNFα (proinflammatory biomarker) in Brain of different experimental groups Data are presented as mean ± S.E. of 15 rats.
a significant difference versus control
b significant difference versus AuNPS
c significant difference versus AuNPS+ omega -3

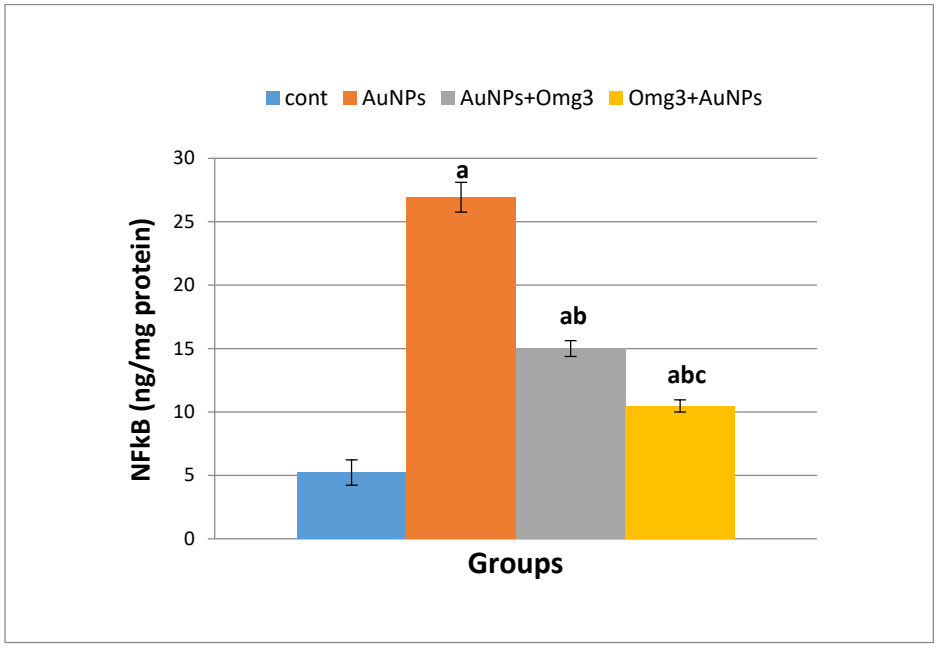

Figure 10: Levels of NFkB (proinflammatory biomarker) in brain of different experimental groups Data are presented as mean ± S.E. of 15 rats.
a significant difference versus control.
b significant difference versus AuNPS
c significant difference versus AuNPS+ omega -3.

In this study, CNS enzymes monoamine oxidase (MAO) and acetylcholinesterase (ACHE) in brain tissues showed significant reduction at $p<0.05$ in GNPs group as compared with the control (figures 11,12). Mitigating effect of omega-3 was recorded in both treatment regimens. However, pre-supplementation with omega-3 recorded pronounced mitigating effect significantly versus other groups at $p<0.05$.

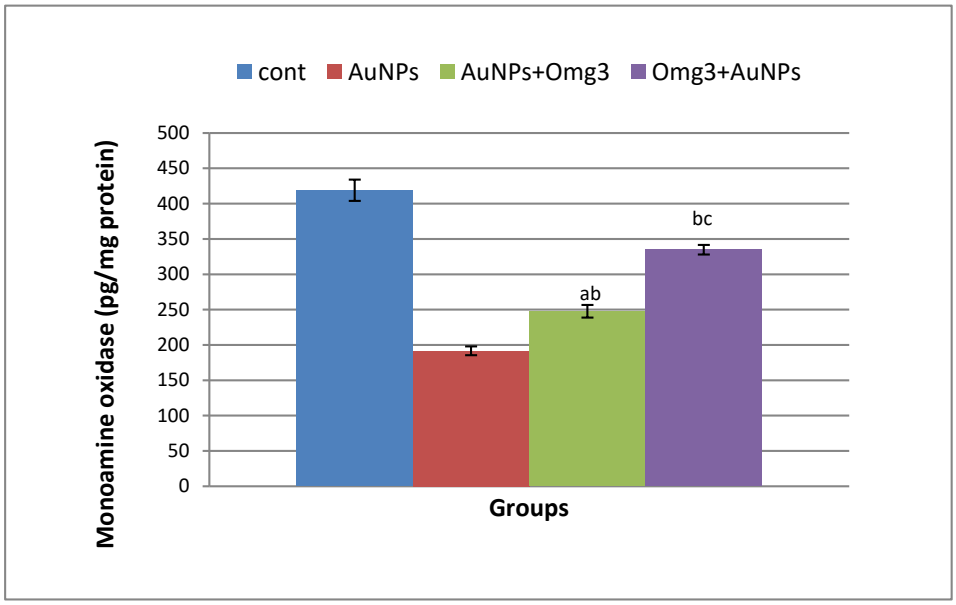

Figure 11: Levels of CNS enzyme monoamine oxidase (MAO) in Brain of different experimental groups
Data are presented as mean ± S.E. of 15 rats.
a significant difference versus control.
b significant difference versus AuNPS
c significant difference versus AuNPS+ omega -3.

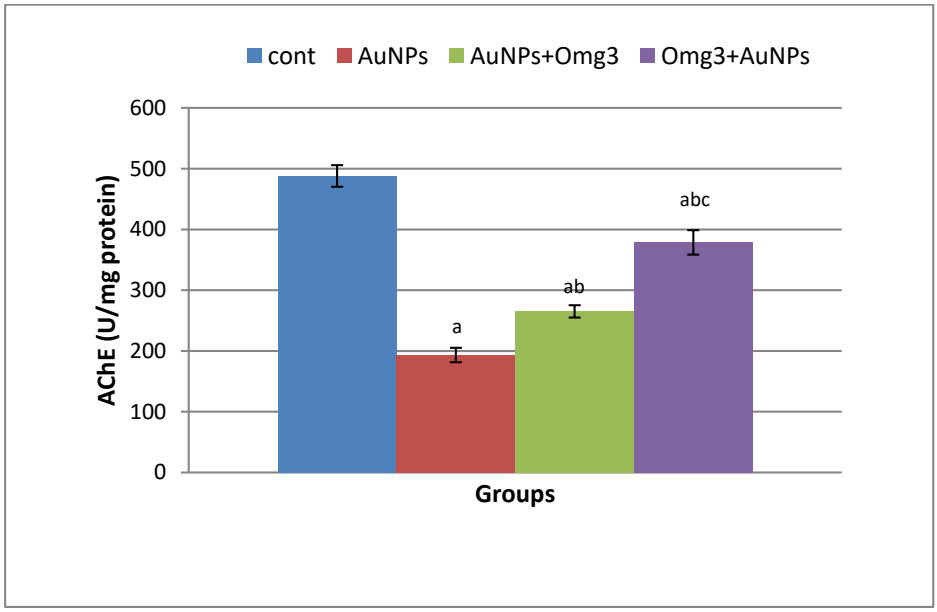

Figure 12: Levels of CNS enzyme acetylcholinesterase (ACHE) in Brain of different experimental groups
Data are presented as mean ± S.E. of 15 rats.
a significant difference versus control.
b significant difference versus AuNPS
c significant difference versus AuNPS+ omega -3.

Histological Study of Brain Tissues

Light microscopic examination of hematoxylin and eosin stained sections of cerebral cortex of adult male rats was illustrated in figures 13 .It was noticed that group 1 (control group) revealed the gray matter with its well six distinguished layers. These layers were; molecular layer, external granular layer, external pyramidal layer, internal granular layer, internal pyramidal layer and finally the layer of polymorphic cells. The normal pattern of the white matter is formed of homogenously stained nerve tracts running down the cortex. Degenerative changes were markedly intensified in different brain regions of GNPs treated rats (group 2). These include marked cortical layers disorganization, vacuolated foci with cellular loss; intense eosinophilic staining of neuropil, non - specific inflammatory cells infiltration, and increased number of apoptotic and red neurons. However, light microscopic examination of brain regions of group 3 and 4 showed return of brain tissues towards normal morphology as evidenced by remarkable regression of the total degenerative changes but still different when compared with control.

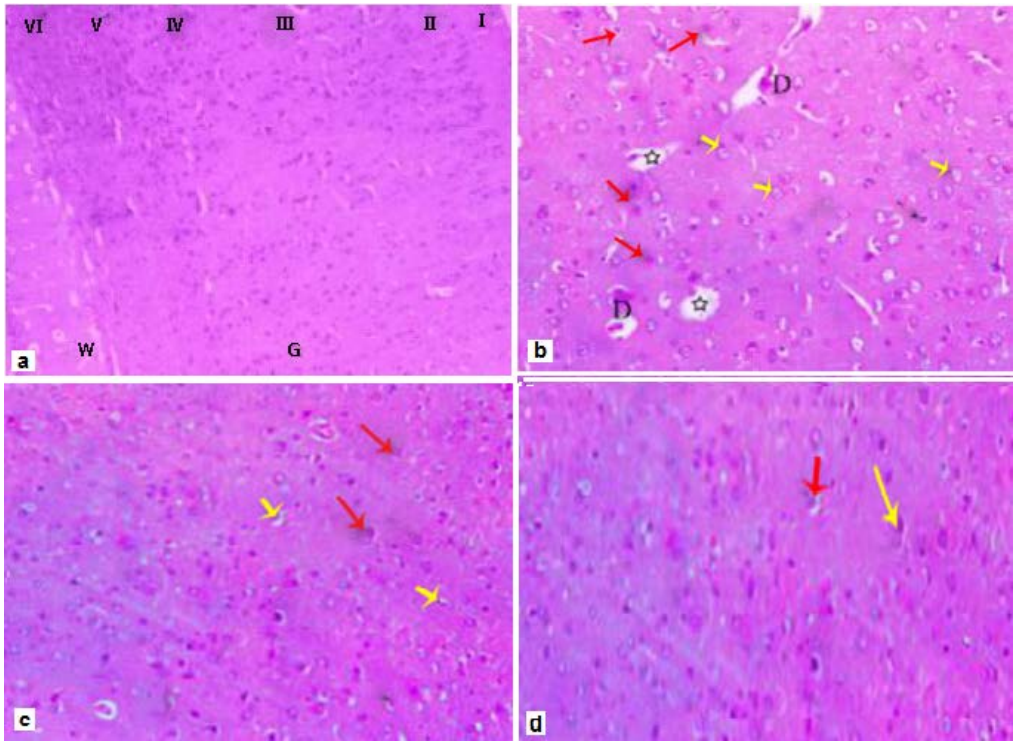

Figure 13: Light photomicrographs of rat brain stained with hematoxylin and eosin (scale bar: 100 μm), representing: a) showing normal brain architecture and Typicall layers of cereberal cortex, b) section of brain from gold nanoparticles injected group showing Disorganization of the cortical layer. Degenerated neurocytes (D) neuronal cells showed pyknotic nuclei and scanty eosinophilic cytoplasm (apoptotic cells) (yellow arrow))and slight vacuolation of neuropirn (*) and few red neurons (red arrowed) . c) .gold nanoparticles+ Omega 3supplemented group (X100) Less degenerative changes in the cereberal cortex (near normal tissue. D) section of pre supplementation of omega 3 + GNPs (X100)showing Regression of the total degenerative changes in the cereberal cortex.

Discussion

GNPs had been steadily carried out inside diagnosis, imaging and drug shipping to the central nervous system. The precept reason behind this lies in their straightforwardness for delivery via those blood brain barrier (BBB), while the mechanisms of NPs delivery throughout BBB has not been elucidated in reality. The NPs might cooperate with the low density lipoproteins (LDL) on the endothelial cells and then get internalized. The NPs can also get into the CNS by manner of totally bypassing the BBB at the median eminence, lamina terminalis, or the location postrema. This type of deposition added about oxidative stress, infection and depleted neurotransmitters in the striatum each one such factors extend the risk closer to the development of neurodegenerative disease [15].

Consequently, 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 [16].

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 [17].

Nanoparticles are recognized to set off reactive oxygen species (ROS) production, leading to an oxidative pressure 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 [18].

Within this study, gold nanoparticles caused substantial technology of oxidative stress inside the brain. This is evident through an extended malondialdehyde manufacturing observed with the aid of a widespread decrease ($p < 0.001$) of glutathione peroxidase inside the brains of GNP intoxicated rats even as compared to control organization.

The apparent manufacturing of ROS and lipid peroxidation took place in the brains of the rats treated with goldnanoparticles indicating that these nanoparticles underwent intense oxidative stress. In addition, Tio2 nanoparticles have extra biological activity to produce ROS in mouse brains. It's been said that nanoscale Tio2 may be phagocytized through neurons and microglia, which then launched ROS [19].

Activity of G-Px become notably inhibited inside the rat brains in nanoparticles handled organization. G-Px has been pronounced to shield the cellular in opposition to peroxidative damage. On this observe the inverse relationship between MDA and G-Px may imply an impairment of antioxidant defenses in the brain by means of the free radicals generation via GNPs [20].

Pre and co-supplementation of omega-3 with AuNPS intoxication recorded significant reduction in MDA and increased GPx levels versus control and non -supplemented one at $p<0.05$, which replicate the useful antioxidant effect of omega-3 in the neural tissues which is more liable to lipid peroxidation. It's far widely recognized that neural tissue is poor in antioxidant enzymes, thus it's far vital to boom antioxidant enzyme ability for primary protection towards free radical injury. Avramovic et al. have shown reduced level of MDA and increased activity of antioxidant enzymes after omega-3 supplementation in elderly brain [21]. It is of the same opinion with our effects where omega-3 supplementation in GNP intoxicated rats ameliorated MDA attention in brain tissue. It's miles widely recognized that DHA is taken up by using the brain and is included into the neuronal membranes [22].

Alternatively pronounced significant reduction in MDA and elevation of GPx were recorded in animals pre- treated with omega-3 before GNPs intoxication versus all other treated groups at $p<0.05$ suggesting the prophylactic effect of omega-3 FA in neuronal tissues.

Equal observation was noticed in traumatic brain damage models supplemented with omega-3 polyunsaturated fatty acids (PUFAs) which confirmed significant reduction of lipid peroxidation, nucleic acid and protein oxidation, thereby selling neuronal and glial cell survival [23]. Therefore, omega-3 FA intake might be considered as a therapeutic choice to lessen the secondary neuronal damages initiated through the usage of nanoparticles.

Concerning to DNA damage biomarkers, significant increase in caspase-3 alongside 8-OHdG might also imply activation of apoptotic machinery within the brain after nanoparticle injection. Zehendner *et al*., (2011) have additionally implicated caspase-3 in numerous neurodegenerative disorders [24].

Oxidative stress may seem as one of the predominant motives for DNA harm. Reactive oxygen species produced over metabolizing cells ought to assault DNA base guanine forming the 8-OHdG lesions, which is understood with want mutagenic opportunity what's extra consequently applied routinely as a biomarker for carcinogenesis [25]. GNPs have been accounted for to actuate DNA harm stated to result in single -strand lesions in human lung fibroblasts [26]. In response to this DNA damage, the cells both initiate DNA restore mechanisms or invoke cell cycle arrest and apoptosis. Caspases are critical mediators of programmed cellular loss of life (apoptosis). Caspase-3 is vital for ordinary brain improvement, and is necessary for apoptotic chromatin condensation and DNA fragmentation in all cell types tested [27].

Pre and co-supplementation with omega-3 to GNPs treated animals alleviate oxidation of DNA in brain tissues, the pronounced mitigating effect was significant versus control and GNPs groups and versus each other at $p<0.05$. However, pre-supplementation with omega-3 has pronounced anti – apoptotic effect on caspase 3 to nearly reach the control level than post-supplementation. Indicating that omega-3 FAs involve in the regulation of apoptotic signaling pathways during stress. The protection provided by omega-3 FAs was associated with their ability to prevent increases in the level of pro-apoptotic basal cell lymphoma protein-2 (Bcl-2)-associated X protein (Bax) in the cerebellum. Omega-3 FAs increased the levels of anti-apoptotic proteins like Bcl-2 and Bcl-extra-large (Bcl-x(L) [28].

Meanwhile, proinflammatory cytokins, Tumor necrosis factor alpha (TNFα) and NFkB showed remarkable significant elevation flowing injection with AuNPs versus control at $p<0.05$. The levels were ameliorated in pre and co-supplemented omega-3 groups. The pronounced anti-inflammatory effect of omega-3 was more significant in pre supplemented groups than co- supplemented one at $p<0.05$ as compared to other groups. It should be noted that pre- supplementation with omega-3 induced marked prophylactic impact.

Nanoparticles also are known to up-modify the transcription of numerous pro-inflammatory genes, inclusive tumor necrosis factor -α and IL(interleukins)-1, IL-6 and IL-8, by way of of enhancement nuclear factor -kappaB (NF-κB) signaling. Those

consecutive molecular and cell occasions are diagnosed to motivate oxidative stress observed through excessive cellular genotoxicity after programmed cellular loss of life [29] that may be clinched alongside facts with our results.

NF-kappa B is one of the most vital transcription factors involved in inflammatory response and upregulation of gene encoding of inflammatory cytokines, adhesion molecules, and COX-2. Evidences indicated that omega-3 decreases expression of adhesion molecules and manufacturing of inflammatory cytokines and COX-2 metabolites, and a common mechanism would be the impact on the NFkB system [30].

Oxidative neurotoxicity induced inhibition in AChE leading to accumulation of AChE in the brain. In the present study GNP injected rats displayed significant reduction $p<0.05$ in the AChE activity compared with the controls and previous studies indicate that antioxidants are reported to exhibit anti AChE inhibitory activity [31]. Similarly, in our study Omega-3 FA intervention effectively restored the altered AChE level to normal which might be due to its antioxidant and free radical scavenging potential [32].

It was recognized that that gold nanoparticles are initially protected by citrate and possess negative charges, and thiocholine is then able to substitute the citrate on the surfaces of gold nanoparticles; as a result, cross linking or aggregation of interparticles could occur because of the electrostatic interactions and gold thiols interplay. Obviously, the degree of cross linking or aggregation of gold nanoparticles is depending on the concentration of thiocholine in the system. This mechanism may additionally describe the lower AChE activity after the injection of properly characterized gold nanoparticles as a result of gold thiols interaction. Similarly, it has been shown that silver and iron oxide nanoparticles extensively inhibited brain AChE; those observations recommended that the metalic nanoparticles would possibly have an effect on the various steps in the metabolic pathways of the neurotransmitters thru quit product inhibition [33].

MAO has the potential to limit the action of many crucial neurotransmitters, oxidatively deaminate neurotransmitter and xenobiotic amines thereby putting in place the basis of speedy repetitive response. MAO assumes a paramount component to catabolizing the neuroactive amines. Within the introduced investigation, MAO in brain tissues expressed significant reduction at $p<0.05$ in GNPs group in comparison with the manipulation. The decreased level of MAO observed in the present study suggests that NPs have the ability to attain the CNS and impair its characteristic [34]. Formerly, it has been shown that the management of titanium dioxide nanoparticles significantly reduced the level of monoamine neurotransmitters within the brain of mice. It also substantially decreased the iron content inside the handled mice which plays an essential physiological role in neuronal procedures together with myelination, synaptogenesis, behavior and synaptic [35].

The improvement in AChE amd MAO levels in the exceptional studied organizations of rat brain treated with omega-3 as a neuroprotective supplement (pre and co- supplementation) towards gold nanoparticles neurotoxicity, could find a help via considering a preceding study which recorded a neurotransmitter regulating and neuroprotective results of omega-3 [36]. Apparently, omega-3 supplementation is associated with will increase in hippocampal serotonin and promotes beneficial outcomes on anxiety, cognitive and depressive-like behaviors in rats subjected to a restraint stress protocol [37].

It is well known that MAO catalyzes the oxidative deamination of many biogenic amines. This may explain the improvement of monoamine oxidase activity in gold nanoparticles injected group supplemented with omega-3 in our study. Degenerative changes were markedly intensified in the brain tissues of GNPs injected rats. These include marked cortical layers disorganization, vacuolated foci with cellular loss, intense eosinophilic staining of neuropil, nonspecific inflammatory cells infiltration, and increased number of apoptotic and red neurons. Treatment with omega-3 post and pre injection of GNPs showed marked improvement of brain tissues which return towards normal morphology as evidenced by remarkable regression of the total degenerative changes but still different when compared with control. Greater improvement of pathological changes is obvious in rats that received pre -treatment of omega-3FA. This result may predict the beneficial synergistic protective impact of omega-3 against brain damage induced by GNPs.

The observed pathological changes inside the specific studied corporations inclusive of neuronal cellar degeneration, irritation and neurotoxicity due to gold nanoparticles injection may be supported via thinking about the preceding study of Bai, *et a*l. [38]. wherein, apparent versions have been determined inside the cerebral cortex and hippocampus tissues after intranasal instillation of copper nanoparticles for 21 days in mice. They mentioned the presence of damaged neurons with shrunken cell bodies, deeply stained pyknotic nucleus with triangular or elongated profile and the absence of nucleoli. It would have been confirmed that surface charge of gold nanoparticles might count on a paramount component in prompting apoptosis. Negative gold nanoparticles, increase the negative charge on the outer membrane, which turns on a disturbance in mitochondrial membrane capacity. The mitochondria compensate via discharging calcium ions that had been stored inside the matrix of the mitochondria, and the spike in calcium induces apoptosis [39]. This final results could probably foresee the beneficial effect of omega -3 as a dietary supplement in the management of diverse disorders wherein oxidant/antioxidant stability is disturbed in brain tissue.

Conclusion

The obtained outcomes have proven omega-3 likely to be responsible for brain for tissue safety in opposition to GNP neurotoxic injury in rats through enhancement resistance to free radical attack and down regulation of immuno-inflammatory mediators, DNA damage, and CNS enzymes. The biochemical results changed into supported by using hestopathological findings.

Curiously, pretreatment with omega-3 is more powerful in modulating most of the studied parameters to near-normal levels compared to co- supplementation.

Acknowledgement
This work was supported by the Deanship of Scientific Research (DSR), King Abdulaziz University, Jeddah, under grant No. D-001-363-1438. The authors therefore gratefully acknowledge the DSR technical and financial support.

Authors' contribution
All the authors have contributed equally. All the authors have read and approved the final manuscript.

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