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# Evaluation of the Potential Neurotoxicity of Gold Nanoparticles in the Different Rat Brain Regions

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**Abstract**

The present study aims to investigate the potential adverse effects of gold nanoparticles (Au NPs) in the cortex, hippocampus, striatum, midbrain, cerebellum and medulla of adult male Wistar rat through the estimation of some oxidative stress parameters and acetylcholinesterase (AChE) activity. Rats were divided into two main experimental groups. Animals of the 1st and 2nd groups were intraperitoneally injected with a single dose (100 µg/kg body wt) of ˷ 20 nm Au NPs and decapitated after 24 hours and two weeks of injection, respectively. Control animals were injected with saline solution and sacrificed simultaneously with the treated groups. The present data revealed that Au NPs induced several significant changes in the levels of GSH and NO and GST activities in the brain areas investigated. These changes were more prominent after 24 hours than after two weeks of injection and varied according to the brain region examined. However, these alterations did not induce lipid peroxidation except for the cerebellum and medulla after 24 hours only. In addition, Au NPs induced significant decreases in cortical and hippocampal AChE activities after 24 hours.

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However, significant increases in cortical and cerebellar AChE activities were recorded after two weeks. In conclusion, although most of the early biochemical changes induced by Au NPs injection were ameliorated after two weeks, careful must be taken into consideration in utilization of gold nanoparticles in biological applications especially with the particle size that can penetrate the BBB.

***Keywords:*** nanoparticles; oxidative stress; AChE; brain; rat.

## 1. Introduction

Recently, nanoparticles (NPs) have been widely utilized in biomedical and biological applications such as deliver of therapeutic agents [1,2,3], photothermal therapy, bioimaging and biosensing [4,5]. However, the biological toxic effects of NPs cannot be ruled out [6, 7], especially, they can pass through the blood-brain barrier (BBB) and enter the central nervous system [8, 9,10].

Gold nanoparticles (Au NPs) have attracted scientific and technological attentions in biomedical applications due to their distinctive physical and chemical properties such as ease of synthesis, chemical stability and excellent optical properties [11,12,13], in addition to biocompatibility and ease of conjugation to biomolecules [14,15,16, 17]. Therefore, Au NPs provide an excellent tool for cancer diagnosis and treatment [18,19, 20], as well as transportation and delivery of therapeutic drugs [21,22,23,24]. Moreover, small size of Au NPs and their ability to cross the BBB are especial characteristics for delivering drugs, genes and other small molecules into the brain [25,26], for treatment of several neurological diseases [27].

Owing to the wide use of Au NPs in the biomedical applications, their cytotoxicity must be carefully investigated. Unfortunately, the results of these studies did not yield univocal reports. Connor and his colleagues [28] reported that Au NPs are taken up by human cells without cytotoxic effects. It may seems that the safety of Au NPs is due to the inert and nontoxic gold core in addition to their high biocompatibility [28]. However, very small gold-cluster may fit into the grooves of DNA-molecules [29], induce oxidative stress [30], and hence cause cytotoxicity. It has been found that Au NPs induced release of nitrogen oxide into serum [31], and cause damage to cells in vitro through oxidative stress [32].

The brain is more susceptible to oxidative stress due to the high utilization of oxygen, high levels of polyunsaturated fatty acids and the abundance of redox-active transition metal ions [33]. In addition, there are relatively low levels of reduced glutathione (GSH), which plays an important antioxidant role in the elimination of free radicals [34].

The present study aims to investigate the early and delayed potential adverse effects of intraperitoneal injection (i.p.) of ~ 20 nm Au NPs in different rat brain regions through estimation of some oxidative stress parameters and acetylcholinesterase (AChE) activity.

## 2. Materials and Methods

### *2.1. Experimental animal*

The experimental animals used in the present study were adult male Wistar rats weighing 150-200 g. They were given standard laboratory diet and water ad libitum. The animals were maintained under fixed appropriate conditions of housing and handling. Experimental protocols and procedures used in this study were approved by the Cairo University, Faculty of Science Institutional Animal Care and Use Committee (IACUC) (Egypt), (CUFS/F/06/13). All the experimental procedures were carried out in accordance with international guidelines for the care and use of laboratory animals.

### *2.2. Chemicals*

Stabilized suspension of Au NPs (˷20 nm diameter) in 0.1 mM PBS was purchased from Sigma-Aldrich Co. Thiobarbituric acid (TBA) and reduced glutathione were purchased from Sigma Aldrich, Germany. 1-Chloro- 2, 4-dinitrobenzene (CDNB) was purchased from Sigma Aldrich, St. Louis, USA. Trichloroacetic acid (TCA) was obtained from SDFCL (SD Fine-Chem Limited), Egypt. Potassium phosphate buffer pH 7.4 (50 mM/L, Triton · 0.1%, EDTA 0.5 ml), potassium phosphate buffer pH 6.5 (100 mM/ L) were obtained from Bio Diagnostic Co., Giza, Egypt. All other reagents were analytical grade reagents purchased from Sigma Chemical Co. (St. Louis, MO, USA).

### *2.3. Characterization of Au NPs*

#### *2.3.1. Dynamic light scattering (DLS)*

By dynamic light scattering (DLS) technique using the Malvern Zeta Sizer model 1000HSa, the hydrodynamic diameter of gold nanoparticles was determined. The instrument calculates the average size of the nanoparticles depending on the measured time-dependent fluctuations of the light scattered by nanoparticles.

#### *2.3.2. Zeta potential analysis*

Using Zetasizer Nano ZS90 (Malvern Instruments, UK), the zeta potentials and electrophoretic mobilities of gold nanoparticles were measured. The gold nanoparticles solution was placed in the sample holder and a laser beam at 633 nm was localized on the sample. Scattered light was detected by a photomultiplier tube.

#### *2.3.3. UV- visible spectroscopy*

Using a double beam UV–vis spectrophotometer (Uv-160A, Shimadzu, Japan) in the range of 400–800 nm, with a resolution of 1 nm using a 1 nm slit width and a 0.3 nm/s scan rate, the absorption spectrum of gold nanoparticles was recorded**.**

#### *2.3.4. Transmission electron microscope (TEM)*

Gold nanoparticles were analyzed by negative stain electron microscopy using (JEOL JEM.1400 Electron microscope). In the center of carbon coated 300-mesh copper grids, 1-2 µl drop of the solution was placed in a formvar support grid and allowed to air dry. The sample was visualized using TEM microscope operated at

200kV and the image was obtained.

### *2.4. Experimental design*

Adult male Wistar rats were divided into two main experimental groups. Each main group was subdivided into two subgroups (control group and treated group). The treated animals of both 1st and 2nd groups were intraperitoneally injected with a single dose (100 µg/kg body wt) of ˷ 20 nm Au NPs. Control animals were injected with saline solution. Animals of the 1st experimental group were suddenly decapitated after 24 hours of injection. Meanwhile, animals of the 2nd group were suddenly decapitated after two weeks of injection. Control animals were sacrificed simultaneously with the treated groups.

### *2.5. Handling of tissue samples*

The animals were suddenly decapitated and the brain of each animal was quickly removed and rapidly transferred to an ice-cold Petri dish. Each brain was dissected to obtain the cortex, hippocampus, striatum, midbrain, cerebellum and medulla. Each brain region was divided into two equal halves, weighed and kept at –20ºC until analyzed. The right half of each brain region was homogenized in 5% w/v 20 mM phosphate buffer, pH 7.4, centrifuged and the supernatant was utilized for the analysis of the levels of malondialdehyde (MDA), reduced glutathione (GSH) and nitric oxide (NO) and activities of glutathione-s-transferase (GST) and acetylcholinesterase (AChE).

#### *2.5.1. Determination of lipid peroxidation*

Lipid peroxidation was measured by determining the level of malondialdehyde (MDA) as indicated by the thiobarbituric reactive species (TBARS) in the tissues according to the method of Ruiz-Larrea and his colleagues [35]. Thiobarbituric acid react with the thiobarbituric acid reactive substances to produce a pink colored complex whose absorbance is read at 532 nm in a Helios Alpha Thermo spectronic (UVA 111615, England).

#### *2.5.2. Determination of reduced glutathione level*

Reduced glutathione (GSH) was estimated according to Ellman's [36]. Ellman´s reagent is reduced by –SH groups of GSH producing 2-nitro-s-mercaptobenzoic acid. The intense yellow color of nitromercaptobenzoic acid anion has an absorbance is measured spectrophotometrically at 412 nm.

#### *2.5.3. Determination of nitric oxide level*

According to the method of Moshage and his colleagues [37], nitric oxide (NO) level was estimated as nitrite using Griess reagent. Nitrite is converted to a deep purple azo compound after the addition of Griess reagent. The absorbance is measured spectrophotometrically at 450 nm.

#### *2.5.4. Determination of glutathione-s-transferase activity*

Glutathione-S-transferase (GST) was determined according to the method of Habig and his colleagues [38]. It measures the conjugation of GSH with 1-chloro-2,4-dinitrobenzene. This conjugation is accompanied by an increase in the absorbance at 340 nm, the GST activity being directly proportional to the rate of absorbance increase.

#### *2.5.5. Determination of acetylcholinesterase (AChE) activity*

The procedure used for the determination of AChE activity was a modification of the method of Ellman and his colleagues [39] as described by Gorun and his colleagues [40]. It measures the thiocholine produced as acetylthiocholine is hydrolyzed. Thiocholine is reacted with the -SH reagent 5,5'-dithiobis-(2-nitrobenzoic acid) (DTNB), which is reduced to thionitrobenzoic acid producing a yellow colored anion whose absorption was read immediately at 412 nm.

### *2.6. Statistical analysis*

The data were expressed as means ± standard error of the mean (S.E.M.) Data were analyzed by Student's t- test. The difference between means was significant at $p < 0.05$.

## 3. Results

### *3.1. Dynamic light scattering (DLS)*

Using DLS, the measured mean diameter of gold nanoparticles was found to be 20 nm ± 1 nm.

### *3.2. Zeta potential analysis*

The mean of the zeta potential for gold nanoparticles was found to be -0.09 mV.

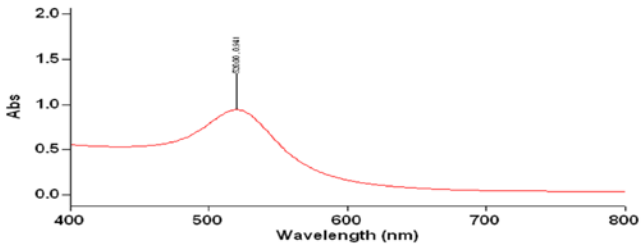


**Figure 1:** Visible absorption spectrum for gold nanoparticles

### *3.3. UV- visible spectroscopy*

Gold nanoparticles showed a specific absorption peak at 520 nm as seen in Figure 1.

### *3.4. Transmission electron microscope (TEM)*

As depicted from Figure 2, using TEM, gold nanoparticles showed uniform spheres of diameter of about 20 nm.

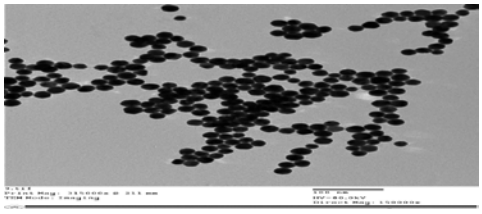

**Figure 2:** Gold nanoparticles ultrastructure

### *3.5. Biochemical parameters*

Tables (1-5) represented the effect of single i.p. injection (100 µg/kg body wt) of ˷ 20 nm Au NPs on some oxidative stress parameters and AChE activity in different brain areas of adult male albino rats after 24 hours (early effect) and two weeks (late effect) of injection.

**Table 1:** Effect of i.p. injection of 20 nm Au NPs on MDA level (nmol/g tissue) in different brain areas of adult male Wistar rat

| Brain area | After 24 hours | | | After 2 weeks | | |
|---|---|---|---|---|---|---|
| | **Control** | **Treated** | **T-test** | **Control** | **Treated** | **T-test** |
| **Cortex** | 4.36 ± 0.13 (7) | 4.38 ± 0.30 (7) | n.s. | 3.61 ± 0.35 (6) | 3.90 ± 0.27 (6) | n.s. |
| **Hippocampus** | 18.61 ± 1.56 (5) | 18.56 ± 0.99 (5) | n.s. | 13.64 ± 0.90 (5) | 15.17 ± 1.50 (5) | n.s. |
| **Striatum** | 14.81 ± 1.26 (6) | 14.52 ± 0.87 (6) | n.s. | 16.84 ± 2.41 (5) | 24.13 ± 2.75 (5) | n.s. |
| **Midbrain** | 10.59 ± 0.42 (5) | 9.02 ± 0.63 (5) | n.s. | 7.42 ± 0.46 (7) | 6.60 ± 0.55 (7) | n.s. |
| **Cerebellum** | 5.47 ± 0.47 (5) | 8.64 ± 0.28 (5) | * | 7.39 ± 0.45 (6) | 7.73 ± 0.57 (6) | n.s. |
| **Medulla** | 6.77 ± 0.25 (7) | 10.30 ± 1.11 (5) | * | 6.34 ± 0.31 (7) | 6.31 ± 0.19 (6) | n.s. |

**Values represent the mean ± S. E. M. with the number of animals between parentheses.**

**n.s.: non-significant. *: $p < 0.05$ significant.**

As shown in Table (1), MDA levels were non significantly changed in the cortex, hippocampus, striatum and midbrain either after 24 hours or two weeks of Au NPs injection. However, significant increases in MDA levels

were observed in the cerebellum and medulla after 24 hours and these increases returned to the normal like values after two weeks of injection. GSH content recorded significant decreases in the hippocampus and striatum after 24 h of injection, on the other hand, the hippocampal and midbrain GSH content recorded significant increases after two weeks of Au NPs injection. However, non significant changes were recorded in the other brain areas (table 2). Table (3) revealed that NO levels showed significant increases in the cortex and medulla while significant decreases were recorded in the hippocampus and midbrain after 24 h of injection. However, the early significant decrease in midbrain NO level was persisted till the two weeks of Au NPs injection and was accompanied by a significant increase in its level in the cerebellum. Regarding GST activity, Table (4) indicated significant decreases in the cortical, striatal and midbrain GST activities after 24 hours. A significant decrease in hippocampal GST activity was recorded after two weeks of Au NPs injection. Au NPs injection induced significant decreases in cortical and hippocampal AChE activities after 24 hours (Table 5). However, late (after twoweeks) significant increases in cortical and cerebellar AChE activities were recorded.

**Table 2:** Effect of i.p. injection of 20 nm Au NPs on GSH level (mg/g tissue) in different brain areas of adult male Wistar rat

| | **After 24 hours** | | | **After 2 weeks** | | |
|---|---|---|---|---|---|---|
| **Brain area** | **Control** | **Treated** | **T-test** | **Control** | **Treated** | **T-test** |
| **Cortex** | 4.56 ± 0.13 (7) | 4.85 ± 0.12 (8) | n.s. | 4.31 ± 0.11 (5) | 4.68 ± 0.12 (8) | n.s. |
| **Hippocampus** | 34.50 ± 0.58 (5) | 25.33 ± 0.72 (6) | * | 24.09 ± 0.55 (6) | 27.19 ± 0.69 (7) | * |
| **Striatum** | 33.14 ± 0.79 (5) | 25.56 ± 1.54 (5) | * | 26.85 ± 0.88 (6) | 28.12 ± 0.77 (6) | n.s. |
| **Midbrain** | 21.12 ± 1.13 (6) | 19.87 ± 0.42 (6) | n.s. | 18.75 ± 1.17 (7) | 24.17 ± 0.66 (6) | * |
| **Cerebellum** | 11.49 ± 0.31 (7) | 10.83 ± 0.41 (7) | n.s. | 7.53 ± 0.45 (7) | 8.30 ± 0.33 (7) | n.s. |
| **Medulla** | 11.14 ± 0.45 (7) | 11.67 ± 0.82 (6) | n.s. | 6.43 ± 0.33 (7) | 7.49 ± 0.35 (6) | n.s. |

**Values represent the mean ± S. E. M. with the number of animals between parentheses. n.s.: non-significant. *: $p < 0.05$ significant**

**Table 3:** Effect of i.p. injection of 20 nm Au NPs on NO level (µ mole/g tissue) in different brain areas of adult male Wistar rat

| Brain area | After 24 hours | | | After 2 weeks | | |
|---|---|---|---|---|---|---|
| | Control | Treated | T-test | Control | Treated | T-test |
| **Cortex** | 0.23 ± 0.01 (7) | 0.30 ± 0.02 (6) | * | 0.50 ± 0.08 (6) | 0.69 ± 0.05 (6) | n.s. |
| **Hippocampus** | 1.12 ± 0.10 (8) | 0.78 ± 0.07 (7) | * | 0.56 ± 0.04 (5) | 0.68 ± 0.09 (5) | n.s. |
| **Striatum** | 1.65 ± 0.14 (8) | 1.70 ± 0.11 (8) | n.s. | 2.28 ± 0.33 (7) | 3.47 ± 0.56 (7) | n.s. |
| **Midbrain** | 1.31 ± 0.06 (6) | 1.09 ± 0.06 (6) | * | 1.34 ± 0.12 (6) | 0.81 ± 0.09 (6) | * |
| **Cerebellum** | 1.26 ± 0.06 (7) | 1.20 ± 0.04 (7) | n.s. | 1.54 ± 0.08 (7) | 1.96 ± 0.07 (7) | * |
| **Medulla** | 1.82 ± 0.13 (6) | 3.19 ± 0.18 (6) | * | 1.12 ± 0.06 (8) | 1.32 ± 0.07 (5) | n.s. |

**Values represent the mean ± S. E. M. with the number of animals between parentheses.**

**n.s.: non-significant. *: $p < 0.05$ significant.**

**Table 4:** Effect of i.p. injection of 20 nm Au NPs on GST (U/g tissue) activity in different brain areas of adult male Wistar rat

| Brain area | After 24 hours | | | After 2 weeks | | |
|---|---|---|---|---|---|---|
| | Control | Treated | T-test | Control | Treated | T-test |
| **Cortex** | 0.28 ± 0.01 (8) | 0.14 ± 0.01 (7) | * | 0.42 ± 0.03 (7) | 0.44 ± 0.02 (7) | n.s. |
| **Hippocampus** | 1.52 ± 0.15 (6) | 1.31 ± 0.09 (8) | n.s. | 1.72 ± 0.06 (6) | 1.33 ± 0.06 (8) | * |
| **Striatum** | 1.61 ± 0.21 (5) | 0.67 ± 0.03 (5) | * | 1.35 ± 0.08 (6) | 1.33 ± 0.05 (7) | n.s. |
| **Midbrain** | 1.23 ± 0.08 (7) | 0.96 ± 0.10 (6) | * | 1.29 ± 0.12 (5) | 1.09 ± 0.04 (8) | n.s. |
| **Cerebellum** | 0.70 ± 0.04 (8) | 0.60 ± 0.02 (6) | n.s. | 0.48 ± 0.04 (7) | 0.56 ± 0.03 (8) | n.s. |
| **Medulla** | 0.64 ± 0.05 (7) | 0.67 ± 0.04 (7) | n.s. | 0.54 ± 0.04 (8) | 0.63 ± 0.03 (6) | n.s. |

**Values represent the mean ± S. E. M. with the number of animals between parentheses.**

**n.s.: non-significant. *: $p < 0.05$ significant.**

**Table 5:** Effect of i.p. injection of 20 nm Au NPs on AChE activity (µmol SH/g/min) in different brain areas of adult male Wistar rat

| Brain area | After 24 hours | | | After 2 weeks | | |
|---|---|---|---|---|---|---|
| | Control | Treated | T-test | Control | Treated | T-test |
| **Cortex** | 11.49 ± 0.44 (7) | 9.16 ± 0.40 (7) | * | 15.98 ± 0.67 (6) | 19.90 ± 0.37 (5) | * |
| **Hippocampus** | 24.60 ± 2.28 (5) | 18.51 ± 0.76 (5) | * | 22.51 ± 1.33 (7) | 24.12 ± 0.53 (6) | n.s. |
| **Striatum** | 50.12 ± 2.63 (5) | 42.16 ± 3.39 (5) | n.s. | 46.72 ± 2.38 (6) | 43.83 ± 2.09 (6) | n.s. |
| **Midbrain** | 27.84 ± 0.98 (5) | 28.44 ± 0.70 (5) | n.s. | 27.95 ± 1.29 (5) | 25.73 ± 0.85 (6) | n.s. |
| **Cerebellum** | 18.05 ± 0.56 (6) | 19.98 ± 1.22 (5) | n.s. | 18.28 ± 0.73 (6) | 21.47 ± 0.70 (7) | * |
| **Medulla** | 28.44 ± 1.40 (5) | 30.08 ± 1.52 (5) | n.s. | 25.95 ± 1.14 (6) | 28.36 ± 0.72 (8) | n.s. |

**Values represent the mean ± S. E. M. with the number of animals between parentheses.**

**n.s.: non-significant. *: $p < 0.05$ significant.**

## 4. Discussion

The present study investigated the potential neurotoxicity of gold nanoparticles in different brain areas after 24 hours (early effect) and two weeks (late effect) of a single i.p. injection of ~ 20 nm Au NPs. Kim and his colleagues [41] investigated the possibility of 20 nm Au NPs to penetrate the mouse blood-retinal barrier and indicated the presence of 20 nm Au NPs in all retinal layers especially neurons (70%). In addition, Studies of Sonavane and his colleagues [42] have demonstrated that 15–50-nm AuNPs can penetrate the BBB, causing their accumulation in the brain. Furthermore, Cardoso and his colleagues [43] found that acute and chronic i.p. injection of 10 and 30 nm Au NPs induced DNA damage in the rat cerebral cortex.

The present data revealed that ~ 20 nm Au NPs induced several significant changes in the levels of GSH and NO and GST activities in the brain areas under investigation. These changes were more prominent after 24 hours than after two weeks of injection and varied according to the brain region examined. However, these alterations did not induce lipid peroxidation except for the cerebellum and medulla which showed an early increase in MDA level then it returned to normal-like value after two weeks.

Therefore, the present data confirmed the ability of ~ 20 nm Au NPs to penetrate the BBB and exert several alterations in the brain areas under investigation. In addition, the present findings supported the previous study of Li and his colleagues [32] as they indicated that Au NPs exert oxidative stress, which damages cells in vitro. Recently, Siddiqi and his colleagues [44] found that i.p injection of 20 nm Au NPs for 3 days induced oxidative

stress which was evident by increase in lipid peroxidation accompanied by deterioration of the antioxidant enzyme, glutathione peroxidase in the rat brain. Therefore, the present study boosts the results of Siddiqi and his colleagues [44] through investigating the oxidative stress parameters in each brain area of adult male rat.

Data of the present study recorded early significant decreases in hippocampal and striatal GSH levels. These decreases were accompanied by significant decreases in NO level and GST activity in the hippocampus and striatum, respectively. In addition, cortical and midbrain GST activities showed early significant decreases. Shrivastava and his colleagues [45] found that oral treatment with Au NPs for 14 days increased ROS formation and decreased GSH:GSSG ratio in the mouse brain. However, their results revealed increase in the GST activity on the contrary of the present data. This controversy may be due to the difference in experimental animal, study protocol and measurement in whole brain rather than different brain regions.

GSH plays a major role as an antioxidant protecting cells against oxidative stress and xenobiotics, as well as preserving the thiol redox state especially in the CNS [46]. GSH, non-enzymatically, scavenges ROS and, enzymatically, acts as an electron donor in the reduction of peroxides catalyzed by glutathione peroxidase producing glutathione disulfide (GSSG) which is reduced by glutathione reductase back to GSH [47]. Glutathione-S-transferases (GSTs) are a family of enzymes that catalyze the addition of GSH to endogenous and xenobiotic substances which have electrophilic functional groups [48].

Therefore, it could be suggested that the present decrease in GSH level may be due to its consumption in scavenging the rapidly liberated free radicals especially NO in the hippocampus as GSH reacts non-enzymatically with NO [49]. In addition, the decrease in GST activity may be due to the decrease in its substrate, GSH as in the striatum or its exhaustion as a result of catalyzing detoxification reactions as in the cortex and midbrain. Fortunately, these early recorded changes in GSH levels and GST activities exerted successful role in counteracting the liberated free radicals which is evident from non significant changes recorded in MDA levels in the cortex, hippocampus, striatum and midbrain.

In contrast, data of the present investigation recorded early significant increase in the cerebellar MDA level in addition to significant increases in both MDA and NO levels in the medulla after 24 hours of Au NPs. These results indicate the variability of brain regions in their tolerance against the free radicals and therefore, may raise concerns about the safety of Au NPs in medical applications.

In vitro study of Jung and his colleagues [50] demonstrated that Au NPs caused hippocampal CA1 neurons to be more excitable in terms of generating more action potentials which might lead to disruptions in neuronal functions and hyperexcitability in pathological conditions such as seizure. In addition, it has been suggested that brain functions may be affected by accumulation of inhaled AuNPs in the hippocampus [51].

Acetylcholinesterase (AChE) has a substantial role in cholinergic neurotransmission because it causes the hydrolysis of acetylcholine released into the synapse [52]. Results of the present study revealed that a single i.p. injection of Au NPs induced early (after 24 hours) significant decreases in AChE activities in the cortex and hippocampus.

The hippocampus has been a focus of attention in research of temporal lobe epilepsy as it contains several neuronal circuits associated with seizure onset and because it develops, in the time course of the disease, a severe loss of pyramidal cells in CA1, CA3 and the dentate gyrus [53]. Epilepsy has been related to release or overproduction of acetylcholine in the brain, due to a neuronal hyperactivity and/or an excitotoxicity, that might induce a neuronal damage during pilocarpine-induced seizure and status epilepticus [54,55].

Therefore, the present decrease in both cortical and hippocampal AChE activities may reflect an increase in cholinergic neurotransmission in these brain regions which could underlie the reported hippocampal hyper-excitability under the effect of Au NPs [50]. Hence, caution must be taken when using Au NPs in epileptic patients or people susceptible to epilepsy.

On the other hand, the study of Chen and his colleagues [56] demonstrated that a single i.p. injection of 17 nm Au NPs decreased the latency time, which was comparable to the effect of scopolamine treatment, while 37 nm Au NPs showed no significant effect. The authors concluded that the ability of Au NPs to impair cognition in mice was size-dependent and related to the ability of the particles to attack the hippocampus. It has been evidenced that high brain acetylcholine levels are associated with cognitive dysfunction [57]. In addition, elevated hippocampal nitrite level was found to be accompanied by learning of spatial task in rats [58]. Moreover, NO - cyclic guanosine mono- phosphate (NO-cGMP) pathway has been involved in the induction of hippocampal long-term potentiation (LTP) and long-term depression (LTD) which are known to be the predominant mechanisms of learning and memory processes [59].

Therefore, the present decrease in the hippocampal AChE after 24 hours of i.p. Au NPs injection may mediate increased acetylcholine level which could interpret the cognition impairment induced by Au NPs [56], especially in the presence of concomitant decrease in hippocampal NO level.

The present study extended to examine the delayed effect of i.p. injection of Au NPs on the oxidative status as well as cholinergic activity of the brain regions under investigation. Surprisingly, data revealed that most of the early changes in oxidative stress parameters has been ameliorated after two weeks of Au NPs injection even the increased levels of cerebellar and medullary MDA, observed in the early stage, were returned to the normal levels after two weeks.

Furthermore, the early significant decrease in the cortical AChE activity was reversed to significant increase after two weeks of Au NPs injection. This could be an attempt of the brain to relief the state of the early increased cholinergic activity as a result of early inhibited AChE activity observed after 24 hours of Au NPs injection.

## 5. Conclusion

Although most of the early biochemical changes induced by a single injection of Au NPs were ameliorated after two weeks, further studies are recommended to investigate the effect of repeated doses of Au NPs. However, care must be taken into consideration when utilizing gold nanoparticles in biological applications especially with the particle size that can penetrate the BBB.

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