Biointerface Research in Applied Chemistry

Original Research Article   **Open Access Journal**

Modulation of rat plasma kynurenine level by platinum nanoparticles and likely association with oxidative stress

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ABSTRACT

In this study, we investigated whether oxidative stress contributes to activation of kynurenine pathway by platinum nanoparticles (PtNPs). Thirty male Wistar rats with an average weight between 126 – 130 g were randomly assigned into six groups. The negative control group was orally administered distilled water while the other treatment groups respectively received oral administration of either PtNPs (25 and 50 mg/kg bw) singly or in combination with ascorbic acid (100 mg/kg bw). Results revealed that oral administration of PtNPs did not cause lipid peroxidation in rat brain and plasma relative to negative control. In contrast, PtNPs elevated protein carbonyl levels in rat plasma relative to negative control. In the meantime, the level of reduced glutathione (GSH) in rat tissues was maintained when compared with negative control and PtNPs alone. However, plasma GSH was significantly ($p<0.05$) increased by PtNPs at both doses used and co-treatment with ascorbic acid. Oral exposure to PtNPs and ascorbic acid elevated kynurenine level in rat plasma. Taken together, data indicated that PtNPs given alone at the doses investigated might not have caused oxidative stress in rat tissues and plasma but co-treatment with ascorbic acid appeared to potentiate capacity to elevate oxidative stress markers. Further, elevation of kynurenine level in rat plasma by PtNPs might be connected with oxidative stress since PtNPs did elevate protein carbonyl level and co-treatment with ascorbic acid modulated the kynurenine level.

Keywords: *Antioxidant; Nanoparticles; Oxidative stress; Tryptophan degradation.*

1. INTRODUCTION

The use of nanoparticles and nanomaterial is globally expanding. Nanoparticles have several applications in biomedicine; drug delivery, cellular delivery, cellular imaging, fluorescence imaging, antimicrobial potential [1-3]. Platinum and silver nanoparticles are among inorganic nanoparticles that are well investigated. For example, the silver nanoparticles have been reported for antimicrobial properties [1], while the antioxidant properties of platinum nanoparticles alongside its application as theranostic agents particularly in cancer therapy have been reported [4].

Small size and a large ratio of surface area to volume are unique characteristics of nanoparticles. These properties enhance reactivity and permit cellular uptake and interaction [5, 6] capable of causing oxidative stress and/or eliciting inflammatory response [7]. Reports have implicated nanoparticles in cellular injury attributable to their ability to produce reactive oxygen species (ROS) directly or indirectly [8]. Though, reports have demonstrated the capacity of nanoparticles to produce free radicals among which is ROS [9, 10], there is no comprehensive understanding of the consequence of nanoparticles at the cellular level. Recently, Adeyemi *et al* [11] suggests that metal nanoparticles may impact multiple cellular targets. Activation of kynurenine pathway was reported as one of them. Kynurenine is a product of tryptophan degradation. The oxidative degradation of tryptophan to kynurenine is catalyzed by the tryptophan 2,3 deoxygenase (TDO) in the liver and/or indoleamine 2,3-dioxygenase (IDO) in extrahepatic tissues. Kynurenine can cyclize to form quinolinate, which can further be converted to nicotinamide adenine dinucleotide (NAD^+). Investigations have shown that oxidative stress, a result of ROS production can affect immune behavior and neurotransmission levels thereby affecting neurotransmitter synthesis [12, 13]. Free radicals can stimulate the adaptive immune response giving rise to an appreciable impact on tryptophan oxidation. If there is an elevated level of pro-inflammatory cytokines, this can lead to increased activity of IDO thereby directing tryptophan into the production of kynurenine [13] and subsequently kynurenic acid, which is neuroprotective. However, kynurenic acid can further generate quinolinic acid, which is neurodegenerative. While reports have linked stress to activation of the kynurenine pathway [12, 13], we do not know whether nanoparticles affect kynurenine pathway activation through ROS production or not. Such knowledge would contribute to our understanding of the cellular mechanism of nanoparticles. Therefore, this study investigates the effect of nanoparticles on rat kynurenine pathway and its link with oxidative stress.

2. EXPERIMENTAL SECTION

2.1. Chemical and reagents. Platinum nanoparticles (PtNPs), Trolox, kynurenine standard, Ehrlich reagent were products of Sigma-Aldrich (St. Louis, MO, USA). All reagents were of analytical grade and used as supplied.

2.2. Experimental animals. Thirty male Wistar rats with an average weight of 126 – 130 g were used in this study. Rats were obtained from the Department of Biochemistry, University of Ilorin, Ilorin, Nigeria, were kept in plastic cages in well aerated atmosphere and acclimated for two weeks before commencement of the study. Rats were given free access to commercial rat pellet and clean water *ad libitum*.

Handling of animals was humane and consistent with the International Guiding Principles for Biomedical Research Involving Animals. Geneva, Switzerland: (CIOMS, 1985), under the observation of the local Institutional Ethics Committee on Scientific Research with approval and protocol 20032018.

2.3. Animal grouping and treatment protocol. The animals were randomly distributed into six (6) groups of five (5) rats in each group. The rats were given the oral administration of PtNPs singly or in combination with ascorbic acid. Further details of the treatment protocols are;

Negative control: Administered 1 ml of distilled water.

PtNPs 25 mg/kg: Administered PtNPs at 25 mg/kg bw only.

PtNPs 50 mg/kg: Administered PtNPs at 50 mg/kg bw only.

PtNPs 25 mg/kg + ASCO: Administered PtNPs (25 mg/kg bw) plus Ascorbic acid (100 mg/kg bw).

PtNPs 50 mg/kg + ASCO: Administered PtNPs (50 mg/kg bw) plus Ascorbic acid (100 mg/kg bw).

ASCO 100 mg/kg: Administered Ascorbic acid (100 mg/kg bw) only.

Platinum nanoparticles were prepared in distilled water and the treatment was daily for seven days. Selection of doses was premised on previous studies [14].

2.4. Preparation of plasma and tissue homogenates. After the last treatment, rats were fasted overnight and sacrificed under mild anesthesia (diethyl ether). Blood samples from rats were collected in clean EDTA bottles and centrifuged at 5000 rpm (model C5, LW Scientific, GA, USA) for 10 min to obtain plasma which was collected in plain sample bottles and stored frozen until needed for analysis. The brain and liver samples were harvested, cleaned and homogenized in ice-cold 0.25M sucrose solution. The tissue homogenates were used for biochemical analysis.

2.5. Biochemical assays. Biochemical measurements in rat plasma and tissue homogenates were carried out using a UV/Vis spectrophotometer (Jenway, Staffordshire, United Kingdom) where applicable. Total protein was determined according to the method of Gornall *et al* [15], protein carbonyl level was estimated by the method of Castegna *et al* [16] and malondialdehyde (MDA) as a byproduct of lipid peroxidation was determined as previously described by Varshney and Kale [17]. Kynurenine level was determined as reported elsewhere [11], reduced glutathione (GSH) level was determined by the method of Bentler *et al* [18] and the diphenylalanine (DPA) was used for the determination of DNA fragmentation according to the method of Perandones *et al* [19].

2.6. Statistical analysis. Data were analyzed using one-way ANOVA (GraphPad Software Inc., San Diego, CA, USA) and presented as mean value of five replicates ± standard error of the mean (SEM). Differences among the group mean values were analyzed by the Tukey's post-hoc test. Mean values at $p < 0.05$ were taken as significant.

3. RESULTS SECTION

3.1. Average rat weight. Oral exposure to PtNPs either singly or in combination with ascorbic acid had no detectable effect on average rat weight (Fig. 1a and b).
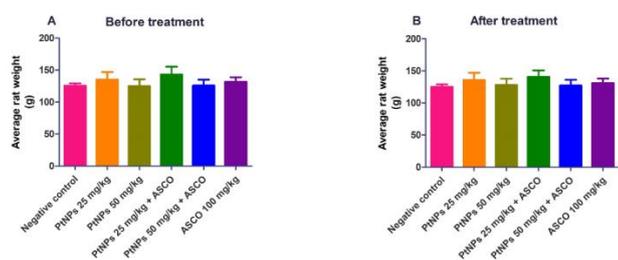

Fig. 1. Effect of oral administration of platinum nanoparticles (PtNPs) on rat average weight; [A] Before experimental treatments and [B] After experimental treatments. Each bar represents a mean value of four replicates ± standard error of the mean (SEM).

3.2. Rat plasma and tissue total protein and kynurenine level. PtNPs at both doses decreased the total protein level of rat plasma when compared to negative control but not significantly. Combination of ascorbic acid with 50 mg/kg of PtNPs visibly raised the total protein level (Fig. 2a). ASCO alone likewise raised the protein level when compared with negative control. In rat liver, PtNPs at all doses used in this study had no effect on the protein level in comparison with negative control (Fig. 2b). However, the combination with ASCO caused a reduction in the protein level. ASCO alone also reduced the protein level when compared with the negative control group.

In rat brain, PtNPs at all doses used in this study reduced total protein level when compared with the control (Fig. 2c). This reduction was sustained even in combination with ASCO. In the meantime, level of kynurenine in rat plasma increased (p<0.05) following oral exposure to PtNPs (25 and 50 mg/kg bw) while co-treatment with ascorbic acid maintained this increase in plasma kynurenine level (Fig. 3a). Ascorbic acid alone also increased the kynurenine level when compared to negative control. Oral exposure to PtNPs (25 and 50 mg/kg bw) as well as co-treatment with ascorbic acid had no appreciable effect on rat liver kynurenine level (Fig. 3b). However, ascorbic acid (100 mg/kg bw) alone elevated kynurenine level in rat liver when compared to negative control (Fig 3b). PtNPs treatment at 25 mg/kg bw did not affect rat brain kynurenine. However, the 50 mg/kg dose reduced the kynurenine level. In addition, co-treatment with ascorbic acid lowered kynurenine level compared with negative control (Fig. 3c).
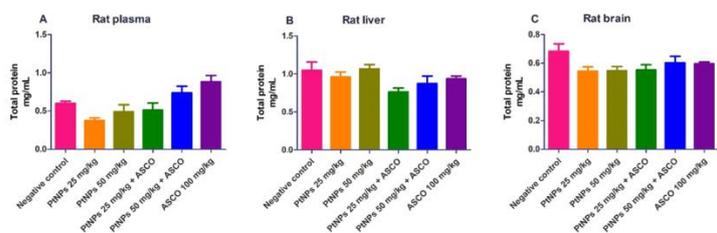
Fig. 2. Effect of oral administration of platinum nanoparticles (PtNPs) on rat total protein level; [A] Rat plasma, [B] Rat liver and [C] Rat brain. Each bar represents a mean value of four replicates ± standard error of the mean (SEM).
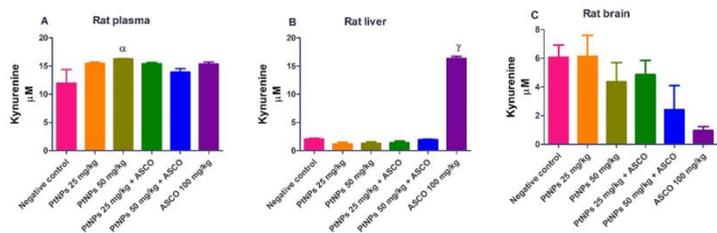

Fig. 3. Effect of oral administration of platinum nanoparticles (PtNPs) on rat kynurenine level; [A] Rat plasma, [B] Rat liver and [C] Rat brain. Each bar represents a mean value of four replicates ± standard error of the mean (SEM). α is significant at $p<0.05$ versus negative control and γ at $p<0.0001$ versus negative control.

3.3. Rat oxidative stress indices. In order to assess if PtNPs predisposes to oxidative stress, markers of oxidative stress such as protein carbonyl, malondialdehyde (MDA) and reduced glutathione (GSH) were assayed for. Results showed that oral administration of PtNPs led to a dose-dependent elevation of rat plasma protein carbonyl level (Fig. 4a) when compared to the negative control (p<0.05). Figure 4b and 4c revealed a significant decrease in protein carbonyl level in the liver and brain (Fig. 4b and c) especially at 50mg/kg dose of PtNPs. Co-treatment with ascorbic did not prevent this reduction. As a further measure of oxidative stress, MDA level as a byproduct of lipid peroxidation in rat tissues and plasma was determined after oral exposure to PtNPs. Results revealed that daily exposure to PtNPs as well as co-treatment with ascorbic acid did not alter MDA level when compared with negative control and PtNPs only respectively in the plasma (Fig. 5a). Lipid peroxidation as shown by the MDA level was increased by both PtNPs and co-treatment with ascorbic acid in the liver while it decreased in the brain (Fig. 5b and c). Meanwhile, ascorbic acid (100 mg/kg bw) alone caused an increase in MDA level of plasma and tissues. Furthermore, the level of reduced glutathione was determined in rat plasma and tissues. Results showed that oral treatment with PtNPs led to significant increases in GSH levels in rat plasma (Fig. 6a). In rat liver and brain, there were no significant changes in GSH level by PtNPs-treatment and ascorbic acid (Fig. 6b and c).

3.4. Discussion. Oral administration of PtNPs as well as co-treatment with ascorbic acid did not affect average rat weight. Likewise, oral exposure to PtNPs had no appreciable effect on rat tissue and plasma. Further, oral exposure to PtNPs might have caused mild oxidative stress in rat plasma and tissues; protein carbonyl in rat plasma increased while lipid peroxidation was elevated in rat brain and liver compared with negative control. The finding does not conform with the antioxidant property of PtNPs as reported elsewhere [14, 20, 21, 22].
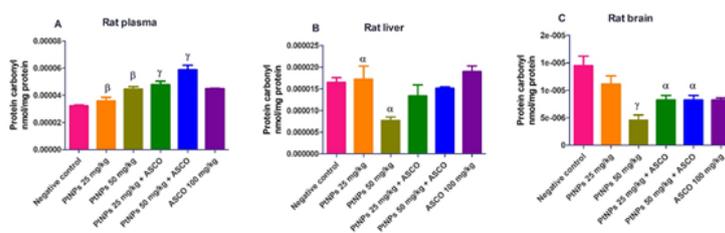

Fig. 4. Effect of oral administration of platinum nanoparticles (PtNPs) on rat protein carbonyl level; [A] Rat plasma, [B] Rat liver and [C] Rat brain. Each bar represents a mean value of four replicates ± standard error of the mean (SEM). α is significant at $p<0.05$ versus negative control and/or PtNPs (50 mg/kg bw), β at $p<0.001$ versus negative control and/or PtNPs (25 mg/kg + ASCO and 50 mg/kg +ASCO), γ at $p<0.0001$ versus negative control.
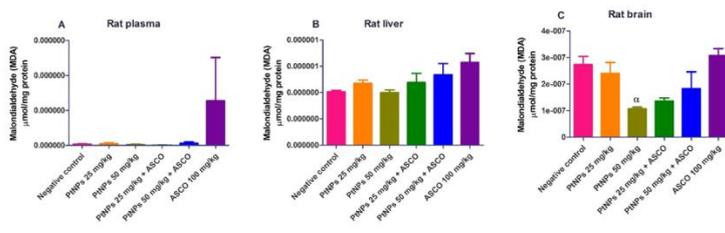

Fig. 5. Effect of oral administration of platinum nanoparticles (PtNPs) on rat malondialdehyde (MDA) level; [A] Rat plasma, [B] Rat liver and [C] Rat brain. Each bar represents a mean value of four replicates ± standard error of the mean (SEM). α is significant at $p<0.05$ versus negative control.
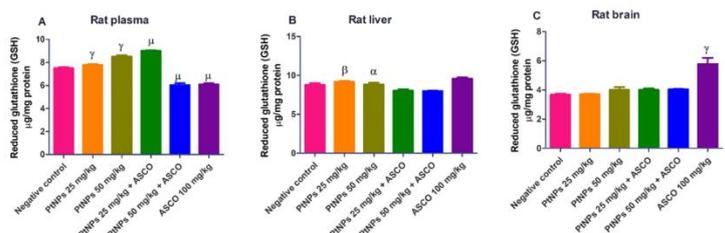

Fig. 6. Effect of oral administration of platinum nanoparticles (PtNPs) on rat reduced glutathione (GSH) level; [A] Rat plasma, [B] Rat liver and [C] Rat brain. Each bar represents a mean value of four replicates ± standard error of the mean (SEM). α is significant at $p<0.05$ versus PtNPs (50 mg/kg bw + ASCO), β at $p<0.001$ versus PtNPs (25 mg/kg + ASCO), γ at $p<0.0001$ versus negative control and/or PtNPs (25 mg/kg + ASCO and 50 mg/kg + ASCO), μ at $p<0.0001$ versus negative control.

Additionally, co-treatment with ascorbic acid raised rat plasma protein carbonyl level in manners that suggest potentiation of oxidative stress. It is possible that ascorbic acid and PtNPs interacts in ways that potentiate capacity to cause oxidative stress. According to Chen et al [23], PtNPs could interact with ascorbic acid blocking its antioxidant activity. Both PtNPs and ascorbic acid have antioxidant properties [22, 23], therefore, it is possible that the duo of PtNPs and ascorbic acid acted in a manner that neutralized their antioxidant potentials. More so, co-treatment with ascorbic acid also altered GSH levels. This may suggest usage of GSH molecules to combat ensuing oxidative stress. Additionally, the fact that oral exposure to PtNPs singly did not lower GSH levels may further underscore that ascorbic acid potentiated PtNPs to cause oxidative stress in rat tissues. Moreover, oral exposure to PtNPs elevated rat plasma kynurenine level and this elevation may be associated with oxidative stress *viz-a-viz* increased protein carbonyls. The finding supports and conforms with earlier investigations which linked oxidative stress to activation of kynurenine pathway [11, 12, 13, 24].

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4. CONCLUSIONS

In conclusion, findings herein revealed that oral exposure to PtNPs might have caused oxidative stress exclusively in rat plasma at the highest dose (50 mg/kg bw) used in this study. Also, the elevation of kynurenine level in rat plasma by PtNPs may be associated with capacity to cause oxidative stress. Additionally, data indicate that co-treatment with ascorbic acid potentiated PtNPs to cause oxidative stress in rat tissues which otherwise was not the case when PtNPs was administered singly. Together, findings contribute to deepening our understanding of the cellular interaction of nanoparticles.

5. REFERENCES

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6. ACKNOWLEDGEMENTS