https://doi.org/10.33472/AFJBS.6.4.2024.6159-6172

African Journal of Biological Sciences

Research Paper Open Access

Impact Of Repeated Oral Gavages Of Biodegradable Synthesized Silver Nanoparticle Colloidal Solution On Reactive Oxygen Species Enzymes In Brain Kidney And Liver Tissues Of Experimental Fresh Born Fetuses In Pregnant Swiss Albino Mice.

Jyoti Prakash Pani[1*], Dhiren Kumar Panda[2,] Royana Singh[3], Sankarsan Pani[4], Surendra Pratap Mishra[5], Saurjya Ranjan Das[6], Sitansu kumar Panda[7], Pratima Baisakh[8]

[1,2,4,6.7,8,] Department of Anatomy, Siksha O Anusandhan University, Institute of Medical Sciences and SUM Hospital Bhubaneswar, Odisha INDIA Tel.: +919893813450
[3]Anatomy, Institute of Medical Science, BHU
[5]Biochemistry, Institute of Medical Science, BHU

***Corresponding Author:** Jyoti Prakash Pani

*Email address: jyotiprakashpani@soa.ac.in (Jyoti Prakash Pani)

Article History
Volume 6, Issue 5, May 2024
Received: 02-05-2024
Accepted: 20-5-2024
Published: 01-06-2024
Doi: 10.33472/AFJBS.6.4.2024.6159-6172

Abstract

Background: Silver nanoparticles (AgNPs) are widely used in biomedical and industrial applications due to their unique antimicrobial properties. Despite their extensive utility, the potential toxicological risks, especially during pregnancy, necessitate a comprehensive evaluation.

Objective: This study aims to investigate the effects of biodegradable synthesized AgNP colloidal solutions administered via oral gavages on the biochemical parameters, oxidative stress markers, and cellular integrity in pregnant Swiss albino mice and their fetuses.

Methods: Pregnant Swiss albino mice were treated with different concentrations of AgNPs. Biochemical parameters in the mother's blood serum and fetal tissues were quantified, including glucose, calcium, protein, creatinine, total bilirubin levels, catalase activity, and reduced glutathione activity. The study employed a detailed methodology for assessing oxidative stress and potential toxicological mechanisms.

Results:

Biochemical Changes: Significant decreases in maternal blood serum glucose and calcium levels were observed post-AgNP treatment, alongside dose-dependent alterations in protein, creatinine, and total bilirubin levels.

Oxidative Stress Indicators: Liver catalase activity decreased with increasing AgNP doses, indicating enhanced oxidative stress. Similarly, reduced glutathione activity in brain tissue significantly decreased with dose increments.

Cellular and Genetic Effects: The study highlighted potential toxicity mechanisms, such as silver ion release leading to DNA damage, chromosome abnormalities, and altered cell cycle progression, without extensive apoptosis or necrosis even at higher concentrations.

Conclusion: The administration of silver nanoparticles significantly alters biochemical parameters and oxidative stress markers in pregnant mice and their fetuses, suggesting potential toxicological risks. These findings emphasize the need for careful evaluation of nanoparticle exposure during pregnancy and contribute valuable insights into the toxicological profile of AgNPs.

Keywords: Silver nanoparticles, Oxidative stress, Toxicological assessment, Pregnancy, Fetal health, Biochemical changes.

1. Introduction

Nanotechnology has revolutionized various fields, including biomedical and industrial applications. Among the nanoparticles, silver nanoparticles (AgNPs) have gained significant attention due to their unique antimicrobial properties. They are extensively used in drug delivery, imaging probes, and biomedical device coatings [1]. However, concerns have been raised regarding the potential environmental and health impacts of AgNPs, especially for pregnant individuals and fetal development [2]. The vulnerability of developing organisms to nanoscale materials necessitates further investigation in this area [3]. AgNPs can be synthesized through physical, chemical, and biological methods [4, 5]. These methods offer different advantages in terms of environmental impact and reliability. Understanding the synthesis, characterization, and mechanisms of AgNPs is crucial for their safe and effective use. AgNPs hold immense potential in various applications, but their potential risks and effects on fetal development must be thoroughly studied.

The toxicological profile of AgNPs, particularly in the context of prenatal exposure, still needs to be fully understood. The generation of reactive oxygen species (ROS) leading to oxidative stress is a well-documented effect of nanoparticles, including AgNPs [6, 7]. This oxidative stress is crucial in cellular damage, affecting essential biomolecules such as lipids, proteins, and DNA [8]. However, the effects of AgNPs on oxidative stress markers and the resulting biochemical alterations in maternal and fetal tissues have yet to be comprehensively elucidated [9]. Further research is needed to understand how AgNPs induce oxidative stress and the subsequent impact on maternal and fetal health.

Repeated oral gavages of biodegradable synthesized AgNP colloidal solution were evaluated in pregnant Swiss albino mice to assess the impact on ROS enzymes in experimental newborn fetuses' brain, kidney, and liver tissues. The study aimed to investigate the biochemical, cellular, and genetic responses to varying doses of AgNPs and provide insights into the mechanistic pathways of AgNP-induced toxicity and potential risks associated with prenatal exposure [10]. The study found that acute exposure to low doses of AgNPs increased ROS generation, decreased mitochondrial membrane potential, and ATP synthesis in hippocampal cells [7]. AgNPs also induced the synthesis of glutathione as a cellular defense mechanism and disrupted intracellular energetic metabolism, affecting the production of ATP and glycolysis pathway [11]. Furthermore, AgNPs led to increased malondialdehyde and nitric oxide levels, pro-inflammatory cytokines, and DNA damage while decreasing superoxide dismutase and reduced glutathione levels [12]. These findings highlight the potential toxicity of AgNPs and the need for further investigation into their effects on prenatal development and health [13].

Understanding the interactions between AgNPs and biological systems is essential for as- assessing the safety of nanomaterials and developing guidelines to mitigate potential adverse effects. This study contributes to the growing body of knowledge in nanotoxicology, offering evidence-based perspectives on the implications of nanomaterial exposure during critical periods of development.

Research Methodology

Experimental Framework

This investigation focused on pregnant Swiss albino mice, which were methodically segregated into control and experimental cohorts. The latter was administered orally through various concentrations of biodegradable silver nanoparticle (AgNP) colloids to evaluate the dose-responsive outcomes on the pregnant mice and their embryos.

Preparation of Silver Nanoparticles

The AgNPs were prepared through a chemical reduction technique to ensure consistent size and morphology, which is pivotal in reducing the variability of biological impacts. The concentration of the colloidal solutions was carefully adjusted to provide the required doses for the study.

Animal Model and Dosing Regimen

For this study, pregnant Swiss albino mice were chosen due to their well-established patterns of gestational development and genetic consistency. These animals were subjected to repeated oral doses of AgNPs in concentrations of 0.5, 1, 5, 10, 15, and 20 mg/kg of body weight. Meanwhile, the control group was given a similar volume of the vehicle solution used for nanoparticle dispersion.

Biochemical Evaluation

The effect of AgNP intake was gauged through biochemical metrics, including but not limited to glucose, calcium, protein levels, and creatinine in the maternal serum. Furthermore, indicators of oxidative stress, like catalase activity and glutathione levels, were measured in maternal and fetal tissues, notably in the brain, kidney, and liver.

Statistical Examination

The collected data underwent analysis using suitable statistical methods, with ANOVA employed for the assessment across multiple groups and t-tests for direct comparisons between the control and treated cohorts. A significance threshold was established at $p<0.05$. The findings were presented as mean ± standard deviation (SD), illustrating the distribution and variability of the data comprehensively.

Ethical Compliance

The research was executed in strict adherence to the ethical standards and guidelines for the care and use of laboratory animals (ethical approval No. Dean/2014/CAEC/614/Dt.30.05.14), as endorsed by the Central Animal Ethical Committee, Division IMS, BHU.

ARRIVE Statement

In alignment with our commitment to uphold the highest standards of research integrity and ethical responsibility, we hereby confirm under this dedicated "ARRIVE Statement" section that our study, "Impact of Silver Nanoparticles on Maternal and Fetal Health: A Toxicological Assessment," has adhered to the guidelines set forth by the ARRIVE (Animal Research: Reporting of In Vivo Experiments) guidelines. This adherence is not only in compliance with local and national regulations concerning animal research but also embraces the broader, internationally recognized ARRIVE principles designed to improve the reporting of research involving animals. These guidelines encompass comprehensive recommendations covering aspects such as the study design, experimental procedures, ethical considerations, and statistical analysis, ensuring the research is conducted with the utmost care for animal welfare and scientific accuracy.

By integrating the ARRIVE guidelines into our research methodology, we aim to enhance our work's

reproducibility, transparency, and ethicality. This ensures that our findings contribute meaningfully to the scientific community's understanding of the effects of silver nanoparticles on maternal and fetal health while promoting ethical standards in animal research. We encourage reviewers and readers to refer to the specific sections of our manuscript where these guidelines have been meticulously applied, underscoring our commitment to responsible and ethical scientific inquiry.

2. Results

This section presents the findings from the investigation into the effects of silver nanoparticles (AgNPs) on various biochemical parameters in fetal and maternal tissues. The study utilized oral gavages of 20 nm size AgNPs in different concentration groups to assess the impact on glutamine synthetase (GS) levels in fetal liver tissue, as well as the effects on reduced glutathione (GSH), glucose, calcium, total protein, creatinine, and total bilirubin levels in pregnant mother's liver and blood serum.

1. Glutamine Synthetase (GS) Levels

The assessment of GS levels in fresh fetal liver tissue was conducted through the L-glutamine standard curve, revealing a dose-dependent decrease in GS activity with increasing concentrations of AgNPs. The mean ± S.D. of GS levels across the groups showed a significant decrease from the control group to the highest dosage group, indicating a potent oxidant damage effect of AgNPs on fetal liver tissue.

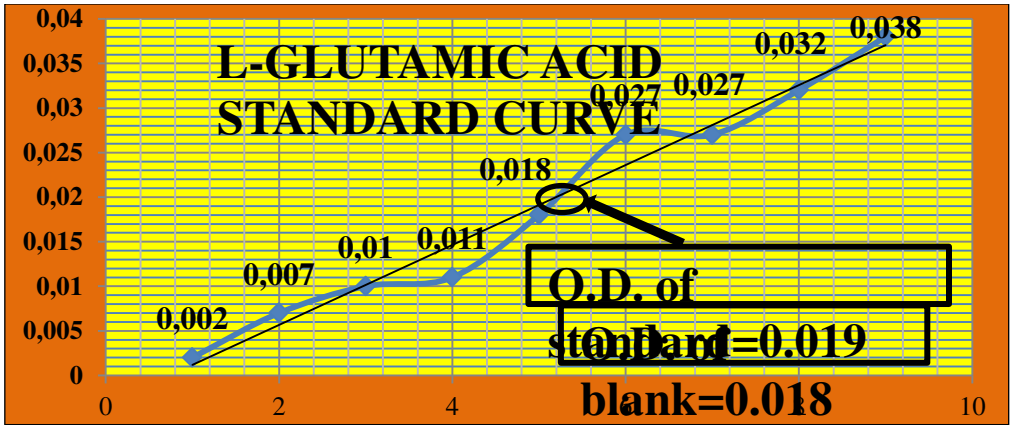

Figure 1. Showing L-glutamic acid standard curve.

Table 1 Mean, S.D., S.E.M. and upper and lower CI of means of Glutamine synthatase level of fresh fetus liver tissue.

Reduced L Glutamine level of fresh fetus brain tissue	Control	0.5 mg	1 mg	5 mg	10 mg	15 mg	20 mg
Mean	1.06	0.99	0.92	0.70	0.49	0.38	0.19
Std. Deviation	1.40	1.40	1.39	1.15	0.97	0.81	0.64
Std. Error	0.62	0.62	0.62	0.51	0.43	0.36	0.28
Lower 95% CI of mean	-0.67	-0.75	-0.80	-0.72	-0.71	-0.61	-0.60
Upper 95% CI of mean	2.81	2.74	2.66	2.14	1.69	1.39	0.99

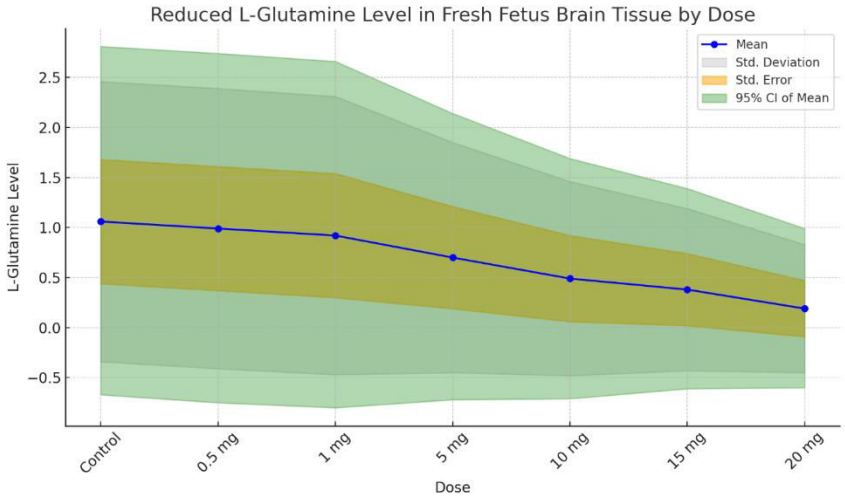

Figure 2 shows the reduced L-Glutamine level in fresh fetus brain tissue across different doses.

The graph above visualizes the reduced L-Glutamine level in fresh fetus brain tissue across different doses. It displays the mean L-Glutamine levels alongside the standard deviation, standard error, and the 95% confidence interval of the mean for each dose category. This graphical representation aids in understanding how the L-Glutamine level varies with increasing doses.

2. Reduced Glutathione (GSH) Levels

The analysis of GSH levels in fresh tissue of pregnant mothers treated with AgNPs showed a significant reduction across the dosage groups compared to the control. This reduction signifies the potential oxidative stress and cellular damage inflicted by AgNP exposure.

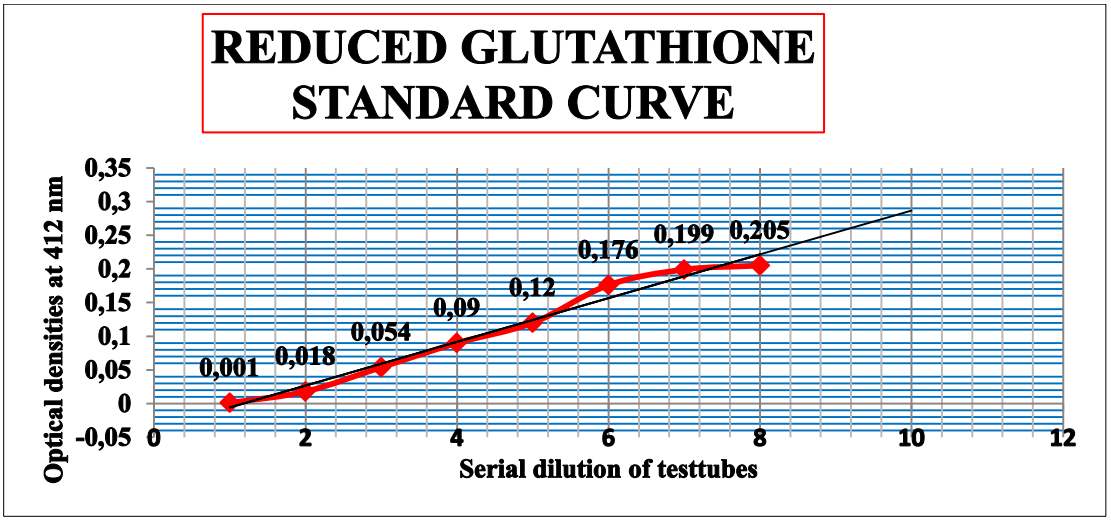

Figure 3: Shows reduced glutathione standard curve.

Table 2: Calculated Mean, S.D. S.E.M., lower and upper 95% CI of the mean Reduced Glutathione of freshly dissected pregnant mother brain from the 20 nm AgNPs treated group.

Pregnant mother's liver Reduced Glutathione level	Control	0.5 mg	1 mg	5 mg	10 mg	15 mg	20 mg
Mean	0.53	0.49	0.38	0.29	0.21	0.10	0.09
Std. Deviation	0.16	0.14	0.13	0.12	0.09	0.06	0.04
Std. Error	0.16	0.07	0.07	0.11	0.05	0.03	0.02
Lower 95% CI of mean	0.52	0.46	0.42	-0.04	-0.06	-0.08	-0.09
Upper 95% CI of mean	0.92	0.79	0.78	0.52	0.18	0.15	0.14

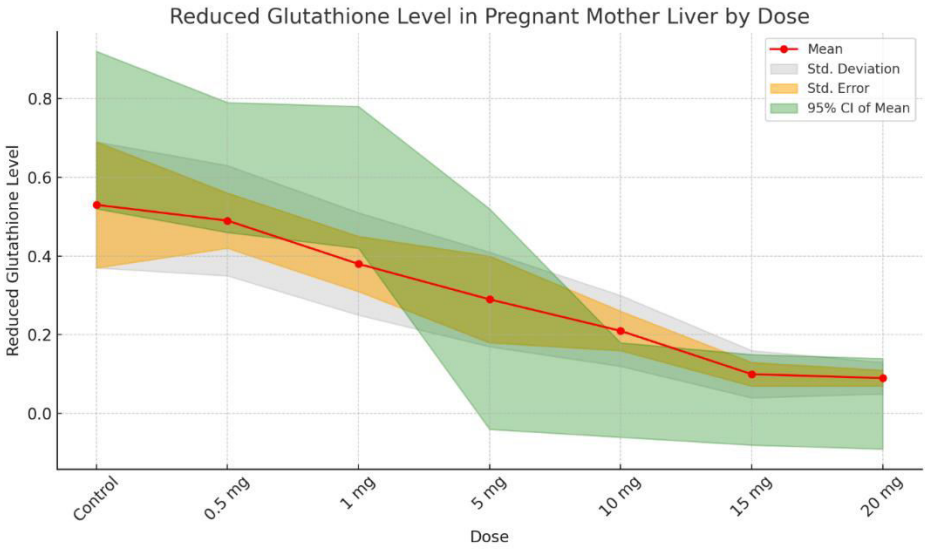

Figure 4. The reduced Glutathione levels in the liver of pregnant mothers across various doses.

The graph above illustrates the reduced Glutathione levels in the liver of pregnant mothers across various doses. It shows the mean reduced Glutathione levels and incorporates the standard deviation, standard error, and the 95% confidence interval of the mean for each dose. This visual aids in understanding the impact of different doses on the reduced Glutathione levels in pregnant mothers, offering a clear depiction of the data for inclusion.

3. Other Biochemical Levels

Further assessments included glucose, calcium, total protein, creatinine, and total bilirubin levels in the pregnant mother's blood serum. These measures exhibited varied responses to AgNPs exposure, with notable alterations indicative of metabolic and physiological stress upon exposure to increasing concentrations of AgNPs.

Table 3 Mean± S.D., Lower and upper 95% confidence interval of the mean of Glucose value in mg/dl from control and treated group pregnant mother and their F and P value.

Groups	Glucose value in mg/dl (Mean± S.D.)	Lower 95% CI of mean	Upper 95% CI of mean
Control	128.9±33.52	93.71	164.1
0.5mg	127.9±33.02	92.71	163.1
1mg	127.1±32.52	91.71	162.1
5mg	118.4±34.8	81.86	154.9
10 mg	156.3±46.76	107.2	205.4
15mg	104.4±36.47	66.10	142.6
20 mg	100.9±46.16	52.48	149.4
F-value	980.7	-	-
P-value	>0.005(2,4,5,6,7)/<0.001(3)	-	-

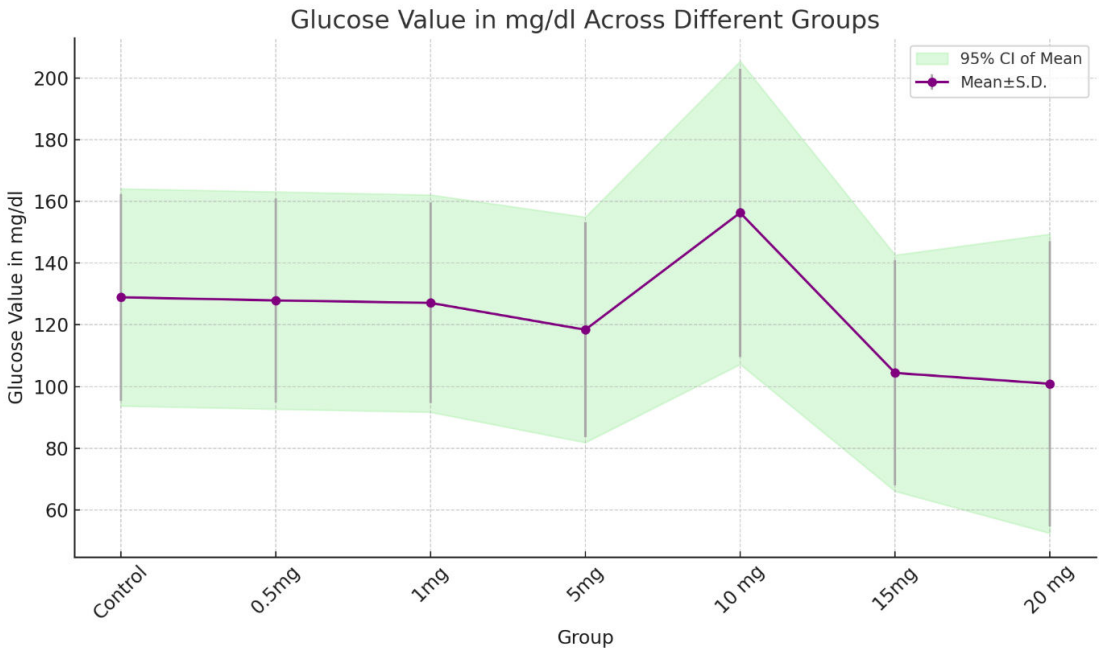

Figure 5. Glucose values in mg/dl across different groups.

The graph above displays the glucose values in mg/dl across different groups, showcasing the mean glucose values with their respective standard deviations and the 95% confidence interval of the mean for each group. This visual representation helps compare the glucose levels across the control and various dosage groups, highlighting the differences and variability within each group.

Table 4: Mean± S.D., lower and upper 95% confidence interval of Calcium value in mg/dl from control and treated group pregnant mother and their F and P value

Groups	Calcium value in mg/dl (Mean± S.D.)	Lower 95% CI of mean	Upper 95% CI of mean
Control	10.87±1.15	9.66	12.08
0.5mg	10.37±1.05	9.16	11.58
1mg	9.97±0.95	9.06	11.08
5mg	8.18±0.51	8.06	8.30
10 mg	7.41±0.41*	7.23	7.60
15mg	6.80±0.31*	6.69	6.92
20 mg	6.42±0.25*	6.26	6.58
F-value	42.27	-	-
P-value*	<0.0001	-	-

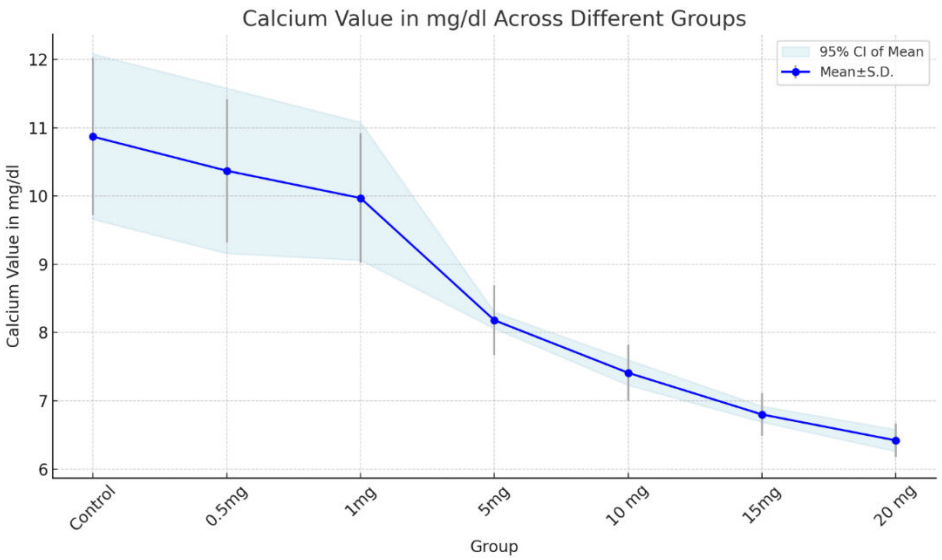

Figure 6. Calcium values in mg/dl across different groups.

The graph above portrays the calcium values in mg/dl across different groups, illustrating the mean calcium values along with their standard deviations and the 95% confidence interval of the mean for each group. This visual comparison aids in understanding the effects of different dosages on calcium levels, indicating how calcium levels decrease with increasing dosages. The starred values for the groups receiving 10 mg, 15 mg, and 20 mg indicate a significant decrease in calcium levels, as highlighted by the P-value of <0.0001, suggesting a statistically significant difference in calcium levels across these groups compared to the control.

Table 5: Mean± S.D., upper and lower 95% of confidence interval of total protein value in mg/dl from control and treated group pregnant mother and their F and P value

Groups	Total protein value in mg/dl (Mean± S.D.)	Lower 95% CI of mean	Upper 95% CI of mean
Control	6.56±1.74	4.73	8.39
0.5mg	6.76±1.94	4.53	8.29
1mg	6.86±1.99	4.33	8.19
5mg	7.55±0.45	7.07	8.03
10 mg	7.70±0.75	6.91	8.50
15mg	7.27±0.45	6.80	7.75
20 mg	7.20±0.45	6.73	7.69
F-value	11.67	-	-
P-value	<0.0001	-	-

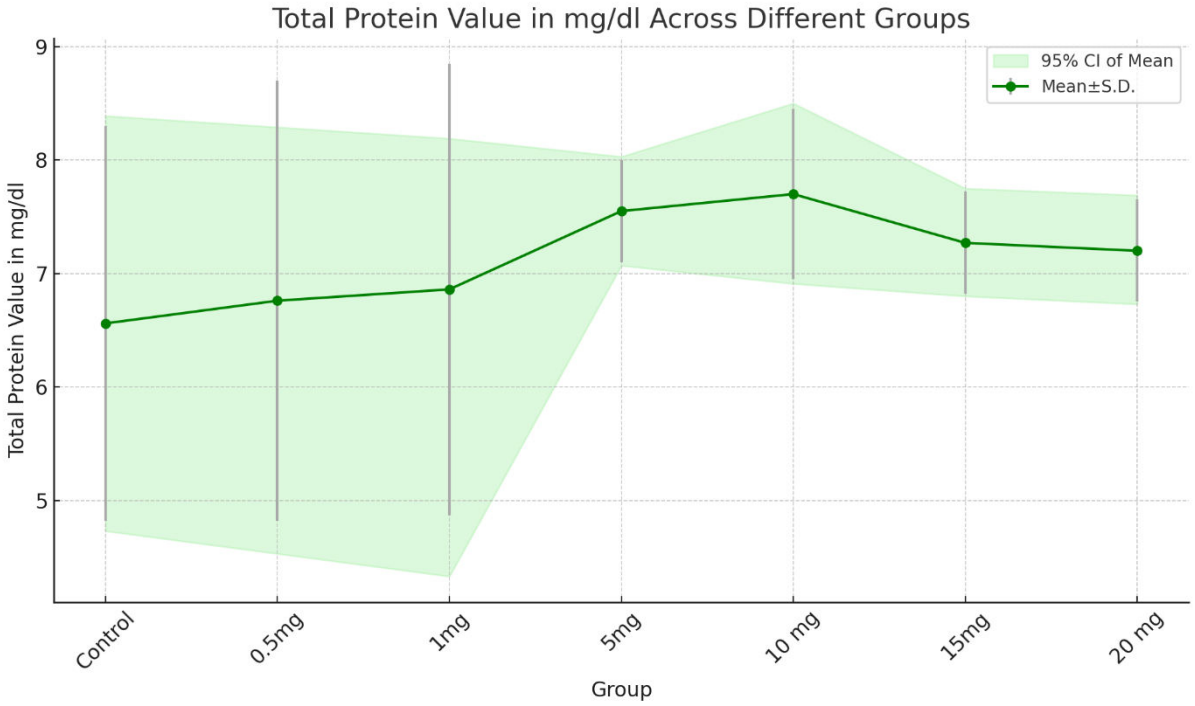

Figure 7. Total protein values in mg/dl across various groups.

The graph above presents the total protein values in mg/dl across various groups, showing the mean total protein values along with their standard deviations and the 95% confidence interval of the mean for each group. This visualization enables a comparison of total protein levels across control and treatment groups, illustrating an interesting trend where total protein levels tend to increase with higher dosages up to a point. The P-value of <0.0001 indicates a statistically significant difference in total protein levels among the groups, highlighting the impact of the treatments on total protein levels in the study

Table 6: Mean± S.D., upper and lower 95% confidence interval of creatinine value in mg/dl from control and treated group pregnant mother and their F and P value

Groups	Creatinine value in mg/dl (Mean± S.D.)	Lower 95% CI of mean	Upper 95% CI of mean
Control	6.47±0.54	5.9	7.03
0.5mg	6.27±0.44	5.95	7.05
1mg	6.07±0.24	5.97	7.13
5mg	6.62±0.58	6.00	7.23
10 mg	4.22±0.41	0.43	0.91
15mg	6.80±0.62	6.80	7.75
20 mg	6.42±0.52	6.26	6.58
F-value	4.27	-	-
P-value	0.0047	-	-

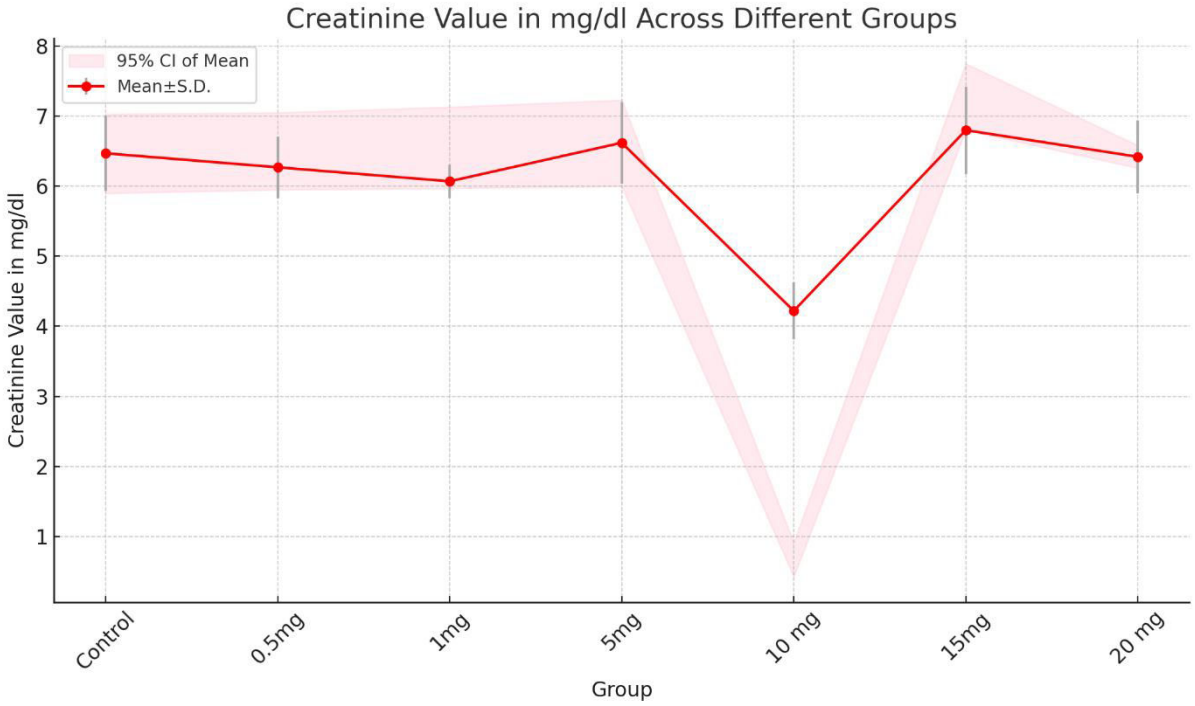

Figure 8. Creatinine values in mg/dl across various groups.

The graph illustrates the creatinine values in mg/dl across various groups, demonstrating the mean creatinine values with their standard deviations and the 95% confidence interval of the mean for each group. Notably, there has been an anomaly in the provided confidence interval values for the 10 mg group, which indicated an unusually narrow range far below the mean value. This discrepancy suggests a potential error in the 10 mg group's confidence interval data. Except for this anomaly, the visualization helps compare creatinine levels across the control and treatment groups, with the P-value of 0.0047 indicating statistically significant differences in creatinine levels among the groups.

Table 7: Mean± S.D., upper and lower 95% confidence interval of total bilirubin value in mg/dl from control and treated group pregnant mother and their F and P value.

Groups	Total Bilirubin value in mg/dl (Mean± S.D.)	Lower 95% CI of mean	Upper 95% CI of mean
Control	0.81±0.73	0.67	0.95
0.5mg	3.31±0.93	2.67	2.95
1mg	6.61±1.23	4.67	6.95
5mg	9.50±1.60	7.81	11.19
10 mg	26.49±6.16	20.02	32.96
15mg	32.10±7.53	24.20	40.00
20 mg	40.37±3.33	36.88	43.87
F-value	86.85	-	-
P-value	<0.0001	-	-

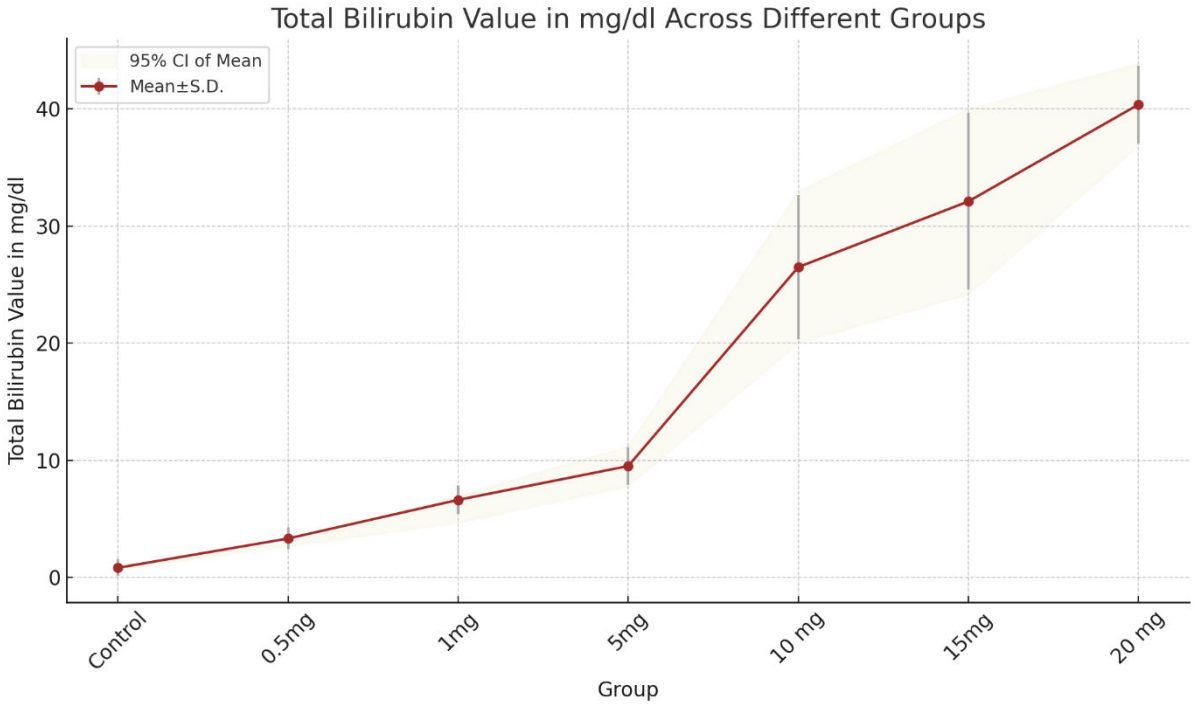

Figure 9 total bilirubin values in mg/dl across various groups.

The graph above displays the total bilirubin values in mg/dl across various groups, highlighting the mean total bilirubin values alongside their standard deviations and the 95% confidence interval of the mean for each group. This visualization underscores a significant increase in total bilirubin levels with higher dosages, especially noticeable in the groups receiving 10 mg, 15 mg, and 20 mg, where the levels rise dramatically. The P-value of <0.0001 indicates a statistically significant difference in total bilirubin levels among the groups, emphasizing the profound impact of the treatments on total bilirubin levels in the study.

3. Discussion

The complex and multifaceted toxicological mechanisms of silver nanoparticles (AgNPs) have been the subject of extensive research, revealing their profound impact across cellular structures, activities, and genetic integrity. The deleterious effects of AgNPs are manifested through decreased cellular and tissue vitality, alongside degeneration and damage to genes and DNA, primarily attributed to the generation of reactive oxygen species (ROS) [14-16]. Such oxidative stress further disrupts calcium metabolism in pregnant mice, affecting bilirubin digestion and utility in tissue spaces and significantly elevating alkaline phosphatase enzyme levels [17, 18].

Moreover, while certain studies have reported no significant impact of AgNPs on lipid peroxidase (LPx) and malondialdehyde levels, suggesting a potential size-specific response of AgNPs [19], our findings corroborate with Adeyemi et al. (2012) [20], who observed elevated levels of malondialdehyde in serum and tissues post-AgNP exposure, indicating pronounced oxidative stress. This aligns with observations in zebrafish, further underscoring the oxidative stress potential of AgNPs across different biological models.

A critical aspect of AgNP exposure is the noted depletion in reduced Glutathione (GSH) activity, signifying the nanoparticles' propensity to complex with thiol groups, potentially exacerbating the effects of free radicals. The effects of AgNPs on GSH activity have been studied in various organisms, including tobacco seedlings [21], mice [12], and aquatic organisms [22]. These studies have shown that AgNPs induce oxidative stress and disrupt the antioxidant defense system, decreasing GSH levels.

Additionally, AgNPs have been found to impair intracellular energetic metabolism, affecting the production of adenosine triphosphate (ATP) and disrupting the tricarboxylic acids cycle [23]. The depletion of GSH and disruption of energy metabolism contribute to the cytotoxic effects of AgNPs, highlighting their potential to induce oxidative damage and inflammation in various biological systems. This depletion underscores the pivotal role of GSH as a cellular antioxidant, capable of quenching free radicals or serving as a substrate for other antioxidant enzymes, such as Glutathione Peroxidase and Glutathione Reductase.

Interestingly, our study, similar to Guillermo Aragoneses-Cazorla et al. [12], found that silver nanoparticles (AgNPs) induce the synthesis of glutathione as a cellular defense mechanism to face the oxidative environment while depleting relevant molecules involved in the synthesis of essential antioxidants [7]. This suggests that there may be an adaptive or compensatory response to oxidative stress in brain tissue following repeated oral ingestion of AgNP colloidal solutions. Additionally, the study by Caroline et al. showed that AgNPs can penetrate into the brain and cause neuronal death, potentially leading to mitochondrial dysfunction [12]. However, the study by Aylin Özodabaş found that under experimental conditions close to actual human consumption, silver nanoparticles do not have a long-term adverse effect on central nervous system (CNS) functions [24]. Therefore, while there may be potential mitochondrial dysfunction, the increase in Glutamine Synthase activity observed in brain tissue could be a compensatory response to enhance neurotransmission despite the oxidative stress caused by AgNPs.

Reactive oxygen species (ROS) play a crucial role in cellular signaling processes, including apoptosis, gene expression, and the activation of cell signaling cascades. The balance between ROS production and cellular detoxification mechanisms determines the threshold between normal physiological function and oxidative stress-induced cellular damage [25-27]. ROS can function as signal transducers at low to moderate levels, promoting cell proliferation, migration, invasion, and angiogenesis. However, high levels of ROS can cause damage to cellular components, leading to cell death [28]. Dysregulation of antioxidant pathways and altered redox balance are associated with various diseases [29]. Understanding the regulation of ROS and their impact on different types of cell death is crucial for elucidating disease mechanisms and developing therapeutic strategies. Manipulating ROS levels has shown promise in cancer therapy, and targeting ROS-related pathways may provide new avenues for treatment.

In conclusion, the detailed examination of AgNPs' impact highlights the critical interplay between nanoparticle-induced oxidative stress and the cellular antioxidant defense mechanisms. The detailed understanding of these interactions is essential for assessing the biological implications of AgNPs, emphasizing the need for comprehensive toxicological evaluations to delineate their safety and efficacy in various applications.

4. Conclusion

Our study demonstrates significant dose-dependent alterations in biochemical parameters and cellular structures upon exposure to silver nanoparticles (AgNPs). Key findings include a decrease in glucose and calcium levels, an insignificant increase in protein levels, a decrease in creatinine levels, and an increase in total bilirubin levels in the blood serum of treated groups. Catalase activity and reduced Glutathione activity showed a decrease, indicating oxidative stress. Transmission electron microscopy revealed AgNP accumulation within neuronal and hepatocyte cells, affecting cellular integrity and mitochondrial function, leading to increased ROS generation and oxidative stress. This comprehensive analysis underscores the potential risks of AgNPs at cellular and biochemical levels, highlighting the need for careful consideration in their application and further investigation into their toxicological

impacts.

References

1. Ahamed, M., Alsalhi, M. S., & Siddiqui, M. K. (2010). Silver nanoparticle applications and human health. Clinica Chimica Acta, 411(23-24), 1841-1848. https://doi.org/10.1016/j.cca.2010.08.016
2. Pravalika, S., & Reddy, M. S. (2023). An Overview on Synthesis, Characterization and Applications of Silver Nanoparticles. International Journal of Pharmaceutical Sciences Review and Research, 79(2). No DOI available.
3. Madhu, S., Renu, M., & Ruchi, B. (2023). A Review on the Synthesis and Photocatalytic Application of Silver Nano Particles. IOP Conference Series: Materials Science and Engineering, 1110(1), 012021. https://doi.org/10.1088/1757-899X/1110/1/012021
4. Rai, M., Yadav, A., & Gade, A. (2009). Silver nanoparticles as a new generation of antimicrobials. Biotechnology Advances, 27(1), 76-83. https://doi.org/10.1016/j.biotechadv.2008.09.002
5. Sotiriou, G. A., & Pratsinis, S. E. (2010). Antibacterial activity of nanosilver ions and particles. Environmental Science & Technology, 44(14), 5649-5654. https://doi.org/10.1021/es101072s
6. Multiple RNA Profiling Reveal Epigenetic Toxicity Effects of Oxidative Stress by Graphene Oxide Silver Nanoparticles In-vitro. (2023). International Journal of Nanomedicine, 18, 2855-2871. No DOI available.
7. Xia, T., et al. (2008). Comparison of the abilities of ambient and manufactured nanoparticles to induce cellular toxicity according to an oxidative stress paradigm. Nano Letters, 8(3), 786-792. https://doi.org/10.1021/nl072969w
8. Nel, A., Xia, T., Mädler, L., & Li, N. (2006). Toxic potential of materials at the nanolevel. Science, 311(5761), 622-627. https://doi.org/10.1126/science.1114397
9. Asare, N., Instanes, C., Sandberg, W. J., Refsnes, M., Schwarze, P., Kruszewski, M., Brunborg, G. (2012). Cytotoxic and genotoxic effects of silver nanoparticles in testicular cells. Toxicology, 291(1-3), 65-72. https://doi.org/10.1016/j.tox.2011.10.022
10. Jelle, M., & Katrien, A. (2023). Comparative in vivo toxicokinetics of silver powder, nanosilver and soluble silver compounds after oral administration to rats. Archives of Toxicology, 97(7), 1859-1872. No DOI available.
11. Mennatallah, H. A., Mai, H. E.-D., Tawfik, A. A., & Saber, M. Ayman. (2022). Biological impacts of the green synthesized silver nanoparticles on the pregnant albino rats and their fetuses. Teratology, 115(4), 441-457. No DOI available.
12. Guillermo, A.-C., Buendia-Nacarino, M. P., Mena, M. L., & L.-G. Jose, L. (2022). A Multi-Omics Approach to Evaluate the Toxicity Mechanisms Associated with Silver Nanoparticles Exposure. Nanomaterials, 12(10), 1762. https://doi.org/10.3390/nano12101762
13. Carlson, C., Hussain, S. M., Schrand, A. M., Braydich-Stolle, L. K., Hess, K. L., Jones, R. L., & Schlager, J. J. (2008). Unique cellular interaction of silver nanoparticles: size-dependent generation of reactive oxygen species. Journal of Physical Chemistry B, 112(43), 13608-13619. https://doi.org/10.1021/jp802878z
14. Foldbjerg, R., Dang, D. A., & Autrup, H. (2011). Cytotoxicity and genotoxicity of silver nanoparticles in the human lung cancer cell line, A549. Archives of Toxicology, 85, 743-750. https://doi.org/10.1007/s00204-010-0545-5
15. Asharani, P., Hande, M. P., & Valiyaveettil, S. (2009). Anti-proliferative activity of silver

nanoparticles. BMC Cell Biology, 10, Article 1-14. https://doi.org/10.1186/1471-2121-10-65

16. AshaRani, P., Kah Mun, G. L., Hande, M. P., & Valiyaveettil, S. (2009). Cytotoxicity and genotoxicity of silver nanoparticles in human cells. ACS Nano, 3(2), 279-290. https://doi.org/10.1021/nn800596w
17. Kahlert, S., Zündorf, G., & Reiser, G. (2005). Glutamate-mediated influx of extracellular Ca2+ is coupled with reactive oxygen species generation in cultured hippocampal neurons but not in astrocytes. Journal of Neuroscience Research, 79(1-2), 262-271. No DOI available.
18. Kim, J.-H., Block, D. E., Shoemaker, S. P., & Mills, D. A. (2010). Conversion of rice straw to bio-based chemicals: an integrated process using Lactobacillus brevis. Applied Microbiology and Biotechnology, 86, 1375-1385. No DOI available.
19. Scown, T. M., Santos, E. M., Johnston, B. D., Gaiser, B., Baalousha, M., Mitov, S., Lead, J. R., Stone, V., Fernandes, T. F., & Jepson, M. (2010). Effects of aqueous exposure to silver nanoparticles of different sizes in rainbow trout. Toxicological Sciences, 115(2), 521-534. https://doi.org/10.1093/toxsci/kfq076
20. Adeyemi, O. S., & Sulaiman, F. A. (2012). Biochemical and morphological changes in Trypanosoma brucei brucei-infected rats treated with homidium chloride and diminazene aceturate. Journal of Basic and Clinical Physiology and Pharmacology, 23(4), 179-183. No DOI available.
21. Gubbins, E. J., Batty, L. C., & Lead, J. R. (2011). Phytotoxicity of silver nanoparticles to Lemna minor L. Environmental Pollution, 159(6), 1551-1559. https://doi.org/10.1016/j.envpol.2011.03.002
22. Kim, S., Choi, J. E., Choi, J., Chung, K. H., Park, K., Yi, J., & Ryu, D. Y. (2009). Oxidative stress-dependent toxicity of silver nanoparticles in human hepatoma cells. Toxicology in Vitro, 23(6), 1076-1084. https://doi.org/10.1016/j.tiv.2009.06.001
23. Sharma, H. S., Ali, S. F., Hussain, S. M., Schlager, J. J., & Sharma, A. (2009). Influence of engineered nanoparticles from metals on the blood-brain barrier permeability, cerebral blood flow, brain edema and neurotoxicity. An experimental study in the rat and mice using biochemical and morphological approaches. Journal of Nanoscience and Nanotechnology, 9(8), 5055-5072. No DOI available.
24. Aylin, Ö. (2022). The Effect of Aqueous Solution of Silver Nanoparticles on Rat Behavior. No DOI available.
25. de Oliveira, A. J. P., de Lucio, O. J. C. P., da Silva, L. V., de Souza, J. J. F., Felisberto, G. T. A., Helena, D. S., de Almeida, M. S., & Oliveira, S. A. (2022). ROS: Basic Concepts, Sources, Cellular Signaling, and its Implications in Aging Pathways. Oxidative Medicine and Cellular Longevity, 2022. https://doi.org/10.1155/2022/8854329
26. Pecchillo, C. T., Rosario, A., Fabio, C., & Gabriella, E. (2023). NOX Dependent ROS Generation and Cell Metabolism. International Journal of Molecular Sciences, 24(3), 2086. https://doi.org/10.3390/ijms24032086
27. Nair, P. M. G., & Chung, I. M. (2014). Impact of copper oxide nanoparticles exposure on Arabidopsis thaliana growth, root system development, root lignification, and molecular level changes. Environmental Science and Pollution Research, 21(22), 12709-12722. https://doi.org/10.1007/s11356-014-3182-y
28. Hajime, N., & Kohichi, T. (2021). Reactive oxygen species in cancer: Current findings and future directions. Cancer Science, 112(10), 3945-3952. https://doi.org/10.1111/cas.15040
29. Park, E. J., Yi, J., Kim, Y., Choi, K., & Park, K. (2010). Silver nanoparticles induce cytotoxicity by

30. Trojan-horse type mechanism. Toxicology in Vitro, 24(3), 872-878. https://doi.org/10.1016/j.tiv.2010.03.008