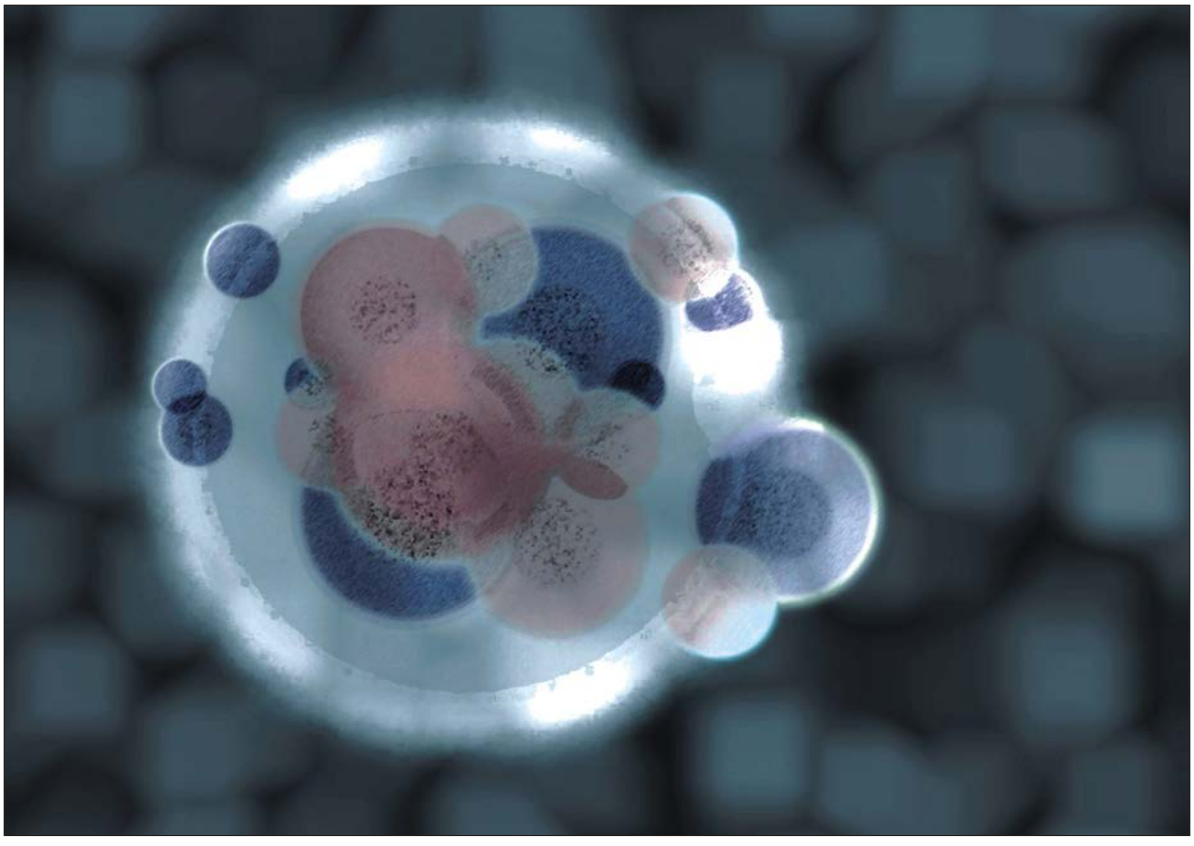

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Research Article
Molecular Investigation of Gold Nanoparticles Toxicity in Mice Model and p53 Activation

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Abstract

Background and Objective: Gold nanoparticles (AuNPs) widely had been used in the medicine and industries, but the safety of the gold NPs exposure remains unclear. The aim of the present study was to evaluate the toxic effects of thioacetamide (TAA) (300 μg kg^{-1}) and gold nanoparticles (AuNPs) (300 μg kg^{-1}) in the organs of mice. **Materials and Methods:** The mice were treated with fixed doses of gold nanoparticles and thioacetamide every day for 5 days. In the blood values of WBCs, RBCs, neutrophils and other blood components were changed as compared to normal values after the treatment in mice groups (1-3 and 4). **Results:** Results indicated that the thioacetamide caused the hepatic damage and caused down regulation of checkpoint gene p53 and also decreased the expression level of apoptotic caspase protein (Casp3, Casp8 and Casp9) which was measured by qRT-PCR. While the gold NPs upregulated the p53 gene which is responsible for the DNA repair and also known as the checkpoint of the cell cycle. The gold nanoparticles were activated and up-regulated the caspase-3, caspase-8 and caspase-9 and induced apoptosis. **Conclusion:** It was concluded that gold nanoparticles activated the apoptotic genes which had the ability to kill the cancer cell. However, further studies are recommended to have better insight.

Key words: Thioacetamide, gold nanoparticles, p53, caspase 3, 8, 9, mice models, cancer, apoptotic genes

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

According to the American cancer society, 1,529,560 cancer cases while 569,490 death cases were estimated in 2010 (American cancer society of Research 2010) while the survival rate depends on the early diagnosis[1]. The diagnosis method of cancer and treatment are much expensive and has an adverse effect on the other organs of the body.

In all aspects of research such as medicine, electronics and energy nanotechnology has played a vital role. The unique property of nanoparticles such as size, absorption and their shape made them unique in the field of medical as well as clinical. Nanoparticles are classified into metallic, nanoparticles, quantum dots and nanowires. Construction of nanofibers, nanowires and nanotubes has growth to achieve the target analytes[2]. The engineering the nanoparticles and customize their physiochemical properties given the way to make the nanomaterials robust and advance for the diagnosis of cancer and binding affinities of drugs targets and biomolecules[3].

In the biological science, NPs have a long history as they have been used in the bioconjugation with DNA, proteins and other biological molecules as labeling, imaging and cellular delivery[4]. In 1980 the specific property of gold was studied by Robert Koch *in vitro* inhibition of *Mycobacterium tuberculosis*. In the 2500 BC the medical use of gold in the medicines can be traced back to ancient Vedic civilization and Chinese civilization they used gold in the ayurvedic medicines after that ancients culture have used gold materials for the medical purpose in the treatment of various diseases such as smallpox and skin ulcer[5]. Recently Gold nanoparticles have been investigated with regard to biocompatibility and cytotoxicity according to their ability to interaction with other cells[6,7]. It was found that gold nanoparticles can combine with heparin-binding glycoprotein and as the result inhibit the endothelial/fibroblast cell angiogenesis and proliferation[8]. Caspases and p53 are responsible for the apoptosis so that the gold nanoparticles could enhance the p53, Casp3, Casp8, Casp9, but the safety of the gold NPs still remains unclear.

Therefore, the present study was designed to evaluate the toxic effects of thioacetamide (TAA) (300 μg kg^{-1}) and gold nanoparticles (AuNPs) (300 μg kg^{-1}) in the mice models.

MATERIALS AND METHODS

Chemical and reagents: Gold nanoparticles were synthesized by the methods[9,10]. Thioacetamide, cDNA kit, primers, ethidium bromide, chloroform, trizma base, PCI, PCR grade water, $MgCl_2$, lysis buffer, PCR kit and proteinase K were purchased from different companies and sources which were available at the Virtual University of Pakistan.

Experiment: On the 1st day mice were treated with the 1st dose of AuNPs (300 μg kg^{-1}) in group 1, TAA (300 μg kg^{-1}) in group 2 and 3 was treated with TAA and AuNPs (300 μg kg^{-1}) and group 4 was controlled (saline). After 8 hours the second dose of AuNPs was injected in group 1 and 3.

On the 2nd day of the experiment, mice were weighted and have observed for their behavior towards the feed and their environment. The third dose of AuNPs was injected in group 1 and 3.

On the 3rd day mice were again weighed and observed for their behaviour and the 4th dose of the AuNPs was injected in group 1 and 3.

Mice were again weighted and treated with a fifth dose of AuNPs in group 1 and 3 as per previous days and it was the repetition of the experiment as per design.

On the 5th day, mice were weighed and slaughtered and collected blood samples from their eyes and compared their organs' weight with the organs of the controlled group.

RNA extraction: For the RNA extraction first of all the sample was homogenized at room temperature only for 5 min and centrifuged it to remove cell debris and transferred the supernatant to the other new tube. Finally RNA was extracted according to the instruction of TRIzol method (Thermo Fisher Scientific, Germany).

Synthesis of complementary DNA: The kit (Qiagen, QuantiTect Reverse Transcription kit) was used for the transcriptional mechanism of the mRNA and other instructions such as transcribed the RNA into a first strand complementary DNA. After that, the reverse transcription sample with hexamer/oligodT was performed in one cycle for 5 min at the 65°C in the PCR Master Cycler gradient. RNAase inhibitor (0.2 μL) reaction buffer (2 μL) and dNTP added and incubated for 5 min at 25°C for 60 min at 42°C and for 5 min at 70°C. After that, the samples were put in storage in the water which was free from the RNAase for further use.

Relative gene expression using real-time quantification polymerase chain reaction: To analyze the expressions of genes caspase-3, caspase-8, caspase-9 qPCR was performed and in this process, primers were mixed with the cDNA and diluted at the ratio of 1:10. In the reaction tube, cyber green master mix and reaction mixture was inserted for attaining the

final 1×concentration. PCR cycles were adjusted as the obligations of denaturation, extension and annealing, while the melt-curve analyzed was completed as per the default parameters of the 7300 P PCR system. The Analysis and measurement of the expressions of the genes were carried out by using the ddCt Relative quantification plate as done in the stuy[11], sequence finding software by Applied Biosystems Corp. The expressions of p53, Caspase-3, 8, 9 which are targeted genes, were standardized with GAPDH.

RESULTS

Complete blood count analysis: As shown in the Table 1, mice were treated with TAA (300 μg kg^{-1}), the CBC results were below the normal values and mice which were only treated with AuNPs (300 μg kg^{-1}), the CBC results were found to be in normal limits. More accurate results were analyzed in those mice which belong to the controlled group and also in those treated with TAA+AuNPs (300 μg kg^{-1}).

Quantitative real-time PCR analyses: In controlled mice expression level of p53 caspase-3, 8, 9 were up-regulated while in TAA (300 μg kg^{-1}) treated mice checkpoint p53 were down-regulated and apoptotic genes, activated by the activation of p53 genes such as caspase-3, caspase-8, caspase-9 were also downregulated so apoptosis process was not observed in them. In mice treated with AuNPs (300 μg kg^{-1}), it was analyzed that the mRNA expression of checkpoint genes and apoptotic genes were upregulated but their regulation was below the normal range. More accurate and normal mRNA gene expression was observed in those mice which were treated with both TAA+AuNPs (300 μg kg^{-1}). In these mice, it was observed that checkpoint p53 expression level and the expression of apoptotic genes was normal as shown in the Fig. 1.

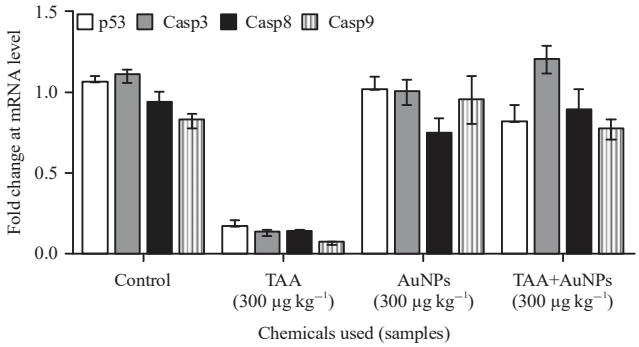

Fig. 1: QRT-PCR analysis of expression level of p53, Casp3, Casp8 and Casp9 genes in control, TAA (300 μg kg^{-1}), AuNPs (300 μg kg^{-1}) and TAA+AuNPs group (300 μg kg^{-1})
p<0.05 is considered statistical significant and mean and standard deviation is represented by columns and bars respectively

Table 1: Comparison analysis of CBC results in mice which were treated with dose of TAA (300 μg kg^{-1}), AuNPs (300 μg kg^{-1}), TAA+AuNPs (300 μg kg^{-1}) and control (saline) group

Statistical data for hematological toxicity	Control (saline)	TAA (300 μg kg^{-1})	AuNPs (300 μg kg^{-1})	TAA+AuNPs (300 μg kg^{-1})
WBCs	6.20	5.30	7.10	7.20
RBCs	7.81	6.17	6.46	5.66
Lymphocytes	80.50	64.70	59.70	4.20
Monocytes	0.10	0.60	6.60	0.20
MCV	50.60	48.50	52.90	40.80
MCH	15.90	15.60	15.90	13.60
HB	12.40	9.60	10.30	7.70
HCT	39.50	29.90	34.20	23.10
PLT	828.00	512.00	756.00	483.00
MPV	7.70	7.50	7.00	10.90
PCT	0.63	0.384	0.529	0.526
PDW	17.80	20.90	15.00	30.20

TAA: Thioacetamide, AuNPs: Gold nanoparticles, WBCs: White blood cells, RBCs: Red blood cells, MCV: Mean corpuscular volume, MCH: Mean corpuscular hemoglobin, HB: Hemoglobin concentration, HCT: Hematocrit, PLT: Platelet count blood test, MPV: Mean platelet volume, PCT: Procalcitonin, PDW: Platelet distribution width

DISCUSSION

Gold nanoparticles have gained attention in the fields of biomedicine and oncology due to its multifunctionality. These particles can be engineered and used as therapeutic agents for different types of cancer. Despite of the vast amount of studies, safe use of gold nanoparticles is not well understood yet. In our preliminary study, we have evaluated the toxic effects of gold nanoparticles (300 μg kg^{-1}) on the different organs of mice such as heart, kidney, spleen, brain and lungs.

We analyzed in our study that mice which were treated with TAA (300 μg kg^{-1}), the CBC results were below the normal values and mice which were only treated with AuNPs (300 μg kg^{-1}), the CBC results were found to be in normal limits. While more accurate results were analyzed in those mice which are controlled and also in those which were treated with TAA+AuNPs (300 μg kg^{-1}). Another study suggested that intraperitoneal injection of gold nanoparticles of diameter 10 nm are not harmful and do not cause inflammation or an infection in the body, generally, in the blood, the WBC increased due to the physiological response to an external agent into the body[12]. Hauck and Anderson suggested that Au nanoparticles caused the inflammation and increased or decreased the activity of the immune system in the living organisms and changed the related hematologic factors[13].

Our results demonstrated that gold nanoparticles to cancer cells cause cytotoxicity. We analyzed the mRNA

expression level of four genes p53, caspase-3, caspase-8, caspase-9 in response to gold nanoparticles injected in TAA treated and controlled mice. Quantitative real-time PCR result showed that AuNPs up-regulated the p53 and pro-apoptotic bax. Furthermore the expression of apoptotic Casp3, Casp8, Casp9 were higher in the gold nanoparticles treated and TAA+AuNPs treated mice while the expression level of p53 and caspase-3, caspase-8, caspase-9 were downregulated in TAA treated mice. Up-regulation of p53 activates the pro-apoptotic members of the bcl-2 family such as bax and it induces permeabilization of the mitochondrial outer member which releases proteins from the intermembrane space into the cytosol and promote the activation of caspases[14,15].

Several groups have reported recently that the use of AuNPs for drug delivery such as doxorubicin for the drug resistance in cancer[16] and peptide functionalized Au nanoparticles for tumor targeting[17]. One of the key challenges in the treatment of cancer is to be targeted delivery in a localized way. AuNPs have the ability to play a vital role to achieve such goals. It is predicted that targeted drug delivery of gold nanoparticles might minimize the dosage of anti-cancer drugs, lower the toxicities and enhanced the efficacy[18].

CONCLUSION

The result of our current research showed that gold nanoparticles produce significant cytotoxicity to cancer cells. Furthermore, quantitative real-time PCR analysis displayed that mRNA levels involved in the apoptosis was altered by gold nanoparticles. Overall, data suggesting that gold nanoparticles may induce apoptosis in cancer cells via p53 and caspase pathway.

SIGNIFICANCE STATEMENT

Gold nanoparticles (AuNPs) are widely used in the medicine and industries now-a-days, but the safety of the gold NPs still remains unclear. Therefore, there was a need to find actual mechanisms of the nanoparticles. This study advances knowledge by showing that effects of AuNPs showed less cytotoxicity on normal body cells as compared to cancer cells in the mice models. However, further studies are recommended to have better insight.

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