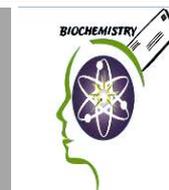




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Silver Nanoparticles Effect on Cytokines in Rat

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ABSTRACT

Background: Silver nanoparticles (AgNPs) was recommended in medicine and veterinary medicine fields, and can be used as drugs for treatment of some diseases. Aim: the toxicity of nanosilver is not well known, it is essential to examine its safety. The experimental albino rats were divided into five groups , each group comprising of ten rats, after adaptation in the animal houses in Faculty of Vet. Medicine Suez Canal University for one week. The groups named as N1, 2, 3, 4 and 5. Group N1, was called control group and was gives deionized water. Blood samples were collected at the end of experiment; serum samples were separated for determination the values of IL-L β , IL-2, IL-6, IL-10 and TNF- α . Ag-NPs administration showed a variable significant increase in the levels of IL-L β , IL-2, IL-6, IL10 and TNF- α . Conclusion : Nanoparticles induce inflammatory immune responses at lower concentrations and chemokines are the major cytokines induced at early stage of exposure to silver nanoparticles..

Introduction:

Most studies have used in vitro systems to test inflammatory responses of nanoparticles, these may not reflect the real biological response of body organ. In fact, certain nanoparticles have provoked opposite effects under in vitro and in vivo conditions. They studied the acute (1 day) and sub-chronic (5 days) effects of gold nanoparticles (10 and 50 nm in diameter) on expression of interleukin-1-beta (IL-1 β), IL-6 and tumor necrosis factor alpha (TNF- α) in rat liver, real-time PCR analysis showed that gold nanoparticles of both sizes

significantly increased cytokine gene expression on day 1, this subsided by day 5. The 50 nm gold nanoparticles produced more severe inflammation than the smaller gold nanoparticle. These findings indicate a possible biocompatibility of medium – sized gold nanoparticles, as they caused only a transient increase in proinflammatory cytokines , followed by normalization during sub- chronic repeated exposure (7) .

The systematic toxicity of the intermediate size (18-37 nm) citrate – capped AG-NPs has been linked to major organ damage in the liver,

spleen, and lungs of mice, but the same nanoparticles have been found to be nontoxic in vitro using Hel-a cell lines (2,11) observed significant induction of IL-6 and TNF- α in mouse macrophages by silver nanoparticles followed by aluminum, carbon black and carbon-coated silver nanoparticles. On the other hand, (20) found that both silver and gold nanoparticles enter the cells but that only the latter (especially those with smaller diameter) upregulate the expression of pro inflammatory genes (IL-1, IL-6, TNF- α).

A significant inflammatory response has been observed in the release of TNF- α and IL-1 β after 24 h of exposure to 15 nm Ag nanoparticles, but there was no detectable level of IL-6 upon exposure to silver nanoparticles (1). A single intratracheal instillation of platinum and silver nanoparticles causes progressive increase in pro-inflammatory cytokines (IL-1, IL-6 and TNF- α) by day 28 post instillation (12).

Rats received for 81 days a standard diet (control) or a standard diet plus 500 mg/dl/ kg B.W. AgNPs, liver inflammatory cytokines TNF- α and IL-6 levels were assayed. Compared with control, AgNPs raised liver TNF- α and IL-6 (3). In toxicity assessment of silver nanoparticles oral administration on immune system in rats, a significant increase on serum TNF- α and IL-6 during 7, 14 and 28th day of treatment (22).

In Ecophysiological study of silver nanoparticles effect on the liver and regulatory cytokine in rabbit, the concentrations of IL-10 were increased in test group than in control while IL-12 concentrations were decreased in test group than control (4); but this disagreement with (13) that found

silver nanoparticles increase IL-12 in mice when repeated doses were used. The effects of silver nanoparticles and gold nanoparticles on IL-2, IL-6 and TNF- α production via pathway in leukemic cell lines, showed that each leukemic cell line treated with nanoparticles exhibited a distinct signaling pathway response to inhibit or stimulate cytokine production, leading to anti-cell proliferation. The effects of AgNPs and AuNPs on leukemic cell lines may have a significant impact on leukemia treatment in the future (17).

Tomasz, (19) investigate the impact of AgNPs on the ability of rat peripheral blood mononuclear cells (PBMCs) to release fever mediating factors after stimulation with lipopoly saccharides (LPs). The presence of the AgNPs resulted in significant increase in levels of IL-1 β , IL-6 and TNF- α (endogenous pyrogens). The aim of this work to study the effect of silver nanoparticles on rats cytokines.

Materials and Methods:

Material:

Silver dispersion – nanoparticles, 20 nm particles size, 0.04 mg/ml in aqueous buffer contain sodium citrate as stabilizer was obtained from sigma – aldrich Co. Fifty (50) male adult albino (200-250g), obtained from National research center Dokki, Egypt were used in this study. Animals were housed in group of ten in cages at 25 \pm 5°C, under 12: 12 hrs light / dark cycle, and 12 hr free access to water and food.

The experimental albino rats were divided into five groups, each group comprising of ten rats, after adaptation in the animal houses in faculty of Vet. Medicine Suez Canal University for one week. The groups

named as N1, 2, 3, 4 and 5. Group N1, was called control group and was given deionized water IP and the other four groups, N2 to 5 were called treated groups. Silver nanoparticles were administered intra peritoneal for 30 days. At a dose of 0.25, 0.5, 1.0 and 2.0 % mg/kg / B.wt.

Methods:

Blood samples were obtained at end of experiment, serum samples obtained were used for evaluation of cytokines in rats.

Determination of serum TNF- α by rat TNF- α ELISA kit according to (6).

Determination of serum IL-1 beta using Rat IL-1 beta ELISA kit according to (6).

Determination of serum IL-2 (Interleukin-2) using rat IL-2 ELISA Kit according to method of (18).

Determination of serum IL-6 (Interleukin-6) using rat IL-6 ELISA Kit according to method of (18).

Determination of serum IL-10 (Interleukin-10) using rat IL-10 ELISA Kit according to method of (9).

Statistical analysis:

The obtained data were analyzed and graphically represented using the statistical package for social science (SPSS, 15.0 software 2009) for obtaining mean and standard deviation and error. The data were analyzed using one way ANOVA to determine the statistical significance of differences among groups. Duncan's test were used for making a multiple comparison among the groups for testing the inter grouping homogeneity.

Results

Table (1) and Figure (1) showed a significant ($p \leq 0.05$) increase in serum

IL-1- β of rats treated with silver nanoparticles in a dose dependent manner when compared with control rats.

Table (2) and Figure (2) showed a significant ($p \leq 0.05$) increase in serum IL-2 of rats treated with silver nanoparticles in a dose dependent manner when compared with control rats.

Table (3) and Figure (3) showed a significant ($p \leq 0.05$) increase in serum IL-6 of rats treated with silver nanoparticles in a dose dependent manner when compared with control rats.

Table (4) and Figure (4) showed a significant ($p \leq 0.05$) increase in serum IL-10 of rats treated with silver nanoparticles in a dose dependent manner when compared with control rats.

Table (5) and Figure (5) showed a significant ($p \leq 0.05$) increase in serum TNF- α of rats treated with silver nanoparticles in a dose dependent manner when compared with control rats.

DISCUSSION

The administration of silver nanoparticles caused significant induction of proinflammatory cytokines in rat serum (Tables 1-5 and Figs. 1-5). (11) observed a significant induction of IL-6 and TNF- α in mouse macrophages by silver nanoparticles followed by aluminum, carbon black and carbon-coated silver nanoparticles. On the other hand, (20) found that both silver and gold nanoparticles enter the cells but that only silver up regulate the expression of proinflammatory genes (IL-1, IL-6 and TNF- α). They speculated that part of the negatively charged gold nanoparticles may adsorb serum protein and enter cells via the more complicated endocytotic pathway,

resulting in higher cytotoxicity and immunological response of gold nanoparticles than silver nanoparticles (9).

A significant inflammatory response has been observed in the release of TNF- α and IL-1 β after 24 hr. of exposure to 15-nm Ag nanoparticles, but there was no detectable level of IL-6 upon exposure to silver nanoparticles (1). The levels of TNF- α and IL-1 β bronchoalveolar lavage fluid at 5 and 24hr. were found to be higher in carbon nanotube – exposed animals than in corresponding air – exposed control (5). **Park and Park (14)** noticed that a single treatment of silica nanoparticles (50 mg/kg, ip) activates peritoneal macrophages and increased blood levels of IL-1 β and TNF- α ; mRNA expressions of inflammation-related genes were also elevated in the cultured peritoneal macrophages harvested from the treated mice. A single intra-tracheal instillation of platinum and silver nanoparticles causes progressive increase in proinflammatory cytokines (IL-2, IL-6 and TNF- α) by day 28 postinstillation (13,15). Several studies also showed that silver nanoparticles increase the anti-inflammatory cytokine IL-10 (10,12). IL-12 concentrations were decreased in test group compare control, this due to the silver nanoparticles increase TH2 by increasing IL-10, IL-12 act as a key factor for activation TH1 and consider one member of immune – regulatory cytokine group that include IL-12-23, 27 and IL-35 (16) but this disagreement with (10) that found silver nanoparticles increased IL-12 in mice when repeated dose were used.

The most important mediators of fever and other symptoms of sickness cytokines such as interleukin–

L β (IL-1 β), IL-6, interferon- γ (INF- γ) and tumor necrosis factor- α (TNF- α) (8). Since it has been known that macrophages stimulated with lipopolysaccharides release the pyrogenic factors (21), and there are data showing that AgNPs may influence some immunologic activities.

CONCLUSION

From these study, it was concluded that the intrapretoneal injection of silver nanoparticles produced changes, in some serum cytokines of rats administered with silver nanoparticles in higher repeated doses.

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Table (1): Interluken-I β level in serum of rats groups treated daily for one month with different doses 0.25, 0.5, 1.0 and 2.0 mg / Kg.B.wt. of silver nanoparticles in comparison with control group.

Group Parameter	Control group	Group 1 treated	Group 2 treated	Group 3 treated	Group 4 treated
IL-1 β (pg/ml)	106 $\pm 7.1^c$	109 $\pm 7.4^c$	111 $\pm 7.5^c$	118 $\pm 0.76^b$	125 $\pm 0.70^a$

Values carry different letters in same row are significantly differ at $P \leq 0.05$

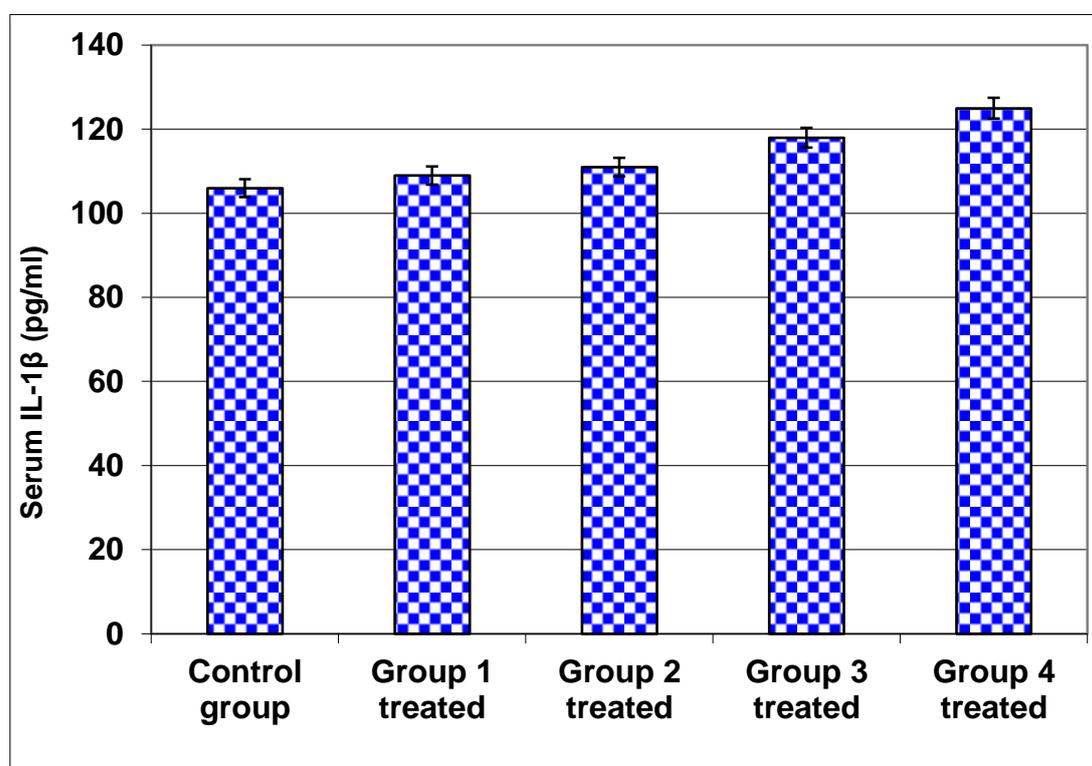


Figure 1: Interluken-I β level in serum of rats groups treated daily for one month with different doses 0.25, 0.5, 1.0 and 2.0 mg / Kg.B.wt. of silver nanoparticles in comparison with control group.

Table (2): Interluken-2 level in serum of rats groups treated daily for one month with different doses 0.25, 0.5, 1.0 and 2.0 mg / Kg.B.wt. of silver nanoparticles in comparison with control group.

Group	Control group	Group 1 treated	Group 2 treated	Group 3 treated	Group 4 treated
IL2 (pg/ml)	135.68 ± 7.7 ^d	137.65 ± 0.79 ^{cd}	139.95 ± 0.92 ^b	142.08 ± 9.1 ^b	155.16 ± 8.61 ^a

Values carry different letters in same row are significantly differ at $P \leq 0.05$.

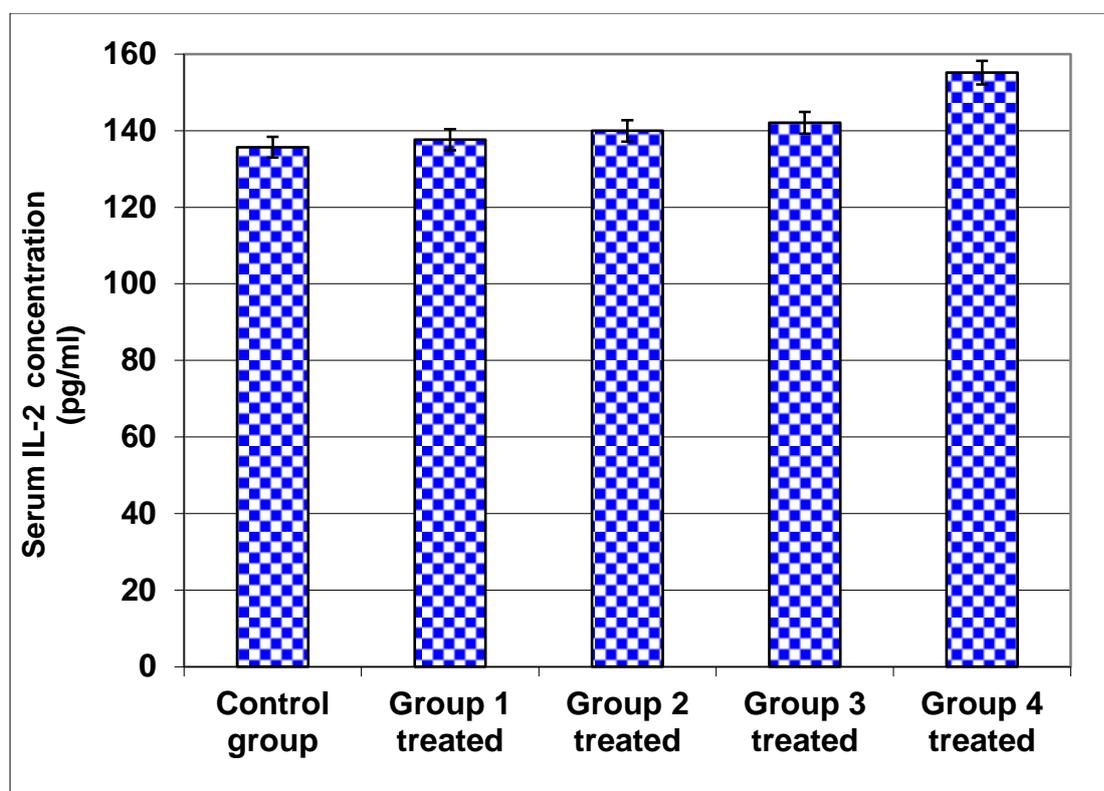


Figure (2): Interluken-2 level in serum of rats groups treated daily for one month with different doses 0.25, 0.5, 1.0 and 2.0 mg / Kg.B.wt. of silver nanoparticles in comparison with control group.

Table (3): Interluken-6 level in serum of rats groups treated daily for one month with different doses 0.25, 0.5, 1.0 and 2.0 mg / Kg.B.wt. of silver nanoparticles in comparison with control group

Group	Control group	Group 1 treated	Group 2 treated	Group 3 treated	Group 4 treated
IL6 (pg/ml)	19.00 ± 0.65 ^d	21.50 ± 0.75 ^c	23.70 ± 0.75 ^b	25.80 ± 0.78 ^b	28.80 ± 0.76 ^a

Values carry different letters in same row are significantly differ at $P \leq 0.05$.

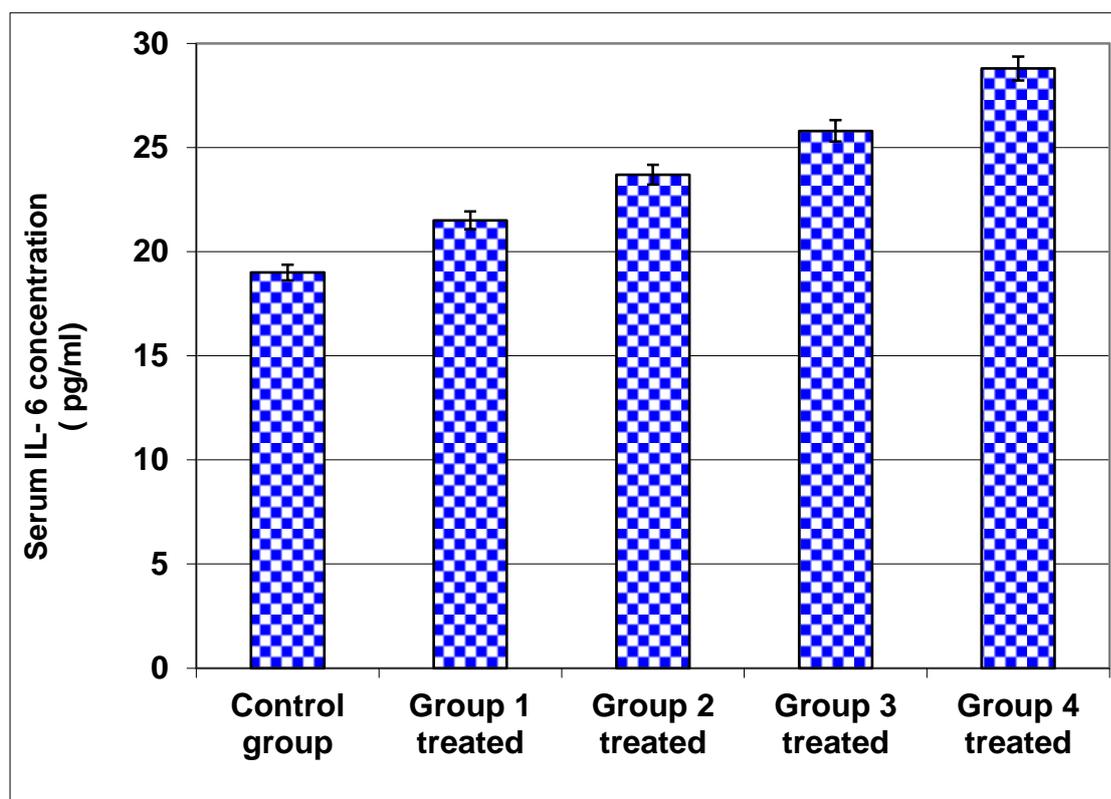


Figure 3: Interluken-6 level in serum of rats groups treated daily for one month with different doses 0.25, 0.5, 1.0 and 2.0 mg / Kg.B.wt. of silver nanoparticles in comparison with control group

Table (4): Interluken-10 level in serum of rats groups treated daily for one month with different doses 0.25, 0.5, 1.0 and 2.0 mg / Kg.B.wt. of silver nanoparticles in comparison with control group.

Group	Control group	Group 1 treated	Group 2 treated	Group 3 treated	Group 4 treated
IL-10(pg/ml)	63.10 ± 0.75 ^d	65.70 ± 0.75 ^c	68.00 ± 0.73 ^b	70.00 ± 0.78 ^b	73.00 ± 0.90 ^a

Values carry different letters in same row are significantly differ at $P \leq 0.05$.

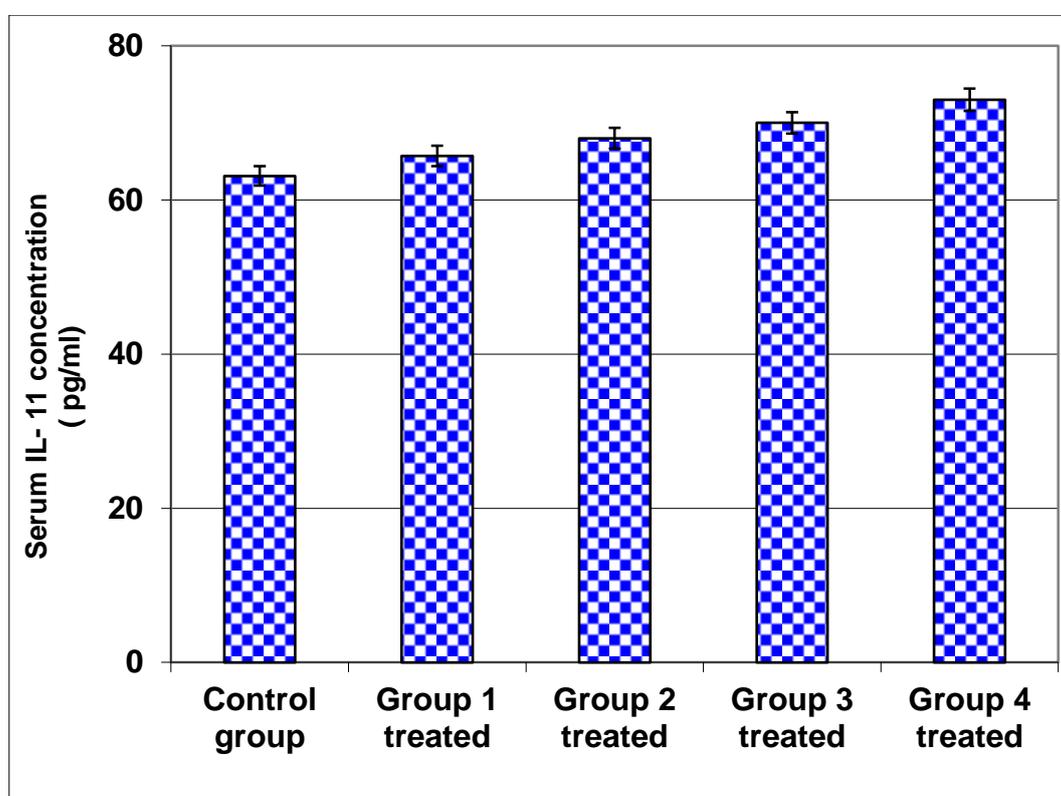


Figure 4: Interluken-10 level in serum of rats groups treated daily for one month with different doses 0.25, 0.5, 1.0 and 2.0 mg / Kg.B.wt. of silver nanoparticles in comparison with control group.

Table 5: Tumor necrotic factor- α level in serum of rats groups treated daily for month with different doses 0.25, 0.5, 1.0 and 2.0 mg / Kg.B.wt. of silver nanoparticles in comparison with control group

Table (5)

Group	Control group	Group 1 treated	Group 2 treated	Group 3 treated	Group 4 treated
TNF- α (pg/ml)	32.7 \pm 1.00 ^d	34.7 \pm 1.05 ^{cd}	37 \pm 1.00 ^{bc}	39.2 \pm 0.94 ^b	42.2 \pm 0.91 ^a

Values carry different letters in same row are significantly differ at $P \leq 0.05$

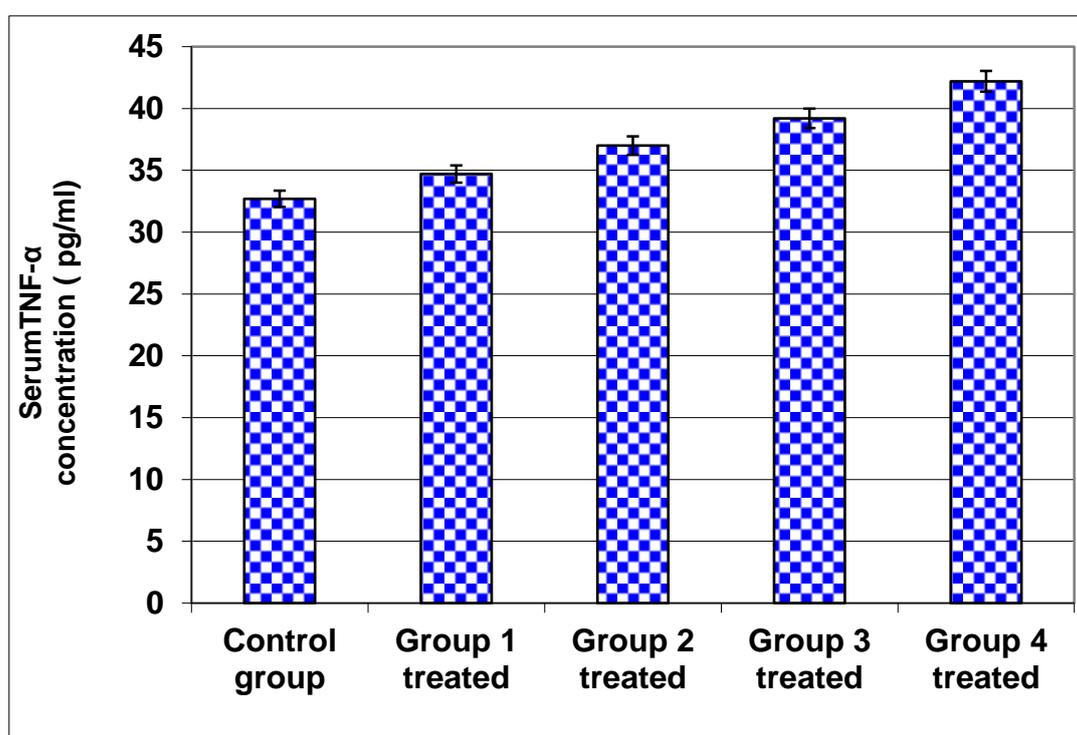


Figure 5: Tumor necrotic factor- α level in serum of rats groups treated daily for month with different doses 0.25, 0.5, 1.0 and 2.0 mg / Kg.B.wt. of silver nanoparticles in comparison with control group