ISSN: 0067-2904

The Effects of Combined toxicity of Silver and Silicon Nanoparticles on Hematological and Biochemical Parameters in Male Albino Mice

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Received: 7/11/2021 Accepted: 18/1/2022 Published: 30/10/2022

Abstract

In this study, the potential combined effects of silver (AgNPs) and silicon dioxide nanoparticles (SiO_2NPs) on male albino mice which were exposed intraperitoneally to the 50 mg/kg and 100 mg/kg and mixed concentrations of nanoparticles (NPs) for periods of 2 and 4 weeks, were evaluated. The evaluation was performed by examining the haematological and biochemical parameters, in addition to the bioaccumulation of NPs in the liver and kidney. The results showed that the platelets count significantly increased in all exposed groups, while red blood cell count (RBCs) decreased in most exposed groups. White blood cells (WBCs) and haemoglobin (Hb) showed different levels in the exposed groups. Serum aspartate aminotransferase (AST) and serum alanine aminotransferase (ALT) levels also showed a significant increase in the exposed groups, except in the groups exposed to 100 mg/kg of NPs for 2 weeks. Whereas blood urea nitrogen (BUN) serum level showed a significant decrease in all exposed groups. However, the alkaline phosphatase (ALP), uric acid (UA) and creatinine (CR) levels did not show any significant alternations in the exposed groups. Significant increase was also noted in thyroid-stimulating hormone (TSH) levels among the exposed groups. An accumulation of AgNPs and SiO_2NPs was observed both in livers and kidneys in the exposed groups. The results showed that SiO_2NPs have a synergistic effect on AgNPs accumulation in the groups exposed to mixed nanoparticles.

Keywords: Bioaccumulation, AgNPs, SiO_2NPs, Creatinine, RBCs, AST

التأثيرات السميةالمشتركة لدقائق الفضةوالسيلكون النانوية على المؤشرات الدموية والكيمياوية لذكور الفئران البيضاء

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الخلاصة

تم تقييم التأثير المشترك المحتمل لدقائق الفضة النانوية (AgNPs) ودقائق ثاني أكسيد السيليكون النانوية (SiO_2NPs) على ذكور الفئران البيضاء،والتي تم تعريضها إلى التركيز 100 ملغرام/ كغم و50ملغرام / كغم والمختلط من الجسيمات النانوية لمدة 2 و4 أسابيع. تم إجراء التقييم من خلال فحص مؤشرات الدم المؤشرات الكيموحيوية والتراكم الحيوي للدقائق النانوية في الكبد والكلى. اظهرت النتائج زيادة ملحوظه في عدد الصفائح

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الدموية في جميع المجموعات المعرضة، بينما انخفض عدد خلايا الدم الحمراء (RBC) في معظم المجموعات المعرضة. أظهرت خلايا الدم البيضاء (WBCs) والهيموكلوبين (Hb) مستويات مختلفة في المجموعات المعرضة. أظهرت مستويات ناقلة أمين الأسبارتات (AST) و ناقل أمين الألنين (ALT) زيادة معنوية في المجموعات المعرضة، باستثناء المجموعات التي تعرضت لـ 100 مجم / كجم من الدقائق النانوية لمدة أسبوعين. بينما أظهر نيتروجين اليوريا في الدم (BUN) انخفاضًا معنويًا في جميع المجموعات المعرضة. ومع ذلك، فإن مستويات الفوسفتيز القلوي (ALP)، حامض اليوريك (UA) ومستويات الكرياتينين (CR) لم تظهر تغيرا كبيرًا في المجموعة المعرضة. فضلا عن ذلك لوحظت زيادة كبيرة في مستويات هرمون الغدة الدرقية (TSH) في المجموعات المعرضة. وتم ملاحظة تراكم دقائق الفضة النانوية ودقائق ثاني أكسيد السيليكون النانوية في كل من الكبد والكلى في المجموعات المعرضة. تظهر النتائج أن لدقائق ثاني أكسيد السيليكون النانوية تأثير تآزري على دقائق الفضة النانوية، حيث لوحظ تراكم المزيد من دقائق الفضة النانوية في المجموعات المعرضة للدقائق النانوية المختلطة.

1. Introduction

The nanotechnology revolution has brought benefits into almost every aspect of life. Several products contain nanoparticles (NPs) in their basic structure, for that the possibility of exposure to nanoparticles (NPs) and nanomaterials (NMs) is high, particularly during product series manufacture, application and waste disposal [1]. However, NMs are defined as materials consisting of nanoparticles that have at least one or more external dimensions between 1 and 100 nanometers [2].NPs enter the body of living organisms through various routes of exposure involving inhalation, ingestion, dermal contact and systematic exposure [3]. The physicochemical properties including size, surface charge, shape, surface area, surface modification, stability and density have a great influence on the absorption, distribution, metabolism and excretion (ADME) of NPs [4, 5**].** Owing to their unique characteristics, silver nanoparticles (AgNPs) and silicon dioxide nanoparticles (SiO_2NP) are among the most commonly used and manufactured engineered nanomaterials (ENMs) [6]. Besides their application in the environment, they are applied in various fields including industrial, agriculture, medical, nutrition, healthcare and consumer applications [7, 8]. Until 2012 about 1.5 million tons of SiO2NPs were released in the global market, leading to SiO_2NPs becoming among the most produced nanoparticles in 2013 [9, 10].The evaluated worldwide production volume of AgNPs was approximately 500 tons per year [11]. With the wide use of NMs , the necessity to understand their adverse effects on public health and the environment has also increased which has led to the emergence a new field called nanotoxicology. It is a study of the toxic effects of NMs interactions with biological systems [12]. For example, some studies found out that an interaction with blood components (plasma proteins, coagulation factors, RBCs, WBCs and platelets) is believed to occur whenever NPs reach the systemic circulation [13]. Moreover, the *in vivo* and *in vitro* studies have demonstrated that exposure to AgNPs not only causes alternation in the blood and biochemical indicators, but also organs inflammations [14, 15]. It was also demonstrated that the toxic effects occur when reactive oxygen species (ROS) levels rise, resulting in oxidative stress damage [16, 17]. Furthermore, the accumulation of AgNPs and SiO_2NPs was observed in the liver, kidney, spleen, lungs and brain of rats [18, 19]. Widespread application of the various types of nanoparticles brings the risk of co-exposure. However, once the combination of nanoparticles enters the organisms bodies, it undergoes different physicochemical interactions which may result in changes in their prime characteristics and affect their bioavailability as a consequence. It also affects their toxicity which could become either less or more toxic [20].

2. Methods and Materials

2.1. Nanoparticles

The studied AgNPs were dark gray nano-powder (20-30 nm) particles spherical in shape and had 99.95% purity with a density of 10.5 g/cm^3. While SiO_2NPs were in white nano-powder form (20-23 nm) with 95.9% purity and a density of 2.4 g./cm 3. These nanoparticles powders were obtained from Areej Al-Furat Company (a chemicals Company in Baghdad, Iraq)

2.2. Animal Housing

Albino male mice (*Mus musculus*), with an average body weight of 30 ± 5 g, were used in the current study. The animals were housed in polypropylene cages in a specially controlled environment with 12 on 12 hours of light and darkness. Temperature was kept at 25°Ċ with a relative humidity of 50-60%. The nutrition sources were available 24 hours a day. The animals were cared for and housed in the animal house at the Biotechnology Research Center, Al Nahreen University.

2.3. Preparation of nanoparticles suspension

The suspension of the NPs was prepared by suspending weighed AgNPs and SiO_2NPs with deionized distilled water and putting the suspension tube in an ultrasonic bath for 60 minutes by using Branson 3510 (Memmert\Germany). The suspension of MiXNPs was prepared by mixing AgNPs and SiO_2NP suspensions.

2.4. Subchronic toxicity test of AgNPs, SiO2NPs, and MiXNPs

Mice were divided into eight groups (each group with 10 animals) and injected intraperitoneally three times a week with two different concentrations (50 and 100 mg/kg) of AgNPs, SiO_2NPs and MiXNPs for 2 and 4 weeks.

2.4.1. Hematological parameters

In order to examine hematological parameters, three animals were taken from each group including the control group. The blood samples, taken by heart puncture at the end of 2nd and 4th week, were then put in ethylenediaminetetraacetic acid (EDTA) tubes. Later complete blood counts (CBC) including red blood cell counts (RBC), white blood cell counts (WBC), hemoglobin (Hb) and platelets (PLTs) were immediately assessed using a fully automated digital hematology analyzer.

2.4.2. Biochemical parameters

The serum alkaline phosphatase activity (ALP), serum alanine aminotransferase activity (ALT) and serum aspartate aminotransferase activity (AST) were also measured, in addition to serum level of blood urea nitrogen (BUN), creatinine (CR) and uric acid (UA). The samples were collected from three members of each group, then placed in a gel tube, were later examined by using an A 15 analyzer (Biosystems/Spain). Thyroid Stimulating Hormone (TSH) was examined by an AFIAS-6 analyzer (Boditech/South Korea). The assessment was performed using specific kits and quality control reagents for validation.

2.4.3. Bioaccumulation study

About 0.25-0.5 g of organ tissues (liver and kidney) was weighed. Samples were prepared by using a microwave digestion system method [21].Concentration assays were performed by using an atomic absorption flame emission spectrophotometer (Perkin Elmer/ USA) for detecting the AgNPs. UV-visible spectrophotometer (Analytic Jena/Germany) was used to detect SiO_2NPs.

2.5. Statistical analysis

Statistical Analysis System- SAS (2012) program was used to detect the effects of various factors in study parameters. In this study, the least significant difference (LSD) test in the context of the analysis of Variance(ANOVA) was used to compare means.

3. Results and Discussion

3.1 Subchronic toxicity

3.3.1. Hematological parameters

Hematological parameters are used to determine the physiological response to an introduced substance. The changes in hematological parameters of the exposed mice are shown in Table 1.

A significant decrease in RBC counts was noticed in all AgNPs treated groups. \L) After exposure to 50 mg/kg for 4 weeks, the lowest mean value recorded was 5.16 ± 2.50 10^{12}\L. While non-significant decrease values were noted in the groups treated with SiO_2NPs, except in the groups exposed to 50 mg/kg for 4 weeks which recorded a significant increase. A significant decrease in RBC count was noted in groups treated with 100 mg/kg of MiXNPs, compared to the control group. The toxicity studies reported toxic effects of nanoparticles on RBCs which could be related to the large surface area of NPs compared to its size. Nanoparticles have the potential to cause hemolysis due to their large surface area that could enable more ions to be released. Both ions released by nanoparticles and the direct interaction of nanoparticles with RBCs result in the production of oxidative stress (ROS), membrane injury, and subsequently hemolysis [22, 23]. A significant decrease in Hb values was indicated after 2 weeks of exposure to AgNPs. The lowest mean value recorded was 10.86 ±1.18 after being exposed to 100 mg/kg of AgNPs for 2 weeks. Whereas, Hb values significantly increased in the groups treated with SiO_2NPs.The highest recorded value was 16.95 ±3.65 g/dL. Hb values also increased 14.70 ±2.46 g/dL after being exposed to 50 mg/kg of MiXNPs for 4 weeks, while the values decreased after 2 weeks of exposure to 100 mg/kg of MiXNPs; compared with the control group (12.72 ±0.75 g/dL). Non-significant changes in Hb value were noted in mice exposed to 100 mg/kg AgNPs, SiO_2NPs and MiXNPs for 4 weeks. The changes in RBCs and hemoglobin (Hb) levels may suggest that NPs are affecting their synthesis in the time of RBCs maturation and the production in the bone marrow [24]. Significant changes were indicated in WBCs counts after being exposed to AgNPs with the highest mean value of 8.96 ± 1.13 10^9/L after being exposed to 50 mg/kg of AgNPs for 4 weeks. The WBCs counts significantly increased in groups treated with SiO_2NPs with the highest count of 14.40 ±2.10 10^9\L observed after being exposed to 50 mg/kg for 2 weeks\). Furthermore, WBCs count significantly increased in groups exposed to MiXNPs for 2 weeks, while it significantly decreased in the groups exposed to MiXNPs for 4 weeks; compared with the control group. The increase in WBCs count indicates an increase in immunogenic response [15, 16].This decrease in the number of WBCs could possibly be because most particles accumulated in the organ tissues or were removed from the body by the liver and kidneys, resulting in a lower number of particles remaining in the blood [15]. The PLTs counts significantly increased in all treated groups, with different concentrations of NPs at all times of exposure, compared to the control group. This increase in PLTs number indicates anemia or other inflammatory diseases. High level of PLTs could lead to the formation of thrombus in the blood vessels which can promote the progression of atherosclerosis [25].

Table 1: Mean±standard of a hematological parameters of albino mice after 2 and 4 weeks of exposed intraperitoneally to 50 and 100 mg\kg concentrations of AgNPs, SiO2NP and MiXNPs.

Period AgNPs	Parameters	100 mg\kg		50 mg\kg		LSD Value
2 WEEKS	WBC 10^9\L	6.61 ±2.54 a	6.13 ±1.65 ab	5.90 ±0.50 b	6.13 ±1.65 ab	0.662 *
	RBC 10^{12}\L	7.53 ±0.77 b	8.10 ±.24 a	6.06 ±0.05 c	8.10 ±0.24 a	0.847 *
	Hb g\dl	10.86 ±1.18 b	12.72 ±0.75a	12.35 ±0.25 a	12.75±0.75a	1.33 *
	PLT 10^9\L	926.67±173.27 a	224.87±107.75 b	955.50 ±42.50 a	224.87±107.75 b	174.38 *
4 WEEKS	WBC 10^9\L	6.29 ±3.31 c	7.82 ±1.67 b	8.96 ±1.13 a	7.82 ±1.67 b	0.807 *
	RBC 10^{12}\L	7.20 ±0.05 b	7.80 ±0.49 b	5.16 ±2.50 b	7.80 ±0.49 a	0.936 *
	Hb (g\Dl)	12.25 ±0.05 b	12.00± 0.30 b	14.30 ±0.75 a	12.00 ±0.30 b	1.735 *
	PLT 10^9\L	833.50±84.50 a	426.25±108.95 b	1227.00±345.50 c	426.25±108.95 b	308.51 *
Period SiO2NPs	**Parameters**	**100 mg\kg**		**50 mg\kg**		**LSD Value**
2 WEEKS	WBC 10^9\L	9.16 ±0.11 b	6.13 ±1.65 c	14.40 ±2.10 a	6.13 ±1.65 c	2.372 *
	RBC 10^{12}\L	7.28 ±0.91	8.10 ±.24	7.91 ±0.46	8.10 ±.24	0.894 NS
	Hb g\dL	10.15 ±0.95	12.72 ±0.75	16.95 ±3.65	12.72 ±0.75	2.375 *
	PLT 10^9\L	1521.50±202.50 a	224.87±107.75 c	1027.00 ±139.00 b	224.87±107.75 c	261.48 *
4 WEEKS	WBC 10^9\L	7.04 ±2.25 c	7.82 ±1.67 b	8.86 ±1.16 a	7.82 ±1.67 b	0.794 *
	RBC 10^{12}\L	7.50 ±0.14 a	7.80 ±0.49 a	8.37 ±1.23 b	7.80 ±0.49 b	0.885 NS
	Hb g\dL	12.26 ±0.91 b	12.00 ±0.30 b	15.80 ±1.80 a	12.00 ±0.30 b	2.074 *
	PLT 10^9\L	836.33 ±86.41 a	426.25±108.95 b	1034.67 ±181.96 a	426.25±108.95 b	277.612 *
Period MiXNPs	**Parameter**	**100 mg\kg**		**50 mg\kg**		**LSD Value**
2 WEEKS	WBC10^9\L	7.72 ±0.93 a	6.13 ±1.65 b	7.15 ±1.35 a	6.13 ±1.65 b	0.709 *
	RBC10^{12}\L	7.62 ±0.57 ab	8.10 ±.24 a	6.74 ±0.34 b	8.10 ±0.24 a	1.026 *
	Hbg\dL	10.90 ±0.66 b	12.72 ±0.75 a	12.75 ±1.65 a	12.72 ±0.75 a	1.69 *
	PLT10^9\L	1056.33±132.51 a	224.87±107.75 b	1273.50 ±332.50 a	224.87±107.75 b	286.94 *
4 WEEKS	WBC10^9\L	6.36 ±1.59 b	7.82 ±1.67 a	6.90 ±1.39 b	7.82 ±1.67 a	0.779 *
	RBC 10^{12}\L	7.70 ±0.36	7.80 ±0.49	7.61 ±0.89	7.80 ±0.49	0.569 NS
	Hbg\dL	11.26 ±0.77 b	12.00 ±0.30 b	14.70 ±2.46 a	12.00 ±0.30 b	2.066 *
	PLT10^9\L	634.33±104.21 ab	426.25±108.95 b	978.67 ±445.33 a	426.25±108.95 b	269.03 *

Means having with the different letters in the same row differed significantly. * (P≤0.05).

3.3.2. Biochemical parameters

The biochemical parameters such as serum enzymes are used (as biomarkers) to indicate the toxic effects that organisms could be exposed to from environment pollution including NPs. Changes in biochemical parameters of exposed mice are shown in Table 2.

The mean value of ALT, ALP and AST levels showed non-significant changes after mice groups were treated with 100 mg/kg of AgNPs, SiO_2NPs, and MiXNPs for 2 weeks. While the ALT, ALP and AST levels significantly changed after 4 weeks of exposure to 100 mg\kg of studied NPs, except in the group treated with AgNPs where the ALP level showed non-significant change. However, after mice groups were treated with 50 mg/kg of AgNPs, SiO2 NPs, and MiXNPs, ALT and AST levels significantly increased in both time exposures (2 and 4 weeks).The noted highest mean value was 47.00 ±0.5 U/L (in mice group treated with 50 mg\kg of SiO_2NPs), and 144.67 ±0.88 U/L (in mice group treated with 50 mg/kg of MiXNPs) respectively. Whereas ALP level showed no change; compared to the control group. High level of AST indicates liver diseases [26]. A low level of AST is likely to occur with either liver or kidney disease, and/or inflammatory diseases [27]. ALT increase mostly indicates liver cells damage. However, ALT release is associated with liver tissue injury [28]. The abnormalities in ALP levels may be an indication of various diseases including hepatitis, gallstones and thyroid disease [29, 30]. However, the histopathological examination of the liver shows various alterations that indicate hepatotoxic effects of AgNPs, including hepatocellular degeneration [31]. The mean value of BUN levels significantly decreased in all treated groups with studied NPs for 2 and 4 weeks, except the group treated with MiXNPs for 4 weeks where no significant change in BUN level was noted. The lowest recorded value was 22.33 ±2.88 mg/dl. The mean value of UA level showed a non-significant change in the treated groups except the group treated with 50 mg/kg of MiXNPs for 2 weeks which observed significant changes with the recorded lowest value of 1.80 ±0.50 mg/dl. The CR level showed a significant decrease after mice were treated with 50 of SiO_2NPs for 2 weeks and 50 mg/kg of MiXNPs for 4 weeks where the observed lowest level was 0.133 ±0.03 mg/dl. While the CR level increased significantly after 2 weeks of MiXNPs exposure, since urea, uric acid and creatinine are waste products produced by the liver and removed by the kidney [32]. The BUN, UA and CR levels are considered as indicators of kidney and liver functions where high levels of BUN and CR indicate kidney disease or organ failure, while the low levels may indicate liver diseases. The histological examination demonstrated adverse effects of NPs in the kidney, for example tissue necrosis of glomerular cells and damage in the epithelium layer of the Henley loop were observed after AgNPs exposure [31].Also pathological change in the kidney tissues was noted after the exposure to SiO2NPs [33]. Regarding TSH levels, the result showed a significant increase of 0.300 ±0.00 µU/ml in the group treated with 50 mg\kg of AgNPs for 4 weeks. Significant increase of 0.250 ±0.05 µU/ml was also noted in the treated groups with 100 mg/kg of SiO_2NPs for 2 weeks. Significant increase in TSH levels was also noted in the groups treated with 100 mg/kg for 2 weeks and 50 mg/kg for 4 weeks of MiXNPs. However, a significant decrease was observed in the groups treated with 100 mg\kg MiXNPs for 4 weeks; compared with the control group. The change in hormone concentrations could be attributed to increased oxidative stress caused by NPs, which results in ROS and altered hormone levels. Besides NPs have an ability to penetrate tissues which causes inflammation or structural and functional disorders of the thyroid gland that is reflected on thyroid hormone (TSH) secretion [34].

Table 2: Mean ±standard of a biochemical parameters of albino mice after 2 and 4 weeks of exposed intraperitoneally to 50 and 100 mg\kg concentrations of AgNPs, SiO2NP and MiXNPs.

Period (Weeks)\ Concentration(mg\kg)	Groups	Parameters						
		ALT U/L	ALP U/L	AST U/L	BUN mg/dl	CR mg/dl	UA mg/dl	TSH µU/ml
2\100	AgNPs	45.33 ±0.88 a	93.00 ±0.57 a	146.00 ±1.15 a	27.67 ±0.88 a	0.200 ±0.05 a	2.40 ±0.11 a	0.133 ±0.03 a
	SiO2NPS	46.00 ±3.00 a	92.00 ±1.00 a	145.50 ±2.50 a	26.50 ±0.50 a	0.250 ±0.05 a	2.20 ±0.20 a	0.250 ±0.05 b
	MiXNPs	48.33 ±1.20 a	94.00 ±0.57 a	148.00 ±2.00 a	26.33 ±1.76 a	0.200 ±0.06 a	2.33±0.23 a	0.233±0.03 c
	Control	46.88 ±6.71 a	96.05 ±2.05 a	142.66 ±7.33 a	32.38 ±3.11 b	0.315 ±0.02 a	2.40 ±0.20 a	0.150 ±0.05 a
	LSD	10.052NS	3.461 NS	11.24 NS	5.996 *	0.196 NS	0.686 NS	0.139 NS
4\100	AgNPs	45.00 ±2.51 a	89.33 ±2.40 a	134.53 ±5.75 a	22.33 ±2.88 a	0.263 ±0.02 a	2.36 ±0.12 a	0.150 ±0.03 a
	SiO2NPS	44.53 ±2.82 a	89.33 ±1.45 a	194.67 ±22.84 b	22.73 ±2.18 a	0.220 ±0.01 b	2.33 ±0.08 a	0.150 ±0.02 a
	MiXNPs	44.16 ±2.09 a	89.00 ±1.15 a	189.33 ±19.67 b	25.26 ±1.88 a	0.233 ±0.02 a	2.26 ±0.08 a	0.100 ±0.00 b
	Control	54.36 ±0.64 b	100.96 ±1.92 b	157.40 ±2.92 c	39.80 ±8.85 b	0.286 ±0.02 a	2.67 ±0.06 a	0.167 ±0.03 a
	LSD	7.12 *	5.860 *	50.28 *	15.894 *	0.0595 *	0.302 NS	0.085 NS
2\50	AgNPs	44.33 ±2.84 a	93.67 ±1.20 a	139.00 ±2.64 a	29.33 ±1.45 a	0.333 ±0.08 a	2.16 ±0.13 a	0.233 ±0.06 a
	SiO2NPS	47.00±0.57 a	92.00±1.52 a	143.00 ±3.21 b	22.52±1.38 b	0.133±0.03 b	2.06 ±0.23 a	0.233 ±0.08 a
	MiXNPs	47.00 ±3.60 a	92.33 ±0.88 a	144.00 ±3.60 b	24.96 ±2.78 b	0.600 ±0.23 c	1.80 ±0.50 b	0.200 ±0.05 a
	Control	21.80 ±8.20 b	94.00 ±1.00 a	50.13±21.46 c	29.07 ±0.27 a	0.320 ±0.02 a	3.10 ±0.20 a	0.205 ±0.01 a
	LSD	12.55 *	4.192NS	25.71 *	6.515 *	0.473 NS	1.103 *	0.236 NS
4\50	AgNPs	46.50 ±0.50 a	92.50 ±2.50 a	144.50 ±4.50 a	26.00 ±4.00 a	0.300 ±0.00 a	2.65 ±0.05 a	0.300 ±0.00 a
	SiO2NPS	46.33 ±1.76 a	93.33 ±0.67 a	143.67 ±3.28 a	27.67 ±1.45 b	0.300 ±0.06 a	2.33 ±0.17 a	0.166 ±0.06 b
	MiXNPs	45.66 ±2.18 a	95.33 ±0.88 a	144.67 ±0.88 a	28.66 ±1.45 b	0.133 ±0.03 b	2.30 ±0.34 a	0.266 ±0.03 c
	Control	31.81 ±11.80 b	99.57 ±3.75 a	84.85 ±36.71 b	29.62 ±0.52 b	0.283 ±0.04 a	2.73 ±0.14 a	0.203 ±0.01 b
	LSD	23.011 *	7.882NS	55.085 *	5.943NS	0.151 *	0.788 NS	0.141 NS

Means having with the different letters in the same row differed significantly (P≤0.05)

3.3.3. Bioaccumulation study

In order to determine, the accumulation of studied NPs (AgNPs, SiO2 NPs, and MiXNPs), the deposition of NPs in the organs of treated animals (liver and kidney) were measured as shown in Table 3.

An accumulation was observed in the livers and kidneys of the treated group with 100 mg/kg of AgNPs (G1). While in the group treated with 100 mg/kg of SiO_2NPs (G2), the accumulation was noted only in the kidneys, compared with the control group. However, the

group exposed to 100 mg/kg of MiXNPs showed more accumulation of AgNPs compared with G1 and the control, and SiO_2NPs showed a different ratio of accumulation compared with G2 and the control group. At 50 mg/kg dose, the group treated with AgNPs showed an accumulation in both livers and kidneys, while accumulation of SiO_2NPs was noted only in the kidneys, compared with the control group. However, the accumulation of AgNPs and SiO_2NPs was noted in the group treated with 50 mg/kg of MiXNPs. After the exposure to nanoparticles and blood circulation, the distribution in many organs including the liver, spleen and kidneys occurs. "Dziendzikowska" [35] suggested that the liver is the first line of defense against NPs, for that the deposition of the non-biodegradable nanoparticles are likely to occur. Although the amount of these particles is translocated to other organs like the kidney and spleen [36, 37]. An accumulation of AgNPs after intraperitoneal exposure in body organs mostly the liver and kidneys, were observed by Razooki [38]. A previous study showed that SiO_2NPs could accumulate in the liver [20]. Furthermore, many researchers indicated that SiO_2NPs can be accumulated in kidney tissue [31, 39].

Table 3: The bioaccumulation in liver and kidney of albino mice after 4 weeks of exposed intraperitoneally to 50 and 100 mg\kg concentrations of AgNPs, SiO2NP and MiXNPs.

Concentration	Organs	AgNPs (G1)	SiO2NPs (G2)	MiXNPs	
				AgNPs	SiO2NPs
100 mg\kg	Liver	1.7 ppm	17 ppm	2.94 ppm	22 ppm
	Kidney	1.9 ppm	23.5 ppm	2.37 ppm	19.5 ppm
Concentration	**Organs**	**AgNPs**	**SiO2NPs**	**MiXNPs**	
				AgNPs	SiO2NPs
50 mg\kg	Liver	2.31 ppm	15	2.17 ppm	18.5 ppm
	Kidney	2.68 ppm	21 ppm	1.34 ppm	19 ppm
Control	Liver	0.00	16.5ppm	0.00	16.5 ppm
	Kidney	0.00	16.5ppm	0.00	16.5ppm
Means having with the different letters in the same row differed significantly. * (P≤0.05)					

Conclusions

The current study concludes that the exposure to nanoparticles (AgNPs, SiO_2NPs, and MiXNPs) induced clear and significant changes in blood parameters in all treated groups. The biochemical parameters of liver and kidney showed more alternations after being exposed to 50 mg\kg of studied nanoparticles compared with 100 mg\kg. However, the effects were more visible after 4 weeks of exposure. ALT, AST, and BUN were the most affected parameters; compared with the control groups. The results suggest that the combination of AgNPs and SiO_2NPs has a synergetic effect on thyroid-stimulating hormone (TSH) levels. The bioaccumulation study showed the capability of the liver and kidney on accumulating the AgNPs and SiO_2NPs respectively. However, the combination of nanoparticles demonstrated a synergetic effect on AgNPs accumulation.

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