Toxicity and Biochemical Impacts of new Pesticides (Potassium Silicate (Sil-Matrix 29 %) and Silica Nano-particles in Rat

H. M. El-bendary

Plant Protection Department, Faculty of Agriculture, Fayoum University, Egypt.

ABSTRACT: The application of silica nanoparticles silica- nanoparticles (SNPs) has wide ranging applications both in scientific research and industries because of their easy preparation and their potential uses in various fields. In spite of the versatile application of silica nanoparticles (SNPs), the potential of these nanoparticles (NPs) in agriculture remains unexplored, silica has become very popular as an insecticidal agent.The Toxic effect of silica nano-particles [SiO_2-NPs] was investigated using male albino rats, at sublethal doses, relative to control. The effects of orally administered at [1/30, 1/60 and 1/90 LD_{50}] for 30 and 60 days on immunoglobulin levels in blood and micro-nucleated polychromatic erythrocytes (MN). The SiO_2-NPs slight significantly reduced the antibodies (IgG, IgA and IgM) content relative to the normal health control rats. On the other hand, results revealed that the micro-nucleated polychromatic erythrocytes (MN) in rat bone marrow were adversely affected by treatment.The histological structure of liver, kidney and spleen samples was assessed in Sprague-Dawley rats (60 animals) after 30, 60 and 90 days, orally administration of Sil-Matrix 29 % and Silica nanoparticles (SiO_2-NPs) at 30 mg/kg twice dose weakly. Histological findings included the appearance of foreign body-type granulomas in the liver and spleen as well as microgranulation in the liver after administration of (NPs). The number of granulomas was significantly lower after administration of (SiO_2-NPs) for 30, 60 and 90 days. In conclusion, silica nanoparticles are relatively biocompatible nano materials, at least when considering acute toxicity.

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Keywords: silica nanoparticles; immunoglobulin levels, histological toxicity.

INTRODUCTION:

Nano molecular (NMs) can assume many different forms, such as tubes, rods, wires, spheres or particles. NMs have been widely used in consumer and industrial applications, such as medicine, cosmetics and foods, because they exhibit unique physicochemical properties and innovative functions Salata O. (2014).

(SNPs) are used as catalysts Banerjee *et al*. (2009), electronic and thin film substrates Jeong *et al*. (2010), electronic and thermal insulators Kim *et al*. (2011).

Recently silica-nanoparticles (SNPs) have shown promising potential in the field of biotechnology. The chemistry of silica provides the opportunity for a variety of surface functionalization with hydroxyl/ amine/ thiol/ carboxyl groups Han *et al*. (2009), which can be used to attach a number of bio targeting molecules.

Bioactive molecules like enzymes, genetic materials and chemotherapeutic drugs can also be incorporated within porous silica-nanoparticles (SNPs). So silica-nanoparticles (NPs) have great potential as DNA Bharali *et al*. (2005) or drug delivery agent Wang *et al*. (2012).

In particular, Kumar *et al*. (2010), showed a complete clearance of organically modified, 20-25 nm SiO_2 - NPs from nude mice. This clearance occurred via hepatobiliary excretion within 45 days after a single intravenous infusion, with no sign of organ toxicity.

In contrast, Xie *et al*. (2010), in a partially analogous experimental model, demonstrated extensive liver injury *(i.e.*, hepatocyte necrosis and mononuclear infiltration) accompanied by silica nanoparticles (SiO_2-NPs) retention in the reticulo-endothelial system (RES) for over 90 days. A similar hepatotoxic effect after either single or repeated (SiO_2-NPs) administration was also reported by others Nishimori H. (2009). The differences in the hepatotoxic effects, apart from other factors, might be accounted for by the distinct characteristics of particle size Cho, M. (2009), surface charge Isoda, K. (2011).

MATERIALS AND METHODS:

1. Silica Nanoparticles characterization: Silicon dioxide nanoparticles (SIO_2-NPs), also known as silica nanoparticles or nanosilica, are the basis for a great deal of biomedical research due to their stability, low toxicity and ability to be functionalized with a range of molecules and polymers.

Chemical Properties of silicon dioxide.

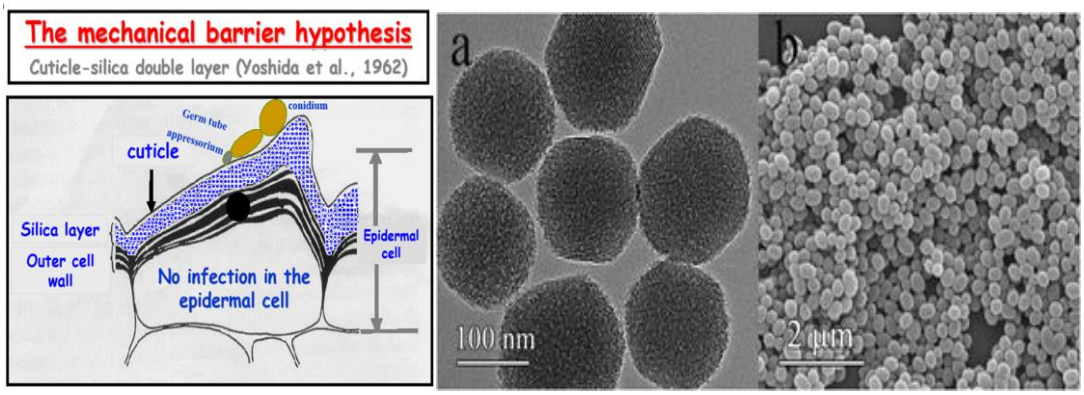
Physical Properties

Silicon dioxide nanoparticles appear in the form of a white powder. The table below provides the physical properties of these nanoparticles.

Properties	Metric	Imperial
Density	2.4 g/cm^3	0.086 lb/in^3
Molar Mass	59.96 g/mol	-

Thermal Properties

Properties	Metric	Imperial
Melting Point	1600°C	2912°F
Boling Point	2230°C	4046°F



2. Sil-Matrix 29 %: For use on vegetables, fruits, nuts, vine crops, field crops, ornamentals and turf for control of fungal diseases, and suppression of spider mites, aphid, whiteflies and other insects**.**

• Active Ingredient: Potassium Silicate 29 %.

• 8 % K_20, 21 % SiO_2.

• Completely Soluble: (Alkaline pH buffered to near neutral when diluted; aids in increasing pH of acid solutions.

• Product stable under all conditions of use and storage.

• Created by mixing Quartz sand (SiO_2) and Potassium Carbonate (K_2CO_3) and melting at 1200 °C.

•Amorphous silica deposits in the leaf apoplast.

• Prevents penetration of fungi, becomes less Palatable to insects, silica acts as a desiccant (used commonly in packaging to reduce humidity).

•Persistence and degradability: Inorganic Soluble silicates, upon dilution, rapidly depolymerize into molecular species indistinguishable from natural dissolved silica.

• Toxicity: Fish (Leuciscus idus) LC_{50} (48 hour) >146 mg/l.

Aquatic invertebrates: (Daphnia magna) EC_{50} (24 hour) >146 mg/l.

3. Sub chronic toxicity: Rats (60 animals) *Rattus norvigicus*, (100-120 gm.), were housed in laboratory animal center, the animal were kept under normal health laboratory conditions for two weeks in their cages prior to experiment of acclimizathion. Rats were housed individually in a room maintained at 20 ^{0}C with a 12 hr. light/12 hr. dark cycle. They were allowed free access to tap water and fed on a diet. All experiments were performed in accordance with the "Guide for the Care and Use of Laboratory Animals (2011), and approved by the local Ethics Committee.

4. Animal treatment schedule: Randomized groups of rats housed in cages containing saw dust as bedding and were allocated into (6 groups, each group contained 10 males) the first (1st), second (2nd), and third (3rd) groups were treated with Sil-Matix 29%, while, fifth (5th), sixth (6th) and seventh (7th) groups were treated with (SiO_2-NPs) at dose 30 mg/kg twice per weak, via oral administration for 30, 60 and 90 days respectively. At the end of the experiment period, the tissues and blood samples were collected according to standard procedures in tubes contained anticoagulant (EDTA solution), than whole blood was centrifuged twice at 3000 rpm for 10 min in order to separate serum at room temperature to obtain the plasma which were kept frozen at -20 ^{0}C until used for analysis.

5. Experimental Protocol:

5.1. Determination of IgG, IgA, IgM (RID):

The kit is intended for measuring plasma (IgG, IgA, IgM) and other biochemical fluids Fahey and Makelvey, (1965). The method involves antigen diffusing radially from a cylindrical well through an agarose gel containing an appropriate mono-specific antibody. Antigen-antibody complexes are formed which, under the right condition, will from a precipitin ring. The ring size will increase until equilibrium is reached between the formulation and breakdown of those complexes, this point being termed 'completion'. At that stage a liner relationship exists between the square of the ring diameter and antigen concentration. In this study, the rings were measured at completion and a references table was provided, which converts ring diameter directly to protein concentration.

Reagent: RID plates: contains monoclonal antibody to (IgG, IgA, IgM) in agarose gel.
Calibrators: are supplied in stabilized liquid from as set of three containing high, medium and low concentration of the stabilized liquid from and included for use as diluent.
Control plasma or serum: supplied in stabilized liquid from the expected concentration for (IgG, IgA, IgM) are marked on the vial label.

Procedure: The method does not require the construction of a calibration curve-sample concentrations corresponding to each ring diameter are read directly off the (RID) reference table. Rings must be allowed to develop to completion which will require a minimum diffusion time of 48 hours (72 hr. for IgM).
The calibrators control and test samples should be gently mixed immediately before use. The required number of wells were filled with 5 ml of high calibrate ore using a micropipette. The remaining wells were then filled 5 Ml of appropriately diluted test samples and controls. Plates were not left open for long period during calibrator/test sample application to avoid excessive drying of the gel.
After samples application, the lid is tightly closed and the plate was stored flat with the (IgA) and for 72 hours for (IgM).
After he required diffusion time, ring diameter was measured to the nearest 0.1 mm, using a jeweler's eyepiece. Results were read off (RID) reference.

5.2. Micronucleus analysis:

5.2.1. Extraction of bone-marrow: The adhering soft tissue and epiphyses of both tibiae were removed after killing rats. The marrow was aspirated from the bone and transferred to centrifuge tubes containing 5 ml fetal calf serum (one tube for each animal).

5.2.2. Preparation of the smears: Bone-marrow smears were made according to Schmid (1975). The tubes were centrifuged at 3000 rpm for 10 min., the supernatant was removed and small drop of the viscous pellet was transferred on the end f a slide and spread by a cover glass held at an angle of about 45 degrees. The preparation was then air-dried.

5.2.3. Staining: The preparation was stained in ordinary vertical staining jar according to the method of Schmid (1973). The slide were fixed in absolute methanol for 5 min., rinsed twice in deionized distilled water, stained for 10 min. in Gimsa (1:6 Gurrs R-66 Giemsa in deionized water), rinsed again thoroughly in deionized distilled water, air-dried, cleaned in xylene for 3 min. and mounted.

5.2.4. Screening of slide: Four thousands polychromatic erythrocytes per group were scored by using a special hand counter. The frequent of micronucleated cells was expressed as a present of micronucleated cells based on the total polychromatic erythrocytes percent. Micronuclei were identified as dark-blue staining bodies in the cytoplasm of polychromatic erythrocytes.

5.3. Histopathological examination:

5.3.1. Sampling: Autopsy samples were taken from livers, kidneys and spleen of experimental groups and placed in fresh fixative 10% formalin saline solution for at least 12 hours, Then washed in a running tap water overnight, dehydrated in ascending grades of serial dilutions of alcohol, then fixed in cleared xylol, fixed tissue samples were processed routinely by paraffin embedding technique. Liquefied Para film, (melting point between 55°C and 90°C) for one and a half hours. After solidification of Para film, wax blocks were cut at section of 5.5 µm in thickness were

trimmed with rotary microtome at 200 µm intervals, and every eight section thought the tissue was collected on the Super Frost Plus slides.

5.3.2. Staining method: (Haematoxylin and Eosin stain). The section were placed in descending grades of alcohol and rinsed in distilled water. The sections were stained in haematoxylin for 1/2 minutes and then placed in tap water for 3-5 minutes. Counter staining was done in 1 % solution of eosin for one minute followed by washing in distilled water. The sections were dehydrated, cleared in xylol and mounted in Canada balsam, (the nuclei will stain and the cytoplasm will take red color).

5.3.3. Histopathological Examination: The resulting sections covered with cover slides to be ready for microscopically examinations.

6. Statistical analysis: The data were analyzed by using SPSS (version 4.0) for windows and expressed as mean ± S.E. Paired sample t-test was used to compare between the data of the control and those of treatments.

RESULTS AND DISCUSSION:

1. Immunoglobulin levels: The effects of SiO_2-NPs on immunoglobulin levels in blood of male albino rats presented in Table (1) and Fig. (1). The SiO_2-NPs slight significantly reduced the antibodies (IgG, IgA and IgM) content relative to the normal health control rats.

Data showed that oral ingestion SiO_2-NPs revealed lowest than control group. Also high dose of the SiO_2-NP 1/30 LD_{50} resulted more influence than the lower dose 1/60 and 1/90 LD_{50}. This means that the reduced values of immunoglobulin were paralleled with dose increasing. The decreased contents of (IgG, IgA and IgM) by ingestation of SiO_2-NPs may be due to the toxic of body metabolism. The present results are in agreement with those of Salah *et al.*, (2010) and Abdou *et al.*, (2010), they reported that the three immunoglobulin (IgG, IgA, IgM) were reduced by Tefluthrin pesticide induction and the formulation pesticide had more influences than the technical ones. Also, Righi *et al.*, (2008) reported that systemic insecticides at certain dosage levels simultaneously induce stress-like symptoms and immunosuppressive effects in albino rats. These observations are in agreement with the present results in which SiO_2-NPs induction decreased the immunoglobulin contents in plasma of intoxicated rats relative to control. These may contents in plasma of intoxicated rats relative to control. These may be due to that the xenobiotic and removal free radical Verma *et al.* (2007). In general immune compounds were changed paralleled with protein biosynthesis processes.

Table (1): The effects of SiO_2-NPs on immunoglobulin levels in blood of male albino rats.

Treatments	Doses LD_{50}	Treatment for 30 days					
		IgG		IgA		IgM	
		mg/dl	%	mg/dl	%	mg/dl	%
Control		2501	100	228	100	346	100
SIO_2-NPs	1/30	2325	92	178	78	284	82
	1/60	2356	94	194	85	299	86
	1/90	2482	99	207	91	311	90

Treatments	Doses LD_{50}	Treatment for 60 days					
		IgG		IgA		IgM	
		mg/dl	%	mg/dl	%	mg/dl	%
Control		2513	100	237	100	339	100
SIO_2-NPs	1/30	2230	88	142	60	248	73
	1/60	2308	91	171	72	282	83
	1/90	2485	98	218	91	326	96

(%) Relative to control. Each value represented the mean of 5 rats (mean ± SD). Statistical differences from the control: *significant at $P \leq 0.05$& highly significant at $P \leq 0.01$.

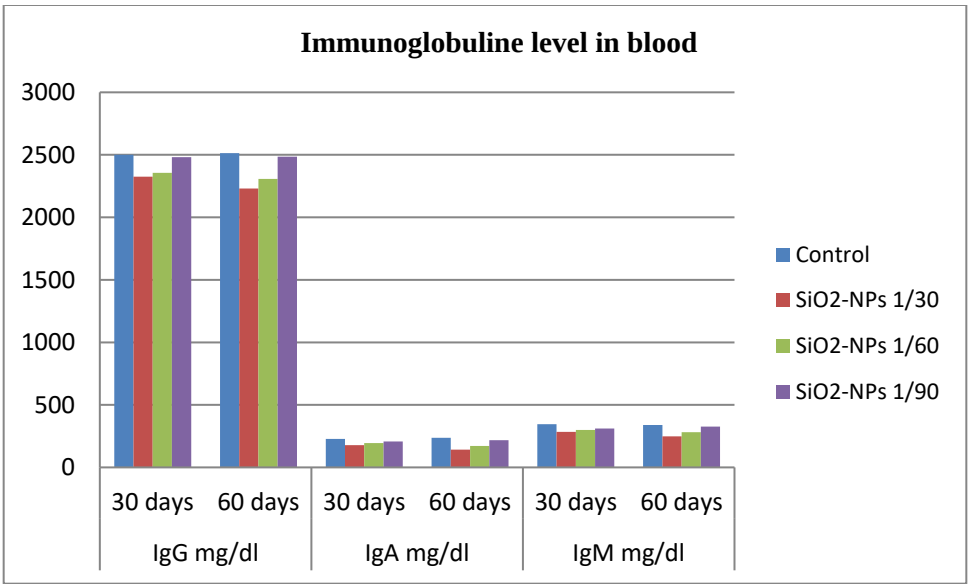


Figure (1): The effects of SiO_2-NPs on immunoglobulin levels in blood of male albino rats.

2. Micronucleus on bone marrow:

A 'micronucleus' is literally a small nucleus. The nucleus is an organelle in the cell contains the genetic materials (DNA) that directs normal cellular function and reproduction. In cells of eukaryotic organism, the nucleus contain (DNA) packaged into chromosomes. Chromosome shape, size, and number are constant for the species. During cell diviSiOn, the genetic material replicates and then divides equally between the two daughter cells that are produced. If the process is disrupted, or the chromosomes are broken or damaged by chemicals, then the distribution of genetic material between the two daughter nuclei during cell diviSiOn may be affected and pieces or entire chromosomes may fail to include in any of the two daughter nuclei. When this occurs, the genetic material that is not incorporated into the new nucleus may from its own 'micronucleus' which is clearly visible with the microscope. Thus in the micronucleus test animals are treated with chemicals and then the frequencies of the micro-nucleated cells are determined. In case of any animals treated group showed significantly higher frequencies of micro-nucleated cells than the untreated one, then the chemical is considered to be capable of inducing structural and/or numerical chromosomal damage Matter and Schmid, (1976). On the other hand, after withdrawal was still significant in the treatment of SiO_2-NPs. In general, the previous results revealed that the micro-nucleated polychromatic erythrocytes (MN) in rat bone marrow were adversely affected by treatment, as illustrated in Table (2) and Fig. (2).

Table (2): Micronucleated polychromatic erythrocytes on bone marrow cells of male rats exposed to SiO_2-NPs

Treatments	Doses LD_{50}	Treatment period 30 days		Treatment period 60 days	
		Micronuleated polychromatic	Micronucleus (MN %)	Micronuleated polychromatic	Micronucleus (MN %)
Control	----	14	0.35	16	0.40
SiO_2-NPs	1/30	34	0.85**	20	0.50*
	1/60	24	0.60*	17	0.43
	1/90	17	0.43	16	0.40

(%) Relative to control. Each value represented the mean of 5 rats (mean ± SD).
Statiistical differences from the control: *significant at $P \leq 0.05$& highly significant at $P \leq 0.01$.

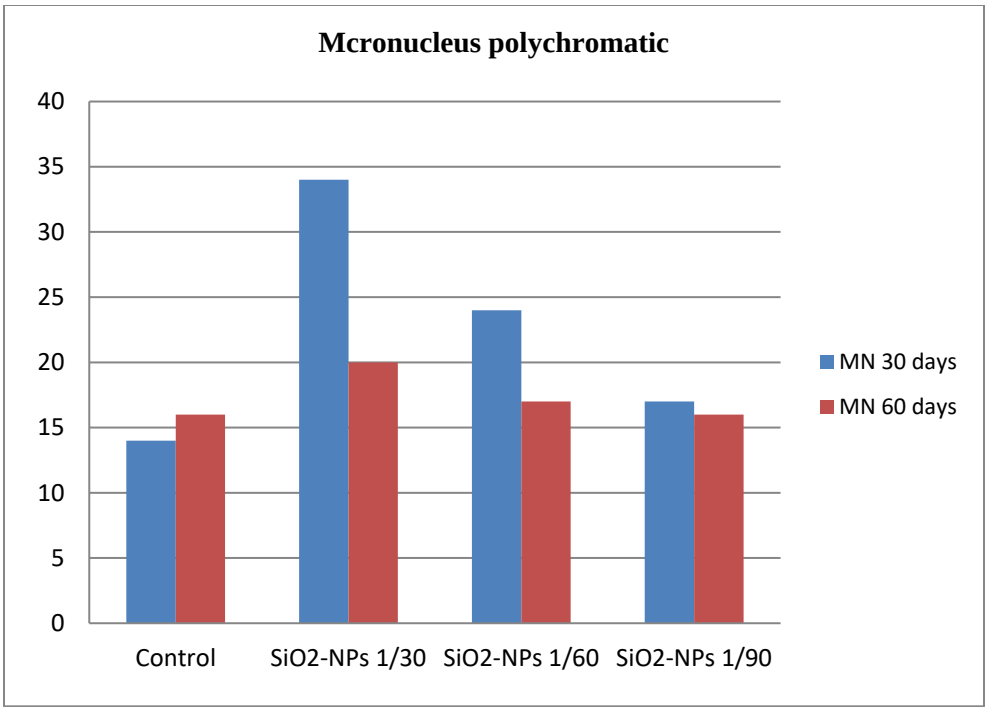


Figure (2): Micronucleated polychromatic erythrocytes on bone marrow cells of male rats exposed to SiO_2-NPs

3. Pathological finding in Liver: Data in Fig. (3) and Table (3) showed that, after three month treatment revealed, congestion of hepatic blood vessels and sinusoids. Other cases revealed congethion of hepatic blood vessels and vacular degeneration of hepatic cells with ruelear changes. The portal areas showed congethion of blood vessels and aggregathion of lymphocytes around the blood vessels and hyperplastic bile ducts proleferation.
After two months, vacular and hydropic degeneration together with focal necrosis of some hepatocytes were noticed, some of cells showed fatty changes with nuclear changes. Besides to the previously mentioned lesions. The portal areas showed neoplastic cells orginate from the cells of bile ducts epithelium.
Liver suffered from necrosis after treatment with (SiO_2-NPs) as a toixc materail reached to the liver via the gastro intestinal tract blood supply, therefore, the necrosed aresa mainly appeared around portal tract.
We cane concluded that, in the liver multiple foreign body-type granulomas and mononuclear infiltrates were identified in (SiO_2-NPs) treated animals after 90 days post-infusion . However, small, dense granulomas as well as microgranulation of hepatocytes appeared in the liver of SiO_2NP-treated animals at the 60 days. Notably, granulomas were not observed in the liver of (SIO_2-NPs) treated animals after 30 days post-infusion.

4. Pathological finding in kidney: As data presented in Fig. (4) and Table (3). The kidney of the sacrified rats after three month showed congestion of renal blood vessels in both cortex and nedulla together with perivascular edema. Degeneration and coagulative necrosis in other renal tubules were detected. After two months the kidney of scarificed rats showed wide spread lesions represented by congestion of renal blood vessels together with interstetial hemorrhage. Aggregation of lymphocytes perivascular. Some slides showed focal medulary hemorrhages and edema, sever cangested glomerular tuft and few round cells could be observed.
In generally, the surrounding hepatocytes appeared moderately dystrophic, starting from the 60 days post-infusion. Inflammatory infiltrates consisting of both mononuclear cells and granulomas were also observed in the spleen in both 60 and 90 days treated animals. The mononuclear inflammatory infiltrates in the spleen were more variable in both size and shape in comparison to those seen in the liver. The amount of granulomas in the liver samples was significantly lower in 60 days treated rats as compared with 90 days treated rats. In addition, the density of granulomas higher in the liver at the 90 days, but should be noted that the number of granulomas at the 60 and 30 days post-infusion. There were apparent histological abnormalities in the kidney samples related to treatment with either treatment period.

5. Pathological finding in Spleen: Histopathological changes of the spleen of sacrified rats after three month showed hyperplasia of the lymphocytes of the white pulp, together with infiltration of the red pulp with lymphocytes. After two months the spleen showed congestion of splenic sinusoids and heamorrhages with depletion of lymphocytes in white pulp, as illustreated in Fig. (5) and Table (3).
At present, there is some evidence that hydrogenated, porous with 90 days treatment are more readily biodegradable than other. This is due to the formation of a back bonded oxygen (Si-O-Si bonds), resulting in the rapid layer by layer nanoparticles (NPs) dissolution in biological fluids Hirsch *et al.,* (2003).

For instance, porous silica nanoparticles silica nanoparticles (SiO_2-NPs) were shown to have a half-life in the blood of approximately only 10 min Moos *et al.,* (2010), requiring either thermal oxidation or thermal hydrocarbonization to increase particle half-life and ensure effective biological applications. Our findings on the persistence of microgranulation in the liver up to the 180 day after administration of (SiO_2NPs) suggest that the rate of biodegradation for colloidal might be lower.

In the present study, multiple foreign granulomas were identified after nanoparticles (NPs) administration in organs, specifically, in the liver and spleen. The amount of granulomas was significantly lower in the animals treated with 90 days compared with those treated with 30 and 60 days.

On the other hand, the lysosomal enzymes cannot digest inorganic material, which leads to the accumulation of (NPs) in macrophages. Activated macrophages secrete proinflammatory cytokines, such as interleukin-1, interleukin-6, and tumor necrosis factor α. The macrophage-derived inflammatory cytokines have two major effects: (1) expression of adhesion molecules on endothelial cells for extravasation of monocytes and lymphocytes; and (2) stimulation of targeted migration of mononuclear cells to the area of inflammation (chemo taxis). Thus, additional mononuclear phagocytes are recruited to the tissue from the intravascular space. Both resident and recruited macrophages and lymphocytes are organized into a specific association of inflammatory cells, or granuloma. Interestingly, the extensive formation of granulomas in the liver and spleen was not associated with any appreciable changes in biochemical serum markers in our experiments. This fact suggests that, despite the presence of multiple granulomas, liver function remained unaltered.

Several factors are thought to have a profound impact on the development of nanoparticle-mediated toxicity. One of the key factors is nanoparticles (NPs) diameter, which, at least for colloidal (NPs), inversely correlates with the surface area. In general, smaller (NPs) with a greater surface area are more toxic both in vitro and in vivo Napierska *et al.,* (2010). Higher doses of (NPs) and/or their repeated administration are associated with higher probability of toxicity.

In vivo toxicity of (SiO_2NPs) after intravenous administration was studied by several groups; Xie *et al*. (2010), reported intracellular persistence of 20 and 80 nm (SiO_2NPs) in the lungs, liver, and spleen for over 45 days after administration in mice at a dose of 20 mg/kg. The amount of 20 nm (SiO_2NPs) in the liver and spleen was higher than that of the 80 nm (NPs). Moreover, both types of (SiO_2NPs) caused periportal mononuclear infiltration in the liver, as well as hepatocyte necrosis. In a more recent study, administered (SiO_2NPs) accumulated mainly in the liver and in the white pulp of the spleen Xie *et al.,* (2012).

The (NPs) in both urinary and hepatobiliary excretion from the organism, with the former being more efficient. Nishimory *et al.* (2009), compared the effects of 70, 450, and 1000 nm SiO_2NPs on liver histology and function in mice. (SiO_2NPs) (70 nm) caused liver injury at a dose of 45 mg/kg, while 450 and 1000 nm (SiO_2NPs) were found to be non-toxic even at 100 mg/kg. Repeated intravenous administration of 70 nm (SiO_2NPs) for 4 weeks resulted in hepatic microgranulation and, at a later stage, liver fibrosis. It was shown in mice that organically modified 20–25 nm (SiO_2NPs) were completely eliminated from the organism within 15 days after intravenous infusion at a relatively low dose of 2 mg/kg Kumar *et al.,* (2010). The LD_{50} of (SiO_2NPs) was found to be greater than 1000 mg/kg in mice [26. No histopathological findings in the liver, spleen, lung, or kidney were observed in mice that received (SiO_2NPs) at single doses ranging from 40 to 190 mg/kg. At the same time, lymphocytic infiltration, microgranulation, and degenerative necrosis of hepatocytes were observed in the liver when hollow (SiO_2NPs) were administered at 500 or 1280 mg/kg. Cho *et al*. (2009), analyzed tissue distribution and excretion of 50, 100 and 200 nm (SiO_2NPs) given to mice at a dose of 50 mg/kg. Surprisingly, the cellular uptake of (SiO_2NPs) increased with their size. Particles of all sizes were excreted via urine and bile; the most efficient urinary clearance was observed for 50 nm (SiO_2NPs). The NPs persisted in liver and splenic macrophages for four weeks post-injection. To summarize, most of the in vivo studies demonstrated the accumulation and persistence of (SiO_2NPs) in macrophages of the liver and spleen, which

was associated with a variable degree of inflammatory response. The determinants of the observed toxicity are complex and include particle size, surface area, dose, and treatment regimen. Our results generally confirmed the above findings. Single orally administration (SiO_2NPs) to rats at a dose of 20 mg/kg resulted in granuloma formation and mononuclear infiltration in the liver and spleen that persisted for 90 days post-ingestion.

Table (3): Histopathological changes in the liver, kidney, and spleen of male rats exposed to Sil-Matrix and (SiO_2-NPs) based on scoring severity of injury in organs.

Treatments/dose 30 mg/kg	Period/day	Summary of histopathological observation								
		Sub-chronic (90 day)			Withdrawal period (60 day)			Withdrawal period (30 day)		
		Renal injury			Renal injury			Renal injury		
		liver	kidney	Spleen	liver	kidney	spleen	liver	kidney	spleen
SiO_2-NPs	30	+	++	+	+	+	+	+	+	-
	60	++	++	++	++	++	+	++	+	+
	90	++++	+++	++	++	+++	++	++	++	++

Scores in terms of numerical values are mentioned in histopathological studies section.

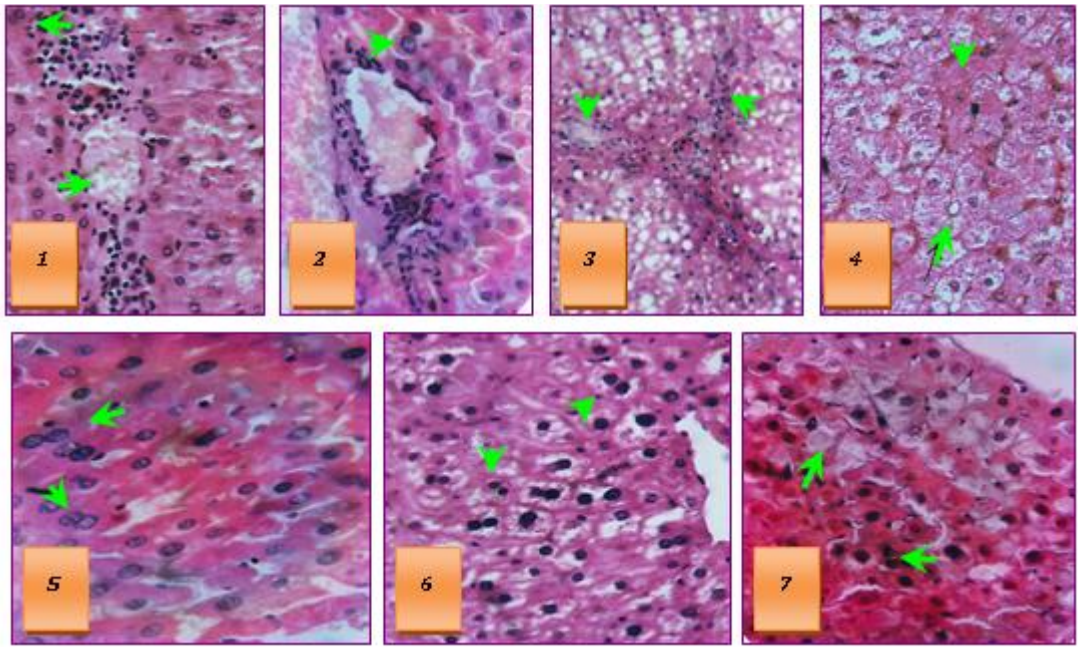


Fig. (3): Liver paraffin section stained with haematoxyline and eosin (H&E) for histopathological changes.Sil-matrix group [1-2-3-4] showing degeneration, necrosis, fatty changes with nuclear changes, neoplastic cells. (SiO_2-NPs) group [5-6-7] showing congestion of blood vessels, congethion, congethion and aggregathion.

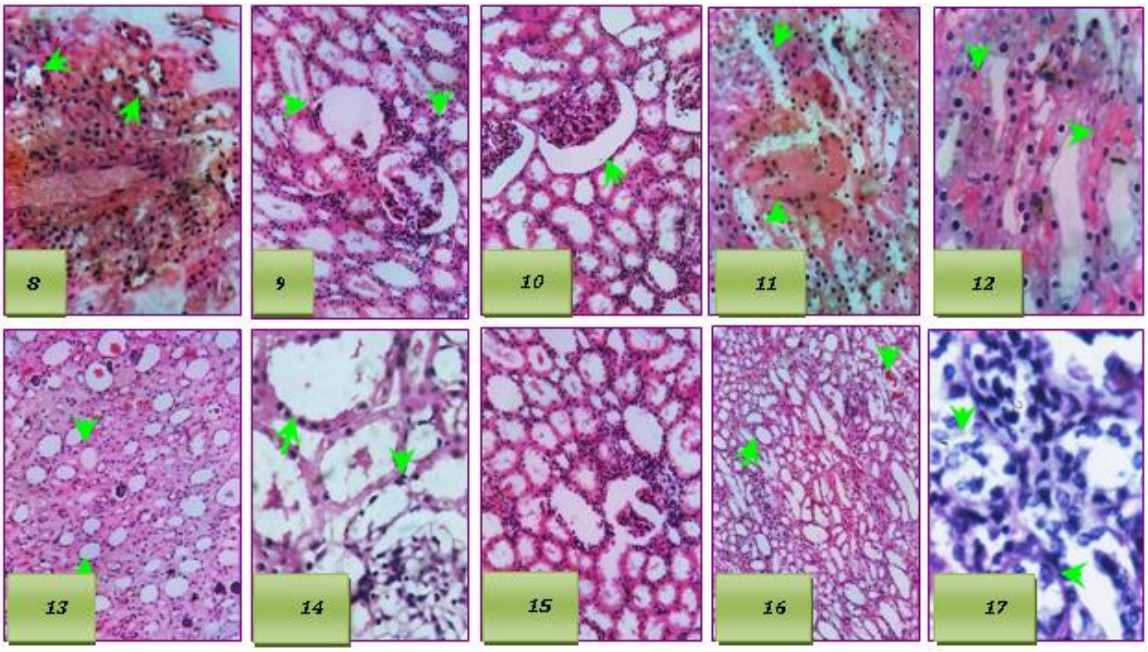


Fig. (4): Kidney paraffin section stained with haematoxyline and eosin (H&E) for histopathological changes.Sil-Matrix group [8-9-10-11-12] showing congestion in both cortex and nedulla, perivascular edema, Degeneration and coagulative necrosis. (SiO_2-NPs) group [13-14-15-16-17] showing congestion, hemorrhage, Aggregation of lymphocytes perivascular, hemorrhages and edema and sever cangested glomerular tuft and few round cells.

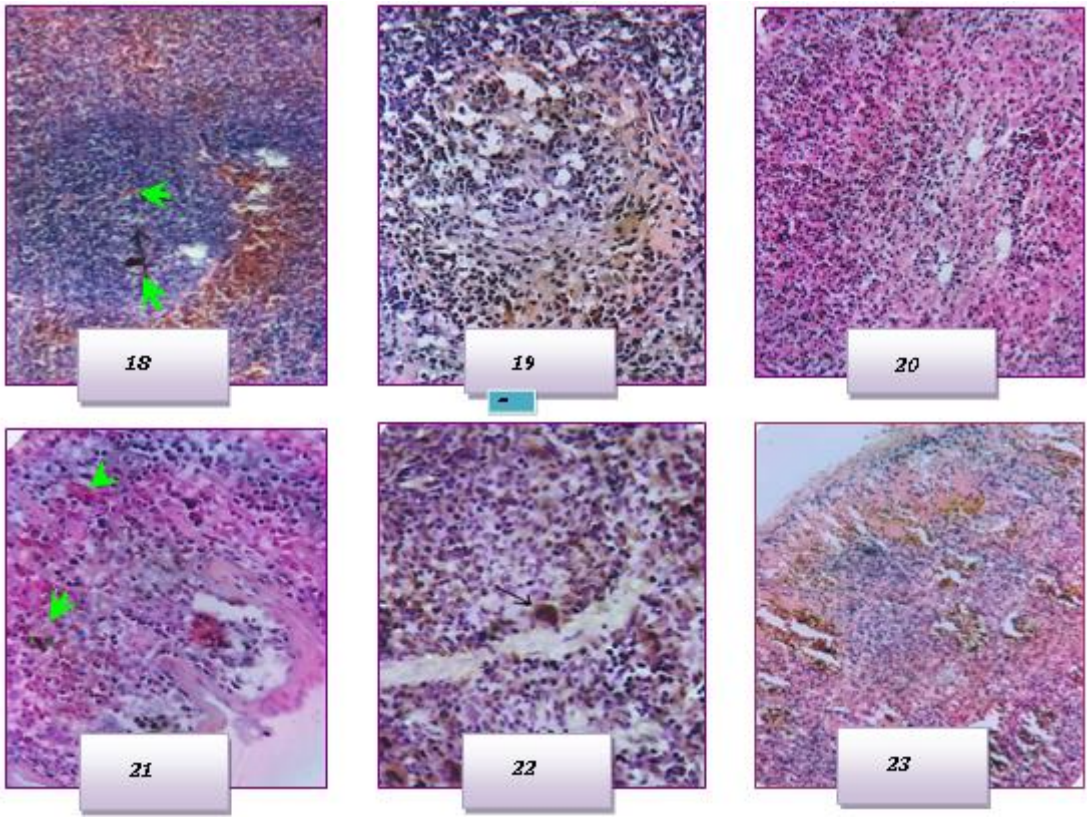


Fig. (5): Spleen paraffin section stained with haematoxyline and eosin (H&E) for histopathological changes. Sil-matrix group [18-19-20] showing hyperplasia, infiltration and lymphocytes. (SiO_2-NPs) group [21-22-23] showing congestion and heamorrhages.

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