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Research Article

Subacute Toxicological Profile Assessment of Silver-Nanoparticles After Topical Mucosal Application in Albino Rats

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Abstract

Aim: The present study aimed to assess the toxicity of topical administration of two concentrations of silver nanoparticles after 28 days exposure.

Materials and Methods: Sixty males albino rats were randomly divided into the following experimental groups; Group 1 (vehicle control group): consisted of 20 healthy rats (given 0.5 ml distilled water once daily for 4 weeks topically on rat's tongue). Group 2: (nano-silver 50 group): consisted of 20 healthy rats (given 0.5 ml nanosilver solution50 µg/ml once daily for 4 weeks topically on rat's tongue in a dose of 10mg/kg). Group 3 (nano-silver 100 group): consisted of 20 healthy rats (given 0.5 ml nanosilver solution100 µg/ml once daily for 4 weeks topically on rat's tongue in a dose of 10mg/kg).By the end of study blood was drawn then analyzed for GOT (glutamic oxaloacetic transaminase), GPT (glutamic pyruvic transminase), CRE (creatinine), and BUN (blood urea nitrogen). Tongue, kidneys, and liver were removed, weighed, fixed, stained, and examined under light microscopy for histopathological study.

Results: The results showed that there was no significant difference in the organs weight of rats after 4 weeks of exposure, no significant changes in all four enzymes tested. Histopathologic examination revealed no pathological abnormalities in all treated animals compared to untreated healthy animals. (no inflammatory cell infiltration, no hyperplasia, no fatty degeneration ,no edema ,as well as no necrosis).

Conclusion: silver nanoparticle (size 20 nm) in concentrations of 50,100 µg/ml in dosing volume 10mg/kg body weight are safe when applied topically in rats for 28 days.

Keywords: Silver Nanoparticle; Topical; Sub-Acute; Toxicity; Rat

Introduction

Nanotechnology is rapidly growing by producing nanoproducts and nanoparticles (NPs) that have novel and size-related physico-chemical properties differing significantly from larger matter [1]. The novel properties of NPs have been exploited in a wide range of potential applications in medicine, cosmetics, renewable energies, and biomedical devices [2-4]. Among them, silver nanoparticles (Ag-NPs or nanosilver) have attracted increasing interest due to their unique physical, chemical and biological properties compared to their macro-scaled counterparts [5].

Nanoparticles have a large surface area to volume ratio which leads to an alteration in biological activity compared to the parent bulk materials. In the past two decades, the use of nanoparticles (NPs) in experimental and clinical settings has risen exponentially due to their wide range of biomedical applications, for example in drug delivery, imaging and cell tracking [6-9]. This highlights the need to consider not only the usefulness of NPs but also the potentially unpredictable and adverse consequences of human exposure thereto. In this context, NP toxicity refers to the ability of the particles to adversely affect normal physiology as well as to directly interrupt the normal structure of organs and tissues of humans and animals. It is widely accepted that toxicity depends on physiochemical parameters such as particle size, shape, surface charge, chemistry, composition, and subsequent NPs stability. The exact underlying mechanism is as yet unknown, however, recent literature suggests cytotoxicity to be related to oxidative stress and pro-inflammatory gene activation [10-12]. Further to particle-related factors, the administered dose, route of administration and extent of tissue distribution seem important parameters in nano-cytotoxicity. Typically, cell-based toxicity studies use increasing doses of the NP in order to observe dose-related cellular or tissular toxicity. Such dose-response correlations are the basis for determining safe limits of particle concentrations for *in vivo* administration [13].

Silver nanoparticles have come up to the market by many industries with diverse medical applications ranging from silver

based dressings to silver coated medical devices in band, pad, gloves, catheter cover, wound dressing etc. [14,15]. As a result of very few data available on the safety of silver nanoparticles, which is increasingly used in industry and its direct contact with the skin, the dermal and systemic toxicity of this nanoparticle should be carefully determined by *in vitro* and *in vivo* models to define its safety thresholds for regulatory purposes [16,17].

The antibacterial activity exhibited by silver in a number of studies [18-21] has resulted in the widespread use of silver nanoparticles in bedding, washing machines, water purification, toothpaste, shampoo and rinse, nipples and nursing bottles, fabrics, deodorants, filters, kitchen utensils, toys, and humidifiers [22,23], where the main body or inner surface of the product is mixed or coated with germ-resistant nano-silver to prevent the growth of fungi and bacteria. Despite such widespread use of silver-nanoparticle-containing products, safety/toxicity data on silver nanoparticles remain rare.

The increase in applications of manufactured nanoparticles, which are composed mostly of metal and metal oxides, is raised the potential for exposure among manufacturers and consumers [24]. The major toxicological concern associated with manufactured nanomaterials is that some are redox active, and some particles are transported across cell membranes then interact with subcellular organelles [16]. Thus, evaluation of their toxicity on acute and subchronic exposure is essential. Silver nanoparticles have recently been recognized as antimicrobial agents and are finding diverse medical applications such as silver-based dressings and, silver-coated medical devices [25,26]. This nanoparticle can damage bacterial cell walls and cause cell death [14]. Although nanosilver magnifies the therapeutic effects of silver, its safety profile has remained controversial compared with silver compounds.

The nano-mucus membrane pathway is the lining of most endodermal cells that cover the epithelium and are involved in absorption and secretion. They line various body cells and cavities that are exposed to the external environment and internal organs. It is continuous with the skin, nostrils, lips, ears, genital and anus. NPs deposited on the various mucus tissues pathway, encounter mucus or epithelial lining fluid. This may trigger phagocytosis or contact fibroblasts cells or endothelial cells resulting into the NPs removal [27,28]. Other report by [29] have shown that NPs can be translocated through the mucosal lining and epithelial cells of the intestine and do associate with GALT and blood circulatory system. According to [30]. NPs deposited on the mucus membranes might lead to various types of interaction forces between mucoadhesive nanomaterials and the mucus surface. These forces include weak Vander Waals forces, strong hydrogen bonding, electrostatic attraction, mechanical interpenetration, and entanglement. Furthermore, methods such as fluorescence probe have been used to evaluate these interactions *in vitro* and *in vivo* to measure mucoadhesive capacity. Depending upon the specific mucus membrane application, NPs exposure may translocate and impart a cytological toxic effect depending on the factors reported earlier.

The aim of this study was for assessment of sub-acute toxicological profile of two concentrations of topical oromucosal administration of silver nanoparticles in rats.

Material and Methods

Chemicals used

Synthesized silver nanoparticles aqueous solution were purchased from Nano-Tech Company (6th October city, Egypt), and prepared in two different concentrations (50 and 100 µg /ml).

Synthesis of silver nanoparticles

Silver nanoparticles were prepared using chemical reduction method [31]. Briefly, 1 x 10^{-3} M of silver nitrate (AgNO3) was added in three necked round-bottom flask in the presence of 0.1 g of polyvinyl-pyrrolidone (PVP). Then 0.6 ml of tri-sodium citrate was added and stirred very well followed by drop wise addition of 1 x 10^{-3} M of sodium borohydride (NaBH4) solution. With the addition of NaBH4, the mixture turned yellow immediately. Continuing the process, the solution became opaque (dark brown) gradually indicating the appearance of silver nanoparticles.

Characterization of silver nanoparticles

The preliminary detection of silver nanoparticles was carried out by visual observation of the color change of the cell filtrates. Also, the optical properties of the prepared silver nanoparticles were investigated by recording the plasmon band for silver nanoparticles using the UV-Visible absorption spectra. The shape and size of the prepared silver nanoparticles were recorded by the transmission electron microscopy (HR-TEM) images.

Instrumentation

The UV absorbance was measured on a PERKIN-ELEMER LAMBDA 4B spectrophotometer using 1-cm matched quartz cells and scanning the spectra between 250 and 800 nm. The transmission electron microscopy (HR-TEM) images were carried out on JOEL JEM-2100 operating at 200 kV equipped with Gatan digital camera Erlangshen ES500.

Animals

Sixty male specific-pathogen free albino rats, obtained from the Center of Experimental Animals, Ministry of Health, Helwan, Cairo, with age of 10-12 weeks, weighting about 200-220g, were used in the current study. Animals were acclimated for two weeks before starting the experiment. During the acclimation and experimental periods, rats were housed in polycarbonate large cages (maximum 5 rats per cage). The rats were given food and water ad libitum at a normal dark/light cycle. All efforts were made to minimize animal

suffering and to reduce the number of animals used.

Administration of synthesized nanoparticles to albino rats

Rats were randomly divided into the following experimental groups; Group 1 (control group): consisted of 20 healthy rats (given 0.5 ml distilled water once daily for 4 weeks topically on rat's tongue). Group 2: (nano-silver 50 group): consisted of 20 healthy rats (given 0.5 ml nanosilver solution100 µg/ml once daily for 4 weeks topically on rat's tongue), in dosing volume 10mg/kg body weight. Group 3 (nano-silver 100 group): consisted of 20 healthy rats (given 0.5 ml nanosilver solution100 µg/ml once daily for 4 weeks topically on rat's tongue).

Clinical signs and measurement of body weight

Observations were made before and 3 hours after dosing and they were recorded as per the guidelines provided by Organization for Economic Co-operation and Development (OECD, 2002) [32]. They included any change in appearance behavior, hair, feces and mortality. Changes in weight, an important toxicity index for rats, were measured before grouping and dosing.

Sample collection

On the last day of the study, the overnight fasted animals were anesthetized with CO2 gas and blood samples for sera preparations were collected from the tail vein of each rat into sterile plain tubes. Serum samples were separated from the clot by centrifugation at 3000 rpm for 15 min using a bench top centrifuge (MSE Minor, England). Serum samples were separated into sterile plain tubes and stored in the refrigerator at -20 °C until analysis.

Biochemical tests

The sub-acute toxicity assay involved, levels of GOT (glutamic oxaloacetic transaminase), GPT (glutamic pyruvic transminase), CRE (creatinine), and BUN (blood urea nitrogen), were analyzed using standard diagnostic test (Point of care, Roche: Refrotron test strips) kits on Automated Clinical Biochemistry analyser using a UV-visible spectrophotometer (UV-1601PC, Schimadzu, Japan).

Gross pathology and histopathology

Tissues harvesting

Signs of hemorrhages, ecchymosis and petechial appearances were assessed after 28 days of drug administration. Tissue specimen from the liver, kidneys and tongue were collected and preserved in 10% buffered formalin. Thereafter, The specimens were processed to be stained using H and E stains to detect histological changes, following the method as described by Ingelheim (2003) [33].

Statistical analysis

Data were collected, tabulated and expressed as mean ± SD. Statistical significance between groups was analyzed at each time point using one-way analysis of variance (ANOVA) followed by Bonferroni's post-hoc test. The statistical package for social sciences (SPSS, version 13.5, Chicago, IL, USA) was employed. Values of $P < 0.05$ were considered statistically significant.

Ethical approval

Approval for animal experiments was granted by the Faculty of Dentistry, Suez Canal University. The care and handling of the animals were in accordance with the Animal Care and Use Committee at the Suez Canal University and the National Institutes of Health guide for the care and use of laboratory animals (Maryland, USA). (n.2013/91).

Results

Characterization of silver nanoparticles

The synthesized silver nanoparticles showed a characteristic surface plasmon peak, at 425 nm (Figure 1-A). The nanoparticles were spherical and the size range of 15-40 nm as revealed by high resolution transmission electron microscopy (HRTEM). The data on particle size distribution revealed the presence of particles with an average size 20 nm. (Figure 1-B) The nanoparticles were found to be stable for over twelve months.

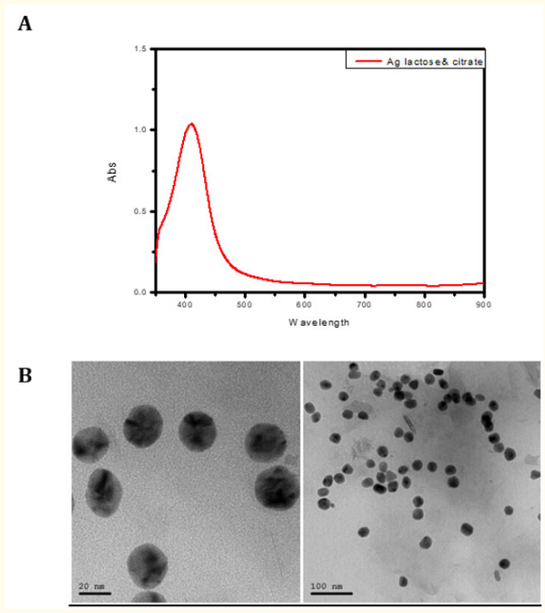

Figure 1: A: UV-Visible absorption spectra of the silver nanoparticles at 425 nm. B: Transmission electron micrograph of the silver nanoparticles used in this work showing spherical in shape with average size of 20 nm.

Clinical observations and body weight measurement for 28 days

Clinical

The administration of dose up to 10 mg/kg of nano-formulation did not lead to mortality. No clinical signs of toxicity were recorded during the 28 days experimental period of all doses of silver nano formulation. The skin, fur, water intake, food intake, mucous membrane, and urination of rats were found to be normal after treatment. Additionally, diarrhea, fast breathing, lethargy, inactivity, liquid secretions from eyes or excessive salivation were not observed.

Body weight

The body weight of control non-treated group varied between 200 to 220 g at the beginning and end of the treatment period, respectively. Following treatment, no significant variations ($p > 0.05$) of body weight was observed between the control groups and the nano formulation treatment groups.

Biochemical tests

Clinically, no mortality or signs of intoxication or any adverse oral mucosal reactions were observed in all rats at the begging of experiment and during the experimental period up to 28 days.

Kidneys and liver are considered to be the most susceptible organs in case of exposure and absorption of silver. The toxicity in kidneys can be evaluated by urea and creatinine measurements. The damaging effect of hepatotoxic substances on the liver is manifested by an increase of aspartate aminotranferase (AST) and alanine aminotransferase (ALT). For assessment of toxic effects for the nanosilver solutions, liver enzymes, blood urea and creatinine were determined. There were no statistically significant differences between all groups of the study ($p > 0.05$) (Table 1).

Groups	ALT (Unit/l) (M± SD)	AST (Unit/l) (M± SD)	Creatinine (mg/dl) (M± SD)	BUN (mg/dl) (M± SD)
Control	20.5 ± 2.8	72.5 ± 13.5	1.4 ± 0.65	6.7 ± 1.08
Silver nanoparticles (50 µg/ml)	21.7 ± 2.7	73 ± 10	1.2 ± 0.016	7.1 ± 2.2
Silver nanoparticles (100 µg/ml)	18.8 ± 2.3	76 ± 12	1.2 ± 0.011	6.8 ± 2.1

Table 1: Effect of silver nanoparticles solution (50 or 100 µg/ml), on serum ALT, AST, creatinine and urea after topical application in rats. (mean ± S.D.).

ALT: Alanine Aminotransferase; AST: Aspartate Aminotransferase; BUN (mg/dl): Blood Urea Nitrogen. Rats were treated with agents for 4 weeks. Results are expressed as mean ± SEM and analyzed using one-way ANOVA. * Significant difference vs. control, $p < 0.05$

Organs	Mean weights		G3 (M ± SD)
	G1 (M ± SD)	G2 (M ± SD)	
Liver	2.91 ± 0.15	2.8 ± 0.14	2.9 ± 0.15
Right Kidney	0.27 ± 0.01	0.28 ± 0.02	0.29 ± 0.03
Left Kidney	0.28 ± 0.02	0.29 ± 0.03	0.28 ± 0.02
Tongue	0.83 ± 0.12	0.79 ± 0.11	0.82 ± 0.12

Table 2: Relative organs weights (g) for male rats after 28-days topical administration of silver nanoparticles (mean ± S.D).

GI = Normal; G2= Nano 50; G3= Nano100 . *Significant difference vs. control, $p < 0.05$.

Organs weights and histopathology

Pathological studies were performed on H and E-stained slides at baseline, and day 28 of study. Results of light microscopic examination of normal and treated tongues G1, G2, G3, showed normal bristle-like tongue papillae. The normal surface epithelium composed of four layers with well-arranged rete ridges. The lamina properia composed of dense collagen bundles overlying well defined muscular tissue. No inflammatory cell infiltration was seen in the lamina properia figure 2.

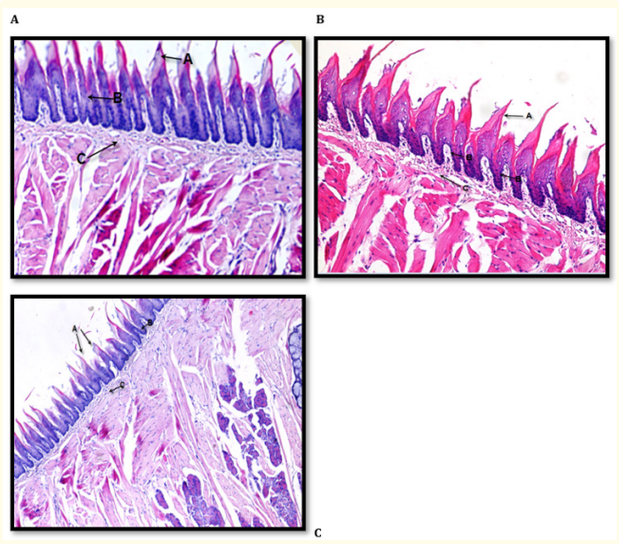

Figure 2: A: (G1) (Normal group) Showing tissue section of normal tongue of a rat at end of the study, (H and E x10). B: (G1) showing normal bristle-like tongue papillae (arrows A). The normal surface epithelium composed of four layers with well arranged rete ridges (arrows. B) The lamina properia composed of dense collagen bundles No inflammatory infiltrate is seen in the lamina properia (arrows C).(H and E x10). C: (G3) showing normal bristle-like tongue papillae (arrows A). The normal surface epithelium composed of four layers with well arranged rete ridges (arrows B). No inflammatory infiltrate is seen in the lamina properia (score 0) (arrows C).(H and E x 10).

No pathological lesion was detected in tested organs including liver, and kidneys of animals during the treatment period. After histopathological assessment of rats organs,no pathological abnormalities were seen in all treated animals compared to untreated healthy animals (no inflammatory cell infiltration, no hyperplasia, no fatty degeneration ,no edema,as well as no necrosis), figures 3-5.

Discussion

A number of factors can influence the toxicity of SNPs. Basic experimental design, including exposure route and conditions, test concentrations, endpoints evaluated, and species or cell type used can affect outcomes of SNPs toxicity tests. Additional parameters such as size and surface area are recognized as important determinants for toxicity [34].

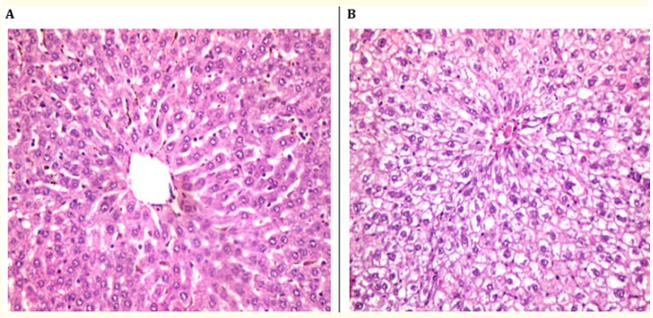

Figure 3: (A). Liver of a normal rat (G1). It is composed of hexagonadal or pentagonadal lobules with central veins and peripheral hepatic triads embedded in connective tissue. Hepatocytes are arranged in trabecules running radiantly from the central vein and are separated by sinusoids containing Kupffer cells. They are regular and contain a large spheroidal nucleus with a distinctly marked nucleolus and peripheral chromatin distribution. Some cells have two nuclei each. (H and E x20). (B). Liver of control rat at the end of experiment. It is composed of hexagonadal or pentagonadal lobules with central veins and peripheral hepatic triads embedded in connective tissue. (H and E x10).

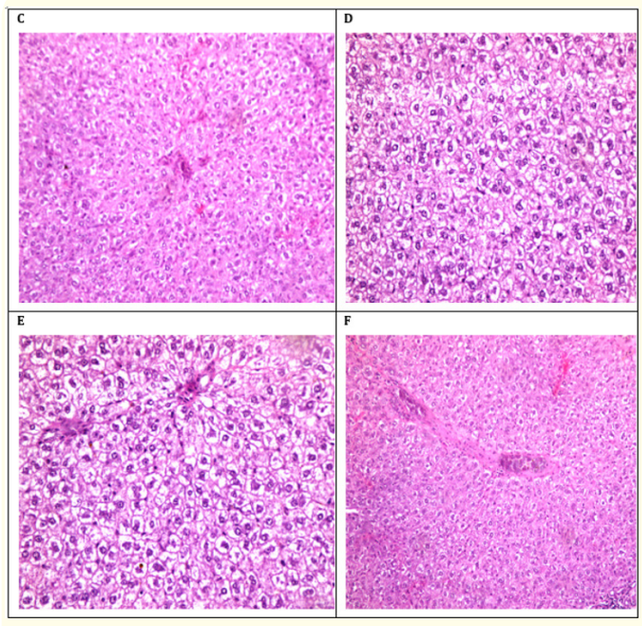

Figure 4: C, D: Histology of rats liver tissues of nano silver G2 at the end of experiment show normal agriculture, central vein with well-preserved hepatocytes. no inflammatory cell infiltration, no hyperplasia, no fatty degeneration, no edema, no necrosis C:H and E(20X), D: H and E (40X).

E, F: Histopathological findings in liver tissues of G3 at the end of experiment: show normal articuture with no inflammation no hyperplasia, no fatty degeneration E:H and E(40X), F: H and E (20X).

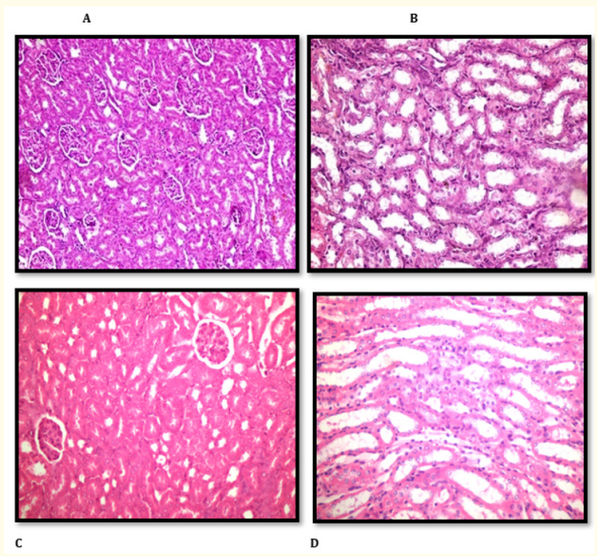

Figure 5: (A): Photomicrograph of left kidney sections of a rat at end of the study from normal group (G1) showing normal histological structure of the glomerulus and tubules in the cortical part. (H and E x20) (B): Photomicrograph of kidney section of a rat from normal group showing normal histological structure of the tubules in the medullary part (H and E x20). (C): G2: cortical part (H and E x20). (D): G3: medullary part (H and E x 40).

The present toxicity results of nanosilver provoked no toxicity in rats, when a single daily dose of 0.5 ml of drugs topically applied on tongues for 28 days (10 mg/kg). This is confirmed by results of liver and kidney tissues as well as their enzymes before and after treatment. There were no significant difference between all groups regarding the main values of liver enzymes (ALT, AST) and blood urea and creatinine. Also the histopathological analysis of rat's liver and kidneys showed that no singns of inflammation or pathological changes up to 10 mg/kg. The doses used in this study were far less than the toxic doses demonstrated by previous studies dealing with toxicological effect of nanosilver in different concentrations in rats. This difference may be due to many factors related to route of administration, size, shape, concentration, dose and chemicals used for nanosilver synthesis [35-41].

Study tested the oral toxicity of silver nanoparticles (60 nm) over a period of 28 days in Sprague-Dawley rats divided into equal four groups: vehicle control, low-dose group (30 mg/kg), middle-dose group (300 mg/kg), and high-dose group (1000 mg/kg). After 28 days of exposure, the blood biochemistry and hematology were investigated, along with a histopathological examination as well as level of silver distribution. The male and female rats did not show any significant changes in body weight relative to the doses of silver nanoparticles during the 28-days experiment [35]. They found that exposure to more than 300 mg of silver nanoparticles may

result in slight liver damage. There was no statistically significant differences in the micronucleated polychromatic erythrocytes or ratio of polychromatic erythrocytes among the total erythrocytes after silver nanoparticle exposure.

Acute dermal toxicity studies on SNPs gel formulation (S-gel) size 7-20nm in Sprague-Dawley rats showed complete safety for topical application. The acute dermal LD50 value of S-gel was found to be greater than 2000 mg/kg body weight. No mortality, signs of intoxication or any adverse skin reactions were observed in the treated rats during the observation period of 14 days following application. At termination of the study, no pathological abnormalities were seen in any organ of sacrificed animals, indicating safety at the highest concentration tested. The authors clearly indicated that silver nanoparticles could provide a safer alternative to conventional topical antimicrobial agents [36].

In 2011, for toxicological assessments, male Guinea pigs were exposed to two different concentrations of nanosilver (1000 and 10,000 µg/mL) in an acute study and three concentrations of nanosilver (100, 1000, and 10,000 µg/mL) in a sub-acute(14days) or chronic study (13 weeks). Toxic responses were assessed by clinical and histopathologic parameters. In all experimental animals the sites of exposure were scored for any type of dermal toxicity compared with negative control and positive control groups. In autopsy studies during the acute test, no significant changes in organ weight or major macroscopic changes were detected, but dose-dependent histopathologic abnormalities were seen in skin, liver, and spleen of all test groups. In addition, experimental animals subjected to subchronic tests showed greater tissue abnormalities than those subjected to the acute tests. It seems that colloidal nanosilver has the potential to provide target organ toxicities in a dose- and time-dependent manner. The results suggested that it is necessary to determine the association between the period of exposure and histopathologic changes with lower doses over different time periods [37].

In a study done on 72 healthy adult male and female albino rabbits with 10nm, 20nm, and 30nm SNPs after 2 weeks of topical skin application, the concentration of nanosilver in the suspension was 8000 mg/L. Solutions containing nanosilver particles were stirred on vortex agitator for 15 min and before each dermal exposure with single dose of 0.5 mL/animal. Authors concluded that dermal exposure to lesser sizes of silver nanoparticles is more disastrous than greater ones, as lesser sizes are more penetrable through skin [38].

Some Authors assessed the toxic effects of SNPs at 70 nm in rat's tissues, with different doses (0.25, 0.5, 1 and 2 mg/kg body weight) on kidney, liver and spleen tissues via oral gavage for 30 days. Adverse impacts on liver, spleen and kidney were observed in a high dose-treated groups (1 and 2 mg/kg), when determined by histopathological analysis. Pathological examination showed tissue changes, cell necrosis and apoptosis from all rats in high dose groups compared to control group [39].

In another study, the repeated-dose toxicity of SNPs (42 nm) was also investigated in male and female mice by oral administration for 28 days. By the administration of SNPs (0.25 mg/kg, 0.50 mg/kg, 1.00 mg/kg), adverse effects on liver and kidney were observed in the high dose-treated group (1.00 mg/kg), as determined by blood chemistry and histopathological analysis. In other groups, histopathological evidence was not observed in liver. BUN and creatinine were not increased. It seemed that the inflammatory responses are too weak in kidney to impair the filtration capacity of kidney [40].

In the present investigation, no significant difference between all groups regarding the main values of liver enzymes (ALT, AST), blood urea and creatinine. The data obtained were lower than those demonstrated by previous studies in rats or mice after using nanosilver solution with systemic routes [40,41].

The oral route is the most popular drug delivery method among patients because it is less expensive, easier to use, and does not require specialized medical assistance. The main drawback is the reduced bioavailability of the drug due to its quick breakdown in the gastrointestinal tract and first-pass liver metabolism. For these reasons, alternative transmucosal administration methods, such as those through the vaginal, ocular, nasal, buccal, and oral mucosae, have received increased attention in recent studies[42-45]. Specifically, the oral mucosa is distinguished by superior drug accessibility, quick absorption due to relatively high blood flow, a robust epithelium, bypass of first-pass metabolism, and less exposure of medicines to the gastrointestinal environment [46,47].

Silver nanoparticles (AgNPs) are diffusely used in food packaging, containers, toothpaste and teeth brushes, nipples and nursing bottles, water purification devices etc [48,49]. These particles are therefore able to come in contact with oral mucosa, whose penetration properties are not completely known. Silver is used for its good antimicrobial properties and its safe profile [50]. Since the spread of nanotechnologies has taken place in many fields of everyday life, there are many available products containing AgNPs but the knowledge on NPs permeation properties through mucosal membranes is still lacking [51].

Our study employed a chemical reduction method to synthesize silver nanoparticles (AgNPs), resulting in spherical nanoparticles with a size range of 15-40 nm. This method is consistent with previous studies [52-54] which also reported the synthesis of AgNPs using similar techniques. The stability of AgNPs over twelve months, as observed in our study, aligns with findings from (Patil., *et al*., 2012., T. Garcia., *et al*. 2016 and Zaki., *et al*. 2020) [52-55] suggesting long-term stability of AgNPs synthesized via chemical reduction.

In the present study, we evaluated the sub-acute toxicity of topically applied AgNPs in albino rats. Contrary to our expectations, we did not observe significant alterations in liver enzyme activities (ALT, AST), serum creatinine, or blood urea nitrogen (BUN) levels across experimental groups. This contrasts with findings from [56] who reported elevated liver enzymes following AgNP exposure in rats but their study was Chronic toxicity was determined in a dosages of 30 mg/kg body weight for 180 days. As well as Notably, our study utilized topical mucosal application, while previous studies often employed oral routes of administration, potentially influencing the biodistribution and toxicokinetics of AgNPs.

On the other hand our findings were similar to findings in study of [57] that was aimed to investigate the effect of subacute intravenous administration AgNP (silver nanoparticles, 10 nm) and AuNP (gold nanoparticles, 12.8 nm) and AgNP/AuNP mixture to blood biochemistry, hematology, and platelet coagulation, subacute toxicity study for 4 weeks was conducted. There were no significant dose-related changes in the hematology and blood biochemical values for the rats. Authors concluded that the subacute injection of AuNP and AgNP or their mixture did not induce any noticeable systemic toxicity.

Moreover, study made by Hendrickson., *et al*. 2016 [58] describes the biodistribution and biological action of 12nm non-coated silver nanoparticles intragastrically administered to male rats after acute (single exposure) and sub-acute (multiple exposures over 30 days) toxicity experiments. The daily doses were 2000 and 250mg/kg of body weight for single and multiple administrations, respectively. Hematological indices and biochemical parameters of the treated rats did not differ from those of the vehicle control animals. Overall, it can be concluded that nanosilver is able to be absorbed from the gastrointestinal tract into the bloodstream and accumulate in the secondary organs of rats. It showed no distinct toxicity under the experimental conditions.

Consistent with our results, Nghilokwa., *et al*. [56] also found no significant changes in organ weights or histopathological alterations in the liver and kidneys of rats exposed to AgNPs of the same dosage of our study. However, discrepancies exist regarding the route of exposure and duration of the study, highlighting the importance of considering these factors in the interpretation of toxicological outcomes. Another related study by T. Garcia., *et al*. 2016 [53] found no significant changes in histopathological alterations in the liver and kidneys of rats exposed to AgNPs of the similar dosage of our study at doses of 50, 100 and 200 mg/kg/day after oral exposure for 90 days.

Ingestion is a relevant route of exposure for AgNPs, whether occurring unintentionally via Ag dissolution from consumer products, or intentionally from dietary supplements. Study done by Wilding., *et al.* [59] evaluated murine gut microbial communities following 28 days of repeated oral dosing of well characterized AgNPs of two different sizes (20 and 110 nm) and coatings (PVP and Citrate). Irrespective of size or coating, oral administration of AgNPs at 10 mg/kg body weight/day did not alter the membership, structure, or diversity of the murine gut microbiome which is support our results.

Remarkably, our study did not observe any signs of oral mucosal reactions or intoxication in rats following topical application of AgNPs. This contrasts with findings from Zaki., *et al*. 2020 [55] who reported mucosal irritation and inflammation in rats exposed to AgNPs via intra-peritoneal systemic route. The absence of adverse oral effects in our study suggests that topical mucosal application may pose minimal risk to oral tissues, although further investigation is warranted to confirm these findings.

Generally, After 28 days of treatment, animals did not show significant reductions in organ weights and in the number of deaths. Likewise, no effects on body weight gain were found. Probably, either the route of administration, the dose used in this study, or both, was not appropriate to induce body weight gain alterations. These results are in agreement with those of a recent study with oral administration for acute 14 days exposure with very high concentration of citrate-AgNPs [60] was given. Similar results were also reported by [61].

The results of study by [53] showed that oral subchronic exposure for 90 days of adult Sprague Dawley rats to PVP-AgNPs caused accumulation of Ag in different tissues at doses of 50, 100 and 200 mg/kg/day. However, Ag accumulation was not sufficient (at any dose) to alter hematological parameters or to induce harmful effects in kidney, spleen, or ileum of treated rats. The ileum of treated rats was the tissue with the highest Ag levels. In liver, the AgNPs were located inside mitochondria and cytoplasm. Again, this accumulation was not sufficient to alter biochemical markers for hepatic status, or general tissue morphology. with no significant effects at cellular level. All these findings were with agreement with the current results.

The results of the present study were correspondence with recent study done by [56] concern about the toxicity of orally administered *Azadirachta indica* AgNPs was assessed in albino rats. Sub-acute toxicity was determined in daily dosages from 0.3-30 mg/kg body weight for 28 days. Chronic toxicity was determined in two dosages 30 and 10 mg/kg body weight for 180 days. The nanoparticles synthesized were 45 nm average sizes. There were no significant differences observed between the packed cell volume animals body weight of the control and treatment groups in sub-acute and chronic toxicity. Portal hepatitis in the liver at dosage of 30mg/kg was noted. Hence, histopathology examinations confirmed the liver damage noted in clinical biochemistry. Kidneys histological organization appeared normal generally with glomeruli and tubules visible. There results demonstrate that silver nanoformulation may be safe at daily dosage of up-to 10 mg/kg.

A recent study by the FDA reported that repeated administration (once/week for 8 weeks) of silica (5 mg.kg−1), gold (10 mg.kg−1)

and silver (5 mg.kg–1) nanoparticles to mice did not saturate the mononuclear phagocytic system [62]. Toxicity was not observed with gold and silver nanoparticles during the entire duration of the study, which took two months [62]. Even though gold nanoparticles are generally reported as biocompatible, a variety of opinions exist regarding their potential harm, especially after long-term exposure, due to their non-biodegradable nature [63]. Particularly, a recent FDA position paper raised a concern that traditional preclinical safety models may overlook secondary effects which may occur due to the durable nature of gold and other similar nanomaterials that retain their particulate state during administration, distribution and accumulation in the body [63].

Conclusion

Silver nanoparticle (size 20 nm) in concentrations of 50,100 µg/ml in dosing volume 10mg/kg body weight are safe when applied topically in rats for 28 days.

Conflict of Interest

None.

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None.

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