

Full Length Research Paper

Sub-acute and chronic toxicity of silver nanoparticles synthesized by *Azadirachta indica* extract

Emma Nghilokwa¹, Judith Sokei¹, Peter Mwitari² and Naomi Maina^{3*}

¹Department of Molecular Biology and Biotechnology, Pan-African University, Institute of Basic Sciences, Technology and Innovation, P. O. Box 62000-00200, Nairobi, Kenya.

²Centre for Traditional Medicine and Drug Research, Kenya Medical Research Institute, P. O. Box 54840-00200, Nairobi, Kenya.

³Department of Biochemistry, Jomo Kenyatta University of Agriculture and Technology, P. O. Box 62000-00200, Nairobi, Kenya.

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In this study, biosynthesis of silver nanoparticles (AgNPs) and its toxicity were investigated. Different functional groups responsible for adsorption, morphology and absorption of the nanoparticle were characterized using UV-Vis, Fourier transmission infrared spectroscopy (FTIR), and scanning electron microscope (SEM) analyses, respectively. The toxicity of orally administered *Azadirachta indica* AgNPs was assessed in Swiss albino rats. Sub-acute toxicity was determined in daily dosages from 30-0.3 mg/kg body weight for 28 days. Chronic toxicity was determined in two dosages 30 and 10 mg/kg body weight for 180 days. Control groups were included and were administered with distilled water. The UV-Vis spectroscopy showed surface plasmon resonance of 430 nm for the silver nanoparticle. The FTIR spectrum showed primary-N-H bond and secondary-C-N amides. The nanoparticles synthesized were 45 nm average sizes. There were no significant differences ($P>0.05$) observed between the packed cell volume (PCV), animals body weight of the control and treatment groups in sub-acute and chronic toxicity. Portal hepatitis in the liver at the 30 mg/kg b. wt was noted. Hence, histopathology examinations confirmed the liver damage noted in clinical biochemistry. Kidneys histological organization appeared normal generally with glomeruli and tubules visible. Our results demonstrate that *A. indica* silver nanoformulation may be safe at daily dosage of up-to 10 mg/kg b. wt. However, it indicates that the *A. indica* silver nanoformulation on daily use at 30 mg/kg may lead to liver damage.

Key words: Silver nanoparticles, toxicity, *Azadirachta indica*, rats.

INTRODUCTION

Therapeutic activities of neem plant (*Azadirachta indica*), a member of the Meliaceae family, originally found in India, Pakistan, Bangladesh, and Nepal (Abdelhady et

al., 2015). Several activities from neem have been reported including antibacterial, antifungal, and anti-inflammatory (Alzohairy, 2016). *A. indica* extracts have

*Corresponding author. E-mail: nmaina@jkuat.ac.ke.

been encapsulated in various nanoparticles (NPs) such as Silver (Ag), Gold (Au) and chitosan Dash et al., (2017). Silver nanoparticles (AgNP) is one of the most used nanoparticles in drug formulation due to its potent broad-spectrum of antibacterial properties, strong permeability and little drug resistance (Wen, 2017). Methods like nano clustered lipid carrier appears to be optionable for delivery to overcome disadvantage of lower loading capacity and drug expulsion in storage Elsaesser and Howard, (2012) however, in recent studies they showed moderate toxicity of elevated alkaline phosphatase (ALP) (Okeahialam et al., 2000). Biosynthesis of silver nanoparticles have been claimed to support optimal green chemistry metrics (Bilal et al., 2017). The green synthesis methods have proven to be consistent, economic and biocompatible, environmental and eco-friendly (Luo et al., 2018). Plant-mediated green chemistry approach indicates numerous advantages, including a significant yield production at a minimal cost (Singh et al., 2016). The study by Nwagbogu (2018) depicted that the ethanolic *A. indica* bark extract had antiproliferative activity against HCT116 human colon cancer cell, MCF7 breast cancer cells and Hep-G2 human liver cell line. In that study, encapsulation of *A. indica* bark extracts with silver nitrate increased its *in vivo* tumor growth inhibitory activities in Swiss albino rats (a popular mammalian model), showing a 71.96% inhibition capability at the dose of 30 mg/kg b.wt. The lethal dose (LD50) of the *A. indica* AgNPs was found to be higher than 160 mg/kg and no organ damage was noted after daily administration for 14 days. This study further determined the sub-acute (28 days) and Chronic (180 days) toxicity effect after administration of *A. indica* silver nanoparticles (NPs) in Swiss albino rats.

MATERIALS AND METHODS

Collection and plant material authentication

The bark of *A. indica* plant was collected in December 2018 from Kiambu county, Kenya. It was authenticated by Mr. John Kamau Muchuku, Department of Botany, Jomo Kenyatta University of Agriculture and Technology (JKUAT). A voucher specimen (ET001ABC) was deposited in the University Herbarium.

Methanolic extraction

The bark specimen of *A. indica* were washed with distilled water, air-dried inside a greenhouse for two weeks and pulverized into coarse powder using grinding machine. The coarse powder was further processed to fine particles by sieving. The powdered bark (50 g) was macerated in 70% Methanol. The mixture was allowed to stand for 72 h with vigorous shaking. Following the method described by Nwagbogu (2018), methanol extract was then filtered using Whatman' filter paper No. 1 and the filtrate was concentrated under pressure at 45°C in a rotary evaporator to remove methanol and the remaining water. The extract was freeze dried using a freeze dryer (mrc # Israel). The resultant extract was weighed, labelled and stored in an airtight container at -20°C prior to use.

Preparation of AgNPs

One millimole of silver nitrate (AgNO₃) was weighed, bark extract prepared and used for synthesis of silver nanoparticles. Methanolic extract of the bark of *A. indica* was added to silver nitrate solution in a ratio of 4:1 for bio-reduction process at room temperature (25°C) following the method discussed by Abdullah et al. (2016) in his study. The progress of the reaction between silver metal ions and the bark extract was monitored by performing periodic sampling (at 3 h intervals for 12 h) using a UV spectroscopy. The Nano-formulated product was purified by centrifugation at 5000 rpm for 5 min. The supernatant was discarded and then the precipitated silver nanoparticles were washed with deionized water 10 times for complete purification. The resultant product was dried in vacuum chamber for 24 h at 35°C to obtain the dry silver nanoparticles (Neran and Lamia, 2016).

Classification and characterization of *A. indica* nanoparticles

The synthesized nanoparticles were subjected to UV- Spectroscopy and Fourier-transform infrared spectroscopy (FTIR) to confirm the successful synthesis of silver nanoparticles and detect the key functional groups present in the extract, which are responsible for capping of silver nanoparticles, respectively. Scanning electron microscope (SEM) was performed for Scanning electron morphological analysis of Silver nanoparticles following the method described by Lalitha et al., (2013).

Animals

Male Swiss albino rats (*Mus musculus*), 8-12 weeks old weighing between 180 and 200 g were bred in the animal house at JICA SAFARI House, Jomo Kenyatta University of Agriculture Technology Kenya. The animals were kept in clean plastic cages placed in a well-ventilated house with optimum condition (temperature: 28 ± 2°C; photoperiod: 12 h natural light and 12 h dark; humidity: 40-45%). They had free access to commercial pelleted rat feed and water *ad libitum*. The floors of the cages were filled with paper cuts while the cage cleaning was done on daily basis.

Administration of synthesized nanoparticles to swiss albino rats

Animals (Male rats) were divided into six groups for sub-acute toxicity study based on the dosage administered Group 1: (30 mg/kg), Group 2 (10 mg/kg), Group 3 (3 mg/kg), Group 4 (1 mg/kg), Group 5 (0.3 mg/kg) of nano-formulation and Group 6: negative control (distilled water). For chronic toxicity study, the animals were divided into three groups based on the dosage administered group1: (30 mg/kg), Group 2 (10 mg/kg) of nano-formulation and group 3: negative control (distilled water). 0.2 ml of the drug was orally administered once daily for 28 and 180 days for sub-acute and chronic toxicity test respectively. Rats were fasted overnight prior to dosing. Following the period of fasting, the animals were weighed and then the test substance was administered orally using 5 ml oral gavage.

Clinical signs and measurement of body weight

Observations were made before and 3 h after dosing and they were recorded as per the guidelines provided by Organization for Economic Co-operation and Development (OECD, 2002). They included any change in appearance behavior, hair, feces and

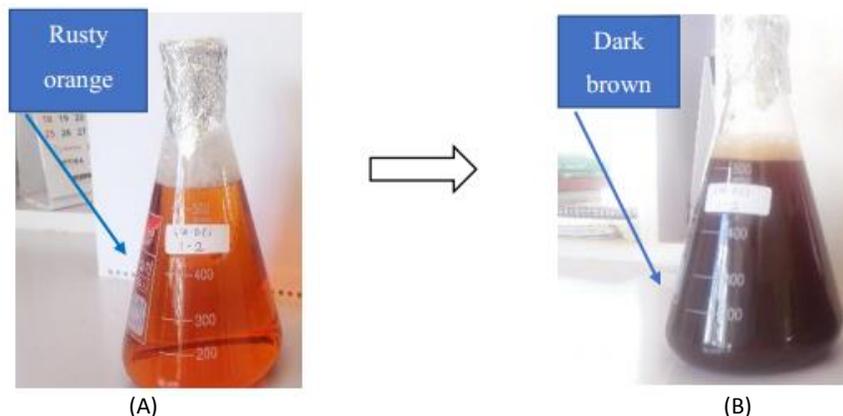


Figure 1. (A) *A. indica* bark extract + AgNO₃ at time T=0 min (Rusty-orange colour) (B) *A. indica* bark + AgNO₃ at time T = 72 h (Dark brown colour).

mortality. Changes in weight, an important toxicity index for rats, were measured before grouping and dosing.

Sample collection

On the last day and after an overnight fast of 8 h, the animals were sacrificed under carbon dioxide asphyxiation. Blood samples were collected by cardiac puncture and collected into clean bottles for hematological and biochemical investigations. The liver and kidneys were excised from dissected rats, after which they were washed using physiological saline and weighed. The liver and kidneys were then fixed in 10% formalin saline for further histopathological processing. Tissues were embedded in paraffin wax and sections of 3 micron were prepared and stained with hematoxylin and eosin according to the procedure described by Slaoui and Fiette (2011).

Packed cell volume determination (PCV)

The PCV was determined using the micro hematocrit method (Shamaki et al., 2014). Briefly, an aliquot of blood sample with anticoagulant from each rat was placed in micro-capillary tubes and then centrifuged at 14000 rpm for 10 min. Samples were analyzed for PCV after centrifugation, using a micro-capillary reader.

Biochemical tests

The sub-acute toxicity assay involved, the levels of Aspartate aminotransferases (AST), Alanine aminotransferases (ALT), Urea, and creatinine levels were analyzed using standard diagnostic test (Point of care, Roche: Refrotron test strips) kits on Automated Clinical Biochemistry analyser (Reflotron Plus System®, model: Cobas 4800 Detection Analyzer; India).

Gross pathology and histopathology

Tissues harvesting

Signs of hemorrhages, ecchymosis and petechial appearances were assessed after 28 days and 180 days of drug administration. Tissue specimen from the liver and kidney were collected and preserved in 10% buffered formalin. Thereafter, the organs were

processed for histopathological examination following the method as described by Ingelheim (2003), after 180 days of *A. indica* silver nanoformulation treatment.

Statistical analysis

Data collected from the biochemical and hematological analysis were expressed as mean \pm SEM. One-way ANOVA and column statistics was used to compare means of treated and control rats for the different parameters assessed. The significance level was set at $P \leq 0.05$.

Ethical approval

Approval for animal experiments was granted by the Jomo Kenyatta University of Agriculture and Technology Animal Ethics Committee. The protocols were approved by the Institutional Animal Care and Use Committee (IACUC) REF: JKU/2/4/896B at JKUAT and conducted in compliance with Kenya's national ethical standards to minimize the suffering of animals.

RESULTS

Characterization of AgNPs

UV Vis spectroscopy

The prepared *A. indica* nano-formulated extracts were isolated to different particle sizes using size fractionation process by a centrifugation at different intervals. The specific speeds and uniform sizes were selected for the study. The UV-Vis spectrum illustrated in Figure 2, depicts a well-defined absorption peaks for the particle sizes. These correspond to the wavelength (500 nm) of the surface plasmon resonance (SPR) of *A. indica* nano-formulated extracts. Colour change from rusty orange at time 0 to dark brown at 72 h and peak formation at about 400-480 nm confirmed bio-reduction of silver ions by the extract and the successful nanoformulation (Figure 1).

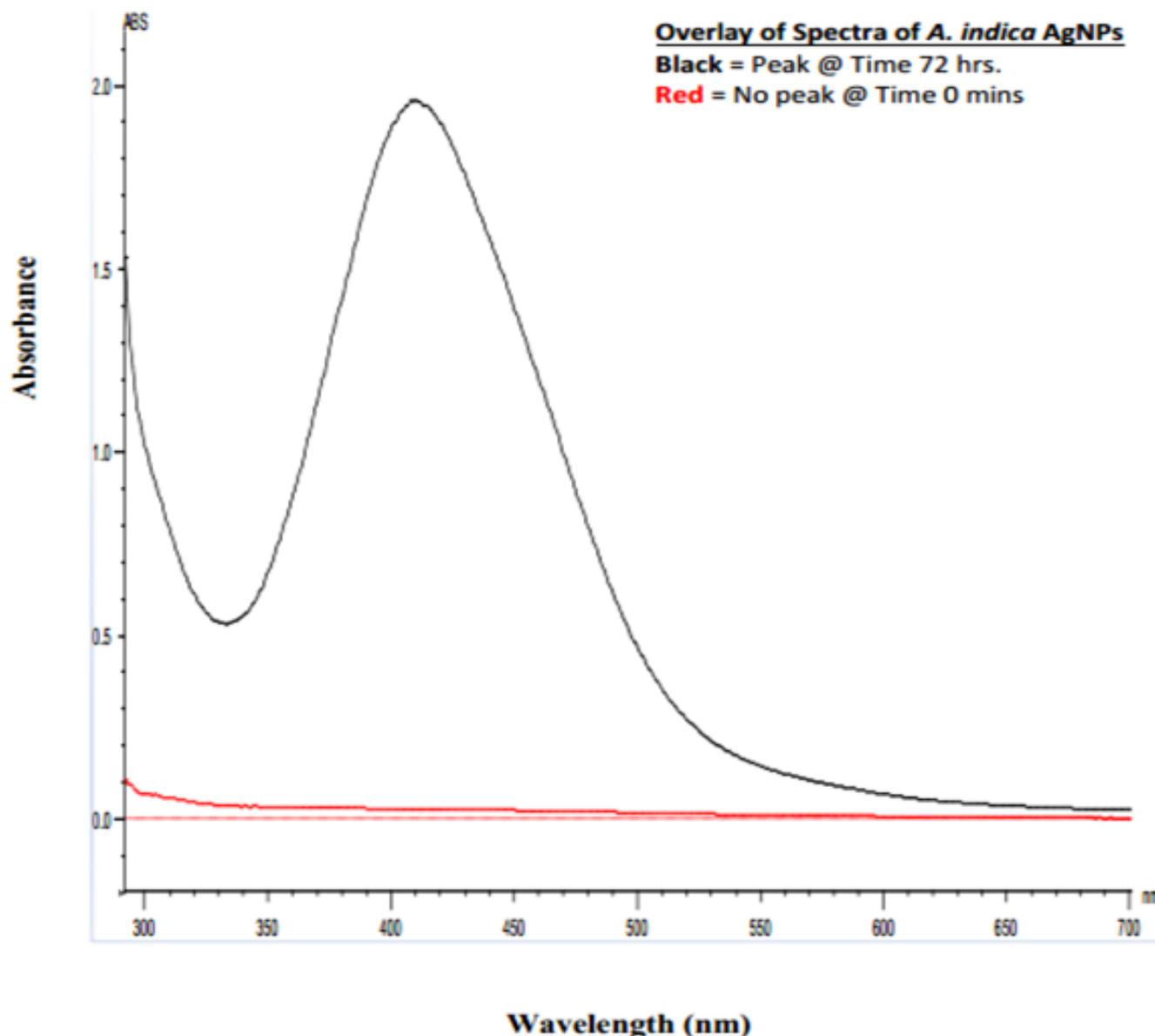


Figure 2. UV/Vis absorption spectrums of silver ions reduction (red colour line) to AgNPs (black colour line), the spectra in the low and high wavelength regions were included. The absorption spectrum was recorded at room temperature after 72 h of the preparation period.

Fourier transform infrared spectrometer (FTIR)

The transmittance bands of different functional groups varying between 4000 and 500 cm^{-1} was recorded for the FTIR spectra, which allows for high reactivity, specificity and compound solubility in water (Coates, 2004) (Figures 3 and 4). The results depict the presence of hydroxyl /alcohol groups at 3679.9 cm^{-1} , alkyne C-H, saturated aliphatic groups of alkane and hydrocarbons including methyl C-H at 2935.5 cm^{-1} and methylene at C-H 1595.0 cm^{-1} , aliphatic fluoro compounds at 1143.7 cm^{-1} and aliphatic phosphates at 1112.9 cm^{-1} . This was

compared to the study of Tensingh (2017) and Amuanyena et al. (2019).

Scanning electron microscopy (SEM) analysis

The result shows the high-density Ag nanoparticles synthesized by *A. indica* bark extracts and further confirmed the presence of silver nanoparticles. It was shown to be relatively spherical in shape and polydisperse with $10\text{ }\mu\text{m}$ width (Figure 5a). As portrayed in the micrographs, *A. indica* silver nanoparticle appears to have

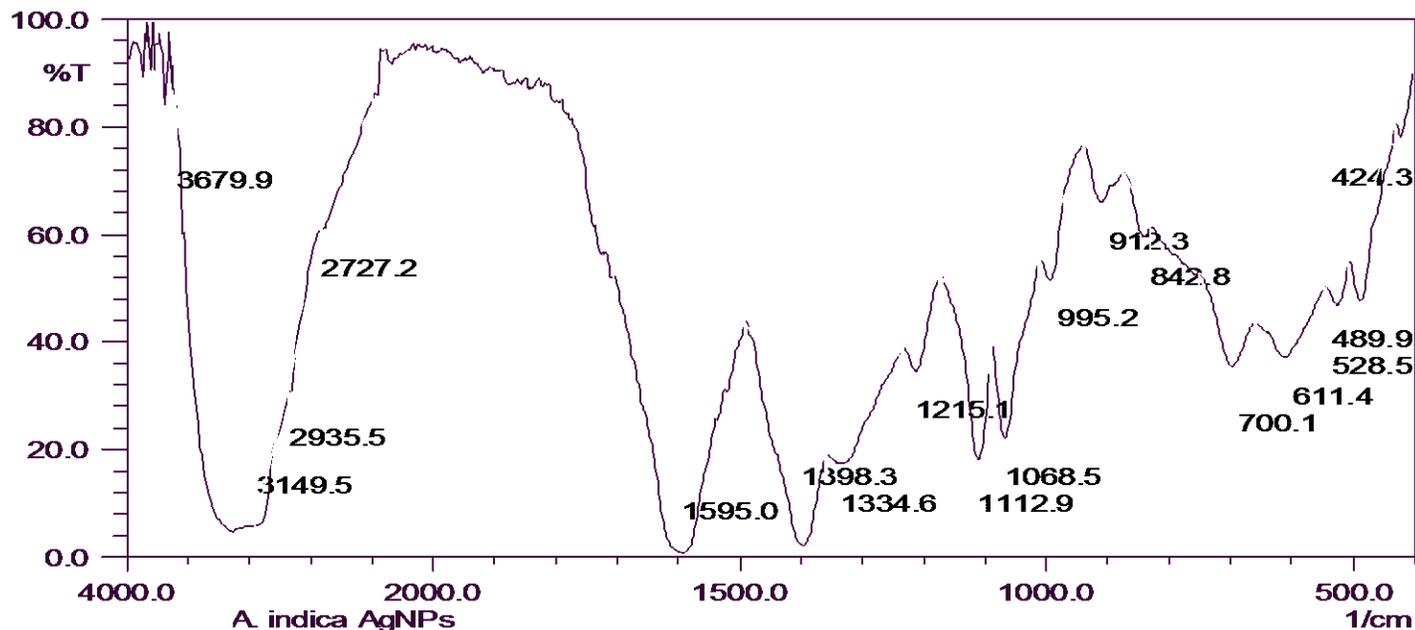


Figure 3. Shows the FTIR absorbance spectra of the *A. indica* silver nano-formulation.

an energy dispersive spectrometers (EDS) pattern which indicates that 13% presence of Ag ion (Figure 6). The EDS spectrum also reveals (C) peak (Figure 6).

Clinical observations and body weight measurement for 28 and 180 days

Clinical

The administration of dose up to 30 mg/kg of *A. indica* nano-formulation did not lead to mortality. No clinical signs of toxicity were recorded during the 28 and 180-days experimental period of all doses of *A. indica* silver nanoformulation. 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 in both sub-acute and chronic toxicity study.

Body weight

The body weight of control non-treated group varied between 144 to 394 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 *A. indica* nanoformulation treatment groups for both sub-acute and chronic toxicity study.

Packed cell volume (PCV) for both sub-acute and chronic toxicity

PCV levels of control groups ranged between 37-50%, the values were within the normal range. There were no significant differences ($P > 0.05$) between the control and the treatment groups in sub-acute and chronic toxicity study.

Biochemical analysis

Hepatic: The serum ALT levels of untreated control groups ranged from 102 to 130 μL . There were no significant differences ($P > 0.05$) in serum ALT levels between the untreated control group and the treatment groups below 10 mg/kg after 28 days treatment period. However, there was a significant increase ($P < 0.05$) in ALT of 30 mg/kg treatment groups. In addition, at 30 mg/kg b. wt, there were significant increase ($P < 0.05$) in ALT after 180 days treatment period. The serum AST levels of untreated control groups ranged from 135 to 188 μL . There was significant increase ($P < 0.05$) in AST of 10 and 30 mg/kg after 28 days treatment period.

Kidney: The blood urea levels of untreated control groups ranged from 5 to 8.88 mmol/L. There were no significant differences in blood urea levels ($P > 0.05$) between the untreated control group and the treatment groups in sub-acute and chronic toxicity experiment. The blood creatinine levels of untreated control groups ranged from 44.20 to 57.7 $\mu\text{mol/L}$. There were no significant

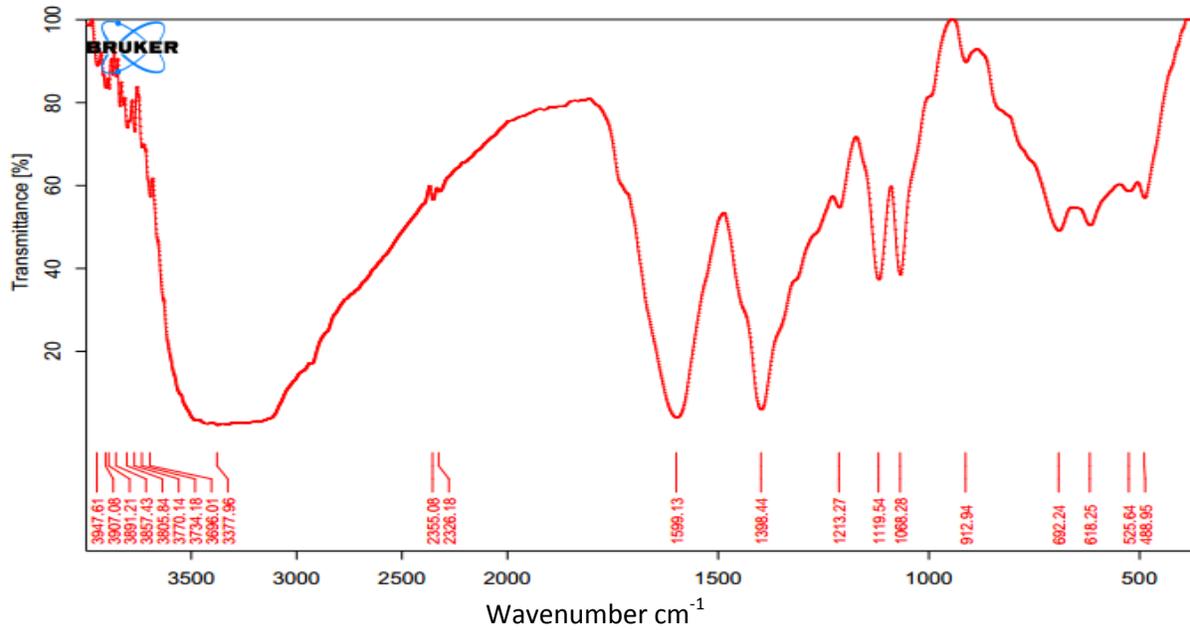


Figure 4. FTIR transmittance (%) spectra of the *A. indica* silver nano-formulation.

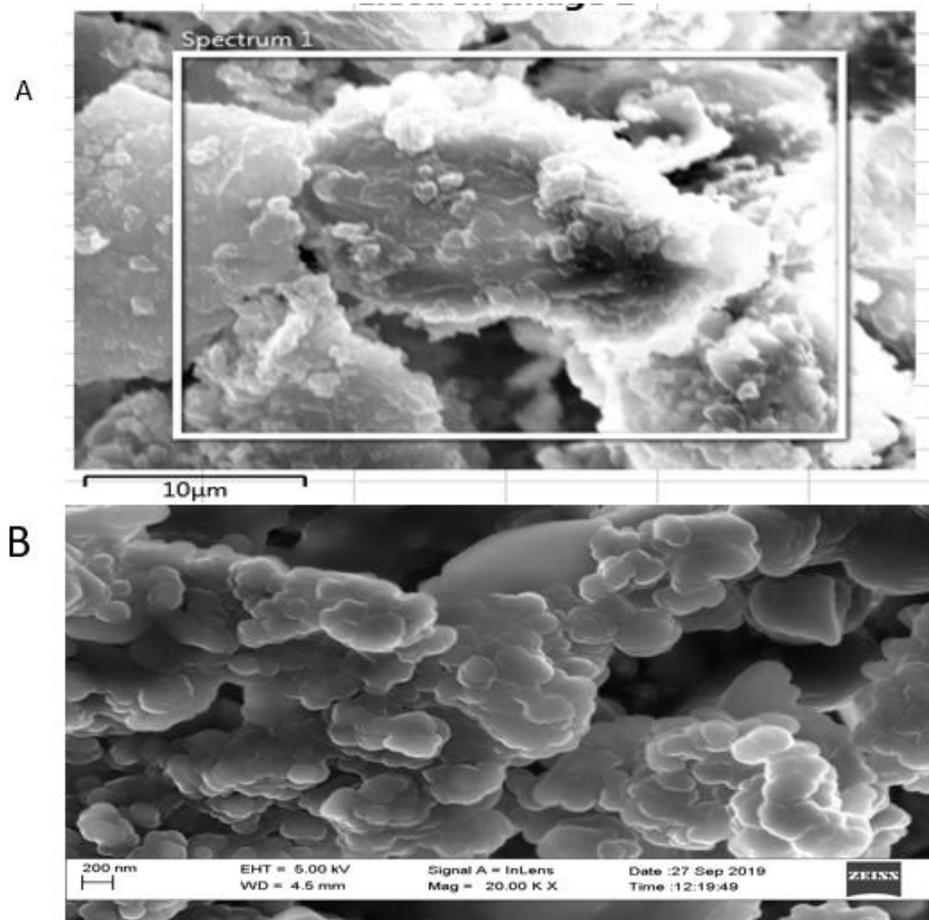


Figure 5. SEM image of dispersed, spherical shaped *A. indica* Ag nanoparticles.

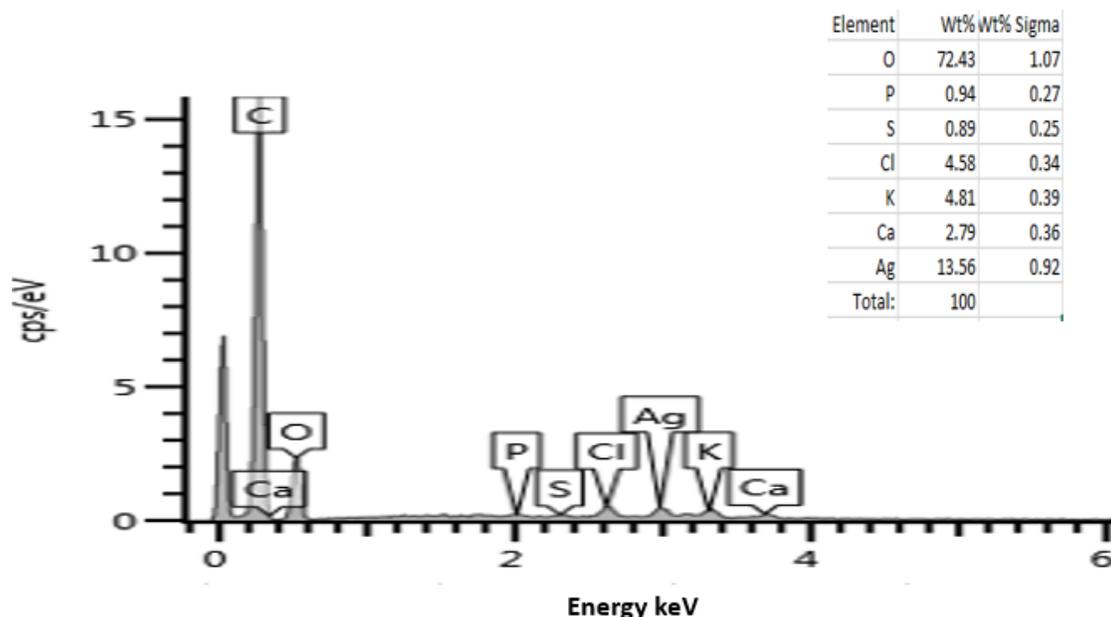


Figure 6. Shows the spectrum peak element in kiloelectron volt (KeV) and their percentage.

Table 1. Biochemical profiles of Swiss albino rats administered with silver nanoformulated *A. indica* after 28 and 180 days of treatment.

Dose (mg/kg)	Sub-acute/chronic toxicity (days)	AST(U/L)	ALT(U/L)	Urea (mmol/L)	Creatinine (μ mol/L)
30	28	300.5 \pm 11.5**	152 \pm 3.04**	9.275 \pm 0.92	46.6 \pm 0.5
	180	ND	168.3 \pm 6.69*	6.767 \pm 0.32	46.77 \pm 1.3
10	28	289 \pm 14**	123.5 \pm 11.5	8.825 \pm 0.80	44.85 \pm 0.65
	180	ND	153.3 \pm 6.76	6.6 \pm 0.20	45.23 \pm 1.03
3	28	236 \pm 18	119 \pm 8	7.705 \pm 0.29	44.8 \pm 0.4
1	28	215 \pm 17	125.5 \pm 4.5	8.505 \pm 0.89	45.2 \pm 1.0
0.3	28	220.5 \pm 13.5	111.5 \pm 4.5	7.23 \pm 0.86	44.7 \pm 0.5
Control	28	161.5 \pm 26.5	104.5 \pm 2.51	7.915 \pm 0.96	48.85 \pm 1.15
	180	ND	103.2 \pm 20.64	5.7 \pm 0.51	50.37 \pm 3.79

Notes: Values are expressed as mean \pm SEM, ND-Not Done, n=3. * P <0.01, ** P <0.001 compared to the control. ALT, alanine aminotransferase; AST, aspartate aminotransferase; control, rat treated with distilled water; SEM, standard error of mean.

differences between blood creatinine levels (P >0.05) of the untreated control groups and the treatment groups in both sub-acute and chronic studies (Table 1).

Gross pathology and histopathology after sub-acute and chronic toxicity

Sub-acute toxicity gross and histopathology: The liver of rats administered with *A. indica* silver nanoformulation showed a smooth surface and were evenly dark red colored. Rats administered the dosage of 0.3 and 3

mg/kg showed normal hepatocellular architecture. Rats administered the highest dosage (30 mg/kg) showed signs of portal and interface hepatitis. Kidney appears smooth surfaced and evenly dark red in color. They presented a normal histological structure with a normal appearance of the renal cortex, glomeruli and tubules in the low dose of 0.3 and 3 mg/kg. Kidneys presented a normal ultrastructure but had some congested blood vessels (BVC) in the group administered 30 mg/kg (Figure 7).

Chronic toxicity gross and histopathology: Similarly,

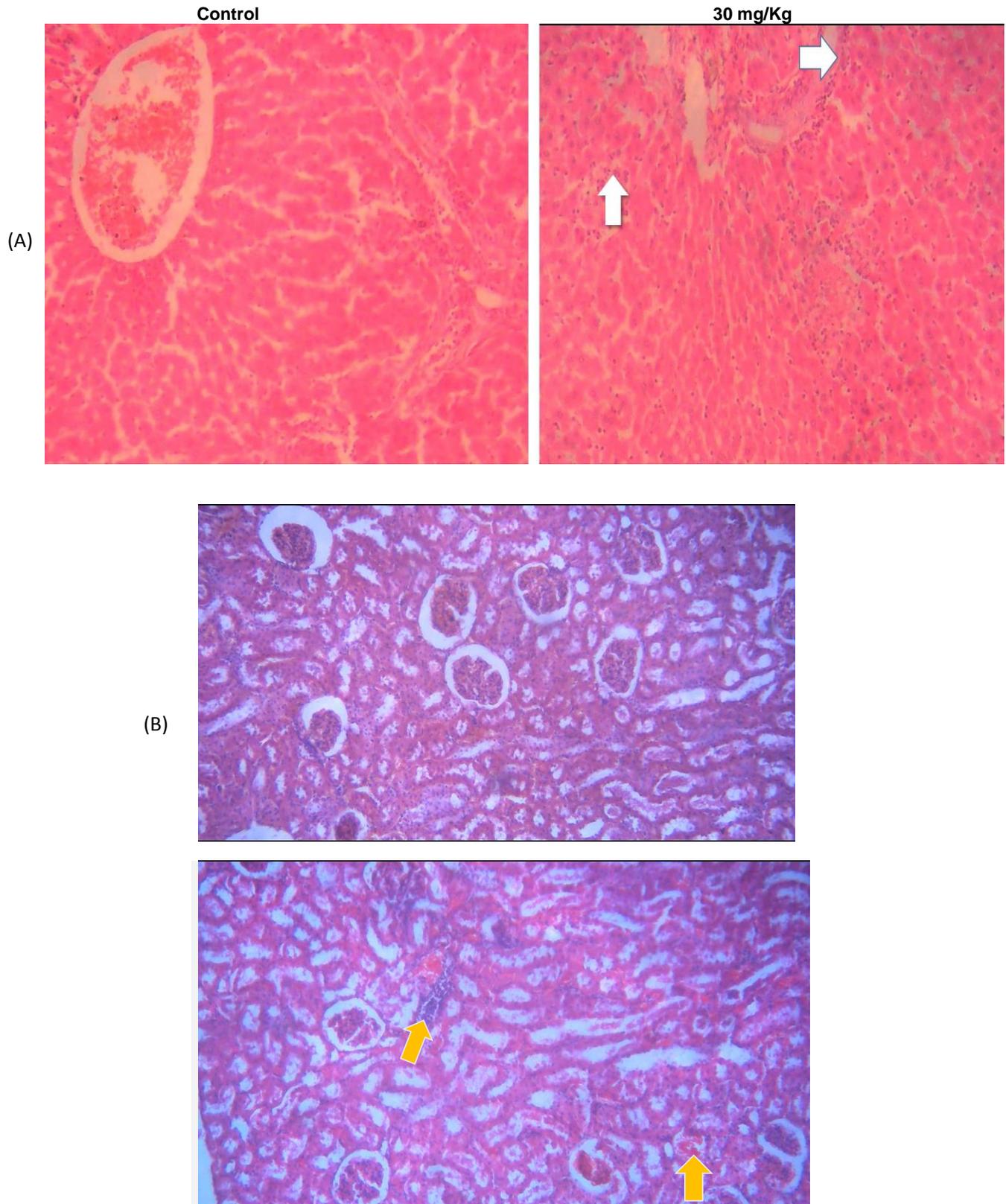


Figure 7. Histopathological examinations of the kidney and liver of rats after 28 days of drug administration. A. Represent liver and B. Kidneys. The arrows make a representation of portal infiltration in the liver treatment group tissues and a presence of congested blood vessels in the kidney treatment group tissues. The sections were stained with hematoxylin and eosin. A. *indica* AgNPs treatment group up to (30 mg/kg), H&E x400.

to the 28 days drug administration effect, the liver of rats administered up to 30 mg/kg showed a smooth surface and evenly dark red colored. Rats administered 10 mg/kg showed normal hepatocellular architecture but with congested blood vessels (CBV). They presented a normal renal architecture with glomeruli and tubules having normal structure. However focal zones of hemorrhage (ZH) were observed in the medullary interstitium. The highest dose of 30 mg/kg was associated with periportal infiltration in the liver indicating portal hepatitis and an infiltration in cortex including glomeruli. Kidneys of rats administered up to 30 mg/kg showed a smooth surface and evenly dark red colored. Further kidneys showed a proliferation of podocytes (PD) limiting bowman capsule space and a renal tubular lumen with exudate (Figure 8).

DISCUSSION

The synthesized particle size had a maximum Surface Plasmon Resonance (SPR) peak at 430 nm. Various reports have established that the resonance peak of silver nanoparticles appears around this region, but the exact position depends on a number of factors such as particles size, and the surface-adsorbed species (Wen, 2017). Absence of absorbance at wavelengths greater than 550 nm indicated their well-dispersed state in solution (Coates, 2004). Scanning electron microscopy (SEM) showed that the AgNPs formed were spherical in shape with an average diameter of 10 μm and an average size of 45 nm. Similar results have been reported by Nwagbogu (2018). The electrostatic relations and hydrogen bond between the bio-organic capping molecule bonds are attributed with the synthesis of silver nanoparticles using plant extract, (Heera et al., 2015). The AgNPs were also characterized using EDS spectroscopy. EDS analysis provides qualitative and quantitative status of elements that may be involved in the formation of Silver nanoparticles (Sulaiman et al., 2017). The EDS spectrum was recorded in the profile spot mode as demonstrated in Figure 6. The outcomes clearly illustrate the presence of strong signals from the NPs which confirmed the successful biosynthesis of AgNPs by methanolic bark extract of *A. indica*. Weaker signals from P, Cl, K, Ca and S atoms were also noted. These weak signals may originate from macromolecules like metabolites and fatty acids present in the plant. A peak related to O atom was observed in the EDS spectrum, which is probably origin of the glass substrate or from the SEM chamber (Ismail et al., 2018). The same was reported and was also explained by Wang et al., (2016). Whereas, other reports depicted that O present in EDS analysis may be applied during preparation processes (Li et al., 2012). However, from the EDS spectrums, it is evident that silver particles were reduced by methanolic bark extract of *A. indica* and had the

weight percentage of silver of 13.56%, as shown in the inset of Figure 6. The inset table also illustrates the atomic weight percentage of other elements. The biomolecules responsible for reducing Ag⁺ ions and capping the bio-fabricated AgNPs were presented at different functional groups such as the alkane, alkene, amine, and carboxylic acid. They have been recently reported to be major reducing agents in the biosynthesis of silver nanoparticles (Amuanyena et al., 2019). This in addition plays majorly the role of indicator of the presence of residues of plant phytochemicals in the synthesized silver nanoparticles, (Taha et al., 2019). Therefore, it shows in the FTIR results the NPs synthesized were possibly surrounded by proteins, fatty acids and some metabolites namely terpenoids having functional groups of amines, alcohols, ketones, aldehydes, and carboxylic acids similar to the recent findings of Taha et al. (2019). The presence of metabolites and proteins act as reducing agent and also as stabilizing agent and may avoid accumulation by binding to AgNPs through free amino groups through electrostatic magnetism of negatively charged carboxylate groups in extracellular enzyme filtrate from *A. indica* (Suresh et al., 2011; Ammar and El-Desouky, 2016). Consequently, the findings observed in FTIR spectrum discovered the presence of different biomolecules that acted as reducing and capping agents that were involved in the AgNPs formation and its stability, (Taha et al., 2019).

In this study, no mortality was noted after oral administration of dosages of up to 30 mg/kg of nanoformulation synthesized using methanolic bark extract of *A. indica* in the rats. According to Nwagbogu (2018) neem bark extracts has a low toxicity and its LD₅₀ (lethal doses) has been evaluated to be greater than 300 mg/kg in mice. This is the lowest toxicity class and according to the Guidance Document on Toxicity (Sharma, 2010), the *A. indica* silver nanoformulation may be assigned to be class 5 (LD₅₀ > 30 mg/kg body weight). The biochemical parameters such as ALT and AST are among the biomarkers for the liver function. In normal rats the ALT level observed in this study was comparable with the finding by Ong et al., (2016) who showed that the ALT level of Albino mice ranged from 55.0-208.2 μL . There was a significant increase (3 times higher) of the ALT at a dosage of 30 mg/kg treatment groups after 28 days and 180 days of drug administration. However, the animals were not further monitored after the experiment. Similar to the ALT, there were significant increase of the AST at 10 and 30 mg/kg treatment group after 28 days of drug administration. Despite our gross pathology results showing evenly dark red and normal smooth surfaced liver, the histopathological findings showed periportal infiltration by inflammatory cells suggesting portal hepatitis. This may be confirming the clinical biochemistry results. It may suggest that the *A. indica* silver nanoformulation on

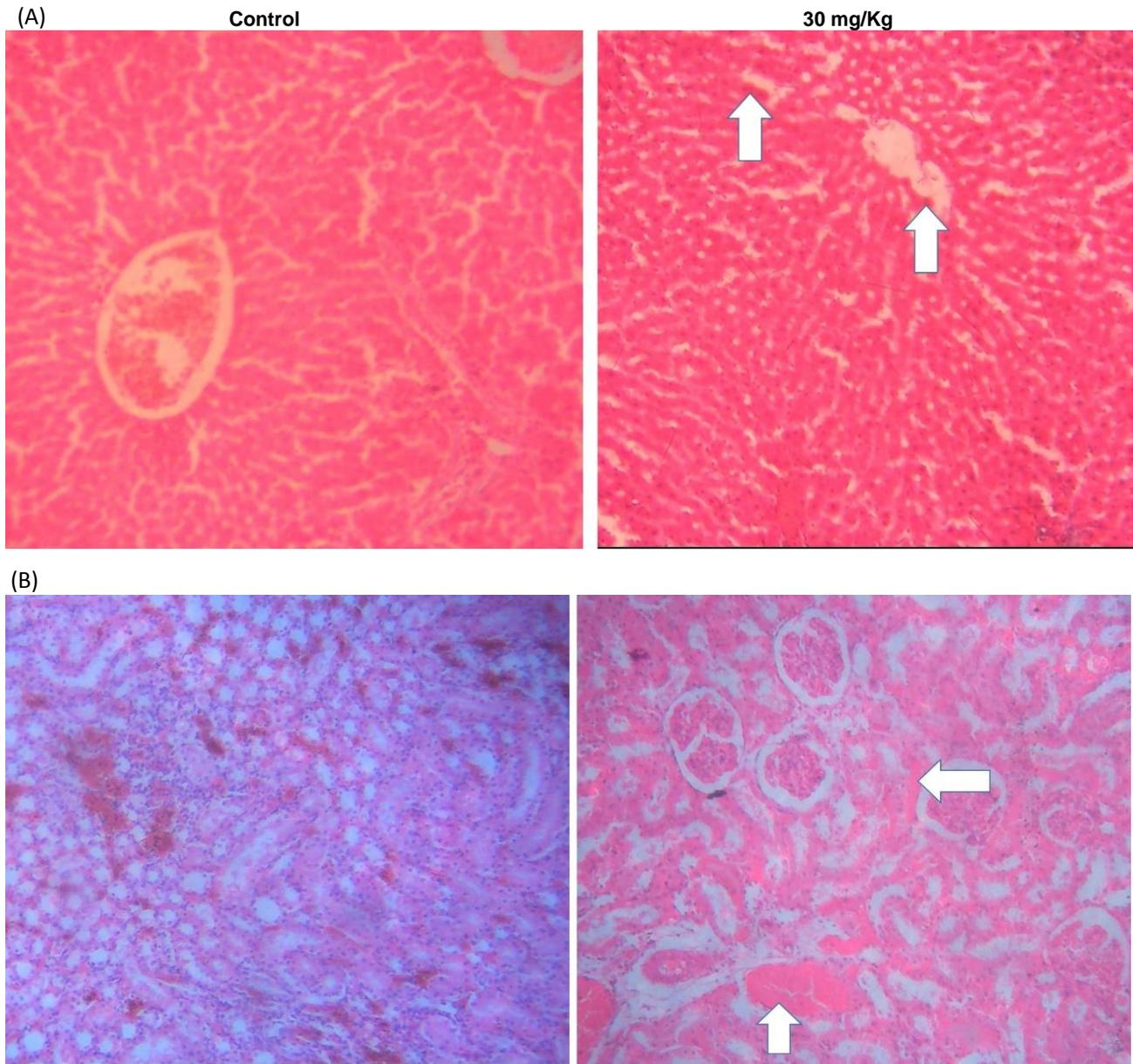


Figure 8. Histopathological examinations of the kidney and liver of rats after 180 days of drug administration. (A) Represent liver and (B) Kidneys. The arrows make a representation of portal infiltration in the liver tissue and BVC (blood vessels congested) of the kidney tissue in treatment groups. The sections were stained with hematoxylin and eosin. *A. indica* AgNPs treatment group (30 mg/kg), H & E x400.

continuous use at 30 mg/kg may lead to damage of the liver, evident in both sub-acute and chronic studies. Portal hepatitis may suggest a chronic hepatitis of which the portal inflammation predominates. Recently, studies described mixed infiltrate of lymphocytes, neutrophils, monocytes, and occasional eosinophils, however the relative numbers of diverse cells and their relationship to disease progression are not known. The portal inflammation degree does not correlate with grade of lobular inflammation, but is associated instead with portal

based changes, such as a ductular reaction (DR) (Gadd et al., 2014). The DR, a reactive lesion at the portal tract interface comprising small biliary ductules with a complementary complex of stroma and inflammatory cells which develops when hepatocyte regeneration is reduced and hepatic progenitor cell (HPC) proliferation takes charge (Gadd et al., 2014). HPCs are bipotential and capable of proliferation and differentiation into hepatocytes, to replace damaged cells. HPC activation and a DR are common responses to chronic liver injury

and are thought to precede progressive, portal fibrosis in which portal tracts, inflammatory cells and their mediators influence the differentiation and fate choice of HPC, which, in turn, may determine the balance between liver repair and fibrogenesis.

Both sub-acute and chronic toxicity studies indicated normal serum creatine and urea values, a share pointer of non-nephrotoxicity. This is in agreement with the study by Shirodkar (2015). The kidney gross pathology and histological organization appeared normal generally. The renal architecture was maintained normal with glomeruli and tubules having normal structure at the dose level of 10 mg/kg. However, at the highest dosage (30 mg/kg), the organs showed an infiltration in cortex including glomeruli. Since the same observation was made for the control group rats, it may be lucid to speculate that the infiltration may not be associated with the drug administered.

The PCV values of rats observed in this study were within the normal range and they were comparable to the 28 days sub-acute values (37.5-47.7%), these corresponds with the recent results reported by Shamaki (2014) for normal albino rats. The observation that, there were no significant differences between the PCV of control nontreated and treatment groups indicates that administration of silver nanoformulated *A. indica* bark extract does not destroy blood cells after both 28 and 180 days of drug administration. These findings are comparable to the findings of Wen (2017) who reported that administration of silver nanoparticles in rats has no effect to the red blood cells after 28 days of drug administration.

Conclusion

The studies in toxicities of *A. indica* nanoformulation *in vivo* it currently gained extensive attention, and more knowledge on AgNP is required for safety evaluation and risk management. Taken our data and previous studies composed, it is rational to speculate that a lower dose of AgNP with extended exposure period could essentially accumulated in targeted organs and produce chronic toxicity. Additionally, the potential of carcinogenicity may intensify. Major concerns on *A. indica* AgNP's safety assessment at present are its disposition in the targeted organs and the subsequent toxicities, which necessitates a further genotoxicity and carcinogenicity study to follow. The distribution of *A. indica* AgNPs in animals can be identified by, for illustration, a recently developed method using gold nanocluster as fluorescence probes Zhang, 2015. In conclusion, this study sheds light on the underlying safety of *A. indica* AgNPs, targeted organs and genotoxicities particularly generated by *A. indica* AgNP in Swiss albino rat models. The specific immunotoxicities and potential carcinogenic effect induced by nanoformulation need to be further investigated in a chronic toxicity study.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

Abbreviations

AgNPs, Silver nanoparticles; **ALT**, Alanine aminotransferases; **AST**, Aspartate aminotransferases; **BWT**, Body weight; **FTIR**, Fourier Transform Infrared Resorption; **KeV**, Kilo electron volts; **kg**, Kilogram; **NM**, Nanometer; **NPs**, Nanoparticles; **PCV**, Packed Cell Volume; **SEM**, Scanning Electron Microscopy; **SPR**, Surface plasmon resonance; **TEM**, Transmission electron microscopy; **UV/Vis**, Ultraviolet visible spectrum; **XRD**, X-ray diffraction analysis.

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