EVALUATION OF ANTIOXIDANT ACTIVITY OF BIOLOGICALLY SYNTHESIZED SILVER NANOPARTICLES USING *ALOE VERA*

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ABSTRACT

Eco-Friendly green synthesis of nanoparticles is a fast-growing area of research in nanotechnology. This study aims to synthesize *Aloe vera* mediated silver nanoparticles (AV-AgNPs) and evaluates in-vitro and in-vivo antioxidant potentials. Also examines the phytochemical screening of aqueous *Aloe vera* leaves extract (AVLE). The AV-AgNPs were characterized by UV–visible spectroscopy and scanning electron microscopy. However, the phytochemical screening of AVLE was performed by standard protocols. In-vitro antioxidant activities were determined through lipid peroxidation inhibition, DPPH (1,1-diphenyl-2-picrylhydrazyl), and reducing power assays. Whereas, in-vivo antioxidant activities were studied in rats (180-220g b.w), received AV-AgNPs (10 mg/kg b.w) via oral gavage for 28 days. After euthanization of rats, blood and kidneys were collected and used for biochemical (urea, creatinine) and antioxidant (MDA, SOD, CAT, and GSH) investigations. Results showed the maximum absorption of AV-AgNPs at 400 nm and 20-24 nm particle size with spherical morphology. Whereas, flavonoid, glycosides, phenols, saponin, tannins, and terpenoids phytochemicals were present in AVLE. Both in-vitro and in-vivo studies exhibited the significant potentials of AV-AgNPs as an antioxidant and free radical scavengers thus it could be proposed for many biomedical and environmental applications such as for drug encapsulation or purification of water.

Key-words: Silver nanoparticles, Green synthesis, Phytochemical screening, DPPH, Lipid peroxidation inhibition, Antioxidant.

INTRODUCTION

For the generation of energy in cells, redox process is carried out due to which free radicals are generated which include reactive oxygen species (ROS), which play a significant role in the maintenance of cellular responses and immune functions (Sivanandham, 2011). When ROS are produced in excess, they generate oxidative stress, thus damaging the cell structure including proteins, lipids, and DNA; and become responsible in the development of various degenerative disorders and chronic diseases (Pham-Huy *et al*., 2008). Excessive and unbalanced free radical generation is combat by compounds called antioxidants, these compounds not only provide the shield to the cells from injuries but also improves immune function and reduces the risk of potential indemnities associated with these radicals thus evading many pathological events in living organisms propagated by the generation of free radicals (Bhattacharya, 2015). Therefore, recent trends are evolving on the potential discoveries of natural antioxidant compounds and their role in the improvement of health care procedures, treatments, and prevention. Plants possess phytochemicals like aldehydes, amides, carboxylic acids, flavones, ketones, and terpenoids, that have been shown to act as effective antioxidants (Rai and Yadav, 2013).

Kidneys are the highly susceptible organs to toxicants due to a high volume of blood flows and filters large quantities of toxins which can concentrate in the kidney tubules. During this, the kidney may become vulnerable to systemic toxicity which affects kidney efficiency to remove body wastes, regulating the body fluid and electrolytes, and synthesis of many biologically important molecules (Oduola *et al*., 2010).

Aloe vera (L.) Burm. f. is a medicinal plant belongs to the family Asphodelaceae (www.efloras.org). It is a cost-effective and easily available plant. It is enriched with a variety of phytoconstituents, which are pharmacologically active compounds. These compounds responsible to provide defense against various infections and used to treat a variety of clinical conditions, therefore, it is prevalently used in many Ayurvedic formulations and provides the base for new pharmaceutical composites (Kumar *et al.,* 2017).

On the other hands, nanotechnology deals with the production of nanoscale products called nanoparticles. Synthesis of the nanoparticles is a growing territory of research nowadays; enormous utilization in diverse fields of human life is the reason behind the fascination of scientists towards the direction of this subject (Kumar *et al.*, 2017). Herbal plants being utilized is a contrasting choice for the development of nanoparticles in an eco-accommodating way and on the other hand, these herbal plants possess phytochemicals with strong antioxidant

properties, thus opening new prospects of medical therapy to use nanoparticles as therapeutic agents (Pavani *et al.,* 2012). Plant-based synthesis of nanoparticles is currently being experimented by the investigators for its effective benefits like avoidance of retaining up the microbial culture, the absence of toxic chemicals and occurrence of natural capping agents (Bangale and Ghotekar, 2019). The production of metal nanoparticles by merging metal salts with bio-molecules found in plants, like amino acids, enzymes, polysaccharides, proteins, and vitamins has been explored (Marchiol, 2012). In this regard, several plants are efficiently used for the biosynthesis of nanoparticles such as *Azadirachta indica* (Shankar *et al.*, 2004), *Camelia sinensis* (Vilchis-Nestor *et al.*, 2008), *Catharanthus roseus* (Kannan *et al.*, 2011), *Moringa oleifera* (Prasad and Elumalai, 2011), *Tinospora cordifolia* (Singh *et al.*, 2014) and *Carica papaya* (Banala *et al.*, 2015).

Certain studies have reported plant constituents for the synthesis and stability of silver nanoparticles (Benelli, 2016; Sadeghi *et al.*, 2015) while fewer studies are found to describe the properties of nanoparticles because of the interactions between plant constituent and metal nanoparticles. In the current study, we have determined the phytochemical screening of *Aloe vera* leaves extract and the antioxidant potential of *Aloe vera* mediated silver nanoparticles (AV-AgNPs) via both in-vitro (DPPH activity, Lipid peroxidation inhibition assay and reducing power ability) and in-vivo studies (malondialdehyde (MDA), superoxide dismutase (SOD), catalase (CAT), and glutathione (GSH) activities).

MATERIALS AND METHODS

Plant Material and Preparation of Aqueous *Aloe vera* Leaves Extract (AVLE):
Fresh and healthy leaves of *Aloe vera* were collected and authenticated by the Karachi University Herbarium. The leaves were washed under tap water and used for extract preparation. The aqueous *Aloe vera* leaves extract (AVLE) was prepared by the method of Chandran *et al.* (2006). Washed leaves were comminuted, immersed (10 g) in water (100 mL) and boiled for 20 min. The extract was filtered and refrigerated for further use.

Phytochemical Screening of AVLE:
Phytochemical screening of AVLE was carried out by using standard methods to detect the existence of alkaloids, carbohydrates, proteins & amino acids, flavonoids, glycosides, phenols, steroids, saponins, tannins and terpenoids (Harborne, 1984; Sofwara, 1993; Trease and Evans, 1989).

Preparation of *Aloe vera* Mediated Silver Nanoparticles (AV-AgNPs):
The formation of AV-AgNPs was directed by the procedure of Chandran *et al.* (2006) with some modifications. Briefly, 10 mL *Aloe vera* leaves extract (AVLE) (10%), 85 mL $AgNO_3$ solution (1 mM) and 5 mL ammonia solution (0.1%) were mixed and incubated for 24 h in dark condition at room temperature. The reaction mixture changed from colorless to reddish-brown indicating the formation of nanoparticles. The resulting phyto-mediated SNPs were repeatedly centrifuged for 10 min at 15,000 rpm, washed, collected and re-dispersed in deionized water.

Characterization of AV-AgNPs:
UV–Visible Spectroscopy Analysis:
The AV-AgNPs formation was primarily characterized by the UV–Visible absorption spectral analysis in the range of 300 to 700 nm. The characteristic peak for AV-AgNPs was monitored to the highest absorbance from 400–450 nm range, which is evidence of the existence of the AgNPs (Chahardoli *et al.*, 2018).

Scanning Electron Microscopy (SEM):
The scanning electron microscopy (SEM) was used to describe the morphology and size of the particle. For SEM, the AV-AgNPs was dried and coated with gold up to 300°A using a JEOL; Japan (JFC-1500). The gold-coated sample was subjected to SEM JEOL; Japan (JSM 6380A) at 15 kV and the photographs were recorded from 15000-35000 magnification range.

In-vitro Antioxidant Study:
Lipid peroxidation inhibition assay:
Kidney homogenate was prepared by taking fresh kidney tissue of a healthy albino rat; the kidney tissue was homogenized with phosphate buffer (pH 7.4, chilled), centrifuged (3000 rpm) for 15 minutes and the clear supernatant was collected for lipid peroxidation inhibition assay. The percent of lipid peroxidation inhibition (% LPOI) of the AV-AgNPs and AVLE were examined in vitro by the scheme of Halliwell and Gutteridge (1999).

DPPH radical scavenging assay:
    1, 1-diphenyl -2-picrylhydrazyl (DPPH) radical scavenging assay for both AV-AgNPs and AVLE was measured relating to scavenge the hydrogen giving ability as described by Kumar *et al.* (2013).

Reducing power assay:
    The reducing power capacity of AV-AgNPs and AVLE was expressed by the process as described by Reddy *et al.* (2014).

***In-vivo* Antioxidant Study:**
Study design:
    The male albino Wistar rats were randomly divided into three groups consisting of 10 rats each. The experimental procedures were approved by the guidelines of the Institutional Animal Ethics Committee. The weight of all treated animals and their food consumption were recorded throughout the study period regularly.
Group 1: Control group: received no treatment and fed on a normal rat diet.
Group 2: AVLE group: received AVLE (100 mg/kg b.w) via oral gavage for 28 days.
Group 3: AV-AgNPs group: received AV-AgNPs (10 mg/kg b.w) dissolve in water via oral gavage for 28 days.

Sample collection:
    Animals were euthanized after 24 hours of the last dose administration, blood was collected in the lithium heparin-coated tubes and was subjected to centrifuge for plasma separation. Whereas, kidneys were excised, rinsed with saline (0.9% NaCl) and connective tissues were detached. Record their weight after dried with the help of filter paper by blotting, and the sample, then reserved in a freezer at −70ºC until analysis.

Preparation of kidney homogenates:
    To prepare kidney homogenate, kidney tissue was minced, homogenized with 10% of 100 mM KCl buffer (pH: 7, containing 0.3 mM EDTA) and allowed to centrifuge at 600 x g for 60 min (4°C). The supernatant was separated and stored for biochemical assays.

Assessment of kidney biochemical parameters:
    Kidney function markers such as urea and creatinine were analyzed using spectrophotometric analysis via monoxime method (Ather and Ronald, 1969) and Jeff's method (Spierto *et al.*, 1979), respectively.

Assessment of renal oxidative stress:
Renal malondialdehyde (MDA) activity:
    The oxidative stress of renal tissues measured in terms of Malondialdehyde (MDA, a product of lipid peroxidation) which was assayed in kidney homogenate by the method of Okhawa *et al.*, (1979) based on the determination of thiobarbituric acid reacting substance (TBARS) which interacted with MDA producing the pink color. The intensity of color was read at 530 nm on UV Spectrophotometer (Schimadzu-1900) and values expressed in nmol/gm of tissue.

Assessment of renal antioxidant status:
Renal catalase (CAT) activity:
    Catalase activity in kidney homogenate was analyzed by the method of Sinha (1972) and expressed in μmol/gm of tissue.

Renal superoxide dismutase (SOD) activity:
    Levels of renal SOD were measured by the method of Kono **(1978)** in the cell-free supernatant, and percent inhibition rate of NBT reduction in the reaction mixture was recorded per minute at 560 nm and expressed in U/gm of tissue.

Renal glutathione (GSH) activity:
    The renal GSH activity was assessed by the method of Carlberg and Mannervik (1985), absorbance was recorded at 340 nm on UV Spectrophotometer (Schimadzu-1900) and expressed in U/gm of tissue.

Statistical analysis:
The results were analyzed by using IBM-SPSS (statistical package for social sciences) version 22 and data were expressed as mean ± SD (standard deviation). The statistical significance between the groups was analyzed using one-way ANOVA followed by Tukey's post hoc test, where $p < 0.05$ was considered significant.

RESULTS

Phytochemical Screening of plant extract:
Phytochemical screening of plant extract (*Aloe vera* leaves extract = AVLE) was tested with its appropriate phytochemical assays to diagnose the existence of secondary metabolites such as alkaloids, carbohydrates, phenols, flavonoids, protein and amino acids, saponins, glycosides, steroids, tannins, and terpenoids. The result of the phytochemical test is depicted in Table **1**. All the tested phytochemicals were found in aqueous *Aloe vera* leaves except alkaloid and steroids.

Preparation of *Aloe vera* mediated silver nanoparticles (AV-AgNPs):
In the bio-green method, AVLE was used as a reducing and stabilizing agent for phyto-mediated SNPs synthesis. The formation of AV-AgNPs was preliminarily confirmed by a change in color from colorless to reddish-brown in silver nitrate solution upon the addition of *Aloe vera* solution after 24 hours of incubation at room temperature (Fig. **1**). The mutual vibration of free electrons found in nanoparticles was responsible for the change in color (Noginov *et al.*, 2007).

Characterization of AV-AgNPs:
UV–Vis Spectral Analysis:
The UV–Visible absorption spectrum demonstrated a precise and specific surface plasmon resonance band with the maximum λ at 400 nm (Fig. **2**) while the broadening of peak indicative of polydispersed nature of particles (Roy and Anantharaman, 2018).

Scanning Electron Microscopy (SEM):
The size and morphology of the AV-AgNPs were examined by SEM at 15kV. The result revealed that the size of the AV-AgNPs was 20-24 nm with spherical morphology (Fig. **3**). Nethradevi *et al.* (2012) suggested that the morphology of nanoparticles belongs to agglomeration property which could be correlated with a capping agent.

***In Vitro* Antioxidant Study:**
The scavenging activity of AV-AgNPs and AVLE toward free radical was determined by using various in-vitro assays, including lipid peroxidation inhibition assay, DPPH scavenging assay and reducing power assay.

Lipid Peroxidation Inhibition Assay:
The lipid peroxidation inhibition activity of AVLE and AV-AgNPs was assayed at several concentrations (100-600 µg/mL). The BHA was used as a positive control. The results obtained against lipid peroxidation activity displayed the capability of both AVLE and AV-AgNPs protecting tissue from peroxidative damage (Fig. **4**). The AVLE showed higher activity while AV-AgNPs also explained its inhibition activity toward lipid peroxidation while the BHA showed maximum activity at a concentration of 600 µg/mL that was 84.2%. The result demonstrated the concentration-dependent lipid peroxidation inhibition activity.

DPPH Radical Scavenging Assay:
The DPPH scavenging activity of AVLE and AV-AgNPs was assayed at various concentrations (100-600 µg/ml), as the antioxidant ability is directly related to reducing capacity. The grade of discoloration specifies the scavenging capacities of both AVLE and AV-AgNPs. The ascorbic acid was used as a control. The silver nanoparticles donate electrons or hydrogen, which was accepted by DPPH. The results attained by DPPH assay displayed efficient scavenging activity by both AVLE and AV-AgNPs (Fig. **5**). The AV-AgNPs exhibited higher DPPH scavenging activity than AVLE and the antioxidant activity was improved with the rise in concentrations while the ascorbic acid showed the highest activity among all (75.4%) at a high concentration of 600 µg/mL. Similar observations have been reported previously (Reddy *et al.*, 2014).

Reducing Power Assay:

The antioxidant activity of AVLE and AV-AgNPs can also be used to express by the measurement of reducing power assay. Significant reducing power was observed in both AVLE and AV-AgNPs and results are shown in Fig. **6**. Higher reducing power was indicated by higher absorbance value. The reducing power of the AVLE and AV-AgNPs was improved by increasing the concentration (100–600 μg/mL) of extract exhibiting dose-dependence. Dipankar and Murugan (2012) also found similar observations in this regard. The AV-AgNPs disclosed high reducing potential than AVLE and the antioxidant activity was augmented as concentrations were getting higher, while the ascorbic acid showed maximum reducing activity (2.53) at 600 μg/mL concentration.

***In Vivo* Antioxidant Study:**
Body Weight, Kidney Weight, and Relative Kidney Weight:

Both the treated groups; AVLE (100 mg/kg b.w) and AV-AgNPs (10 mg/kg b.w), showed no observable changes in body weight, kidney weight and relative kidney weight of rats as compared with the control group (Table **2**).

Renal Biochemical Parameters:

The kidney function markers (urea and creatinine) showed no significant difference in both the AVLE and AV-AgNPs treated groups (Table **3**).

Assessment of Renal Oxidative Stress:
MDA levels:

Renal tissue MDA levels were significantly ($p<0.001$) decreased in both the AVLE and AV-AgNPs treated groups compared with control (Fig. **7**).

Assessment of Renal Antioxidant Status:
Catalase levels:

Renal tissue Catalase levels were increased nonsignificantly in both the AVLE and AV-AgNPs treated groups compared with control (Fig. **8**).

SOD levels:

Renal tissue SOD levels were increased nonsignificantly in both the AVLE and AV-AgNPs treated groups compared with control (Fig. **9**).

GSH levels:

Renal tissue GSH levels were increased nonsignificantly in both the AVLE and AV-AgNPs treated groups compared with control (Fig. **10**).

Table 1. Phytochemical screening of plant extract (AVLE).

Phytoconstituents	Phytochemical Test	**Result**
Alkaloids	Mayer's Test	-
	Hager's Test	-
Carbohydrates	Molisch's Test	+
	Benedict's Test	+
Flavonoids	Alkaline Reagent Test	+
	Lead Acetate Test	+
Glycosides	Legal's Test	+
Phenols	Ferric Chloride Test	+
Proteins & Amino acids	Xanthoproteic Test	+
	Ninhydrin Test	+
Saponins	Froth Test	+
Steroids	Salkowski's Test	-
Tannins	Ferric Chloride Test	+
	Potassium Dichromate Test	+
Terpenoids	Lieberman Bouchard Test	+

+: present, −: absent

Table 2. Effects of AVLE and AV-AgNPs on Body Weight, Kidney Weight, and Relative Kidney Weight.

Parameters	Control	AVLE	AV-AgNPs
Mean Body Weight (g)	211.525 ± 5.19	229.50 ± 6.59 [ns]	219.36 ± 7.66 [ns]
Kidney weight (g)	0.61 ± 0.19	0.566 ± 0.21 [ns]	0.666 ± 0.38 [ns]
Relative Kidney weight (g/100g of b.w)	0.288 ± 0.221	$0.252 \pm 0.7.28$ [ns]	0.238 ± 0.222 [ns]

Values are mean $\pm$ SD (n=10).
***p<0.05, ***p<0.01, ***p<0.001, ns= non-significant compared with control group by One-way ANOVA followed by Tukey's post hoc test.
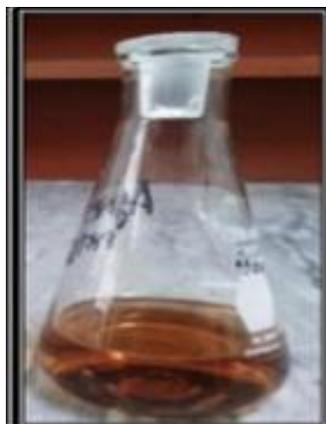
Table 3. Effects of AVLE and AV-AgNPs on Renal Biochemical Parameters.

Parameters	Control	AVLE	AV-AgNPs
Urea (mg/dL)	35.503 ± 3.522	36.30 ± 4.032 [ns]	35.334 ± 3.601 [ns]
Creatinine (mg/dL)	0.440 ± 0.028	0.45 ± 0.026 [ns]	0.438 ± 0.378 [ns]

Values are mean $\pm$ SD (n=10).
***p<0.05, ***p<0.01, ***p<0.001, ns= non-significant compared with control group by One-way ANOVA followed by Tukey's post hoc test.
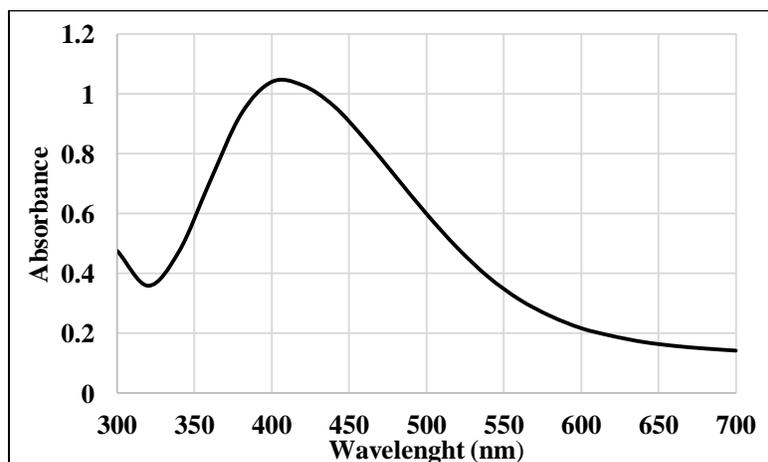

Fig. 1. Formation of AV-AgNPs.

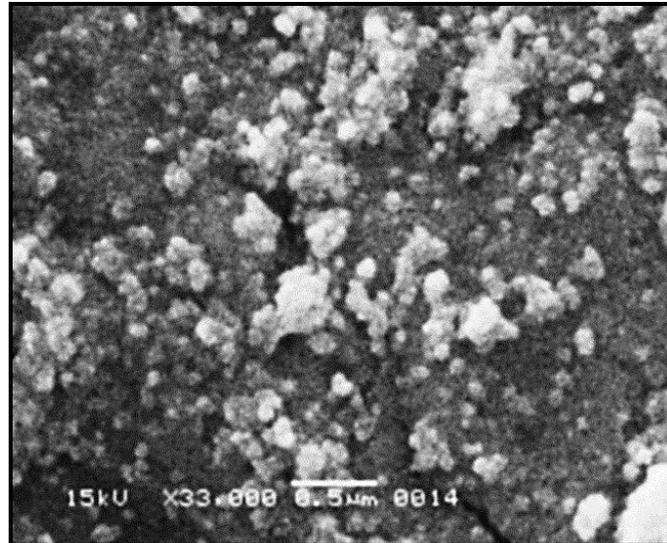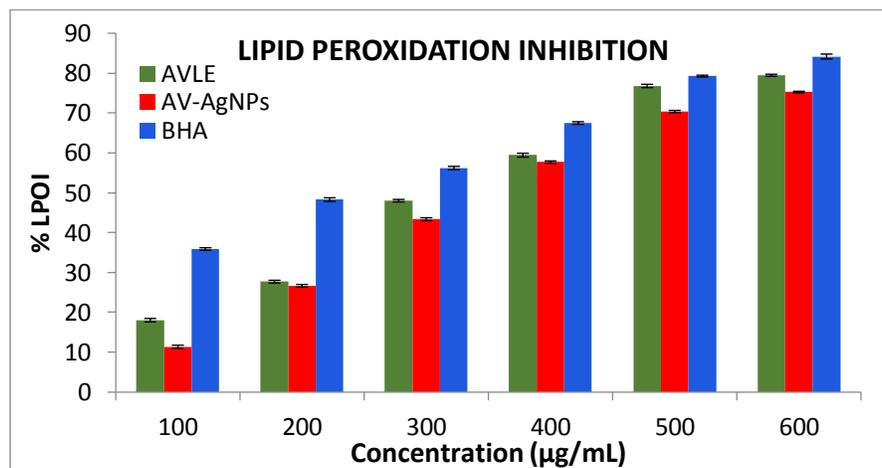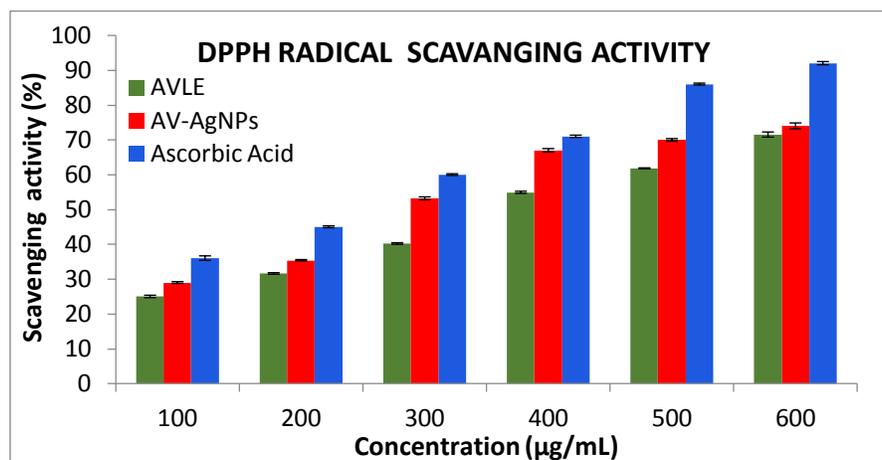Fig. 2. UV–Visible absorption spectra of AV-AgNPs.

Fig. 3. SEM image of AV-AgNPs.

Fig. 4. Lipid Peroxidation Inhibition Assay of AVLE, AV-AgNPs, and BHA. Values are mean ± SD (n=3).

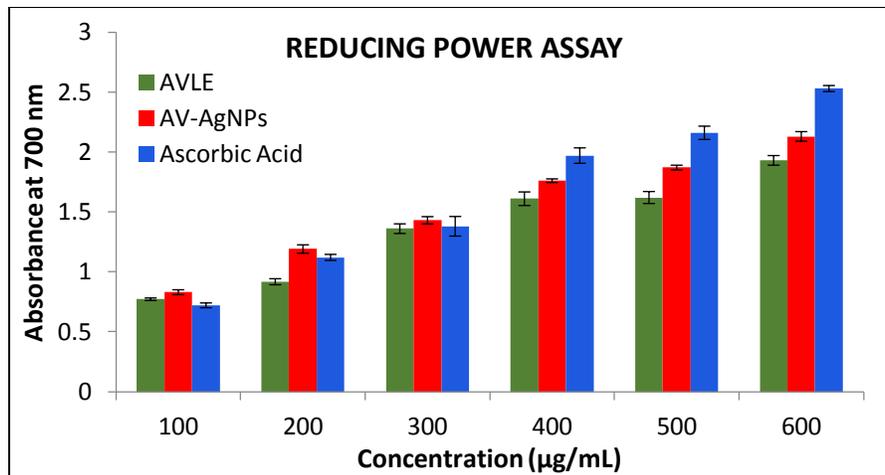Fig. 5. DPPH Radical Scavenging Assay of AVLE, AV-AgNPs, and Ascorbic Acid. Values are mean ± SD (n=3).

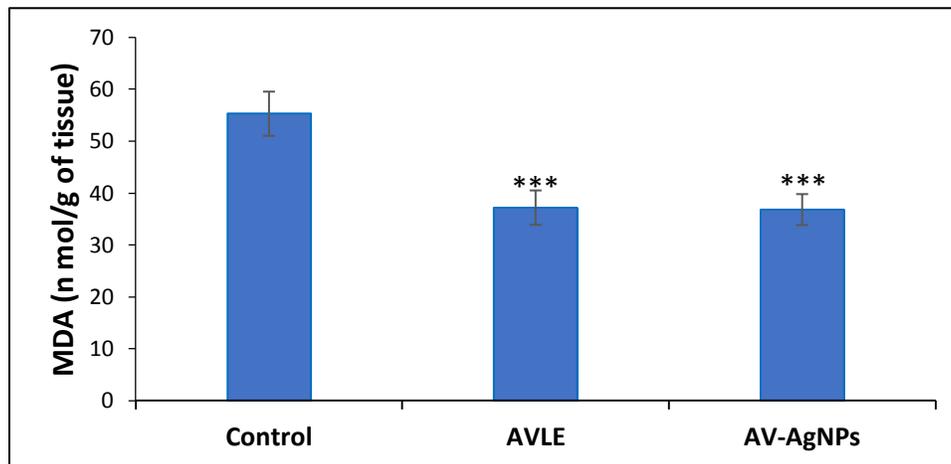Fig. 6. Reducing Power Assay of AVLE, AV-AgNPs, and Ascorbic Acid. Values are mean ± SD (n=3).

Fig. 7. Effects of AVLE and AV-AgNPs on Renal Oxidative Stress (MDA).
Values are mean ± SD (n=10); ***$p<0.001$ compared with the control group by One-way ANOVA followed by Tukey's post hoc test.
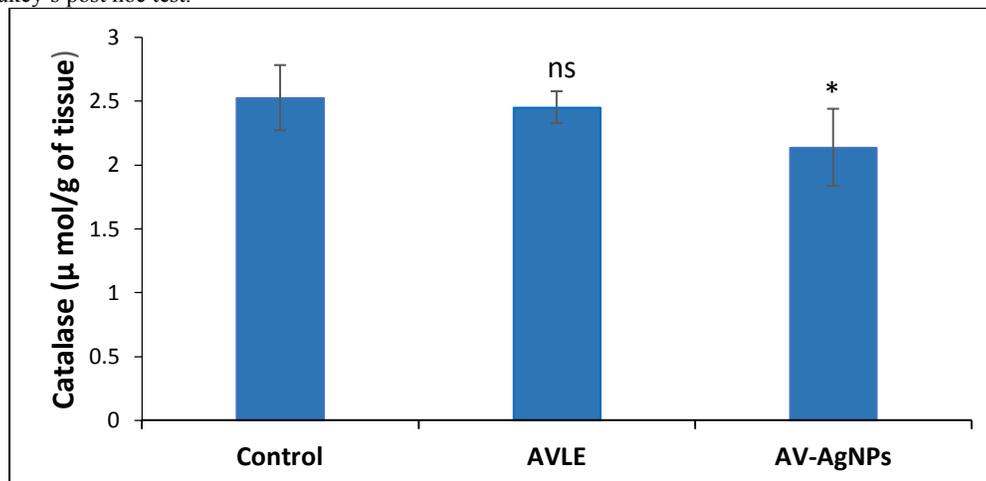

Fig. 8. Effects of AVLE and AV-AgNPs on Renal Antioxidant Parameters (A) Catalase.
Values are mean ± SD (n=10); ns= non-significant, *$p<0.05$ compared with the control group by One-way ANOVA followed by Tukey's post hoc test.
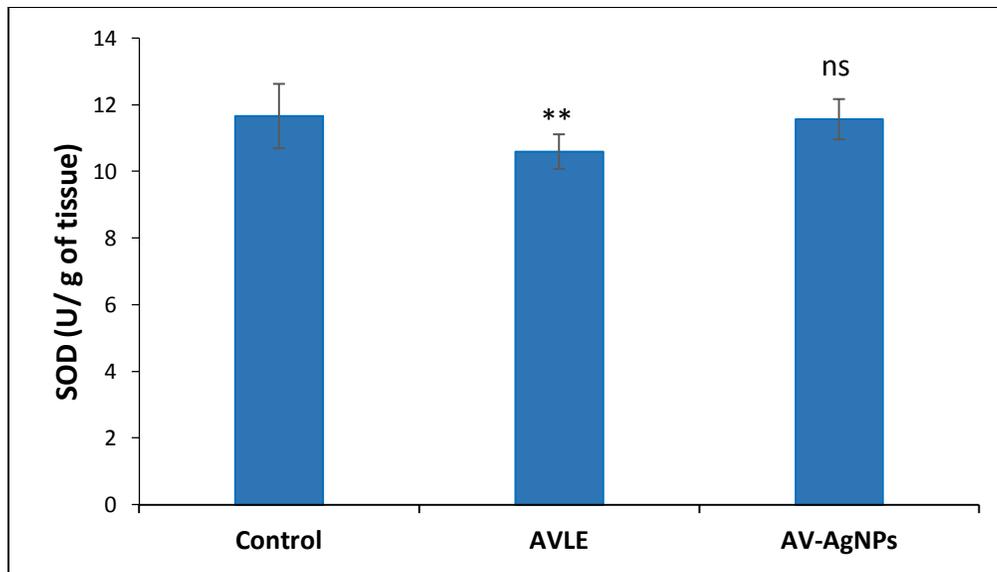
Fig. 9. Effects of AVLE and AV-AgNPs on Renal Antioxidant Parameters (B) SOD.
Values are mean ± SD (n=10); **p<0.01, ns= non-significant compared with the control group by
One-way ANOVA followed by Tukey's post hoc test.

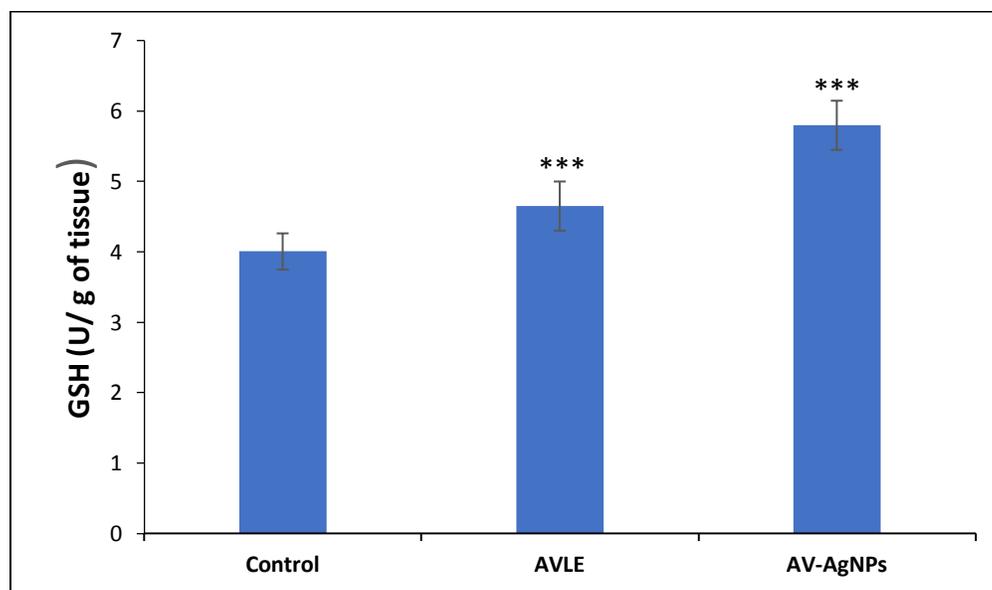Fig. 10. Effects of AVLE and AV-AgNPs on Renal Antioxidant Parameters (C) GSH.
Values are mean ± SD (n=10); ***p<0.001compared with the control group by One-way ANOVA followed by
Tukey's post hoc test.

DISCUSSION

Plants are the source of natural antioxidants called phytochemicals, which combat the free radicals and their reactive derivatives (ROS). Phytochemicals have a beneficial role to modulate the metabolism for the prevention of chronic and degenerative diseases in humans (Dipankar and Murugan, 2012). Therefore, plants are broadly in the study for the formation of biogenic AgNPs (Zhang *et al.*, 2015). In the present study, *Aloe vera* is used to prepare AV-AgNPs. The AVLE were subjected to the preliminary phytochemical screening like alkaloids, carbohydrates, flavonoids, glycosides, phenols, protein and amino acids, saponins, steroids, tannins, and terpenoids. These metabolites contribute to significant biological activities (Saratale *et al.*, 2018). Phytochemical analysis of plants has

a great interest in producing new drugs to cure various diseases. The presence of various medicinally important phytoconstituents in the AVLE rationalizes the participation in the production of AV-AgNPs.

Preliminary identification of nanoparticle synthesis was carried out by perceiving the color change of the reaction solution. Synthesis of AV-AgNPs from $AgNO_3$ solution and AVLE was confirmed by UV-Vis spectral analysis. The change in color of the reaction mixture to reddish-brown is specified to the SPR band due to excitation and of $AgNO_3$ (Atanasov *et al.*, 2015). SEM provides morphology and size of the nanoparticles. It is discovered that the AV-AgNPs were 20-24 nm in size and spherical in morphology.

In the present study, AVLE and AV-AgNPs utilized in-vitro as an antioxidant agent by analyzing lipid peroxidation inhibition assay, DPPH scavenging activity, and reducing power assay. In conclusion, both AVLE and AV-AgNPs were found to be effective to hinder or diminish the lipid peroxidation chain reaction, free radical scavenging and reducing power. The lipid peroxidation has been proposed to progress via a free radical chain reaction, that is accompanied with cell damage in living organisms, leading to different diseases like cancer, cardiovascular diseases, and diabetes (Ahmed and Ikram, 2015). The AVLE and AV-AgNPs in this study exhibited effective against LPOI activity while in comparison, AVLE revealed high LPOI compared to AV-AgNPs. Whereas, the increase in LPOI activity showed a concentration-dependent manner.

Similarly, AVLE and AV-AgNPs showed effective DPPH scavenging activity in all concentrations. DPPH scavenging assay is a very convincing procedure for the determination of in vitro antioxidant activity. DPPH quickly responds with substances containing phenolic structures. The dark purple color of DPPH solution contains unpaired electrons which are responsible for allocation of an electron from antioxidant to DPPH radical, due to which purple color of DPPH solution is converted into yellow; this color change is determined by spectrophotometric analysis (Phaniendra *et al.*, 2015). AV-AgNPs displayed maximum DPPH scavenging activity compared to AVLE. The antioxidant activity of both AV-AgNPs and AVLE usually increases with the rise in concentration (Fig. 5). The reducing power assay is also another method to determine in-vitro antioxidant activity. In this assay, substances which have a possible ability to reduce, react with Fe^{+3} and converts it into Fe^{+2} which leads to the formation of a ferrous complex by reacting ferric chloride. The intensity of color may determine by its absorbance at 700 nm, the high value is indicated as high reduction ability to shift the hydrogen atom to the free radical chain (Usmani, 2013). AVLE and AV-AgNPs were also investigated to determine the reducing power. AV-AgNPs showed greater reducing ability compared to AVLE. Both AVLE and AV-AgNPs displayed concentration-reliant proposal of reducing capacity.

The effects of AV-AgNPs on experimental rats for 28 days at a dose of 10 mg/kg b.w, was evaluated in terms of renal oxidative stress and kidney histology. The body weight and organ weight elucidate the normal physiological functions of the body. In our study, the effect of AV-AgNPs administration leads to no observable changes in rats' body, kidney, and relative kidney weights. The effects of silver nanoparticles administration in rats depend on the concentration and duration of treatment. Earlier studies found that the AgNPs did not have any adverse effects on body weights of the animals following oral administration of AgNPs (Espinosa-Cristóbal *et al.*, 2013; van der Zande *et al.*, 2012). The kidney is the primary organ, accounting for the elimination of by-products and their metabolites. Urea and creatinine are considered as the primary indicators of kidney malfunction. Under normal physiological conditions, these biochemical levels remain normal, whereas as the kidney undergoes any malfunction, it affects the levels of these biochemical parameters (Van Hung *et al.*, 2019). Effects of AV-AgNPs administration on kidney biochemical parameters (urea and creatinine) showed steadiness of these parameters, thus exhibiting the nontoxic nature of AV-AgNPs dependent on dose, size, and duration of exposure.

Oxidative stress has a strong association with the progression of various diseases ultimately leads to disease complications. Whereas, in our study, the decline in the level of MDA was observed with improvement in kidney Catalase, SOD and GSH levels that showed AV-AgNPs have been found to decrease oxidative stress and work as an antioxidative agent. With our findings, the improved antioxidant status could be correlated with the phytochemicals reside in *Aloe vera* that capped AV-AgNPs (Ighodaro and Akinloye, 2018). Improvement in the activity of total antioxidant status, CAT, SOD, and GSH progress the oxidant-antioxidant equilibrium thus boost the organism antioxidant system proficiency. The findings of this study suggest that AV-AgNPs is an effective antioxidant mediator. Earlier studies have reported that the toxicity of silver nanoparticles associated with the release of silver ions from silver nanoparticles (Kuppusamy *et al.*, 2016). This study proposed that *Aloe vera* not only reduces the silver into nanoparticles but also capped these particles, consequently hinder the release of silver ions, thus eliminating or reducing the chance of oxidative stress and protects cells from toxicity.

In summary, the bioactive silver nanoparticles were synthesized using AVLE by a green method in an echo accommodating way. The nanoparticles possessed the additional benefit of dynamic phytoconstituents combined with them. The AV-AgNPs are authenticated by their significant potential quenching effects on free radicals. The antioxidant property of AV-AgNPs is attributed due to capped phytochemical compounds thus illustrates its broad

range of applications in biomedical as a therapeutic agent by combating free radical-mediated damage. Moreover, this study also exhibited the role of AVLE administration on oxidative stress and antioxidant enzymes levels of the kidneys in rats. Results of AVLE were identical to AV-AgNPs, that showed the renoprotective effects by decreasing MDA with increasing the antioxidant enzymes. These results correlated with larger amounts of phytochemicals such as polyphenolic compounds, flavonoids, tannins, and saponins present in *Aloe vera* leaves extract. It is established from the previous reports that the antioxidant effects of *Aloe vera* leaf extract are due to free radical elimination (Khanam and Sharma, 2013; Tabatabaei *et al.*, 2017).

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