

Synthesis of Selenium Nanoparticles and Their Effect on Pancreatic Functions and Acute Pancreatitis in Rats

Juan Shu, Yusheng Liao, Jian Wang, Yuanjie Zhang, Weilai Zhou, and Heng Zhang*

Department of Gastroenterology, Central Hospital of Wuhan, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, CHINA

Abstract: Acute pancreatitis (AP) have been documented to have severe impact on pancreatic function. Frequent incidence of AP can result in chronic pancreatitis and thereby it can increase the probability of pancreatic cancers. This study intended to examine the effect of selenium nanoparticles (Se-NPs) synthesized from *Coleus forskohlii* leaf extract on pancreatic function and AP in rat. Primarily, Se-NPs was fabricated using the *C. forskohlii* leaf extract. The synthesized nanomaterial was characterized through UV-visible, XRD, and FTIR spectroscopies. Notably, the zeta potential of Se-NPs was found to be -32.8 mV with a polydispersity index (PDI) of 0.18. Morphological analysis on SEM unveiled the spherical shape of Se-NP with an average particle size of 12.69 nm. Strikingly, cytotoxicity analysis on pancreatic cancer and normal cells unveiled the concentration-dependent toxicity profile. However, IC_{50} value is lower in normal pancreatic cell lines in comparison to pancreatic cancer cells lines. Assessment of Se-NPs on AP rats revealed the positive impact of Se-NPs. It effectively decreased the amount of lipase, amylase, IL-1 β , MDA, NO, and Bcl-2 while increased the glucose, insulin, HOMA- β and antioxidant potential in AP rats. In addition, an evaluation of Se-NPs in the pancreatic functions revealed the non-harmful effect of Se-NPs.

Key words: selenium nanoparticles, phytochemicals, acute pancreatitis, histopathology analysis

1 Introduction

The chemical and physical methods are predominantly employed to build nanoparticles, through top-down (bulk material is broken down to nano size) or bottom-up (materials are synthesized from atom level) approaches. However, the growing problem of carcinogenicity and environmental toxicity from nanoparticle synthesis has necessitated the need to find a most reliable and environmental-friendly alternative technique. This was made possible by the green manufacturing process (green nanotechnology) which uses different raw materials from living organisms for the generation of nanomaterials¹⁻³. From the periodic table, several elements such as silver, gold, zinc, copper, titanium, cadmium, selenium, gadolinium, etc., are used for generation of nanoparticles. But the high cost and toxic nature of some metals has restricted its use in the synthesis process^{4,5}. Selenium nanoparticles (Se-NPs) have gained interest because of low toxicity, high biocompatibility, biodegradability, and unique bioactivity properties, as well as for potential use in nutritional supplements. Antimicrobial activi-

ty of selenium has been spotted in a wide range of bacteria and fungi^{6,7}. Selenium (Se) is a trace mineral that is requisite for human health. Recent research in selenoproteins has revealed a hitherto unsuspected role for this element. Selenium can be classified into organic and inorganic selenium. The organic form is naturally present in bacteria, humans, and animals as selenocysteine (Sec) and selenomethionine while the inorganic form is found in plants as selenite and selenate. Selenoproteins such as glutathione peroxidases (GPX) and thioredoxin reductases (TrxR) comprise a single secondary amino acid residue at their active site. Other selenoproteins such as selenoprotein-P (regulates inflammatory cytokines) and selenoprotein-S (inducer of homeostasis) contain about 10 secondary residues. It has been shown to function in antioxidant, anti-inflammatory, thyroid regulation, wound healing, and male-fertility enhancement actions⁸.

For the past four decades, selenium has been deliberated to exert an anticancer potential (directly or indirectly) through antioxidant activity that alters cellular redox ho-

*Correspondence to: Heng Zhang, Department of Gastroenterology, Central Hospital of Wuhan, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, CHINA

E-mail: hengzhang0@outlook.com

Accepted December 20, 2023 (received for review September 27, 2023)

Journal of Oleo Science ISSN 1345-8957 print / ISSN 1347-3352 online

<https://www.jstage.jst.go.jp/browse/jos/> <https://mc.manuscriptcentral.com/jjocs>



meostasis. Nonetheless, a plethora of research dedicated to understand the antitumor property of organic and inorganic selenium unveiled the difference in the mechanism. Inorganic selenium has been shown to exhibit high genotoxic stress. On the other hand, organic selenium has been shown to exhibit significant antitumor property with the ability to avoid the dissemination of metastatic cancer. Diverse nucleophiles present in the organoselenium compounds are demonstrated to improve the antitumor property by enhancing the efficacy, specificity, and selectivity against cancer cells^{9, 10}. Apart from that, Se-NPs have been documented for antitumor property against breast, prostate, colon, liver, ovarian, esophageal, and colorectal cancer cells^{10–12}.

Acute pancreatitis (AP) is a gastrointestinal disease distinguished by inflammation in the pancreas. Symptoms include severe upper abdominal pain. The disease usually resolves within one week. However, mortality rate ranges from 20 to 40% in moderate and severe AP patients. The common causes of AP are gallstone, alcohol abuse, endoscopic retrograde cholangiopancreatography (ERCP), biliary disease, and hypertriglyceridaemia (HTG). Increase in serum amylase/lipase, abdominal pain and imaging are the common diagnostic methods. Treatment options include antibiotic regimen, radiological, surgical, and endoscopic interventions. In the last two decades, the incidence of AP has increased more than 20%^{13, 14}. To enhance the treatment options of AP through nanotechnology, the present study was attempted to synthesize Se-NPs using plant extract and subsequently to evaluate the effect of Se-NPs in rat developed with pancreatitis as well as the functions of the pancreas.

The present study reports the synthesis of Se-NPs from *C. forskohlii* leaf extract. The generated Se-NPs were characterized through UV-visible, XRD, FTIR, other spectroscopic and microscopic techniques. Further, this study intended to examine the effect of synthesized Se-NPs on pancreatic function and AP in rat.

2 Materials and Methods

2.1 Reagents

The analytical grade reagents chemicals, assay kits, and Antibodies were purchased from Sigma-Aldrich, Shanghai.

2.2 Plant collection and authentication

The plant material was collected, identified, and authenticated by the expert.

2.3 Plant extract preparation

Leaves from *C. forskohlii* were collected, washed and dried. After shade drying, the leaves were finely crushed into powder using mortar and pestle. Around 20 g of leaf

powder was weighed and dissolved in 200 mL sterile deionized water and mixed through magnetic stirrer for 20 h at 37°C. The mixture was filtered using Whatman filter paper (grade 1) and the filtrate containing aqueous extract was collected and kept at 4°C while the residual biomass was resuspended in fresh deionized water. The extraction process was repeated until the deionized water became colorless.

2.4 Synthesis and characterization of Se-NPs

The aqueous extract (100 mL) was mixed with 50 mM selenous acid (H₂SeO₃, 40 mL) and stirred for 20 h at room temperature. The mixture was set apart and observed for color change. After intense color change the synthesized nanoparticles were characterized through spectroscopic and microscopic techniques. The reduction in selenium ions was measured through a UV-visible spectrophotometer (SHIMADZU-1800). The phase formation, crystalline size and purity of Se-NPs was recorded through D8 ADVANCE X-ray diffractometer (XRD, Bruker). The Fourier transform infrared spectroscopy (FTIR, PerkinElmer Spectrum 100) technique was utilized to analyze functional groups. Zeta potential and size of Se-NPs was examined through Zetasizer (Nano S90). Morphological view of elemental selenium nanoparticles was detected through scanning electron microscope (SEM, VEGA 3).

2.5 In vitro cytotoxicity

2.5.1 Cell lines

Panc-1 and H6-C7 cell lines were obtained. Panc-1 cell line was grown in DMEM harboring FBS and antibiotic while H6-C7 cell line was maintained in keratinocyte medium (serum-free). H6-C7 cells were additionally added with epidermal growth factor and bovine pituitary extract. Both the cell types were grown at 37°C/5% CO₂.

2.5.2 In vitro cytotoxicity assessment of Se-NPs

Cytotoxicity of Se-NPs was examined in both cancer (Panc-1) and normal (H6-C7) pancreatic cell lines. In brief, 1 × 10⁴ cells were seeded in well plate and incubated at stated conditions for 24 h. Later the medium in the well plate was replenished with respective medium containing different concentrations of Se-NPs (10 to 80 µg/mL) at each well and grown for 48 h. Around 20 µL of MTT dye was added and kept apart for 4 h. The resulting insoluble formazan was collected, suspended in DMSO and the absorbance was gauged.

2.6 Acute pancreatitis studies

2.6.1 Animals

The experiments in rats were performed after obtaining consent from Ethical committee (Central Hospital of Wuhan, Tongji Medical College, Huazhong University of Science and Technology). Animal maintenance was followed according to the recommendations outlined and ex-

periments were conducted in accordance with good laboratory practice. Eight to nine weeks old male albino rats weighing 180 ± 20 g was obtained. The rats were accustomed to standard laboratory conditions (12 h light/dark cycle; temperature: 25 ± 2 ; humidity: 50 ± 2) and fed with necessary food and water.

2.6.2 Animal experiments

Rats were separated into six groups. Group1 is vehicle control in which rats receive distilled water. Group2 and Group3 rats received only 50 and 100 mg/kg Se-NPs, respectively. Doses of Se-NPs were administered orally. Group4 is AP in which the animals received L-arginine, prepared in distilled water. L-Arginine (2.5 g/kg) is used to induce AP in rat¹⁵ and it was injected twice intraperitoneally (with 1 h interval). Group5 and Group6 rats were treated with 50 and 100 mg/kg Se-NPs. The animals were orally administered with Se-NPs for 10 successive days, 1 h before L-Arginine injection. The rats were observed routinely during the experiment. Average food intake and weight were monitored weekly.

2.6.3 Serum collection and biochemical analysis

On the last day, animals were anaesthetized and decapitated. Blood samples were collected from each experimental group. Serum was gathered from blood samples and evaluated for amylase, lipase, and glucose content through colorimetric assays whereas insulin and interleukin-1 β (IL-1 β) content was estimated through kits. Evaluation of homeostasis model analysis (HOMA) of β -cell was carried out as described¹⁶.

2.7 Statistics

Experiments were performed in triplicates. Error bars are denoted as mean $\pm$ SD. One-way ANOVA was employed to analyze the data. $P < 0.05$ is considered statistically significant.

3 Results and Discussion

3.1 Se-NPs fabrication and characterization

In this work, Se-NPs were successfully fabricated using the *C. forskohlii* leaf extract as reducing and stabilizing agent. The change of color of reaction solution to red after 20 h incubation period suggested the synthesis of nanoparticles. The obtained result was substantiated through absorption spectrum of UV-visible spectrophotometer in which the surface plasmon resonance peak (SPR) was observed at 262 nm (Fig. 1). Few studies have described the Se-NPs synthesis by utilizing other reducing agents through natural methods. For instance, Se-NPs synthesis with the leaf extract of *Withania somnifera* displayed the SPR peak at 320 nm¹⁷. Another study relating to the Se-NPs green synthesis with the garlic clove extract from¹⁸ revealed the SPR peak at 260 nm. Likewise, Se-NPs gener-

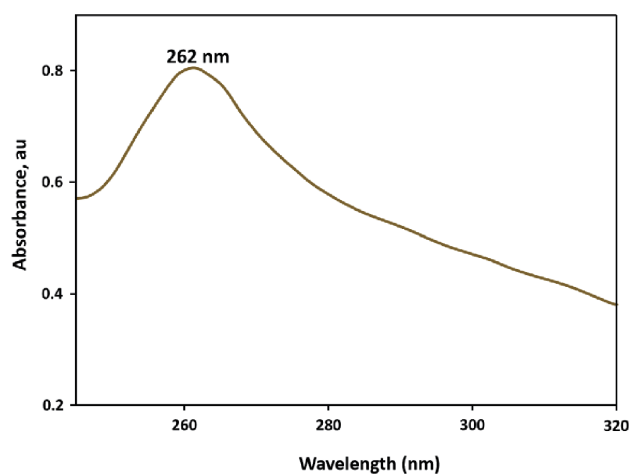


Fig. 1 UV-visible spectrum of Se-NPs.

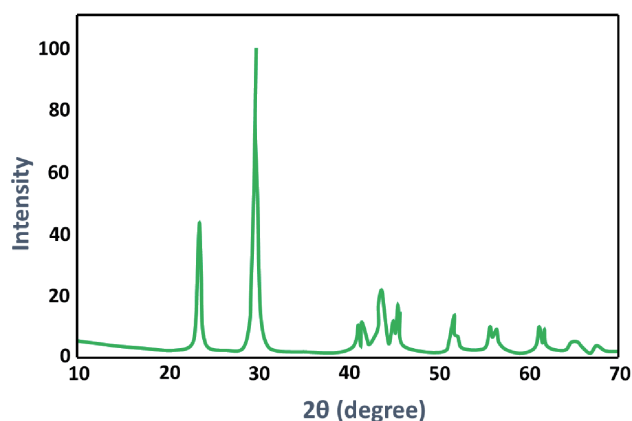


Fig. 2 XRD spectrum of Se-NPs.

ated from *Ceropegia bulbosa* tuber aqueous extract showed SPR at 277.5 nm¹⁹.

The crystallinity of fabricated Se-NPs was examined through XRD. The spectrum resulting from XRD is provided in Fig. 2. The diffractions peaks (2θ) were observed correspond to the standard values of JCPDS (card number 06-0362). Nonetheless, new peaks emerged at other regions could be due to the occurrence of bioactive constituents from *C. forskohlii* leaf extract^{20–22}. Altogether, the result suggests the crystallinity of fabricated Se-NPs and moreover it was in congruent with reports from previous studies describing the Se-NPs synthesis^{19, 20}.

Further, to know the functional groups that could be attributed to the reduction and stabilization process during the synthesis of nanoparticles, FTIR spectrum was recorded for both leaf extract and Se-NPs synthesized from the leaf extract. FTIR spectra revealed the bond stretching at various positions between 4000 and 500 cm^{-1} (Fig. 3), indicating the occurrence of abundant functional groups in bioactive constituents, which can be accountable for reduction and stabilization of the generated nanomaterials. Diverse bioactive molecules from phytoconstituents have

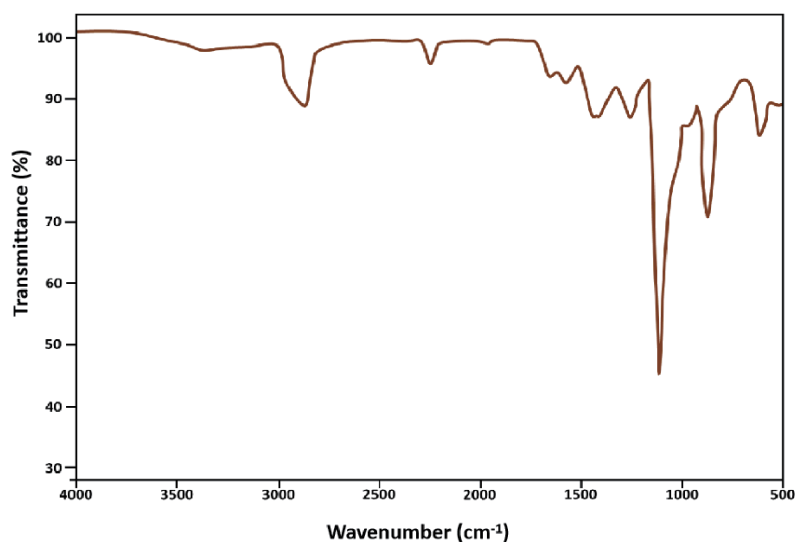


Fig. 3 FTIR spectrum of Se-NPs.

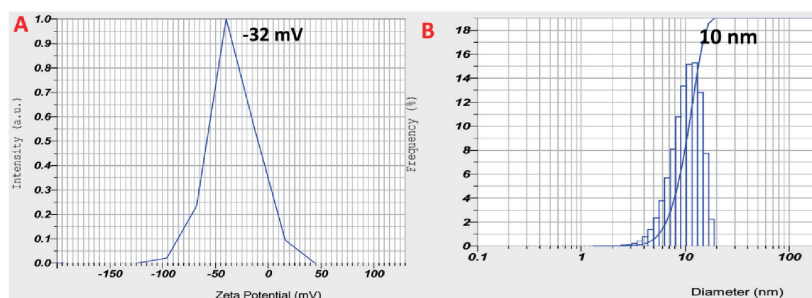


Fig. 4 Zeta potential (A) and DLS analysis (B) for prepared Se-NPs.

been reported to serve as capping and stabilizing vehicles during the nanomaterial synthesis²³. Here, the absorption peak at 2867 cm^{-1} and 1600 cm^{-1} relates to C-H (alkynes), and C=O (aldehydes, alkanes) stretch, respectively. Similarly, the spectra around 1500 and 1418 cm^{-1} relate to the N-O (nitro compounds) and C-C (aromatics), stretch respectively. Formation of peak at 1348 cm^{-1} attribute to C-H (alkanes) stretching. 1128 cm^{-1} peak relate to C-N (amine) stretch; 2851 and 3368 cm^{-1} relates to C-H and N-H stretch, respectively.

Particle size and surface charge are indispensable characters of nanoparticles. In fact, zeta potential is an effective quantification of surface electric charge and it provide information regarding stability of nanomaterial. Thus, DLS and zeta potential quantification was performed using Zetasizer. The zeta potential of Se-NPs synthesized from *C. forskohlii*. leaf extract was found to be -32.8 mV , a magnitude lower than the previous reports. It indicates the highly negatively charged surface of fabricated Se-NPs. Here, higher zeta potential magnitude might be due to capping of negatively charged bioactive molecules, present in the leaf extract, over the surface of Se-NPs. In general, nanoparticles with higher zeta potential values displayed

greater stability by allowing the molecule to exist in dispersion form. As a result of increased electrostatic repulsion, it prevents the nanoparticles from forming aggregation. DLS studies unveiled the size of fabricated Se-NPs particles in the nano range (10-15 nm) with a polydispersity index (PDI) of 0.18 (Fig. 4). PDI describes the distribution of nanoparticles in the solution based on the size. Several studies were reporting zeta potential magnitudes in the range of $+25\text{ mV}$ to -25 mV with different particle size and PDI (less than 0.7). A study on biogenic synthesis of Se-NPs from the *Emblica officinalis* fruit extract resulted in the zeta potential value of -32 mV with size ranges of 20 to 60 nm and PDI less than 0.2²⁴. Likewise, a study from²⁵ described the zeta potential of Se-NPs synthesized from *Ephedra aphylla* extract as -5.61 mV . Synthesis of Se-NPs from hawthorn fruit extract displayed -24.5 mV zeta potential with a size of 113 nm ²⁶. Remarkably, Menon *et al.* have shown a higher zeta potential of -36 mV with a size of 100 to 120 nm during the synthesis of Se-NPs from ginger extract²⁷. Strikingly, biogenic synthesis of Se-NP from citrus fruit exhibited lower PDI value (0.127), indicating the monodispersed sized particles²⁸.

The SEM images of Se-NPs synthesized from *C. for-*

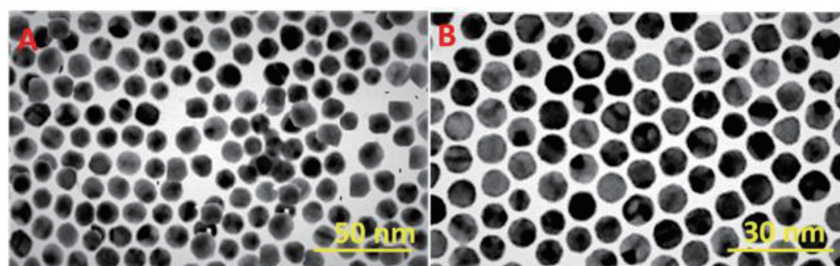


Fig. 5 Morphological view of Se-NPs on SEM.

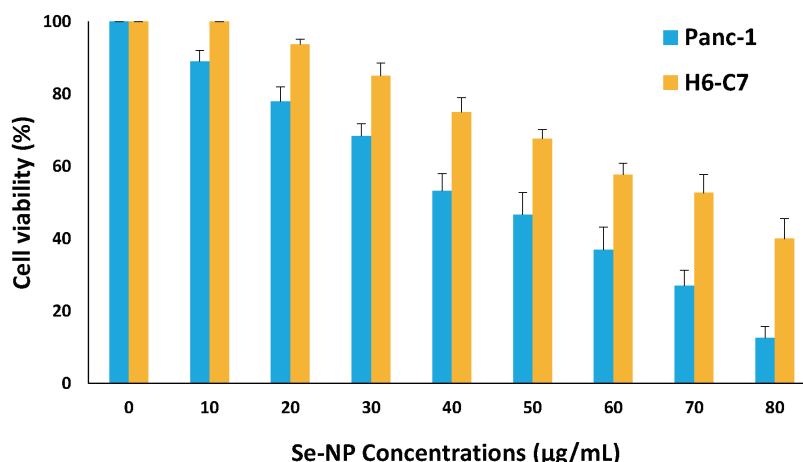


Fig. 6 Effect of Se-NPs on cell proliferation of normal and cancer pancreatic cell lines. Error bars represent mean $\pm$ SD.

skohlaii leaf extract displayed a size from 5 nm to 12 nm with an average size of 10 nm (Fig. 5). The nanoparticles displayed a spherical shape. Se-NPs generated from the *Clausena dentata* leaf extract exhibited spherical shape with 46 nm to 79 nm size²⁹. Whereas, Vennila *et al.*, have shown rod-shaped Se-NPs with a size of 120 ± 15 nm, synthesized using *Spermacoce hispida* plant extract³⁰.

3.2 *In vitro* cytotoxicity analysis

Figure 6 represents the *in vitro* toxicity of Se-NPs synthesized from *C. forskohlii* leaf extract. Concentration-dependent antiproliferative activity was noticed. The cytotoxicity of Se-NPs evaluated on both cancer and normal pancreatic cells unveiled the toxic effect at higher concentrations. However, normal cells exhibited lower cytotoxicity when compared to the cancer cells at respective concentrations. The IC_{50} of Se-NPs against pancreatic cancer cell was 40 µg/mL while IC_{50} of Se-NPs against normal pancreatic cell lines was around 70 µg/mL. Similar results were observed in toxicity analysis of Se-NPs against normal and breast cancer cell lines in which cytotoxic effect was higher in cancer cell when compared to normal cells¹⁹.

3.3 Effect of AP with and without Se-NPs

The effect of AP in the presence (50 and 100 mg/kg) and absence of Se-NPs on rats were studied and provided in Table 1. The intensities of lipase, amylase and IL-1 β were

pointedly higher in all rat groups in comparison to group 1 control rats. However, rats provided with Se-NP (group 2 and 3) and treated with Se-NPs (group 5 and 6) showed reduced lipase, amylase and IL-1 β level when compared to group 4 rats which received only L-Arginine. The increased lipase, amylase and IL-1 β level indicates the characteristic evidence of AP in group 4 rats^{31, 32}. Strikingly, oral administration of 100 mg/kg expressively reduced the serum lipase, amylase as well as the IL-1 β quantities, but considerably higher than group 1 control, when compared to rats administered with 50 mg/kg Se-NPs.

Similarly, the effect of AP on glucose, insulin and HOMA- β in the presence (50 and 100 mg/kg) and absence of Se-NPs were studied. The rats from group 4 showed decreased glucose, insulin and HOMA- β levels, indicating the progression of AP in rats³³. On the other hand, rats treated with Se-NPs showed a marked increase in the glucose, insulin and HOMA- β levels. Among the two concentrations examined, 100 mg/kg showed a prominent increase in glucose, insulin and HOMA- β in comparison to 50 mg/kg. However, the increase in Se-NPs subjected rat groups were considerably lesser than control group.

An evaluation of MDA, NO, and Bcl-2 in AP rats showed abrupt increase in MDA, NO, and Bcl-2 levels in comparison to control group^{34–36}. Strikingly, rats treated with Se-NPs exhibited decreased MDA, NO, and Bcl-2 levels. Interestingly, rats treated with 100 mg/kg Se-NPs displayed

Table 1 Evaluation of biochemical parameters between rats treated with and without Se-NPs.

Parameters	Control	Se-NPs (mg/mL)		AP	AP + Se-NPs (mg/mL)	
		50	100		50	100
Lipase (U/L)	542.12 ± 21.03	544.21 ± 13.64	548.78 ± 24.32	1532.94 ± 64.51	856.32 ± 36.94	749.68 ± 26.97
Amylase (U/L)	22.31 ± 0.84	24.16 ± 1.54	25.02 ± 1.27	58.94 ± 2.21	46.25 ± 0.62	33.04 ± 1.87
Glucose (mg/dL)	92.05 ± 3.13	94.27 ± 2.09	96.05 ± 3.13	45.67 ± 2.59	98.26 ± 1.23	102.77 ± 0.91
Insulin (μIU/mL)	9.02 ± 0.28	9.04 ± 0.08	9.12 ± 0.11	5.96 ± 0.20	7.14 ± 0.19	7.23 ± 1.34
IL-1β (pg/mL)	14.59 ± 0.35	14.68 ± 0.65	14.98 ± 0.58	28.36 ± 1.09	18.64 ± 0.47	17.02 ± 0.13
HOMA-β	129.31 ± 2.31	133.86 ± 3.41	141.37 ± 3.19	25.98 ± 2.86	76.31 ± 2.45	89.92 ± 2.97
MDA (pg/mg)	60.21 ± 1.34	59.68 ± 2.09	60.24 ± 1.47	175.32 ± 8.26	70.54 ± 2.44	64.52 ± 1.65
NO (μMol/mg)	1.79 ± 0.05	1.81 ± 0.21	1.72 ± 0.08	6.24 ± 0.72	3.09 ± 0.43	2.16 ± 0.12
Bcl-2 (ng/mg)	1.21 ± 0.03	1.24 ± 0.04	1.16 ± 0.02	2.54 ± 0.08	1.67 ± 0.09	1.27 ± 0.07
Antioxidant potential (μM/mg)	84.21 ± 4.20	91.64 ± 2.54	94.46 ± 3.84	31.69 ± 1.58	82.31 ± 2.46	85.64 ± 3.47

dropped MDA, NO, and Bcl-2 which is almost equal to control levels. Examination on antioxidant potential in AP groups revealed the decrease in antioxidant activity due to oxidative stress than control rats. However, the levels have been significantly enhanced in the presence of Se-NPs. Although not equal to the control group, the significant increase in antioxidant potential is evidenced in comparison with AP rats.

No significant difference was observed in control (group1) and Se-NPs fed (group 2 and 3) rats except in the antioxidant potential. The rats fed with Se-NPs showed moderate increase in the antioxidant potential when compared to normal control rats³⁷. Altogether, these results suggest that Se-NPs at tested concentration were safe and non-toxic as well as have significant activity against AP.

4 Conclusions

Altogether, the study reports the synthesis of Se-NPs from *C. forskohlii* leaf extract. The generated Se-NPs were depicted through UV-visible, XRD and FTIR spectroscopies. Interestingly, the zeta potential of fabricated Se-NPs was found to be -32.8 mV, indicating the highly negatively charged surface of the nanoparticles. Moreover, the lower PDI value indicated the monodispersed sized particles. The investigation on effect of Se-NPs in pancreatic function and AP in rat unveiled the normal function of pancreas and significant recovery from AP, respectively. The synthesized Se-NPs decreased the lipase, amylase, IL-1β, MDA, NO, and Bcl-2 while increased the glucose, insulin, HOMA-β. In addition, it enhanced the antioxidant potential in AP rats. Evaluation of Se-NPs on pancreatic function did not show any deleterious effect on rats. Thus, Se-NPs may find a way to become potential remedy for AP in future.

Author's Contribution

Juan Shu: Experimental work, Literature survey, Manuscript drafting, Data analysis. Yusheng Liao: Manuscript drafting, Data analysis. Jian Wang: Literature survey, manuscript drafting. Yuanjie Zhang: Experimental work, Literature survey. Weilai Zhou: Manuscript drafting, Data analysis. Heng Zhang: Experimental design, Manuscript drafting, Data analysis, Supervision.

References

- 1) Zambonino, M.C.; Quizhpe, E.M.; Jaramillo, F.E.; Rahman, A.; Santiago Vispo, N. *et al.* Green synthesis of selenium and tellurium nanoparticles: Current trends, biological properties and biomedical applications. *Int. J. Mol. Sci.* **22**, 989 (2021).
- 2) Zhang, D.; Ma, X.L.; Gu, Y.; Huang, H.; Zhang, G.W. Green synthesis of metallic nanoparticles and their potential applications to treat cancer. *Front. Chem.* **8**, 799 (2020).
- 3) Ijaz, I.; Gilani, E.; Nazir, A.; Bukhari, A. Detail review on chemical, physical and green synthesis, classification, characterizations, and applications of nanoparticles. *Green Chem. Lett. Rev.* **13**, 223-245 (2020).
- 4) Sohal, I.S.; O'Fallon, K.S.; Gaines, P.; Demokritou, P.; Bello, D. Ingested engineered nanomaterials: state of science in nanotoxicity testing and future research needs. *Part. Fibre Toxicol.* **15**, 29 (2018).
- 5) Zuverza-Mena, N.; Martínez-Fernández, D.; Du, W.; Hernandez-Viezcas, J.A.; Bonilla-Bird, N. *et al.* Exposure of engineered nanomaterials to plants: Insights into the physiological and biochemical responses-A review. *Plant Physiol. Biochem.* **110**, 236-264 (2017).
- 6) Filipović, N.; Ušjak, D.; Milenković, M.T.; Zheng, K.; Liverani, L. *et al.* Comparative study of the antimicrobial activity of selenium nanoparticles with different

- surface chemistry and structure. *Front. Bioeng. Biotechnol.* **8**, 624621 (2021).
- 7) Hashem, A.H.; Khalil, A.; Reyad, A.M; Salem, S.S. Biomedical applications of mycosynthesized selenium nanoparticles using *Penicillium expansum* ATTC 36200. *Biol. Trace Elem. Res.* **199**, 3998-4008 (2021).
- 8) Hariharan, S; Dharmaraj, S. Selenium and selenoproteins: Its role in regulation of inflammation. *Inflammopharmacology* **28**, 667-695 (2020).
- 9) Gandin, V.; Khalkar, P.; Braude, J; Fernandes, A.P. Organic selenium compounds as potential chemotherapeutic agents for improved cancer treatment. *Free Radic. Biol. Med.* **127**, 80-97 (2018).
- 10) Tan, H.W.; Mo, H.Y.; Lau, A; Xu, Y.M. Selenium species: Current status and potentials in cancer prevention and therapy. *Int. J. Mol. Sci.* **20**, 75 (2018).
- 11) Shi, X.D.; Tian, Y.Q.; Wu, J.L; Wang, S.Y. Synthesis, characterization, and biological activity of selenium nanoparticles conjugated with polysaccharides. *Crit. Rev. Food Sci. Nutr.* **61**, 2225-2236 (2020).
- 12) Hu, Y.; Liu, T.; Li, J.; Mai, F.; Li, J. *et al.* Selenium nanoparticles as new strategy to potentiate $\gamma\delta$ T cell anti-tumor cytotoxicity through upregulation of tubulin- α acetylation. *Biomaterials* **222**, 119397 (2019).
- 13) Bálint, E.R.; Fűr, G.; Kiss, L.; Németh, D.I.; Soós, A. *et al.* Assessment of the course of acute pancreatitis in the light of aetiology: A systematic review and meta-analysis. *Sci. Rep.* **10**, 1-17 (2020).
- 14) Boxhoorn, L.; Voermans, R.P.; Bouwense, S.A.; Bruno, M.J.; Verdonk, R.C. *et al.* Acute pancreatitis. *Lancet* **396**, 726-734 (2020).
- 15) Dawra, R; Saluja, A.K. *L-Arginine-induced experimental acute pancreatitis*, Pancreapedia: The Exocrine Pancreas Knowledge Base (2012).
- 16) Matthews, D.R.; Hosker, J.P.; Rudenski, A.S.; Naylor, B.A.; Treacher, D.F; Turner R.C. Homeostasis model assessment: insulin resistance and β -cell function from fasting plasma glucose and insulin concentrations in man. *Diabetologia* **28**, 412-419 (1985).
- 17) Alagesan, V; Venugopal, S. Green synthesis of selenium nanoparticle using leaves extract of *Withania somnifera* and its biological applications and photocatalytic activities. *Bionanoscience* **9**, 105-116 (2019).
- 18) Anu, K.; Singaravelu, G.; Murugan, K; Benelli, G. Green-synthesis of selenium nanoparticles using garlic cloves (*Allium sativum*): Biophysical characterization and cytotoxicity on vero cells. *J. Clust. Sci.* **28**, 551-563 (2017).
- 19) Cittrarasu, V.; Kaliannan, D.; Dharman, K.; Maluven-then, V.; Easwaran, M. *et al.* Green synthesis of selenium nanoparticles mediated from *Ceropegia bulbosa* Roxb extract and its cytotoxicity, antimicrobial, mosquitoicidal and photocatalytic activities. *Sci. Rep.* **11**, 1-15 (2021).
- 20) Alam, H.; Khatoon, N.; Raza, M.; Ghosh, P.C; Sardar, M. Synthesis and characterization of nano selenium using plant biomolecules and their potential applications. *BioNanoSci.* **9**, 96-104 (2019).
- 21) Khatoon, N.; Ahmad, R; Sardar, M. Robust and fluorescent silver nanoparticles using *Artemisia annua*: Biosynthesis, characterization and antibacterial activity. *Biochem. Eng. J.* **102**, 91-97 (2015).
- 22) Tripathy, A.; Raichur, A.M.; Chandrasekaran, N.; Prathna, T.C; Mukherjee, A. Process variables in biomimetic synthesis of silver nanoparticles by aqueous extract of *Azadirachta indica* (Neem) leaves. *J. Nanopart. Res.* **12**, 237-246 (2010).
- 23) Gupta, A.; Pandey, S; Yadav, J.S. A review on recent trends in green synthesis of gold nanoparticles for tuberculosis. *Adv. Pharm. Bull.* **11**, 10 (2021).
- 24) Gunti, L.; Dass, R.S; Kalagatur, N.K. Phyt fabrication of selenium nanoparticles from *Embllica officinalis* fruit extract and exploring its biopotential applications: antioxidant, antimicrobial, and biocompatibility. *Front. Microbiol.* **10**, 931 (2019).
- 25) El-Zayat, M.M.; Eraqi, M.M.; Alrefai, H.; El-Khateeb, A.Y.; Ibrahim, M.A. *et al.* The antimicrobial, antioxidant, and anticancer activity of green synthesized selenium and zinc composite nanoparticles using *Ephedra aphylla* extract. *Biomolecules* **11**, 470 (2021).
- 26) Cui, D.; Liang, T.; Sun, L.; Meng, L.; Yang, C. *et al.* Green synthesis of selenium nanoparticles with extract of hawthorn fruit induced HepG2 cells apoptosis. *Pharm. Biol.* **56**, 528-534 (2018).
- 27) Menon, S.; KS, S.D.; Agarwal, H; Shanmugam, V.K. Efficacy of biogenic selenium nanoparticles from an extract of ginger towards evaluation on anti-microbial and anti-oxidant activities. *Colloid Interface Sci. Commun.* **29**, 1-8 (2019).
- 28) Alvi, G.B.; Iqbal, M.S.; Ghaith, M.M.S.; Haseeb, A.; Ahmed, B; Qadir, M.I. Biogenic selenium nanoparticles (SeNPs) from citrus fruit have anti-bacterial activities. *Sci. Rep.* **11**, 1-11 (2021).
- 29) Sowndarya, P.; Ramkumar, G; Shivakumar, M.S. Green synthesis of selenium nanoparticles conjugated *Clausena dentata* plant leaf extract and their insecticidal potential against mosquito vectors. *Artif. Cells Nanomed. Biotechnol.* **45**, 1490-1495 (2017).
- 30) Vennila, K.; Chitra, L.; Balagurunathan, R; Palvannan, T. Comparison of biological activities of selenium and silver nanoparticles attached with bioactive phytoconstituents: Green synthesized using *Spermaceoce hispida* extract. *Adv. Nat. Sci.: Nanosci. Nanotechnol.* **9**, 015005 (2018).
- 31) Zhang, Z.; Wang, Y.; Dong, M.; Cui, J.; Rong, D; Dong, Q. Oxymatrine ameliorates L-arginine-induced acute

- pancreatitis in rats. *Inflammation* **35**, 605-613 (2012).
- 32) Atshan, D.A.; Al-Shawi, N.N.; Fadhil, A.A; Sahib, H.A. The effects of vitamin D on L-arginine-induced acute pancreatitis in rats. *Int. J. Pharm. Sci. Rev. Res.* **46**, 208-213 (2017).
- 33) Jia, D.; Yamamoto, M; Otsuki, M. Effect of endogenous cholecystokinin on the course of acute pancreatitis in rats. *World J. Gastroenterol.* **21**, 7742-7753 (2015).
- 34) Czakó, L.; Takács, T; Varga, I.S.; Hai, D.Q.; Tiszlavicz, L. *et al.* The pathogenesis of L-arginine-induced acute necrotizing pancreatitis: Inflammatory mediators and endogenous cholecystokinin. *J. Physiol.* **94**, 43-50 (2000).
- 35) Takács, T.; Czakó, L.; Morschl, E., László, F.; Tiszlavicz, L. *et al.* The role of nitric oxide in edema formation in L-arginine-induced acute pancreatitis. *Pancreas* **25**, 277-282 (2002).
- 36) Abdel-Hakeem, E.A.; Abdel-Hamid, H.A; Hafez, S.M.N.A. The possible protective effect of nano-selenium on the endocrine and exocrine pancreatic functions in a rat model of acute pancreatitis. *J. Trace Elem. Med. Biol.* **60**, 126480 (2020).
- 37) Long, J.; Song, N.; Liu, X.P.; Guo, K.J; Guo, R.X. Nuclear factor-kappaB activation on the reactive oxygen species in acute necrotizing pancreatitis rats. *World J. Gastroenterol.* **11**, 4277 (2005).

CC BY 4.0 (Attribution 4.0 International). This license allows users to share and adapt an article, even commercially, as long as appropriate credit is given. That is, this license lets others copy, distribute, remix, and build upon the Article, even commercially, provided the original source and Authors are credited.

