Pakistan J. Zool., vol. 55(5), pp 2317-2323, 2023 DOI: https://dx.doi.org/10.17582/journal.pjz/20210901220942

Assessment of the Biogenic Effects of Selenium Nanoparticles Prepared from *Citrus paradise* Peel Extract against Bisphenol A-Induced Liver Injury in Male Rats

A.M. Abdul Azeem[1], A.N. El shahat[1]* and Mohamed H.M. Abd el Megid[2]

[1]Food Irradiation Research Department, Egyptian Atomic Energy Authority, 3 Ahmed El-Zomor St., El-Zohour District, 8th District, Nasr City, Cairo
[2]Natural Products Department, National Centre for Radiation Research and Technology, Atomic Energy Authority, Cairo, Egypt.

Article Information
Received 01 September 2021
Revised 13 May 2022
Accepted 06 June 2022
Available online 04 August 2022 (early access)
Published 01 September 2023

Authors' Contribution
AMAA performed animal experiments. ANES and MHMAM performed the biological study and collected blood samples. AMAA, ANES and MHMAM wrote the manuscript

Key words
Selenium nanoparticles, Grapefruit peel, Naringin, Bisphenol A, Naringenin

ABSTRACT

Citrus peel constitutes an important series of flavonoids such as naringin and its aglycone naringenin that play significant roles against various physiological threats. Synthesis of nano-selenium particles (SeNPs) using a natural source as a reducing agents might have more advantages as an alternative to physical and chemical methods because it is inexpensive and considered environmentally friendly. Therefore, the objective of this study was to synthesize selenium nanoparticles from *Citrus paradise* peels extract (SeNPs-*CP*), their general characterization, and their assessment for antioxidant activities against liver injury induced by bisphenol A (BPA) in male rats. In this work, *Citrus paradise* peels extract used for reduction of sodium selenite (Na_2SeO_3) to reduce Se ions to Se^0 confirmed by changing the colour of the solution from light yellow to red. The synthesized SeNPs were characterized by Ultraviolet-visible spectroscopy (UV-Vis spectra), Fourier transform infra-red (FTIR) spectroscopy analysis and Transmission Electron Microscope (TEM). The rats (28 rats) used in this study randomly divided into 4 groups each of 7 animals; Control group, SeNPs-*CP* group (0.5 mg/kg B.Wt. / day /30 days orally), BPA group (50 mg/kg B.Wt. / day /30 days orally) and BPA- SeNPs-*CP* group. The results showed that BPA injection led to an obvious increase in the activities of liver enzymes, elevation of hepatic oxidative stress (a significant increase in lipid peroxidation concomitant with a significant decrease in glutathione content and antioxidant enzyme activities), tumor necrotic factor-α (TNF-α) and apoptotic marker Caspase-3 compared to the control group. Rats received BPA along with SeNPs-*CP* showed significantly less severe damage and remarkable improvement in all the measured parameters when compared to BPA-rats. In this study it was concluded that the synthesis of selenium nanoparticles using a natural source such as grapefruit was effective in combining the antioxidant and anti-inflammatory properties of both the flavonoids present in grapefruit and selenium and increasing the efficiency of the selenium nanoparticles.

INTRODUCTION

Synthesis of nanomaterials by using plant materials is known as green synthesis of nanoparticles that has more advantages as alternative to the physical and chemical methods, because it is inexpensive and does not need any special conditions and considered as eco-friendly synthesis (Alvi *et al*., 2021). Furthermore, the green synthesis is an environmentally friendly technique that uses the extracts of plant parts such as peels, leaves, flowers, roots, stem and seed (Rao *et al*., 2015). Selenium nanoparticles can be used in the medicinal and biological fields because it highly effective at low doses. Also, these nanoparticles exhibit antimicrobial, anticancer and antioxidant activity (Alvi *et al*., 2021). Number of different plant extracts, microorganisms and enzymes has been utilized as a source for production of selenium nanoparticles of variable size and morphology (Alvi *et al*., 2021).

Grapefruit (*Citrus paradisi*) is belonging to family *Rutaceae* and contains different type of secondary metabolites that have physiological effects such as flavonoids including naringenin, tangeritin, auranetin, quercetin, nobiletin, limonene, myrcene, β- sitosterol and adrenergic amines including synephrine, octopamine and tyramine.

* Corresponding author: amrshahat22@yahoo.com
0030-9923/2023/0005-2317 $ 9.00/0

These active constituents have antioxidant, anti-diabetic and anti-inflammatory activities. Also, grapefruit been used for cancer prevention and heart health maintenance (Seleim *et al*., 2019). Moreover, citrus peel extract represents a good source of ascorbic acid that can reduce sodium selenite to selenium nanoparticles (Sasidharan *et al*., 2015).

Bisphenol A (2, 2-bis (4- hydroxyphenyl) propane; BPA), an estrogenic endocrine disrupting chemical, is employed in several plastic consumer products, like toys, water tubes, drinking containers, eyeglass lenses, sport equipment, dental monomers, and medical (Mahdavinia *et al*., 2019). BPA induced oxidative stress within the liver of by inhibition of antioxidant enzymes and increasing peroxide and lipid peroxidation (Mahdavinia *et al*., 2019). The objective of this study was to synthesize selenium nanoparticles from *Citrus paradise* (grapefruits) peels extract, their general characterization, and their assessment for antioxidant activities against BPA in male rats.

MATERIALS AND METHODS

All experiments were carried out during 2020 at the Egyptian Atomic Energy Authority, Food Irradiation Research Department.

Fresh grapefruits (*Citrus paradise)* were purchased from local market (Cairo, Egypt). Chemicals and reagents were purchased from Sigma Chemical Co. (St. Louis, MO, USA).

Preparation of citrus peel powder and its extract

Fruits samples were thoroughly washed with distilled water to get rid of impurities and dust particles followed by drying of fruits with sterilized paper towels. The peel powder was prepared firstly by peeling the grapefruits and then sun dried followed by grinding to a fine powder using grinder.

For preparation peel extract, about 70 g of the sterilized grapefruit dried peels (GDP) were added in 300 mL of the double distilled water and boiled for 10–20 min in a water bath. After boiling the peel extract was filtered through a series of Whatman filter papers with different pore sizes and the resultant filtrate was stored for the further synthesis of nanoparticles (Alvi *et al*., 2020).

Synthesis of selenium nanoparticles from dried peel extract of Citrus paradise *(SeNPs-CP)*

Dried peel extract of *Citrus paradise* (50 ml) was added in flask and taken on a magnetic stirrer. 5 ml of sodium selenite (Na_2SeO_3) is added a concentration of 0.1M in drops under constant stirring up to achieve pH of solution became 7 and until the formation of selenium nanoparticles is confirmed by the formation of red color. The mixture was subjected to constant stirring for 3 h continuously at room temperature. The presence of selenium nanoparticles was characterized by UV-spectroscopy at 200 - 800nm and Fourier transform infra-red (FTIR) spectroscopy analysis (Rao *et al*., 2015).

The morphological analysis of the size, shape and the SeNPs-*CP* state were monitored by usins (TEM) analysis (JEOL JEM-1230) at the national research center (Dokki, Giza, Egypt). A drop of aqueous selenium nanoparticle sample was loaded on carbon-coated copper grid, and it was allowed to dry completely for an hour at room temperature. The clear microscopic views were observed and documented in different ranges of magnifications.

Animal groups

Male albino rats Sprague Dawley (10 ± 2 weeks old; 120 ± 20 g) were purchased from the Egyptian Holding Company for Biological Products and Vaccines (Cairo, Egypt) and used for the different investigations carried out in the present study. Rats were acclimated to controlled laboratory conditions for two weeks. Rats were maintained on rodent diet and tap water ad libitum. All animals' procedures were carried out in accordance with the Ethics Committee of the National Research Centre conformed to the Guide for the Care and Use of Laboratory Animals, published by the US National Institutes of Health (NIH Publication No. 85-23, 1996).

Experimental design

Animals were divided into four groups of eight rats for each experimental procedure of cognition.

Group I (Control group): Animals received 1 ml of emulsion of olive oil orally for 30 days.

Group II (SeNPs-*CP* group): Rats were administrated orally by gavage SeNPs-*CP* (0.5 mg/kg body weight per day alone for 30 days orally (Sasidharan *et al*., 2015).

Group III (BPA group): Rats were administrated orally by gavage BPA (50 mg/kg) (Mahdavinia *et al*., 2019) in olive oil (1 ml) orally for 30 days.

Group IV (BPA- SeNPs-*CP* group): Animals treated with BPA (50 mg/kg) and SeNPs-*CP* (0.5 mg/kg body weight per day alone for 30 days orally (Sasidharan *et al*., 2015).

At the end of the experiment, rats were fasted for 24 hours and anaesthetized with diethyl ether. Blood samples were collected through heart puncture and allowed to coagulate and centrifuged for to obtain serum for biochemical analysis.

Biochemical analysis

Serum alanine transaminase (ALT) and aspartate transaminase (AST) activities were assayed according to

the methods of Bergmeyer *et al*. (1978) and Gella *et al*. (1985), respectively. Serum gamma-glutamyl transferase (GGT) activities were measured according to the methods of Szasz *et al*. (1974) and lactate dehydrogenase (LDH) activity was determined according to the method of Pesce (1984).

Liver was dissected, thoroughly washed with ice-cold 0.9% NaCl, weighed, minced and homogenized (10% w/v) using 66 mmol/L chilled phosphate buffer (pH 7.0). The tissue homogenates were centrifuged at 6000 rpm for 15 min and the supernatants were used to estimate TBARS (Yoshioka *et al*., 1979), GSH (Beutler *et al*., 1963), superoxide dismutase (SOD) activity (Minami and Yoshikawa, 1979) and Catalase (CAT) activity (Johansson and Borg, 1988).

The liver homogenate content of tumor necrotic factor--α (TNF-α, pg/ml) as inflammatory marker was estimated by solid-phase enzyme-linked immunosorbent assay (ELISA) based on the sandwich principle using test reagent kits (Biodiagnostics, Egypt) and liver homogenate content of caspase-3 as apoptotic marker was also estimated by the method described by Wolf and Green (1999).

Statistical analysis

Results were presented as mean ± SE (n = 6). Experimental data were analyzed using one-way analysis of variance (ANOVA). Duncan's multiple range test was used to determine significant differences between means. Data were statistically analyzed by the aid of Statistical Package of the Social Sciences, SPSS version 25 (copyrighted by IBM SPSS software, USA). Differences between means were considered significant at $P < 0.05$.

RESULTS

A visible color red color was observed after addition of sodium selenite to the dried peel extracts of *Citrus paradise* which confirmed the synthesis of SeNPs (Fig. 1). A peak was observed at 340 nm (Fig. 2) in a UV- Vis spectrophotometer from 200-800nm after observation confirmed the presence of selenium in the samples.

FTIR spectroscopy of the synthesized SeNPs in this study exhibited peaks between 1600–1700 cm^{-1} and 3200–3300 cm^{-1} (Fig. 3). The results revealed that SeNPs bio-synthesized by *Citrus paradise* appeared in the nanoscale size and the particles appeared as rounded sphere particles with diameter range (45-70 nm) (Fig. 4).

The results of this study revealed that administration of SeNPs-*CP* alone has a non-significant effect on the activity of liver enzymes, antioxidative and apoptotic status of the liver compared to the control group (Table I).

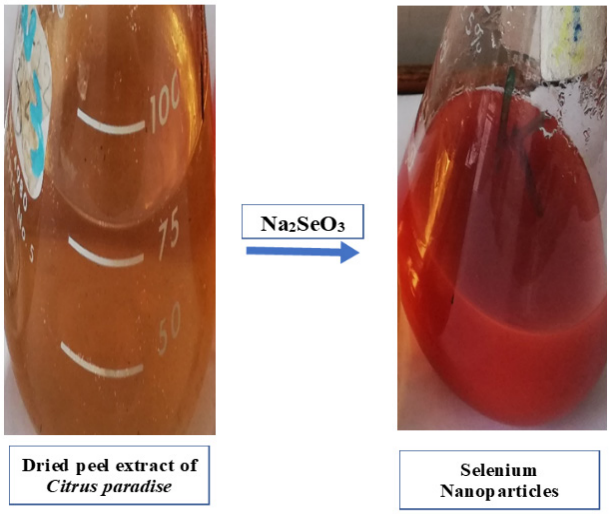

Fig. 1. Formation of red color during synthesis of SeNPs.

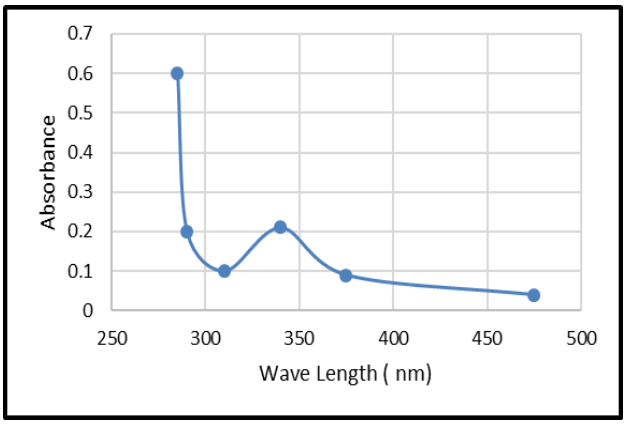

Fig. 2. Absorbance curve of SeNPs at a wavelength between 250 to 500nm.

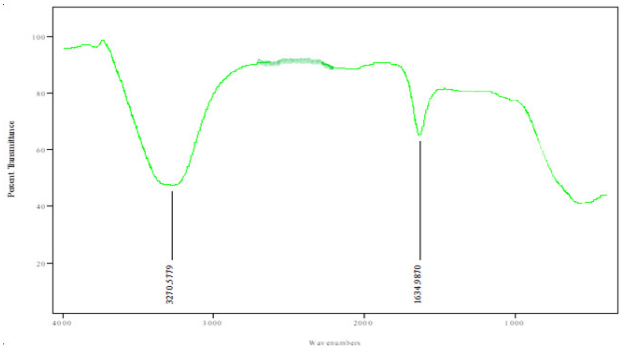

Fig. 3. FTIR band spectrum study of SeNPs synthesized from *Citrus paradise* dried peels extract.

Table I. Effect of BPA and SeNPs-*CP* on the liver function enzymes, hepatic oxidants and antioxidant status, hepatic

TNF-α and Caspase-3 in rats.

Parameters	Control	SeNPs-CP	BPA	BPA-SeNPs-CP
Liver function enzymes				
AST (U/ml)	20.81±1.87^{c}	19.58±1.74^{c}	79.92±1.49^{a}	41.23±1.27^{b}
ALT (U/ml)	26.32±1.12^{c}	25.42±1.17^{c}	88.39±1.95^{a}	36.56±1.62^{b}
γGT (U/ml)	2.82 ± 0.13^{c}	2.75 ± 0.12^{c}	5.62 ± 0.19^{a}	3.96 ± 0.11^{b}
LDH (U/ml)	423.96±38.2^{c}	415.96± 40.2^{c}	911.84± 64.5^{a}	603.55± 40.2^{b}
Hepatic antioxidants				
TBARS (n mol/g tissue)	71.47±11.83^{c}	252.45±10.73^{c}	435.25±15.64^{a}	320.28±12.44^{b}
GSH (mg/g tissue)	20.12±0.93^{a}	21.33±0.86^{a}	8.41±0.72^{c}	15.98±0.92^{b}
SOD (U/mg protein)	49.65 ±2.76^{a}	50.82±3.11^{a}	29.55±2.84^{c}	43.17 ±3.17^{b}
CAT (U/mg protein)	54.38±1.45^{a}	55.88±1.62^{a}	30.97±1.87^{c}	47.55±1.70^{b}
Inflammatory and apoptotic factors				
TNF-α (pg/gm tissue)	35.71 ± 3.23^{c}	34.62 ± 3.48^{c}	78.49 ± 4.77^{a}	50.71 ± 3.11^{b}
Caspase-3 (ng/mg protein)	5.11±0.25^{c}	5.02±0.21^{c}	13.25±1.60^{a}	8.16±0.48^{b}

Means in the same row with different superscripts are significantly different at (P<0.05), Values are expressed as mean ± S.E. (n=7). SeNPs-*CP,* Selenium nanoparticles synthesized from dried peel extract of *Citrus paradise* (SeNPs-*CP*); BPA, Bisphenol A.

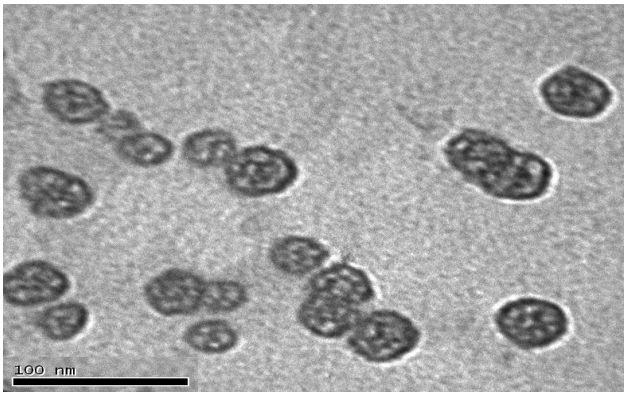

Fig. 4. Transmission Electron Microscopic (TEM) micrograph of SeNPs synthesized from *Citrus paradise* dried peels extract.

The serum level of ALT, AST, GGT and LDH was significantly increased in BPA-treated rats which is compared to the control group (P<0.05). In contrary, a significant decrease was observed in these parameters in rats administered SeNPs-*CP* together with BPA (P<0.05) compared to BPA-treated rats (Table I). Also, BPA cause significant increase in the hepatic tissue level of TBARS, TNF-α and Caspase-3 (P<0.05) associated with significant decrease in hepatic tissue level of GSH and SOD (P<0.05) relative to control group. Supplementation of BPA-treated rats with SeNPs-*CP* significantly improved the level of GSH and SOD and reduced the level of TBARS, TNF-α and Caspase-3 (P<0.05) (Table I).

DISCUSSION

Presence of several bioactive components in the grapefruit increases its nutritional and therapeutic value (Alvi *et al*., 2021). Also, nano-selenium particles have a great role in several biomedical applications. Therefore, it is expected that using a natural source such as grapefruit in the green synthesis of nano-selenium particles will have an effective role in combining the therapeutic properties of both grapefruit and selenium and amplify the efficiency of the nano-selenium particles (Sasidharan *et al*., 2015).

In this study, a visible color change from light yellow to red was observed after adding sodium selenite (source of Se) to dried peel extracts of *Citrus paradise* (reducing agent) at optimum pH and temperature which confirming formation of SeNPs (Alvi *et al*., 2021; Vyas and Rana, 2017). Singh *et al*. (2014) indicated that the formation of red color during synthesis of SeNPs due to reduction of selenite to elemental selenium.

The synthesized SeNPs from *Citrus paradisi* peels was characterized by UV-Vis spectroscopic analysis between wavelengths of 200 to 800nm. The peak was seen at 340 nm which represents the presence of selenium nanoparticles formed by the reduction of selenite ion to elemental selenium (Singh *et al*., 2014). Alvi *et al*. (2021) observed that the synthesized SeNPs from *Citrus paradisi* and *Citrus limon* showed absorbance peaks between a range of 300–550 nm.

Then functional groups involved in the reduction of sodium selenite into SeNPs were evaluated via FTIR spectroscopy of all the synthesized SeNPs. Absorbance

reading of Infra-red radiations was checked for each sample between the ranges of 1000–3500 cm^{-1}. The samples synthesized SeNPs in this study exhibited peaks between 1600–1700 cm^{-1} and 3200–3300 cm^{-1}. The absorbance peaks between 3200 and 3300 cm^{-1} confirmed the presence of O–H bonded stretching and strong vibrations due to presence of alcohol and phenol functional groups. N–H stretching of Amide A in proteins was also detected in that region. Similarly, for the peaks in the region of 1600–1700 cm^{-1}, C=C stretching, C– N stretching and Amides functional groups of amides I and II with C-H bonds with the presence of alkenes. Fardsadegh and Jafarizadeh-Malmiri (2019) indicated that the synthesized SeNPs from aloe vera leaf extract exhibited FTIR spectroscopy peaks at 1635.52 cm^{-1} and 3454.3 cm^{-1} which confirmed the presence of amide group at the peak in region between 1600–1700 cm^{-1} and O–H vibrations at the peak between 2900 and 3200 cm^{-1}.

In this work, TEM micrograph revealed that SeNPs synthesized from *Citrus paradise* dried peels extract appeared in the nanoscale size (45-70 nm) with rounded spherical shape. The results agree with the study of Sasidharan *et al*. (2015) who confirmed that SeNPs synthesized from orange peel extract have spherical shape with 70nm in size.

The results of rats received BPA (50 mg/kg) revealed that BPA-induced hepatic tissue damage associated with oxidative stress and lipid peroxidation evidenced by an obvious increase in the activities of liver enzymes (ALT, AST, GGT and LDH) and elevation of hepatic TBARS, TNF-α and apoptotic marker caspase-3. Korkmaz *et al*. (2010) revealed that BPA-treatment elevated the activities of ALT, AST and LDH, and caused marked defects in liver morphology. Eweda *et al*. (2020) reported that BPA treatment induced the severe disruption of the liver's architecture and the integrity of cellular membranes and leading to leakage of cytoplasmic liver enzymes. Also, BPA treatment Increased levels of hepatic TBARS, decreased activities of SOD and CAT and decreased levels of GSH indicating that there were increased levels of oxidative stress in liver cells (Maćczak *et al*.,2017). The reduction of GSH levels may be due to conjugation of GSH with BPA-toxic metabolites and its oxidation to oxidized glutathione (Bukowska, 2004). Vahdati *et al*. (2018) reported that BPA reacts with oxygen radicals, decomposing them to various reactive metabolites that have potent oxidant activity, inhibit the activity of antioxidative enzymes and increase H_2O_2 and thiobarbituric acid reactive substance levels. Apoptosis allows inactive caspase to become active, and the activation of caspase-3 results in apoptosis reaching an irreversible stage. This study has shown that BPA increases the level of hepatic caspase-3 indicating the occurrence of apoptosis in hepatic cells. In addition, BPA can cause apoptosis by the induction of adenylate kinase activation, TNF-alpha gene expression and dysregulation of ROS (Kovacic, 2010).

The findings in this study show that administration of SeNPs-*CP* significantly counters hepatotoxicity, oxidative damage, inflammation and apoptotic effect induced by BPA which might be due to the therapeutic efficiency of grapefruit and selenium nanoparticles. Ebaid *et al*. (2021) provided that SeNPs treatment against CCl_4 toxicity produced significant improvement in liver function and histological architecture and restored normal oxidative stress balance. In addition, SeNPs possess antioxidant and anti-inflammatory effects and might scavenge free radicals which in turn lowers lipid peroxide, TNF-α and apoptotic marker Caspase-3 (Steinbrenner *et al*., 2015). Moreover, grapefruit peel has a wide array of nutraceutical moieties such as naringin and naringenin that possess the distinct bitter taste of grapefruit juice (Alam *et al*., 2014). The study of Jain and Parmar (2011) showed that naringin normalized the elevated TNF-α concentration in an air-pouch model of inflammation which in turn evidenced the anti-inflammatory activity of this flavonoid compound.

Renugadevi and Prabu (2010) suggested that naringenin (4′, 5, 7-trihydroxyflavanone) scavenge free radicals by donating hydrogen to ROS and allowing acquisition of stable structure. Reportedly, the hepatoprotective action of naringenin has been attributed to its potential in inhibiting hepatic oxidative stress, neutralizing the ROS and increased expression and activation of antioxidant enzymes (Chetia *et al*., 2012). Furthermore, naringenin could be able to improve gamma-glutamylcysteine synthetase (GGT) enzyme activity, increase GSH synthesis (Zeng *et al*., 2018) and reduce caspase-3 and -9 levels (Yen *et al*., 2007).

CONCLUSION

In conclusion, *Citrus paradisi* were used as green reducing agent in the synthesis of SeNPs, and the color change to red was selected as an initial indicator of SeNPs synthesis. The confirmation and characterization tests of these SeNPs were. The UV visible spectrophotometer analysis and FTIR spectroscopy were performed for confirmation and characterization of synthesized SeNPs.

Moreover, the synthesized SeNPs-*CP* nanoparticles in this study play an important role as hepatoprotective, antioxidant, and antiapoptotic agent in ameliorating BPA toxicity-triggered inflammation and apoptosis which could be attributed to the potent effects of both selenium and the active ingredients of *Citrus paradise*.

Statement of conflict of interest

The authors have declared no conflict of interest.

REFERENCES

Alam, M.A., Subhan, N., Rahman, M.M., Uddin, S.J., Reza, H.M., and Sarker, S.D., 2014. Effect of citrus flavonoids, naringin and naringenin, on metabolic syndrome and their mechanisms of action. *Am. Soc. Nutr. Adv. Nutr.*, **5**: 404–417. https://doi.org/10.3945/an.113.005603

Alvi, G.B., Iqbal, M.S., Ghaith, M.M.S., Haseeb, A., Ahmed, B., and Qadir, M.I., 2021. Biogenic selenium nanoparticles (SeNPs) from citrus fruit have anti-bacterial activities. *Sci. Rep.*, **11**: 4811. https://doi.org/10.1038/s41598-021-84099-8

Bergmeyer, H.U., Scheibe, P., and Wahlefeld, A.W., 1978. Optimization of methods for aspartate aminotransferase and alanine aminotransferase. *Clin. Chem.*, **24**: 58–73. https://doi.org/10.1093/clinchem/24.1.58

Beutler, E., Duron, O. and Kelly, B.M., 1963. Improved method for the determination of blood glutathione. *J. Lab. Clin. Med.*, **61**: 882-888.

Bukowska, B., 2004. Glutathione: Its biosynthesis, induction agents and concentrations in selected diseases. *Med. Pr.*, **55**: 501-509. (In Polish).

Chetia, P.P., Bala, A., Khandelwal, B. and Haldar, P., 2012. Comparative in vitro free radical scavenging property of â-carotene and naringenin with respect to vitamin C and N-acetyl cysteine. *Pharmacologia*, **3**: 724–728. https://doi.org/10.5567/pharmacologia.2012.724.728

Ebaid, H., Al-Tamimi, J., Hassan, I., Habila, M.A., Rady, A.M., Alhazza, I.M., and Ahmed, A.M., 2021. Effect of selenium nanoparticles on carbon tetrachloride-induced hepatotoxicity in the Swiss albino rats. *Appl. Sci.*, **11**: 3044-3057. https://doi.org/10.3390/app11073044

Eweda, S.M., Newairy, A-S.A., Abdou, H.M. and Gaber, A.S., 2020. Bisphenol A-induced oxidative damage in the hepatic and cardiac tissues of rats: The modulatory role of sesame lignans. *Exp. Ther. Med.*, **19**: 33-44. https://doi.org/10.3892/etm.2019.8193

Fardsadegh, B. and Jafarizadeh-Malmiri, H., 2019. Aloe vera leaf extract mediated green synthesis of selenium nanoparticles and assessment of their *in vitro* antimicrobial activity against spoilage fungi and pathogenic bacteria strains. *Green Process. Synth.*, **8**: 399–407. https://doi.org/10.1515/gps-2019-0007

Gella, F.J., Olivella, T., Pastor, M.C., Arenas, J., Moreno, R., Durban, R., and Gomez, J.A., 1985. A simple procedure for the routine determination of aspartate aminotransferase and alanine aminotransferase with pyridoxal phosphate. *Clin. chim. Acta*, **153**: 241–247. https://doi.org/10.1016/0009-8981(85)90358-4

Jain, M., and Parmar, H.S., 2011. Evaluation of antioxidative and anti-inflammatory potential of hesperidin and naringin on the rat air pouch model of inflammation. *Inflamm. Res.,* **60**: 483–491. https://doi.org/10.1007/s00011-010-0295-0

Johansson, L.H., and Borg, L.A.H., 1988. A spectrophotometric method for determination of catalase activity in small tissue samples. *Anal. Biochem.*, **74**: 331. https://doi.org/10.1016/0003-2697(88)90554-4

Korkmaz, A., Ahbab, M.A., Kolankaya, D., and Barlas, N., 2010. Influence of vitamin C on bisphenol A, nonylphenol and octylphenol induced oxidative damages in liver of male rats. *Fd. Chem. Toxicol.*, **48**: 2865-2871. https://doi.org/10.1016/j.fct.2010.07.019

Kovacic, P., 2010. How safe is bisphenol A? Fundamentals of toxicity: Metabolism, electron transfer and oxidative stress. *Med. Hypotheses*, **75**: 1–4. https://doi.org/10.1016/j.mehy.2010.03.002

Lee, J.H., Li, Y.C., Ip, S.W., 2008. The role of Ca2+ in baicalein induced apoptosis in human breast MDA-MB-231 cancer cells through mitochondria- and caspase-3-dependent pathway. *Anticancer Res.*, **28**: 1701–1711.

Maćczak, A., Cyrkler, M., Bukowska, B., and Michałowicz, J., 2017. Bisphenol A, bisphenol S, bisphenol F and bisphenol AF induce different oxidative stress and damage in human red blood cells (*in vitro* study). *Toxicol. in Vitro*, **41**: 143-149. https://doi.org/10.1016/j.tiv.2017.02.018

Mahdavinia, M., Ahangarpour, A., Zeidooni, L., Samimi, A., Alizadeh, S., Dehghani, M.A. and Alboghobeish, S., 2019. Protective effect of naringin on bisphenol a-induced cognitive dysfunction and oxidative damage in rats. *Int. J. Mol. Cell Med. Spring*, **8**:142-153.

Minami, M. and Yoshikawa, H., 1979. A simplified assay method of superoxide dismutase activity for clinical use. *Clin. chim. Acta*, **92**: 337-342. https://doi.org/10.1016/0009-8981(79)90211-0

Pesce, A., 1984. Lactate dehydrogenase. In: *Clinical Chemistry* The CV Mosby Co., St Louis, Toronto, Princeton. p. 1124-117.

Rao, K.G., Ashok, C., Rao, K.V., Chakra, C.S. and Rajendar, V., 2015. Synthesis of Tio2 nanoparticles

from orange fruit waste. *Int. J. Multi. Adv. Res. Trends*, **Ii**: 82-90.

Renugadevi, J., and Prabu, S.M., 2010. Cadmium-induced hepatotoxicity in rats and the protective effect of naringenin. *Exp. Toxicol. Pathol*., **62**: 171–181. https://doi.org/10.1016/j.etp.2009.03.010

Rönn, M., Kullberg, J., Karlsson, H., Berglund, J., Malmberg, F., Orberg, J., Lind, L., Ahlström, H., and Lind, M.P., 2013. Bisphenol A exposure increases liver fat in juvenile fructose-fed Fischer 344 rats. *Toxicology*, **303**: 125-132. https://doi.org/10.1016/j.tox.2012.09.013

Sasidharan, S., Sowmiya, R., and Balakrishnaraja, R., 2015. Biosynthesis of selenium nanoparticles using *Citrus reticulata* peel extract. *World J. Pharm. Res*., **4**: 1323-1330.

Seleim, M.A., Manal, A.M., Hassan, A.S.M., Saleh and Nadia, H.A., 2019. Physico-chemical evaluation of white and pink grapefruit (*Citrus paradisi*) juice. *Assiut. J. agric. Sci*., **50**: 112-122. https://doi.org/10.21608/ajas.2019.52742

Singh, N., Saha, P., Rajkumar, K. and Abraham, J., 2014. Biosynthesis of silver and selenium nanoparticles by *Bacillus* sp. JAPSK2 and evaluation of antimicrobial activity. *Der Pharm. Lett*., **6**: 175-181.

Steinbrenner, H., Al-Quraishy, S., Dkhil, M.A., Wunderlich, F., and Sies, H., 2015. Dietary selenium in adjuvant therapy of viral and bacterial infections. *Adv. Nutr*., **6**: 73–82. https://doi.org/10.3945/an.114.007575

Szasz, G., Persijn, J.P., and Coll, E., 1974. Kinetic method for quantitative determination of gammaglutamyl transpeptidase. *Z. Klin. Chem. Klin. Biochem*., **212**: 228.

Vahdati, H.F., Abnous, K., Mehri, S., Jafarian, A., Birner-Gruenberger, R., Yazdian, R.R., and Hosseinzadeh, H., 2018. Proteomics and phosphoproteomics analysis of liver in male rats exposed to bisphenol A: Mechanism of hepatotoxicity and biomarker discovery. *Fd. Chem. Toxicol*., **112**: 26-38. https://doi.org/10.1016/j.fct.2017.12.021

Vyas, J. and Rana, S., 2017. Antioxidant activity and green synthesis of selenium nanoparticles using *Allium sativum* extract. *Int. J. Phytomed*., **9**: 634. https://doi.org/10.5138/09750185.2185

Wolf, B.B., and Green, D.R., 1999. Suicidal tendencies: Apoptotic cell death by caspase family proteinases. *J. bsiol. Chem*., **274**: 20049-20052. https://doi.org/10.1074/jbc.274.29.20049

Yen, F.L., Wu, T.H., Lin, L.T., Cham, T.M., and Lin, C.C., 2007. Naringenin-loaded nanoparticles improve the physicochemical properties and the hepatoprotective effects of naringenin in orally-administered rats with CCl_4-induced acute liver failure. *Pharm. Res*., **26**: 893–902. https://doi.org/10.1007/s11095-008-9791-0

Yoshioka, T., Kawada, K., Shimada, T. and Mori, M., 1979. Lipid peroxidation in maternal and cord blood and protective mechanism against activated-oxygen toxicity in the blood. *Am. J. Obstet. Gynecol*., **135**: 372-376. https://doi.org/10.1016/0002-9378(79)90708-7

Zeng, W., Jin, L., Zhang, F., Zhang, C. and Liang, W., 2018. Naringenin as a potential immunomodulator in therapeutics. *Pharm. Res*., **135**: 122–126. https://doi.org/10.1016/j.phrs.2018.08.002