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Antioxidant activity assessment of different dietary forms of selenium for fortification in functional foods

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Abstract
Antioxidants have the potential ability to prevent the oxidation in cells and tissue molecules. Plants and animals maintain complex systems of multiple types of antioxidants, such as glutathione, vitamin C and vitamin E as well as enzymes such as catalase, superoxide dismutase and various peroxidases. Antioxidants can scavenge free radicals and other Reactive Oxygen Species that causes oxidative damage which leads to cancer, cardio vascular diseases and other degenerative disorders. Dietary intake of antioxidant compounds is necessary because of the incompetent natural antioxidant mechanism. It is a novel approach to evolve plant based green synthesized nanoparticle that are rich in antioxidant properties in neutraceuticals. The objective of this study is to analyse the antioxidant activity of different forms of dietary selenium *in vitro* to find out the optimum inclusion of Se for fortification in foods. Green synthesis Nano Selenium (NaSe) was used in this research. Samples with NaSe showed significantly higher antioxidant activity while comparing with organic and inorganic selenium. Hence it can be concluded that the NaSe rich in antioxidant activity can be further validated in *in vivo* models to assess the suitability of incorporation in food additives for fortification in food.

Keywords: Nanoselenium, antioxidant activity, cell proliferation, dietary forms of selenium

Introduction
Free radicals are those which are natural by-products synthesized by our own metabolism. It can cause lipid peroxidation in foods which leads to deterioration [1, 2]. Low levels of antioxidants or inhibition of the antioxidants enzymes, where the chain reaction starts which leads to the oxidative stress that may cause damage to the cells, proteins and DNA [3]. Free radicals are highly reactive with unpaired electrons that attack cells all the way through cellular membranes to react with the nucleic acids, proteins and enzymes present in the body. But, the excess or uncontrolled free radical production can directly damage the cell macromolecules or liberation of toxic byproducts. Antioxidant is the substances that scavenge the free radicals and detoxify the physiological system. Current research in the free radical biology has validated that foods and beverages mounted with antioxidants play an essential role in the prevention of cardiovascular and neurodegenerative diseases and cancer [1].
Selenium is a micronutrient that is considered as a functional part of the antioxidant system, which acts via selenoproteins. Selenoenzymes which have been identified in animals and humans include: glutathione peroxidases, thio redoxin reductases, and iodo thyronine deiodinases. Glutathione peroxidase is a Se-dependent enzyme involved in the antioxidant system; it plays a major role to prevent the free radical formation via reduction of hydrogen peroxide and lipid peroxide to water and alcohol. In the current research there are various methods to find out the antioxidant activity, each of which has its specific target [4].Cultured cells have been used increasingly as a substrate to reveal the mechanisms of antioxidant agents against various oxidative stressors [5, 6]. The use of cultured muscle progenitor cells has the advantage of allowing the study of intense skeletal muscle contractile activity. The generation of high ROS flow in mitochondria makes skeletal muscle tissue a candidate for antioxidant activity analysis where ROS holds a particular relevance [5].

Materials and Methods
Nano Selenium (NaSe) was achieved by green synthesis of a biological procedure using the reducing power of fenugreek seed extract with minor modification [7]. Organic source of selenium was procured from (SelenoPrecise®) and inorganic source (sodium hydrogen selenite) from Hi-media. Chicken muscle samples were collected immediately after slaughter from Chennai Corporation slaughter house, Perambur in normal saline with antibiotic solution

and processed for further analysis in the BSL II facility at Centre for Stem cell Research and Regenerative Medicine, Madras Veterinary College, Chennai-07. The samples were kept in room temperature and processed within 30 minutes. Muscle tissues were weighed by using an electronic weighing balance and washed 4 to 5 times with equal volume of normal saline containing antibiotic solution.

Isolation of muscle derived progenitor cells

The isolation of muscle progenitor cells was done by [8] with some modifications by. Briefly, after thorough washing in PBS with antibiotic, muscle tissue was dissected from the tendon, nerve, major blood vessels, fat and connective tissue in a sterile 35 mm petri dish. Mechanical digestion of the trimmed muscle tissue was followed by enzymatic digestion using 2% collagenase at 37°C for 30-50 min. After digestion the content was filtered in a 70µm cell strainer. Equal volume of media was added and centrifuged at 1200 rpm for 10 min. The supernatant was aspirated and the cell pellet is re-suspended in 1mL culture medium and seeded in culture flask. After 1 hour of incubation the supernatant is centrifuged again at 1200 rpm for 10 min and the pellet is re-suspended in 1mL culture medium and the cells are seeded in cell culture flask. Cell viability and total cell density were determined by 0.4% trypan blue dye. 20µl of cell suspension was added to 20µl of trypan blue and from that mixture 20µl was taken to check the viability of cells. The cells were counted manually in hemocytometer. The total cell density for seeding was calculated using the formula (Total cell number x diluted factor)/10^{-4}.

Selenium concentration for antioxidant activity

The various form of selenium (NaSe, organic and inorganic selenium) concentration was analysed by seeding muscle progenitors at a density of 5000 cells/cm^2. The cells with 90% of confluence in control group were taken as standard to compare the cell proliferation during the supplementation of different selenium concentration ranging from 25µg/ml, 50 µg/ml, 75 µg/ml and 100µg/ml with different time intervals [9].

Antioxidant activity in chicken muscle progenitor cells

DPPH, 2, 2-diphenyl-1-picrylhydrazil from Hi –Media was used for the antioxidant analysis [10, 11]. DPPH in its radical form has an absorption peak at 515 nm, which disappeared on reduction by an antioxidant compound. An aliquot (100 µl) of the sample was added to 200µlof BHT (0.2g/10ml methanol) and volume was make up to 3ml using methanol. Then 150 µl of freshly prepared DPPH solution (4.3mg/3.3ml methanol) was added and the absorbance was measured after 15 min using methanol as a blank. Radical scavenging activity (RSA) of the extract was calculated using the formula

$$\%RSA = 100 \text{ X} \frac{A_{blank} - A_{sample}}{A_{blank}}$$

Results and Discussion

The present work was extended to investigate the cell proliferation and antioxidant profile of the different forms of selenium (NaSe, Organic Selenium and Inorganic selenium) using chicken progenitor cells. Muscle progenitor cells (MPC) exhibited a fibroblastoid morphology on day five and had a confluent monolayer over 15 days of culture as represented in Figure 1. The proliferating and differentiating cultures of avian myoblast grown under prescribed conditions promoted skeletal muscle cell proliferation and differentiation highly suitable to study the effects of various bioactive compounds like nutrients, antioxidants, hormones, and growth factors as reported earlier [12]. Preliminary experiments were performed to assess the suitable concentration of different selenium forms for cell supplementation. The 60-120 µg/mL concentration did not cause any cytotoxic effect in basal condition and that concentration was taken for antioxidant activity analysis.

The results indicated that the antioxidant activity of Org Se and Inorg Se in the treated group is significantly lower than in the NaSe groups. It was observed that at the optimum proliferation dosage of 80µg/ml concentration in mpc the antioxidant level of NaSe was 33 percent, Inorg Se was 29 percent and Org Se was 30 percent while it was 28 percentages in control without selenium supplementation. Hence the *in vitro* results suggested that the optimum level of NaSe that induces proliferation in mpc has reported higher antioxidant activity compared to others forms of dietary selenium analysed in the present study.

Exposure of muscle progenitors to different selenium forms significantly increased the antioxidant activity showing high significance ($P \leq 0.01$) within the treatments (Figure 2).

The ROS production and antioxidant enzyme expression activity plays a significant role in maintaining muscle homeostasis. The antioxidant enzymes were deliberated to quantify the regulation of fibrosis of skeletal muscles and during the first phase of skeletal muscle regeneration ROS played a crucial role in the induction of necrotic muscle fibre removal and decreases the muscle injury. ROS can accumulate to toxic levels in cells under aerobic conditions. The antioxidant enzymes and the ROS could also affect proliferation; differentiation and final maturation of muscle progenitor cells [13]. In due course, the antioxidant enzyme expression increased to detoxify ROS [14].

The results obtained in the present study indicated that the nanoparticles exhibited free radical scavenging activity against DPPH. The findings of the present study suggested that NaSe could be a potential source of natural antioxidant that would have great importance as therapeutic agents in preventing or slowing the progress of reactive oxygen species and associated oxidative stress related degenerative diseases. Many plant extract with antioxidant activities act as protective agents against these radicals. In the present investigation potent antioxidant activity of NaSe from fenugreek plant extract was observed. It has been reported that the phytonutrient present in the fenugreek extract, inhibited lipid peroxidation due to the enriched antioxidant in *in vitro* model [15]. NaSe with higher antioxidant property can be used effectively in the antioxidant packaging materials to prevent against the oxidation and to enhance the shelf life of nuts, potato chips and other fat rich foods [16].

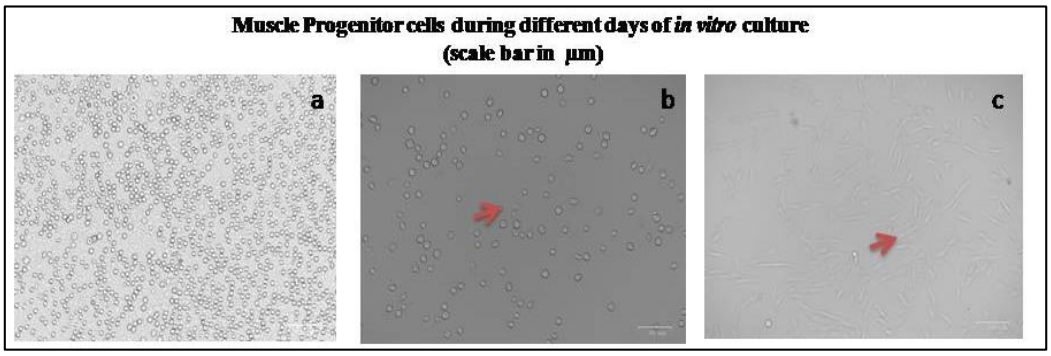

Fig 1: Morphology of muscle progenitor cells a) Day 1 with glistening spheroid morphology (40µm) b) day 7 with spindle morphology (84µm) c) day 15 with elongated monolayer (100µm)

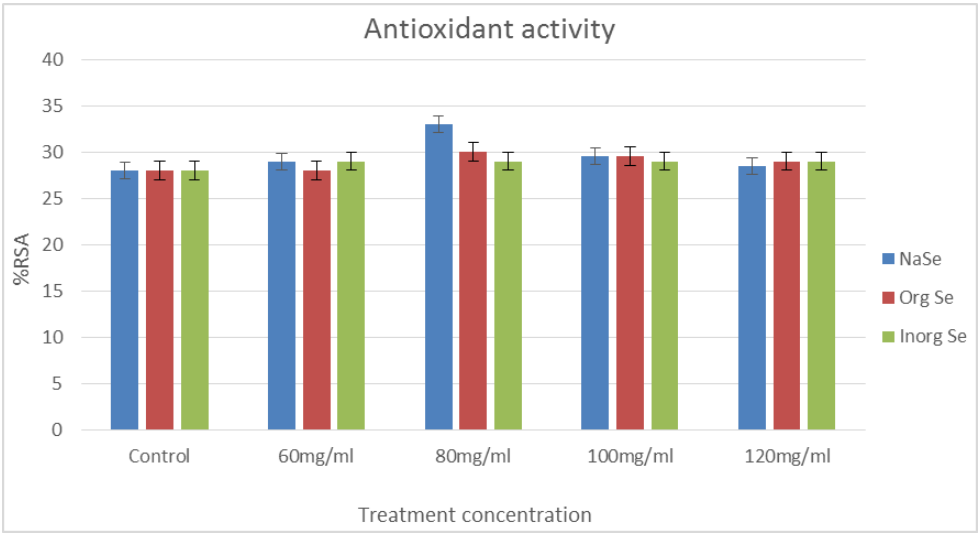

Fig 2: Antioxiant activity of different forms of selenium at varying treatment concentration

Conclusion

Free radicals are molecules which contains one or more unpaired electrons. As the free radical accumulate in cells it can brings about many adverse reactions leading to extensive tissue damage. One of the most important factors that affect antioxidant capacity *in vivo* in humans is the bioavailability of antioxidant rich substances. The antioxidants need to be absolutely absorbed, transported and distributed, and retained properly in the biological fluids, cells, and tissues. Henceforth, nanoparticles with increased bioavailability in comparison with other dietary selenium forms can be the suitable source of antioxidants in food additives. The *in vitro* results suggested that the NaSe did contain compounds that could be capable of donating hydrogen to a free radical in order to remove the odd electron which is responsible for the radical reactivity.

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Reference

1. Mohammad hassanzadeh-khayyat, Mehdi mojarab and Seyyed ahmad emami, Antioxidant activity of methanolic extracts of different species of Artemisia from Irab, Pharmacology online. 2009, 2:797-807, 797.
2. Naphade SS *et al*. Antioxidant Activity of different extracts of plant Tricholepsi Glaberrima DC (Asteraceae); International Journal of Pharm Tech Research. 5, 502-505.
3. Vaibhav Aher D, Arunkumar Wahi, Abhijit Pawdey M. Antioxidants as Immunomodulator: an expanding research avenue Review article; International journal of current pharmaceutical research. 2011; 3(1):8-10.
4. Carocho M, Ferreira ICFR. A review on antioxidants, prooxidants and related controversy: Natural and synthetic compounds, screening and analysis methodologies and future perspectives. Food Chem Toxicol, 2013; 51:15-25.
5. Niki E. Assessment of antioxidant capacity in vitro and in vivo. Free Radical Bio Med. 2010; 49:503-15.
6. Piga R, Saito Y, Yoshida Y, Niki E. Cytotoxic effects of various stressors on PC12 cells: involvement of oxidative stress and effect of antioxidants. Neurotoxicology. 2007; 28:67-75.
7. Ramamurthy KS, Sampath P. Arunkumar M, Suresh Kumar, Sujatha V, Premkumar K *et al*. Green synthesis and characterization of selenium nanoparticles and its augmented cytotoxicity with doxorubicin on cancer cells, Bioprocess Biosyst Eng. 2013; 36:1131-1139.
8. Lavasani M, Lu A, Thompson SD, Robbins PD, Huard J and Niedernhofer LJ., "Isolation of Muscle- Derived Stem/Progenitor Cells based on adhesion characteristics to collagen- coated surfaces", Methods Mol. Biol. 2013; 976:53-65,
9. Priya SA, Mangala Gowri, Sujatha G, Gnanalaksshmi, D.

Baskaran KS. Chicken muscle derived progenitors as a source for testing free radical scavenging activity. Adv. Biomed. Pharma. 2016; 3(4):205-211.
10. Nanjo F, Goto K, Seto R, Suzuki M, Sakai M, Hara Y. Scavenging effects of tea catechins and their derivatives on 1,1-diphenyl-2-picrylhydrazyl radical, Free Radic. Biol. Med. 1996; 21:895-902,
11. Yokozawa T, Dong E, Liu ZW, Shimidzu M. Antioxidant activity of flavones and flavonols *in vitro*, Phytother. Res. 1997; 11:446-449,
12. Hembree JR, Hathaway MR, Dayton WR. Isolation and culture of fetal porcine myogenic cells and the effect of insulin, IGF-I, and sera on protein turnover in porcine myotube cultures, J Anim. Sci. 1991; 69:3241-3250,
13. Bentzinger CF, Wang YX, Dumont NA, Rudnicki MA. Cellular dynamics in the muscle satellite cell niche, EMBO Rep. 2013; 14:1062–1072.
14. Lee SH, Lillehoj HS, Jang SI, Jeong MS, Xu SZ, Kim JB *et al*. Effects of in ovo injection with selenium on immune and antioxidant responses during experimental necrotic enteritis in broiler chickens. Poultry Science. 2014; 93:1113-1121.
15. Zhang S, He B, Ge J, Zhai C, Liu X, Liu P. Characterization of chemical composition of *Agaricus brasiliensis* polysaccharides and its effect on myocardial SOD activity, MDA and caspase-3 level in ischemia-reperfusion rats. Int. J. Biol. Macromol. 2010; 46:363-366.
16. Paula Vera, Elena Canellas, Cristina Nerín. New Antioxidant Multilayer Packaging with Nanoselenium to Enhance the Shelf-Life of Market Food Products. Nanomaterials. 2018; 8:837.