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# Effect of Dietary Nano-Selenium Supplementation on Selenium Content and Oxidative Stability in Table Eggs and Productive Performance of Laying Hens

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**Abstract:** The main target of this study was to evaluate the effect of dietary Nano-Selenium (Nano-Se) supplementation on selenium (Se) content and oxidative stability in table eggs and productive performance of laying hens. One hundred and eighty silver Montazah laying hens (Egyptian local developed strain) aged 32 weeks were housed in individual cages in a semi-open house. Birds were divided randomly into six treatments and fed a basal diet (vitamins and minerals mixture without Se). The experiment involved a 2 x 3 factorial arrangement, 2 Se sources (sodium selenite and Nano-Se) and 3 levels of each source (0.10, 0.25 and 0.40 ppm). Feed and water were provided *ad libitum* throughout the experimental period (three month). The prepared 80 nm Se nano particles were synthesized by chemical reduction method and characterized by Transmission Electron Microscope, X-ray diffraction and spectrophotometry. Different Se levels of sodium selenite or Nano-Se did not affect egg weight, feed intake and most of egg quality. Egg production percentage and egg mass increased and the feed conversion ratio significantly improved, by adding Nano-Se in layer diets. Increasing Se level from 0.10 up to 0.40 ppm either sodium selenite or Nano-Se significantly increasing Se content in eggs and the highest concentration was recorded with high level (0.40 ppm) of Nano-Se. Moreover, increased glutathione peroxides (GSH-Px) activity, with reduction of Malondialdehyde (MDA) content in yolk of stored eggs at room temperature for 15 days. Adding 0.25 ppm of Nano-Se recorded the lower saturated to unsaturated fatty acids ratio thus improved the fatty acid profile and oxidative stability during storage. Nano-Se significantly reduced total lipids, total cholesterol and increased HDL-cholesterol to total cholesterol ratio in maternal hens (plasma and yolk). The main histopathological findings of livers for all treatments were fatty liver with focal aggregation of inflammatory cells. While the spleen showed congestion of blood vessels. Conclusions: It can be concluded that, supplemental layer diets with 0.25 ppm of Nano-Se was effective in improving the productive performance and GSH-Px activity of layer and producing Se enriched egg which could supply 50% (35 µg) of the human Se Recommended Daily Allowances. This give a hand in solving the problem of Se deficiency in food for human.

**Key words:** Nano selenium, productive performance, egg selenium enrichment, antioxidant

## INTRODUCTION

Selenium is an essential trace element that is indispensable for normal functioning of the body and thus plays a critical role in the maintenance of optimal health. It is known to have important role in a number of biological functions, such as antioxidant defense, immune function, reproduction and thyroid hormone metabolism (Surai, 2002). The low concentrations of zinc and Se in the thyroid tissue confirm their participation in the carcinogenic process, (Kucharzewski *et al*., 2003). The United States and Canada Recommended Daily Allowances (RDA) of Se is 55 µg/day for adult males and females (Institute of Medicine, 2000). Moreover, the American Health Organization calculated RDA of Se as 70 µg for adult man and 55 µg for adult woman (Surai and Sparks, 2001). This recommendation is based on the amount of dietary Se required to maximize the activity of plasma GSH-Px enzyme, which protects cells from damage, caused by free radicals and lipid peroxides. Glutathione peroxidase destroys peroxides before they have a change to damage the liposomal membranes (Combs and Combs, 1986).

Selenium is an integral part of the enzyme GSH-Px, which serves as an antioxidant enzyme that helps to control levels of hydrogen peroxide and lipid peroxides that are produced during normal metabolic activity (Underwood and Suttle, 1999). Sodium selenite is

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converted initially to seleno glutathione trisulfide and then degraded in liver to form selenide. The selenide is finally used for seleno protein synthesis, such as GSH-Px enzyme (Schrauzer, 2000).

Increasing the Se intake by 100 to 200 μg/day may have possible health benefits (Schrauzer, 2009). This increased Se intake could be achieved either through supplementation or through the consumption of Se-enriched foods. However, in many countries worldwide the Se consumption is below those recommended levels. Human food ingredients can contain inadequate levels of Se and deficiency this element in human nutrition is a global problem.

In humans, Se deficiency is associated with a compromised immune system and increased susceptibility to various diseases, including arthritis, cancer, cataracts, cholestasis, cystic fibrosis, diabetes, immunodeficiency, lymphoblastic anemia, macular degeneration, muscular dystrophy and stroke, (Surai, 2006).

Eggs and meat are considered to be good sources of Se in human diet. Egg Se content can easily be manipulated to give increased levels, when organic selenium as selenomethionine is included in layer diets at levels that provide 0.3-0.5 ppm. Resulting in egg delivering 50% (30-35 μg) of the human selenium RDA (Payne *et al*., 2005; Chantiratikul *et al*., 2008).

Sodium selenite has been the most common form of Se supplementation in poultry and animals feeds. However, the use of this form has been questioned because of some negative characteristics, such as toxicity, interactions with other minerals and vitamins, poor retention and low efficiency in the transference into egg, meat; milk and poor ability to maintain Se reserves in the body (Underwood and Suttle, 1999).

Recently, Nano-Se which is bright red, highly stable, soluble has attracted widespread attention because nanometer particulates exhibit novel characteristics such as a large surface area, high surface activity, high catalytic efficiency, strong adsorbing ability, high bioavailability and low toxicity (Wang *et al*., 2007; Zhang *et al*., 2008). In addition, they reported that Nano-Se are more efficient than selenite, selenomethionine and methyl selenocysteine in up regulating seleno enzymes in mice, while exhibiting lower acute toxicity. Nano-Se was studied in male Kunming mice, sizes of 20 to 60 nm, the bioavailability of Nano-Se was shown to be similar in increasing the activities of GSH-Px and thioredoxin reductase, at the same time Nano-Se had a much lower toxicity compared with selenomethionine (Wang *et al*., 2007). Moreover, Zhou and Wang (2011) recommended that supplemented 0.30 ppm of Nano-Se in broilers diet was effective in increasing weight gain, Se content in tissues and improving feed conversion ratio and meat quality. Furthermore, dietary supplementation of Nano- Se was effective in enhancing the serum and hepatic GSH-Px activities of chicken. Also, Cai *et al*. (2012) suggested that dietary Nano-Se enhanced the antioxidant ability and oxidative stability and the optimum level of Nano-Se supplementation was ranged from 0.3 to 0.5 ppm and the maximum supplementation could not be more than 1.0 ppm in broilers. The range between optimal and toxic dietary levels of Nano-Se was wider than that of sodium selenite and Nano-Se was more efficiently retained in the body than sodium selenite (Hu *et al*., 2012; Mohapatra *et al*., 2014).

Regarding layers, no literatures has been found to study the effect of supplementing Nano-Se in diet on productive performance. Therefore, the objective of this study evaluated the effects of dietary Nano-Se supplementation on selenium content and oxidative stability in table eggs and productive performance of laying hens.

## MATERIALS AND METHODS

**Birds, management and experimental design:** The present study was carried out at Inshas Poultry Research Station, Animal Production Research Institute, Agricultural Research Center, Egypt. A total number of one hundred and eighty silver Montazah laying hens (Egyptian local developed strain), 32 weeks old were randomly taken from the farm flock, to be similar in weight and productivity. Birds were randomly divided into six treatments, 30 hens each in treatment and then subdivided into three replicates (10 hens/replicate). Birds were fed a basal diet containing vitamins and minerals mixture without Se and supplemented with 0.10, 0.25 and 0.40 ppm Se from sodium selenite ($T_1$, $T_2$ and $T_3$) and Nano-Se ($T_4$, $T_5$ and $T_6$), sources respectively. The first treatment considered as a control diet. All birds were housed individually in layer's cages and maintaining similar managerial and conditions environment with a photoperiod length of 17 h daily. Feed and water were provided *ad libitum* throughout the experimental period (32-45 weeks of age). Experimental diets were formulated to be isonitrogenous and isocaloric to cover the nutrients requirements (Table 1) recommended by Agriculture Ministry Decree (1996).

**Selenium nano particles synthesis:** Selenium nano particles (SeNPs) were prepared according to Zhang *et al*. (2004), with little modifications. In brief, 100 ml of 1 mM sodium selenite heated under stirring condition until boiling then 2.5 mL of 1% Ascorbic Acid was added drop wise until the color change to the characteristic yellowish orange and left to cool with stirring for 30 min, then proceeds for characterization. All used chemicals were obtained from Sigma-Aldrich and used as purchased without any modification. The prepared SeNPs was tested for concentration determination by inductively coupled plasma (ICP).

**Characterization of selenium nano particles:** Synthesis and characterization of the synthesized SeNPs was

done at Nanotechnology and Advanced Materials Central Lab, Agricultural Research Center, Giza, Egypt. UV-Vis spectrophotometer, (Cary 5000, Varian, UK) monitoring SeNPs from 400 to 800 nm with a path-length of 10 mm at 12 nm/s scanning speed and 1 nm bandwidth. High Resolution Transmission electron microscopy (HR-TEM, Tecnia, G20, 200 Kv, FEI, Netherland). Sample was prepared by placing a droplet of the colloidal solution onto a carbon-coated grid and were allowed to dry for 45 min. Bright field imaging mode at electron accelerating voltage 200 Kv using lanthanum hexaboride (LaB6) electron source gun was performed. Eagle CCD camera with (2k*2k) image resolution was used to acquire and collect transmitted electron images. TEM Imaging software was used. Dynamic Light Scattering (ZS-nano, Malvern, UK) used for SeNPs particle size distribution determination. For phase analysis, X-ray diffraction (XRD) patterns of SeNPs were obtained using Panlytical X'pert Pro X-ray diffractometer using Cu Ka (1.54059 A°) radiation with the X-ray generator operating at 45 Kv and 30 mA. The total Se concentration was measured by Inductively coupled plasma (ICP) technique (optical emission spectrometer optima 2000 DV, Perkin elmer).

**Mixing selenium nano particles in diet:** After complete the characterization of the synthesized SeNPs solution, the suggested examined levels of Se were 0.10, 0.25 and 0.4 ppm in layer diet. So there was a necessary to suggest more applicable and accurate method to use SeNPs solution in diets. Three carriers of SeNPs solution were examined (colloidal silica, skimmed milk and wheat bran) and the best one was wheat bran. Three SeNPs diet concentrations (0.10, 0.25 and 0.4 ppm) were prepared by mixing the selected concentrations of SeNPs to wheat bran. Samples were mixed well for homogeny and dried at 60°C overnight. The prepared SeNPs in dry form were added to the final layer diet.

**Measurements:** Daily egg number and egg weight were recorded for each hen and feed intake was recorded weekly. Egg production were calculated for monthly intervals during the production period as egg number/hen/period for each replicate and calculated the average of the whole experimental period. Egg mass was calculated by multiplying egg number by average egg weight. Feed conversion was calculated as g feed consumption divided by g egg mass (g feed/g egg mass). Egg quality traits was measured at the end of experimental period, in which 6 eggs were randomly taken from each treatment (2 eggs from each replicate). Eggs were individually broken and egg shell, yolk and albumen were weighed, shell, yolk and albumen percentages were calculated. Egg shell thickness, including shell membranes, was measured using a micrometer at the equator. Egg shape index and yolk index values were measured according to Sauter *et al*. (1951); Haugh unit score was applied from a special chart using egg weight and albumin height which was measured by using a micrometer according to Haugh (1937), Kotaiah and Mohapatra (1974) and Eisen *et al*. (1962). The egg yolk visual color score was determined by matching the yolk with one of the 15 bands of the Roche Improved Yolk Color Fan. Concentrations of Se were determined in albumen and yolk of eggs. The samples were weighted and digested using mixture of concentrated Nitric acid and Perchloric acid (ratio 3:1). The digested samples were diluted with deionized water then introduced for measurement using the inductively coupled argon plasma spectroscopy to evaluate the Se concentrations according to the method of Cottenie *et al*. (1982). Also, Lipids were extracted from yolk according to Folch *et al*. (1957).

At the end of the experiment, three hens were chosen randomly from each treatment and slaughtered. Two blood samples were collected from each hen in heparinized tubes (1 complete blood sample and 1 plasma sample/hen). The heparinized plasma samples kept at -20°C until the time of chemical analyses of Aspartate amino transferase (AST), Alanin glutamyl transferase (ALT) and alkaline phosphatase enzymes and creatinine. Also, total lipids, total cholesterol, High Density Lipoprotein (HDL)-cholesterol and Low Density Lipoprotein (LDL)-cholesterol, were determined in both of plasma and yolk by colorimetric methods using commercial kits, following the same steps as described by manufactures. The complete blood samples kept at 4°C then directly determined activity of glutathione peroxidase (GSH-Px) in red blood cells.

At the end of the experiment, three yolk samples from each treatment were separated from the broken fresh eggs were directly stored in deep freezer at -20°C. The remaining six eggs from each treatment were kept at room temperature (16-13°C) with relative humidity (65-60%) for 15 days. Three yolk samples from each treatment were separated from the broken storage eggs and stored in deep freezer at -20°C. All yolk samples from fresh and storage eggs were analyzed Malondialdehyde (MDA) by colorimetric methods of Satoh (1978). While, the other yolk samples were separated from the broken storage eggs to analyze fatty acid composition in yolk extract according to AOAC (2000).

**Histopathological examination:** Liver and spleen were removed from each slaughtered bird and immersion-fixed in 10% formalin solution at room temperature. Autopsy samples (4 µ tissue sections) were fixed in 10% formalin saline for twelve hours. Serial dilutions of alcohol (methyl, ethyl and absolute ethyl) were used. Specimens were cleared in xylene embedded in paraffin

at 56°C in hot air oven for 24 h. Paraffin bees wax tissue blocks were prepared for sectioning at 4 micron thickness by sledge microtome. The obtained tissue sections were collected on glass slides, deparaffinized and stained by Haematoxylin and Eosin (H and E) and subsequently processed for histopathological examination under light microscope (Banchroft *et al.*, 1996).

**Statistical analysis:** Data of experimental treatments were analyzed by using two way analysis of variance to detect the effect of selenium source and supplemental level. Also data of all experimental treatments, were analyzed by using one way analysis of variance to detect the best treatment between them. Variables showed significant differences at F-test ($p \leq 0.05$) were compared to each other's using Duncan's Multiple Range Test (Duncan, 1955). The statistical procedures were computed using SAS (1999).

## RESULTS AND DISCUSSION

### Characterization of selenium nano particles

**A-UV-Vis spectrophotometer:** The optical properties of the synthesized SeNPs have been characterized by their Plasmon absorbance band in the visible region of the electromagnetic wave particles have peaks at 265 nm (Fig. 1) with Gaussian distribution indicating formation of spherical GNPs with no aggregation of size that indicated uniformity and excellent dispersion of colloidal Se nanoparticles.

**B-Transmission electron microscopy(TEM):** The high resolution-TEM (HR-TEM) image of SeNPs was synthesized by ascorbic reduction method with monodisperse spherical shape with average size 80 nm. SeNPs was clearly evident that particles were spherical and uniform in size demonstrating homogeneity and monodispersity (Fig. 2).

**C-Particles size distribution:** The particle size distribution of SeNPs was measured by dynamic light scattering (DLS) where the average measured size was 80 nm with perfect Poly Dispersity Index (PDI) 0.077 which confirmed the result obtained by TEM imaging and the corresponding characterized absorption peak (Fig. 3). XRD measurement were employed to investigate the phase and structure of the synthesized sample. Figure 4 shows the XRD pattern of the as prepared PEG-SeNPs shows a broad peak at $2\theta = 29.682^{\circ}$ corresponding to the (101) reflection of selenium. Broad suggesting that the sample in nano size which confirm the results obtained from TEM and DLS. This all the new diffraction peaks of the PEG-SeNPs sample matched well with the data from the JCPDS card (1-086-2246) for SeNPs (the diffraction angles at $2\theta$): $23.499^{\circ}$, $41.305^{\circ}$, $43.618^{\circ}$, $45.337^{\circ}$, $51.684^{\circ}$ and $65.197^{\circ}$ can be assigned to (100), (110), (012), (111), (201), and (210) of the crystal planes of SeNPs.

Table 1: Composition and calculated analysis of the basal diet

| Ingredients | (%) |
|---|---|
| Yellow corn | 63.15 |
| Soybean meal (44%) | 23.29 |
| Corn gluten meal (60%) | 3.02 |
| Mono calcium phosphate | 1.39 |
| Lime stone | 8.40 |
| NaCl | 0.40 |
| Vitamins and minerals mixture* | 0.30 |
| DL-methionine | 0.05 |
| Total | 100.00 |
| **Calculated analysis** | |
| Crude protein (%) | 17.00 |
| Metabolizable energy (Kcal ME/kg diet) | 2748 |
| Available phosphorus (%) | 0.42 |
| Calcium (%) | 3.41 |
| Lysine (%) | 0.868 |
| Methionine (%) | 0.377 |
| Methionine+Cystine (%) | 0.666 |

Each 3 kg of Vitamins and Minerals mixture * contains: Vit. A 10000,000 IU; Vit. $D_3$ 2000,000 IU; Vit. E 10,000 mg; Vit. $K_3$ 1000 mg; Vit. $B_1$ 1000 mg; Vit. $B_2$ 5000 mg; Vit. $B_6$ 1500 mg; Vit. $B_{12}$ 10 mg; Pantothenic acid 10,000 mg; Niacin 30,000 mg; Folic acid 1000 mg; Biotin 50 mg; Choline 250,000 mg; Manganese 60,000 mg; Zinc 50,000 mg; Copper 4,000 mg; Iron 30,000mg; Iodine 300 mg; Cobalt 100 mg; $CaCO_3$ to 3,000gm.

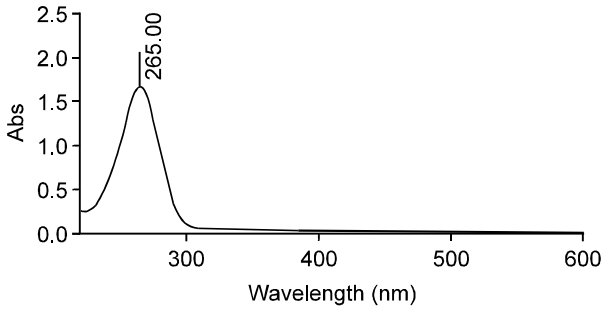


Fig. 1: Shows the UV-Vis spectrum of SeNPs with absorption peak at 265 nm

**Productive performance:** The effect of Se source or level and their interaction on productive performance for the whole experimental period are shown in Table 2. Nano-Se Supplementation in layer diets significantly increased egg production percentage and egg mass and improved feed conversion ratio compared with sodium selenite. Concerning effect of Se level, adding 0.25 ppm in the diet significantly improved productive performance of layer compared with other levels. The improvement attributed to Se supplementation, Se is an important auxiliary factor for the key enzyme of 5-deiodinase. The iodothyronine deiodinase enzymes convert the pro-hormone thyroxine (T4) to the active form triiodothyronine (T3). Triiodothyronine is a main

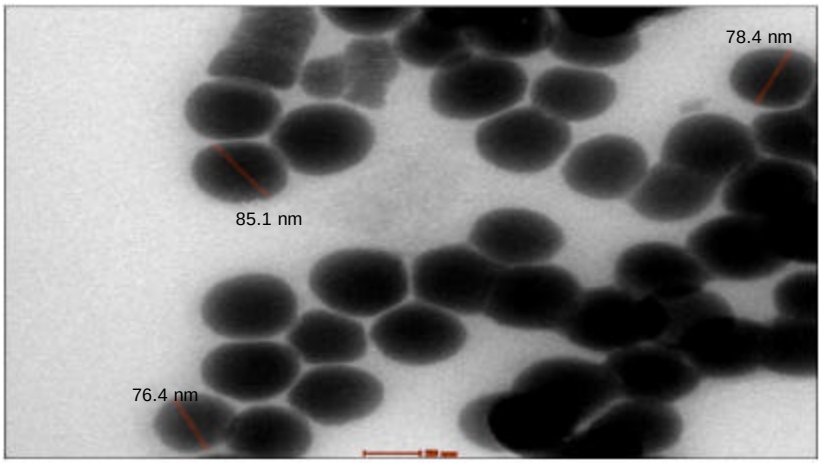


Fig. 2: HR-TEM image of SeNPs with average size 80 nm

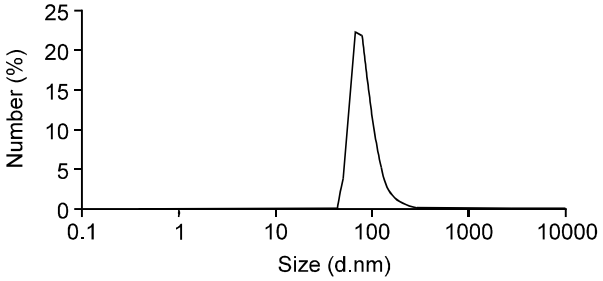


Fig. 3: Particle size distribution of SeNPs

hormone that regulates growth by controlling the body's energy and protein anabolism (Arthur *et al*., 1999; Preter, 2000). Selenium is an integral part of GSH-Px, which eliminates some of free radicals from metabolic activity. The increase in free radicals has been correlated with reductions in productive performance (Underwood and Suttle, 1999). Hens receiving 0.25 ppm of Nano-Se significantly had higher both egg production percentage and egg mass and improved feed conversion ratio than the other treatments. While, egg weight, feed intake and body weight change of hens, did not affect neither by Se source nor level or their interaction (Table 2).

The present results agree with the report of Cai *et al*. (2012) who, found that increasing the level of Se supplementation does not affect feed intake of broilers fed various concentrations (0 to 2.0 ppm) of Nano-Se. However, Nano-Se at the level of 0.30 ppm significantly improved feed conversion ratio (Zhou and Wang, 2011; Cai *et al*., 2012). Egg production percentage was increased with supplementation of organic selenium in layer diets (Gjorgovska *et al*., 2012). While, Attia *et al*. (2010) indicated that egg weight and egg mass significantly increased and feed conversion ratio improved by Se supplementation compared with hens fed the control diet. Nevertheless, the egg production percentage was not affected neither by Se source nor level (Leeson *et al*., 2008; Reis *et al*., 2009; Attia *et al*., 2010).

**Egg quality:** External and internal egg quality parameters were not affected neither by Se source nor level or their interaction, except of Yolk index which was significantly affected (Table 3). Selenium supplementation at 0.40 ppm of Nano-Se or sodium selenite significantly increased Yolk index. The highest value of Yolk index was recorded for eggs produced by hens fed a diet with 0.40 ppm of Nano-Se (43.34 vs 40.96 as control diet, respectively). While, Hough unit insignificantly increased by increasing dietary Se level. The highest value recorded for 0.40 ppm of Nano-Se (78.91 vs 76.25 as control diet, respectively). Attia *et al*. (2010) found that supplementation of inorganic or organic selenium in the diets, had no significant effect on any traits of egg quality, showing that Se content of the basal diet was adequate to support egg production of good quality. Gjorgovska *et al*. (2012) reported that, percentage of yolk, albumen and shell egg were not affected by different levels or sources of Se. As well as, Paton *et al*. (2000) reported that inorganic or organic selenium supplementation at 0.3 ppm had no effect on Hough unit values compared with eggs from hens fed the basal diet. Conversely, Payne *et al*. (2005) and Gajcevic *et al*. (2009) indicated that eggs produced by hens fed a diet with organic selenium had higher Hough unit values than eggs of hens fed the control diet.

**Biochemical parameters:** The effect of different dietary levels or sources of Se and their interaction did not affect liver and renal functions (Table 4). The results are in agreement with that reported by Yang *et al*. (2012) who found that liver enzymes didn't affected by organic or inorganic selenium. Moreover, Mohapatra *et al*. (2014)

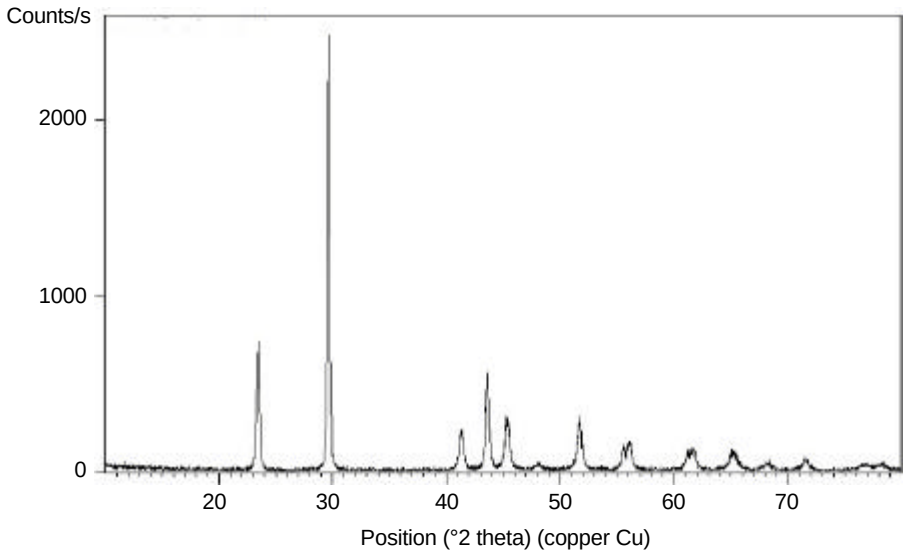


Fig. 4: XRD Pattern of SeNPs

observed also liver enzymes didn't differ significantly between sodium selenite (0.3 ppm) and Nano-Se (0.15, 0.30 and 0.60 ppm) treated. Selenium supplementation caused a significant reduction in plasma and yolk total lipid, total cholesterol and LDL-cholesterol. The greatest reduction was observed when added 0.25 ppm of Se to the diet and had insignificant effect as compared to 0.40 ppm. Selenium could lower plasma total cholesterol and LDL-cholesterol plus very LDL-cholesterol, also increase the level of HDL-cholesterol (Poirirer *et al*., 2002). Because of Se has a crucial role in controlling the effects of thyroid hormone on fat metabolism (Masukawa *et al*., 1983). Hypercholesterolemia have been shown to be associated with Se deficiency was related to increased 3-hydroxy-3-methylglutaryl coA(HMG-coA) reductase activity in liver microsomes (Nassier *et al*., 1997). Selenium forms the active center of GSH-Px that plays a role as an antioxidant may be effective on cholesterol decrease. According to Brown and Jessup (1999), who observed that, cholesterol concentration decreases with increasing antioxidants in the diet. Among the experimental treatments in the present study, Se supplementation at 0.25 or 0.40 ppm of Nano-Se significantly decreased total lipids, total cholesterol and LDL-cholesterol, in addition increased HDL-cholesterol to total cholesterol ratio in maternal hens (plasma and yolk) compared with that in hens fed the control diet (Table 4). Surai (2006) reported that Se supplementation increased HDL-cholesterol to total cholesterol ratio with low cholesterol in plasma. Attia *et al*. (2010) observed that significant decrease of cholesterol with increased HDL-cholesterol concentration in plasma by supplementation of 0.25 ppm of inorganic or organic selenium or 0.40 ppm of organic selenium compared with that in hens fed the control diet. On the other hand, Abaza (2002) found that the plasma cholesterol concentration was significantly increased by Se supplementation or the combination of Se and vitamin E. Changes in enzymes responsible for regulating cholesterol synthesis, may be responsible for lowering the cholesterol synthesis in chickens (Konjufca *et al*., 1997). The mechanism of cholesterol decrease may be the inhibition of sterol biosynthesis by oxysterols. Selenium increases the production of 15-deoxy- $\Delta$-12, 14-prostaglandin J2 (Touyz and Schiffrin, 2006; Vunta *et al*., 2007) a known peroxisome proliferator-activated receptor-$\gamma$ ligand. Activation of peroxisome proliferator activated receptor-$\gamma$ can reduce the concentration of sterol regulatory element-binding protein-2, resulting in a reduction of cholesterol synthesis (Klopotek *et al*., 2006).

**Selenium egg:** The effect of different dietary levels or sources of Se and their interaction on Se content in the albumen; yolk and whole egg are presented in Table 5. Nano-Se significantly increased Se content in the albumen; yolk and whole egg compared with that in eggs produced by hens fed on diet supplemented sodium selenite. This results indicated that Nano-Se led to a higher deposition of Se in egg contents when compared with sodium selenite, likely because of a faster transference Nano-Se into egg. There was a linear correlation between dietary Se level and deposition of Se in egg contents. In addition, a linear increase Se content in the albumen; yolk and whole egg within each source with increasing Se level. Furthermore, both levels of Nano-Se had greater values than the corresponding levels of sodium selenite source. This difference was probably related to the

Table 2: Effect of different selenium sources and levels on productive performance parameters of laying hens from 32 to 45 weeks of age

| Treatments | Egg production (%) | Egg weight (g) | Egg mass (g/d) | Feed intake (g/d) | Feed conversion (g feed/g egg mass) | Live body weight change (g) |
|---|---|---|---|---|---|---|
| **Effect of selenium sources** | | | | | | |
| SS | 71.09$^{b}$ | 48.69 | 34.62$^{b}$ | 117.44 | 3.40$^{a}$ | 266 |
| Nano-Se | 74.02$^{a}$ | 48.49 | 35.90$^{a}$ | 118.65 | 3.30$^{b}$ | 278 |
| SEM | ±0.633 | ±0.137 | ±0.349 | ±0.798 | ±0.022 | ±5.12 |
| p. value | 0.001 | N.S | 0.01 | N.S | 0.01 | N.S |
| **Effect of selenium levels (ppm)** | | | | | | |
| 0.10 | 71.27$^{b}$ | 48.53 | 34.60$^{b}$ | 117.90 | 3.41$^{a}$ | 266 |
| 0.25 | 74.89$^{a}$ | 48.94 | 36.65$^{a}$ | 120.06 | 3.27$^{b}$ | 279 |
| 0.40 | 71.50$^{b}$ | 48.29 | 34.54$^{b}$ | 116.16 | 3.36$^{a}$ | 271 |
| SEM | ±0.633 | ±0.137 | ±0.349 | ±0.798 | ±0.022 | ±5.12 |
| p. value | 0.001 | N.S | 0.001 | N.S | 0.01 | N.S |
| **Interaction between selenium sources and levels** | | | | | | |
| 0.10 SS | 69.25$^{c}$ | 48.41 | 33.53$^{c}$ | 116.45 | 3.47$^{a}$ | 255 |
| 0.25 SS | 72.90$^{b}$ | 49.06 | 35.77$^{b}$ | 119.12 | 3.33$^{bc}$ | 273 |
| 0.40 SS | 71.12$^{bc}$ | 48.60 | 34.57$^{bc}$ | 116.74 | 3.38$^{ab}$ | 270 |
| 0.10 Nano-Se | 73.29$^{b}$ | 48.66 | 35.67$^{b}$ | 119.36 | 3.35$^{bc}$ | 277 |
| 0.25 Nano-Se | 76.89$^{a}$ | 48.82 | 37.52$^{a}$ | 121.00 | 3.22$^{c}$ | 285 |
| 0.40 Nano-Se | 71.89$^{bc}$ | 47.99 | 34.50$^{bc}$ | 115.58 | 3.34$^{bc}$ | 272 |
| SEM | ±0.633 | ±0.137 | ±0.349 | ±0.798 | ±0.022 | ±5.12 |
| p. value | 0.001 | N.S | 0.001 | N.S | 0.01 | N.S |

a,b,c: Means in the same column with different superscripts, differ significantly (p<0.05)
N.S: Not Significant (p>0.05); SEM: Standard Error of Means; SS: Sodium selenite

Table 3: Effect of different selenium sources and levels on external and internal egg quality

| | External egg quality | | | Internal egg quality | | | | |
|---|---|---|---|---|---|---|---|---|
| Treatments | Shell weight (%) | Shell thickness (mm) | Egg shape index | Albumen weight (%) | Yolk weight (%) | Yolk color score | Yolk index | Hough unit |
| **Effect of selenium sources** | | | | | | | | |
| SS | 13.02 | 0.372 | 75.42 | 55.21 | 31.75 | 6.06 | 42.13$^{b}$ | 77.03 |
| Nano-Se | 13.36 | 0.362 | 75.68 | 54.56 | 32.08 | 6.33 | 43.07$^{a}$ | 78.05 |
| SEM | ±0.131 | ±0.004 | ±0.219 | ±0.292 | ±0.270 | ±0.102 | ±0.268 | ±0.408 |
| p. value | N.S | N.S | N.S | N.S | N.S | N.S | 0.04 | N.S |
| **Effect of selenium levels (ppm)** | | | | | | | | |
| 0.10 | 13.40 | 0.365 | 75.24 | 55.14 | 31.45 | 6.04 | 41.77$^{b}$ | 76.70 |
| 0.25 | 13.09 | 0.382 | 75.39 | 54.49 | 32.42 | 6.12 | 42.78$^{ab}$ | 77.58 |
| 0.40 | 13.08 | 0.355 | 76.02 | 55.03 | 31.87 | 6.43 | 43.25$^{a}$ | 78.34 |
| SEM | ±0.131 | ±0.004 | ±0.219 | ±0.292 | ±0.270 | ±0.102 | ±0.268 | ±0.408 |
| p. value | N.S | N.S | N.S | N.S | N.S | N.S | 0.04 | N.S |
| **Interaction between selenium sources and levels** | | | | | | | | |
| 0.10 SS | 13.63 | 0.366 | 74.91 | 55.31 | 31.05 | 6.00 | 40.96$^{b}$ | 76.25 |
| 0.25 SS | 12.77 | 0.390 | 75.16 | 55.26 | 31.97 | 6.08 | 42.28$^{ab}$ | 77.08 |
| 0.40 SS | 12.67 | 0.360 | 76.19 | 55.08 | 32.23 | 6.11 | 43.16$^{a}$ | 77.77 |
| 0.10 Nano-Se | 13.17 | 0.363 | 75.57 | 54.97 | 31.85 | 6.08 | 42.58$^{ab}$ | 77.16 |
| 0.25 Nano-Se | 13.41 | 0.373 | 75.63 | 53.72 | 32.86 | 6.17 | 43.28$^{a}$ | 78.08 |
| 0.40 Nano-Se | 13.50 | 0.350 | 75.84 | 54.99 | 31.52 | 6.75 | 43.34$^{a}$ | 78.91 |
| SEM | ±0.131 | ±0.004 | ±0.219 | ±0.292 | ±0.270 | ±0.102 | ±0.268 | ±0.408 |
| p. value | N.S | N.S | N.S | N.S | N.S | N.S | 0.05 | N.S |

a,b: Means in the same column with different superscripts, differ significantly ($p \leq 0.05$)
N.S: Not Significant (p>0.05); SEM: Standard Error of Means; SS: Sodium selenite

different absorption process for Nano-Se than sodium selenite. Liao *et al.* (2010) suggested that the superior performance of nano particles may be attributed to their smaller particle size and larger surface area, increased mucosal permeability, thus improved intestinal absorption and tissue depositions. Among the experimental treatments in the present study, the highest Se content in edible part of egg and whole egg were recorded for eggs of hens receiving 0.40 ppm Se from Nano-Se. This results indicated that supplementation of 0.25 and 0.40 ppm of Nano-Se to the layer diets resulted in threefold and fourfold more accumulated Se in albumen compared to eggs produced by hens fed a control diet. In addition, Se content in yolk of eggs treated to be fourfold and fivefold higher than in yolk of eggs produced by hens fed a control diet (Table 5). Really, Se transfer to the egg depends on source and level of Se in the diet. In other experiments, increased dietary Se level from 0.15 to 0.30 ppm led to a significant increase in Se concentration in yolk (Zdunczyk *et al*., 2013). Also, Valentic *et al.* (2003) showed that the provision of organic selenium in concentrations ranging from 0.3 to 0.5 ppm in the diet resulted to accumulate only 30% more Se in the egg when compared with the same concentrations of inorganic selenium. On the other hand, supplementation

Table 4: Effect of different selenium sources and levels on liver and renal functions and some constituents of plasma and yolk extract

| | Liver and renal functions | | | | Plasma | | | | | Yolk extract | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Treatments | Alkaline phos (U/L) | ALT (U/L) | AST (U/L) | Creatinin (mg/dl) | T. lipid (mg/dl) | T. Cho (mg/dl) | LDL (mg/dl) | HDL (mg/dl) | HDL/ T. Cho | T. lipid (mg/g) | T. Cho (mg/g) | LDL (mg/g) | HDL (mg/g) | HDL/ T. Cho |
| **Effect of selenium sources** | | | | | | | | | | | | | | |
| SS | 27.57 | 37.42 | 62.06 | 1.04 | 592$^{a}$ | 173$^{a}$ | 92.61 | 61.12 | 35.34$^{b}$ | 37.95$^{a}$ | 15.72$^{a}$ | 9.30$^{a}$ | 5.25 | 33.52$^{b}$ |
| Nano-Se | 28.82 | 40.48 | 63.88 | 1.00 | 553$^{b}$ | 161$^{b}$ | 86.70 | 62.82 | 39.10$^{a}$ | 35.03$^{b}$ | 14.80$^{b}$ | 8.19$^{b}$ | 5.52 | 37.68$^{a}$ |
| SEM | ±0.493 | ±0.939 | ±2.043 | ±0.012 | ±10.62 | ±3.03 | ±2.54 | ±1.05 | ±1.02 | ±0.802 | ±0.244 | ±0.295 | ±0.100 | ±1.164 |
| p-value | N.S | N.S | N.S | N.S | 0.03 | 0.03 | N.S | N.S | 0.02 | 0.02 | 0.02 | 0.01 | N.S | 0.01 |
| **Effect of selenium levels (ppm)** | | | | | | | | | | | | | | |
| 0.10 | 28.21 | 38.76 | 61.98 | 0.99 | 606$^{a}$ | 177$^{a}$ | 99.62$^{a}$ | 59.12 | 33.50$^{b}$ | 39.41$^{a}$ | 16.16$^{a}$ | 9.94$^{a}$ | 4.99$^{b}$ | 30.92$^{b}$ |
| 0.25 | 29.18 | 40.90 | 66.54 | 1.03 | 544$^{b}$ | 160$^{b}$ | 82.77$^{b}$ | 63.97 | 40.00$^{a}$ | 34.23$^{b}$ | 14.59$^{b}$ | 7.76$^{b}$ | 5.74$^{a}$ | 39.54$^{a}$ |
| 0.40 | 27.20 | 37.20 | 60.39 | 1.04 | 567$^{ab}$ | 164$^{ab}$ | 86.58$^{b}$ | 62.82 | 38.17$^{a}$ | 35.83$^{b}$ | 15.04$^{b}$ | 8.54$^{b}$ | 5.43$^{a}$ | 36.35$^{a}$ |
| SEM | ±0.493 | ±0.939 | ±2.043 | ±0.012 | ±10.62 | ±3.03 | ±2.54 | ±1.05 | ±1.02 | ±0.802 | ±0.244 | ±0.295 | ±0.100 | ±1.164 |
| p-value | N.S | N.S | N.S | N.S | 0.02 | 0.03 | 0.01 | N.S | 0.01 | 0.01 | 0.01 | 0.001 | 0.001 | 0.001 |
| **Interaction between selenium sources and levels** | | | | | | | | | | | | | | |
| 0.10 SS | 27.43 | 38.16 | 60.80 | 1.01 | 623$^{a}$ | 182$^{a}$ | 102.29$^{a}$ | 58.47 | 32.27$^{c}$ | 40.75$^{a}$ | 16.42$^{a}$ | 10.23$^{a}$ | 4.94$^{c}$ | 30.06$^{c}$ |
| 0.25 SS | 28.85 | 38.60 | 65.44 | 1.03 | 575$^{abc}$ | 167$^{abc}$ | 84.78$^{b}$ | 62.93 | 37.48$^{abc}$ | 35.66$^{bc}$ | 15.17$^{abc}$ | 8.30$^{bc}$ | 5.65$^{ab}$ | 37.22$^{ab}$ |
| 0.40 SS | 26.45 | 35.52 | 59.94 | 1.08 | 580$^{abc}$ | 170$^{abc}$ | 90.76$^{ab}$ | 61.94 | 36.27$^{bc}$ | 37.42$^{abc}$ | 15.57$^{ab}$ | 9.36$^{ab}$ | 5.17$^{bc}$ | 33.28$^{bc}$ |
| 0.10 Nano-Se | 29.00 | 39.36 | 63.17 | 0.98 | 590$^{ab}$ | 173$^{ab}$ | 96.95$^{ab}$ | 59.78 | 34.73$^{bc}$ | 38.06$^{ab}$ | 15.90$^{a}$ | 9.65$^{a}$ | 5.05$^{c}$ | 31.76$^{c}$ |
| 0.25 Nano-Se | 29.50 | 43.20 | 67.64 | 1.04 | 514$^{c}$ | 153$^{c}$ | 80.76$^{b}$ | 65.02 | 42.51$^{a}$ | 32.80$^{c}$ | 14.00$^{c}$ | 7.22$^{c}$ | 5.83$^{a}$ | 41.87$^{a}$ |
| 0.40 Nano-Se | 27.96 | 38.88 | 60.84 | 1.00 | 555$^{bc}$ | 158$^{bc}$ | 82.40$^{b}$ | 63.68 | 40.07$^{ab}$ | 34.23$^{bc}$ | 14.50$^{bc}$ | 7.72$^{c}$ | 5.70$^{a}$ | 39.42$^{a}$ |
| SEM | ±0.493 | ±0.939 | ±2.043 | ±0.012 | ±10.62 | ±3.03 | ±2.54 | ±1.05 | ±1.02 | ±0.802 | ±0.244 | ±0.295 | ±0.100 | ±1.164 |
| p-value | N.S | N.S | N.S | N.S | 0.04 | 0.05 | 0.05 | N.S | 0.02 | 0.02 | 0.01 | 0.001 | 0.01 | 0.001 |

$^{a,b}$Means in the same column with different superscripts, differ significantly ($p<0.05$).

N.S: Not Significant ($p>0.05$), SEM: Standard Error of Means; SS: Sodium selenite, T. Cho: Total Cholesterol; HDL/ T. Cho: HDL to Total Cholesterol ratio

Table 5: Effect of different selenium sources and levels on glutathione peroxidaes activity in blood and (malondialdehyde and selenium content) in egg

| | | ----- Malondialdehyde (nmol/ml) ----- | | | Selenium conc. (µg/g) | | ---- Selenium content (µg/egg) ---- | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Treatments | Glutathione peroxidaes (mU/mL) | Fresh | After 15 days | Over all | Yolk | Albumin | Yolk | Albumin | Whole egg | Selenium content (%) from RDA* |
| **Effect of selenium sources** | | | | | | | | | | |
| SS | 2.36$^{b}$ | 10.05$^{a}$ | 11.90$^{a}$ | 10.98$^{a}$ | 0.792$^{b}$ | 0.182$^{b}$ | 12.52$^{b}$ | 5.05$^{b}$ | 17.57$^{b}$ | 25.10$^{b}$ |
| Nano-Se | 3.13$^{a}$ | 9.15$^{b}$ | 10.88$^{b}$ | 10.02$^{b}$ | 1.558$^{a}$ | 0.232$^{a}$ | 26.11$^{a}$ | 6.51$^{a}$ | 32.62$^{a}$ | 46.60$^{a}$ |
| SEM | ±0.165 | ±0.202 | ±0.265 | ±0.223 | ±0.135 | ±0.020 | ±2.376 | ±0.587 | ±2.851 | ±4.073 |
| p. value | 0.001 | 0.01 | 0.01 | 0.002 | <0.0001 | 0.01 | <0.0001 | 0.02 | <0.0001 | <0.0001 |
| **Effect of selenium levels (ppm)** | | | | | | | | | | |
| 0.10 | 2.25$^{b}$ | 10.34$^{a}$ | 12.47$^{a}$ | 11.40$^{a}$ | 0.667$^{c}$ | 0.116$^{c}$ | 10.67$^{c}$ | 3.20$^{c}$ | 13.86$^{c}$ | 19.80$^{c}$ |
| 0.25 | 3.17$^{a}$ | 9.35$^{b}$ | 10.96$^{b}$ | 10.15$^{b}$ | 1.30$^{b}$ | 0.205$^{b}$ | 21.25$^{b}$ | 5.82$^{b}$ | 27.06$^{b}$ | 38.66$^{b}$ |
| 0.40 | 2.82$^{a}$ | 9.11$^{b}$ | 10.75$^{b}$ | 9.93$^{b}$ | 1.55$^{a}$ | 0.300$^{a}$ | 26.02$^{a}$ | 8.33$^{a}$ | 34.36$^{a}$ | 49.09$^{a}$ |
| SEM | ±0.165 | ±0.202 | ±0.265 | ±0.223 | ±0.135 | ±0.020 | ±2.376 | ±0.587 | ±2.851 | ±4.073 |
| p. value | 0.01 | 0.01 | 0.001 | 0.001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 |
| **Interaction between selenium sources and levels** | | | | | | | | | | |
| 0.10 SS | 1.90$^{c}$ | 10.65$^{a}$ | 13.26$^{a}$ | 11.96$^{a}$ | 0.47$^{e}$ | 0.090$^{c}$ | 7.21$^{d}$ | 2.42$^{d}$ | 9.64$^{e}$ | 13.77$^{e}$ |
| 0.25 SS | 2.45$^{cb}$ | 9.90$^{ab}$ | 11.55$^{bc}$ | 10.72$^{b}$ | 0.86$^{d}$ | 0.160$^{b}$ | 14.07$^{c}$ | 4.90$^{bc}$ | 18.98$^{d}$ | 27.11$^{d}$ |
| 0.40 SS | 2.743$^{b}$ | 9.60$^{bc}$ | 10.90$^{bc}$ | 10.25$^{bc}$ | 1.05$^{c}$ | 0.296$^{a}$ | 16.26$^{c}$ | 7.83$^{a}$ | 24.09$^{c}$ | 34.42$^{c}$ |
| 0.10 Nano-Se | 2.60$^{cb}$ | 10.02$^{ab}$ | 11.68$^{b}$ | 10.85$^{b}$ | 0.86$^{d}$ | 0.143$^{bc}$ | 14.12$^{c}$ | 3.96$^{cd}$ | 18.08$^{d}$ | 25.83$^{d}$ |
| 0.25 Nano-Se | 3.90$^{a}$ | 8.80$^{c}$ | 10.37$^{c}$ | 9.59$^{c}$ | 1.74$^{b}$ | 0.251$^{a}$ | 28.42$^{b}$ | 6.72$^{ab}$ | 35.15$^{b}$ | 50.21$^{b}$ |
| 0.40 Nano-Se | 2.90$^{b}$ | 8.62$^{c}$ | 10.60$^{bc}$ | 9.62$^{c}$ | 2.07$^{a}$ | 0.304$^{a}$ | 35.78$^{a}$ | 8.84$^{a}$ | 44.63$^{a}$ | 63.75$^{a}$ |
| SEM | ±0.165 | ±0.202 | ±0.265 | ±0.223 | ±0.135 | ±0.020 | ±2.376 | ±0.587 | ±2.851 | ±4.073 |
| p. value | 0.001 | 0.01 | 0.001 | 0.001 | <0.0001 | 0.0001 | 0.0001 | 0.0002 | <0.0001 | <0.0001 |

a,b,c,d: Means in the same column with different superscripts, differ significantly (p<0.05)
N.S: Not Significant (p>0.05); SEM: Standard Error of Means; SS: Sodium selenite; RDA*: 70 µg

of 0.20 and 0.40 ppm of Se from organic source to the layer diet resulted in four and eight times more accumulated Se in albumen if compared to eggs produced by hens fed commercial diets. Moreover, Se content in yolk of eggs treated to be two and three times higher than in yolk of eggs produced by hens fed on a commercial diet (Surai and Sparks, 2001; Gajcevic *et al*., 2009). The linear relationship between Se supplementation and Se content in the albumen; yolk and whole egg (Table 5) agreed with the previous studies on broilers (Cai *et al*., 2012; Hu *et al*., 2012; Zhou and Wang, 2011), those indicated that Se concentrations in serum, liver and breast muscle significantly increased linearly as the dietary Se level increased for either Nano-Se and sodium selenite. Increasing dietary Se of organic source significantly increased Se content in the albumen and yolk linearly, thus resulting the production of Se enriched eggs, two eggs from the hens fed a diet containing 0.40 ppm of Se could supply 100% of RDA (Surai, 2006; Gajcevic *et al*., 2009; Reis *et al*., 2009; Attia *et al*., 2010; Gjorgovska *et al*., 2012). The results of this study indicated that hens would require a diet containing 0.25 ppm Se from Nano-Se to produce Se enriched eggs (35 µg Se/egg) for human health benefits. Consumption of only one egg daily could supply around 50% of the daily required of Se, accordance to American Health Organization calculated daily allowances of Se as70 µg for adult man and 55 µg for adult woman (Surai and Sparks, 2001).

**Glutathione peroxidase activity:** Glutathione peroxidase (GSH-Px) activity significantly higher in blood of hens fed on diet supplemented Nano-Se than sodium selenite source (Table 5). Mohapatra *et al*. (2014) found the same result in blood of layer chicks. Glutathione peroxidase activity in the hens blood is taken as an indicator of Se absorption efficiency from diet. When compared Nano-Se with selenomethionine, it showed similar efficacy in increasing the activities of GSH-Px and thioredoxin reductase, but has much lower toxicity (Zhang *et al*., 2005). Sodium selenite exhibits strong cytotoxicity because in reaction with gluthatione it enables synthesis of super-hydrogen radicals (Seko and Imura, 1997). Concerning effect of Se level, adding 0.25 ppm in the diet increased GSH-Px activity in blood of hens compared with the low level (0.10 ppm) of Se. Among the present experimental treatments, the highest GSH-Px activity in blood of hens was recorded with the hens receiving 0.25 ppm of Nano-Se (3.90 vs 1.90 as control diet, respectively). This improvement may be due to higher availability of Nano-Se. Gajcevic *et al*. (2009) and Wang and Xu (2008) indicated that increased GSH-Px activity in blood as a result of Se dietary, when supplemented Nano-Se at levels between 0.15 and 1.2 ppm in broiler diets.

**Lipid oxidation:** Freshness of eggs is a quality parameter influenced by storage time and environment (temperature and relative humidity). The most common indicators of egg freshness is a parameter of oxidation intensity of yolk lipids. Determination of Malondialdehyde (MDA) concentration indicates the level of fatty acid peroxidation. If concentration of MDA in egg is high, the level of lipid peroxidation will be also high. The MDA content in yolk of fresh eggs and eggs were kept at room temperature(16-13°C) with relative humidity (65-60%) for

15 days are presented in Table 5. The influence of Se source or level and their interaction on the lipid oxidation in yolk was significant. The MDA content in yolk significantly decreased with increasing Se level in the diet for fresh eggs and after 15 days of storing eggs. Selenium supplementation at 0.25 ppm in the diet significantly decreased the MDA content in yolk of the fresh and storage eggs compared with the low level (0.10 ppm). While, showed insignificant difference between 0.25 and 0.40 ppm treatments. A decrease of the MDA content in yolk is related to increase Se content in egg (Table 5), may be due to the enhancement of GSH-Px activity resulting from increasing supplemental dietary Se. The GSH-Px is a major free radical enzyme scavenger, consequently increase in GSH-Px activity enhance the ability to eliminate free radicals (Huang *et al*., 2003). Adding 0.25 ppm Se from Nano-Se significantly reduced the MDA content in yolk of the fresh and storage eggs compared with the control group. In the previous studies, Wang *et al*. (2010) reported that adding 0.3 ppm Se from Se yeast to the hens diet significantly increased GSH-Px activity and reduced the MDA content in yolk compared with the treatment without Se supplementation. Increasing supplement of Se up to 0.4 ppm from Se yeast to layer diets resulted in production of eggs enriched with Se and a prolonged period of egg freshness (Gajcevic *et al*., 2009; Wang *et al*., 2010). Zhou and Wang (2011) and Cai *et al*. (2012) reported that glutathione peroxidase activity and glutathione content significantly higher, in addition to free radical inhibition and lower MDA content in blood of broiler chicks receiving 0.3 ppm Se of Nano-Se compared with the control group. Conversely, Xia *et al*. (2005) reported that no differences were observed in the activities of GSH-Px, blood Se and MDA between the treatments of Nano-Se and sodium selenite when added Se up to 0.30 ppm in broiler diets. But, the chicken had higher activities of GSH-Px and blood Se and lower MDA significantly at concentration range of 0.40-1.0 ppm Se from Nano-Se than sodium selenite. The overall a period, indicated that 0.25 ppm Se from Nano-Se significantly reduced MDA content in yolk (Table 5), thus less the rate of lipid oxidation and improved oxidative stability in the eggs. A reduction of MDA level in yolk can be explained by Huang *et al*. (2003) who indicated that small size Nano-Se with the large surface area had greater ability to transfer electrons to radicals, with a high efficacy for scavenging various free radicals.

**Fatty acid profile of yolk lipids:** The influence of Se source or level and their interaction on fatty acid profile of yolk lipids for storage eggs at room temperature (16-13°C) with relative humidity (65-60%) for 15 days are presented in Table 6. Fatty acid profile of yolk lipids was significantly influenced by Se source. Nano-Se significantly increased concentration of long chain polyunsaturated fatty acids and monounsaturated fatty acids with decreased saturated fatty acids in yolk. As a result, the ratio of saturated to unsaturated fatty acids (SFA/USFA) was significantly decreased. The percentage of arachidonic acid (C20:4$n_6$) in the total fatty acid pool and the ratio between C18:2 $n_6$ and C18:3 $n_3$ were significantly decreased in eggs produced by hens fed on diet supplemented Nano-Se compared to sodium selenite source. These results indicated that, the Nano-Se improved lipid profile in yolk for storage eggs, because of Nano-Se led to higher deposition of Se and reduction of MDA content in yolk (Table 5). Malondialdehyde is one of the final products of polyunsaturated fatty acid peroxidation and is a marker of oxidative stress (Gawel *et al*., 2004). The breast muscle lipid profile can be modified by increased unsaturated fatty acid and decreased saturated fatty acid by adding *Aspergillus awamori* and Nano-Se to broiler diets (Saleh, 2014). Decreasing arachidonic acid level in yolk due to high dietary Se. Selenium increase activity of GSH-Px and enhanced biosynthesis of prostaglandins with the involvement of precursors including arachidonic acid (Hong *et al*., 1989). A higher dietary Se (0.40 ppm) significantly increased total unsaturated fatty acid pool and decreased total saturated fatty acid pool in yolk, consequently reduce SFA/USFA ratio.

In addition to, the ratio between C18:2 $n_6$ and C18:3 $n_3$ was significantly decreased compared with the other levels. These results are in agreement with (Zdunczyk *et al*., 2013) who found the same results in yolk lipids. Also, Haug *et al*. (2007) and Kralik *et al*. (2012) in both breast and thigh muscles tissue. It was explained that higher content of Se in diet affected the activity of $\Delta 6-, \Delta 5-$ and $\Delta 4-$desaturase, which catalyze elongation and desaturation of short-chain fatty acids into long-chain fatty acids, or Se led to reduced speed of long-chain fatty acids degradation in peroxidation processes (Kralik *et al*., 2012). Among the present experimental treatments, Nano-Se at a level 0.25 ppm improved oxidative stability and the lipid profile in yolk during storage through increased unsaturated fatty acid and decreased saturated fatty acid. Furthermore, both of C18:2 $n_6$/C18:3 $n_3$ and SFA/USFA ratios were significantly decreased. Reducing intensity of lipid peroxidation in yolk as shown, attributed to increase Se content in yolk (Table 5). Increased Se caused higher level of antioxidant enzymes may lead to decrease lipid peroxidation, which reduce the rate of polyunsaturated fatty acids degradation by peroxidation (Saleh, 2014). Nano-Se have strong antioxidant properties, the ability to act as an antioxidant with reduced risk of Se toxicity (Wang *et al*., 2007).

**Histopathological examination:** No mortality was resulted during overall the experimental period in any of

Table 6: Effect of different selenium sources and levels on fatty acids (%of the total fatty acid peak area) profile of yolk lipids from storage eggs at room temperature for 15 days

| Treatments | C 8:0 | C 10:0 | C 12:0 | C 14:0 | C 16:0 | C 16:1 n9 | C 16:1 n7 | C 17:0 | C 16:3 n4 | C 18:0 | C 18:1 n9 | C 18:2 n6 | C 18:3 n3 | C 20:4 n6 | SFA | MUFA | PUFA | SFA/ USFA | C18:2 n6 /C18:3 n3 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Effect of selenium sources** | | | | | | | | | | | | | | | | | | | |
| SS | 0.64 | 1.52b | 0.74 | 1.93$^{b}$ | 25.04$^{a}$ | 0.49$^{b}$ | 2.06 | 0.30 | 0.22 | 9.25 | 47.00$^{b}$ | 7.47$^{b}$ | 0.45$^{b}$ | 0.62$^{a}$ | 39.44$^{a}$ | 49.56$^{b}$ | 8.78$^{b}$ | 0.67$^{a}$ | 16.75$^{a}$ |
| Nano-Se | 0.65 | 1.63a | 0.81 | 2.01$^{a}$ | 24.35$^{b}$ | 0.59$^{a}$ | 2.24 | 0.32 | 0.29 | 8.69 | 47.86$^{a}$ | 7.79$^{a}$ | 0.49$^{a}$ | 0.55$^{b}$ | 38.48$^{b}$ | 50.70$^{a}$ | 9.12$^{a}$ | 0.64$^{b}$ | 15.93$^{b}$ |
| SEM (±) | 0.025 | 0.021 | 0.016 | 0.021 | 0.145 | 0.024 | 0.077 | 0.008 | 0.020 | 0.187 | 0.232 | 0.096 | 0.011 | 0.019 | 0.239 | 0.285 | 0.102 | 0.01 | 0.209 |
| p-value | N.S | 0.001 | N.S | 0.02 | 0.005 | 0.01 | N.S | N.S | N.S | N.S | 0.01 | 0.03 | 0.01 | 0.01 | 0.02 | 0.005 | 0.01 | 0.003 | 0.01 |
| **Effect of selenium levels (ppm)** | | | | | | | | | | | | | | | | | | | |
| 0.10 | 0.60 | 1.52$^{b}$ | 0.74 | 1.91$^{b}$ | 25.14$^{a}$ | 0.47$^{b}$ | 1.99 | 0.29 | 0.21 | 9.56$^{a}$ | 46.54$^{b}$ | 7.26$^{b}$ | 0.42$^{b}$ | 0.66$^{a}$ | 39.77$^{a}$ | 49.01$^{b}$ | 8.56$^{b}$ | 0.69$^{a}$ | 17.09$^{a}$ |
| 0.25 | 0.64 | 1.58$^{ab}$ | 0.78 | 1.98$^{ab}$ | 24.63$^{ab}$ | 0.53$^{b}$ | 2.21 | 0.31 | 0.25 | 8.82$^{ab}$ | 47.59$^{a}$ | 7.66$^{a}$ | 0.47$^{a}$ | 0.58$^{b}$ | 38.75$^{b}$ | 50.33$^{a}$ | 8.98$^{a}$ | 0.65$^{b}$ | 16.11$^{b}$ |
| 0.40 | 0.69 | 1.63$^{a}$ | 0.82 | 2.02$^{a}$ | 24.34$^{b}$ | 0.62$^{a}$ | 2.26 | 0.33 | 0.31 | 8.53$^{b}$ | 48.16$^{a}$ | 7.96$^{a}$ | 0.50$^{a}$ | 0.53$^{b}$ | 38.37$^{b}$ | 51.04$^{a}$ | 9.32$^{a}$ | 0.63$^{b}$ | 15.82$^{b}$ |
| SEM (±) | 0.025 | 0.021 | 0.016 | 0.021 | 0.145 | 0.024 | 0.077 | 0.008 | 0.020 | 0.187 | 0.232 | 0.096 | 0.011 | 0.019 | 0.239 | 0.285 | 0.102 | 0.01 | 0.209 |
| p-value | N.S | 0.01 | N.S | 0.04 | 0.02 | 0.01 | N.S | N.S | N.S | 0.05 | 0.003 | 0.002 | 0.001 | 0.004 | 0.02 | 0.001 | 0.001 | 0.001 | 0.01 |
| **Interaction between selenium sources and levels** | | | | | | | | | | | | | | | | | | | |
| 0.10 SS | 0.60 | 1.48$^{c}$ | 0.70 | 1.87$^{c}$ | 25.37$^{a}$ | 0.43$^{c}$ | 1.85 | 0.28 | 0.18 | 9.83$^{a}$ | 46.20$^{c}$ | 7.08$^{c}$ | 0.41$^{c}$ | 0.69$^{a}$ | 40.14$^{a}$ | 48.48$^{c}$ | 8.36$^{c}$ | 0.70$^{a}$ | 17.44$^{a}$ |
| 0.25 SS | 0.63 | 1.51$^{c}$ | 0.75 | 1.94$^{bc}$ | 24.95$^{ab}$ | 0.48$^{bc}$ | 2.06 | 0.29 | 0.21 | 9.27$^{ab}$ | 47.09$^{bc}$ | 7.48$^{bc}$ | 0.44$^{bc}$ | 0.64$^{ab}$ | 39.35$^{ab}$ | 49.64$^{bc}$ | 8.78$^{bc}$ | 0.67$^{ab}$ | 16.81$^{ab}$ |
| 0.40 SS | 0.70 | 1.58$^{bc}$ | 0.79 | 1.98$^{abc}$ | 24.82$^{ab}$ | 0.55$^{abc}$ | 2.27 | 0.33 | 0.28 | 8.65$^{ab}$ | 47.73$^{ab}$ | 7.87$^{ab}$ | 0.49$^{ab}$ | 0.55$^{bc}$ | 38.84$^{abc}$ | 50.55$^{ab}$ | 9.20$^{ab}$ | 0.65$^{bc}$ | 15.99$^{bcd}$ |
| 0.10 Nano-Se | 0.61 | 1.56$^{bc}$ | 0.78 | 1.95$^{abc}$ | 24.90$^{ab}$ | 0.51$^{bc}$ | 2.13 | 0.31 | 0.23 | 9.30$^{ab}$ | 46.89$^{bc}$ | 7.45$^{bc}$ | 0.45$^{bc}$ | 0.63$^{ab}$ | 39.40$^{ab}$ | 49.53$^{bc}$ | 8.75$^{bc}$ | 0.67$^{ab}$ | 16.74$^{abc}$ |
| 0.25 Nano-Se | 0.65 | 1.65$^{ab}$ | 0.80 | 2.02$^{ab}$ | 24.32$^{bc}$ | 0.57$^{ab}$ | 2.35 | 0.33 | 0.29 | 8.37$^{b}$ | 48.09$^{ab}$ | 7.85$^{ab}$ | 0.51$^{a}$ | 0.53$^{c}$ | 38.15$^{bc}$ | 51.02$^{a}$ | 9.19$^{ab}$ | 0.63$^{c}$ | 15.40$^{d}$ |
| 0.40 Nano-Se | 0.68 | 1.69$^{a}$ | 0.85 | 2.08$^{a}$ | 23.86$^{c}$ | 0.68$^{a}$ | 2.25 | 0.32 | 0.35 | 8.42$^{b}$ | 48.60$^{a}$ | 8.06$^{a}$ | 0.52$^{a}$ | 0.52$^{c}$ | 37.92$^{c}$ | 51.53$^{a}$ | 9.44$^{a}$ | 0.62$^{c}$ | 15.66$^{cd}$ |
| SEM (±) | 0.025 | 0.021 | 0.016 | 0.021 | 0.145 | 0.024 | 0.077 | 0.008 | 0.020 | 0.187 | 0.232 | 0.096 | 0.011 | 0.019 | 0.239 | 0.285 | 0.102 | 0.01 | 0.209 |
| p-value | N.S | 0.01 | N.S | 0.05 | 0.01 | 0.01 | N.S | N.S | N.S | N.S | 0.01 | 0.01 | 0.002 | 0.01 | 0.03 | 0.002 | 0.004 | 0.001 | 0.01 |

[a,b]Means in the same column with different superscripts, differ significantly ($p<0.05$).

N.S: Not Significant ($p>0.05$); SEM: Standard Error of Means, SS: Sodium selenite; SFA: total saturated fatty acids, MUFA: total monounsaturated fatty acids; PUFA: total polyunsaturated fatty acids. SFA/USFA: Saturated to unsaturated fatty acids ratio, C18:2 n6/C18:3 n3: linoleic acid to linolenic acid ratio

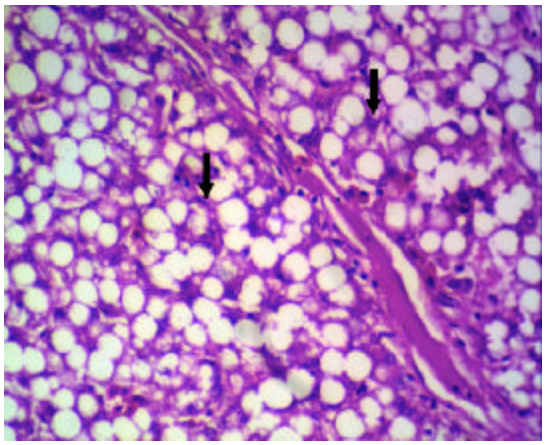

Fig. 5: Liver of treatment 1 showing fatty change in hepatocytes. H and EX400

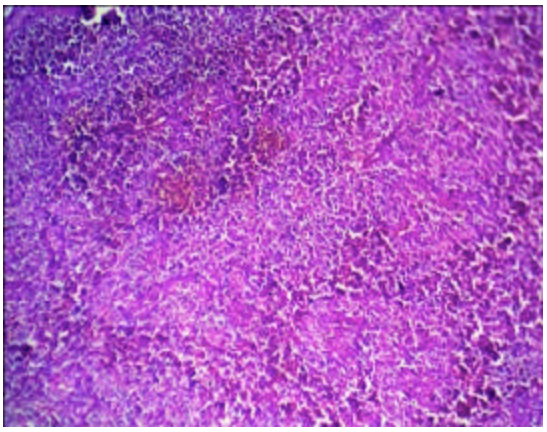

Fig. 6: Spleen of treatment 1 showing normal histological appearance. H and EX400

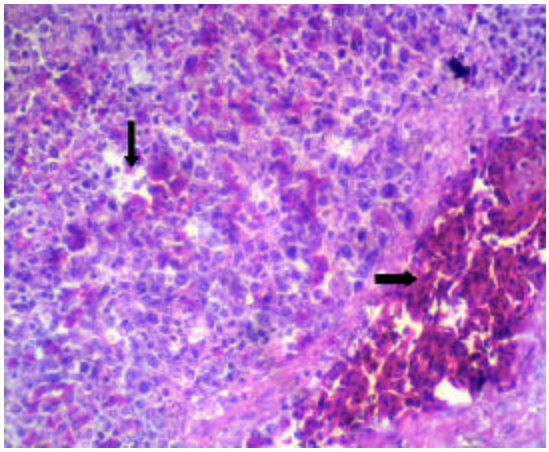

Fig. 7: Liver of treatment 2 showing congestion of blood vessels and sinusoids.H and EX400

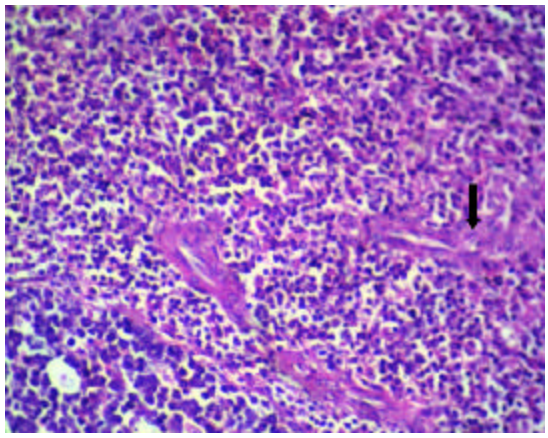

Fig. 8: Spleen of treatment 2 showing depletion of germinal center. H and E X 400

the experimental treatments and no alterations were observed in the behavior hens of all treated groups compared to the control group. Tissues of liver and spleen were chosen for histopathological examination for all experimental treatments. The histopathological examination can be summarized as a following; using sodium selenite as Se source in layer diets resulted that the liver of hens in control group, showed fatty changes in most of hepatocytes.

The hepatocytes appeared enlarged with presence of large vacuole occupy the cytoplasm, (Fig. 5). While, the spleen did not showed any pathological changes, (Fig. 6). The liver of treatment 2, showed congestion of blood vessels and sinusoids. The hepatic plates appeared dissociated from each other, (Fig. 7). While their spleen showed slight depletion of lymphoid follicles, (Fig. 8). For treatment 3, the liver showed different pathological changes represented by fatty change, (Fig. 9). The spleen showed severe congestion in the red pulp, while white pulp showing slight depletion of lymphoid follicles, (Fig. 10). On the other side using Nano-Se as Se source in layer diets resulted in treatment 4, the liver showed congestion of central vein. Most of hepatocytes were suffered from fatty changes, (Fig. 11). While, the only change observed in the spleen of this group was congestion of blood vessels in red pulp, (Fig. 12). The liver of treatment 5, showed fatty changes in most of hepatocytes and focal aggregation of inflammatory cells around the portal area, (Fig. 13) and the only change observed in the spleen of this group was congestion of blood vessels in red pulp, (Fig. 14). Finally, there was no pathological changes observed in liver of treatment 6, except focal aggregation of inflammatory cells replaced some hepatocytes, (Fig. 15) and no pathological changes could be detected in the spleen of these treatment, (Fig. 16). In conclusion, the main histopathological findings of livers for all Nano-Se

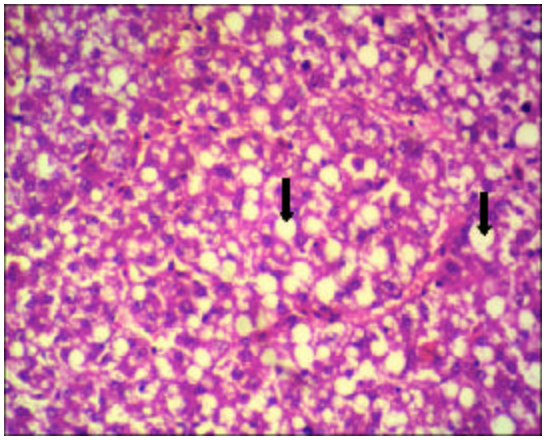

Fig. 9: Liver of treatment 3 showing fatty change in hepatocytes. H and EX400

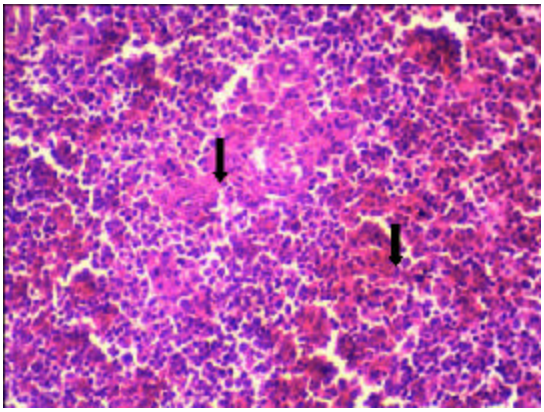

Fig. 10: Spleen of treatment 3 showing congestion of blood vessels and slight depletion of lymphoid follicles. H and E X 400

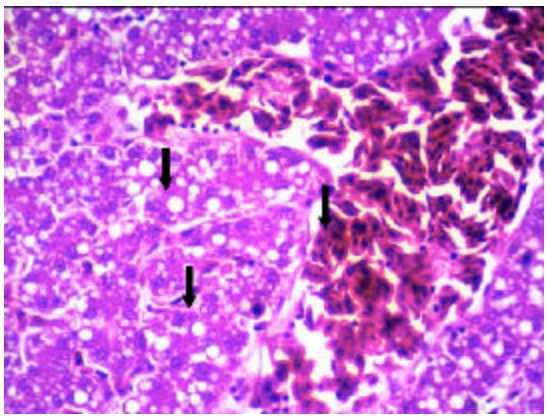

Fig. 11: Liver of treatment 4 showing congestion of central vein and fatty change in most of hepatocytes. H and E X 400

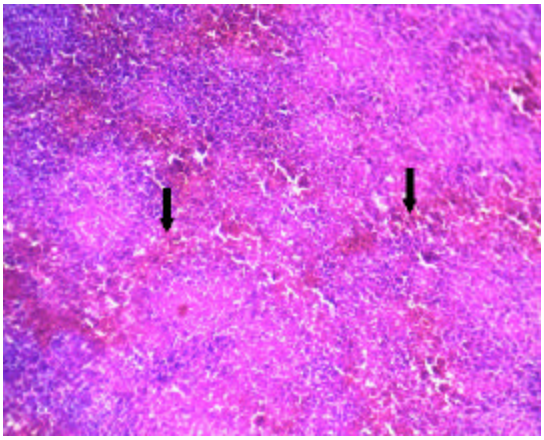

Fig. 12: Spleen of treatment 4 showing congestion of blood vessels. H and E X 400

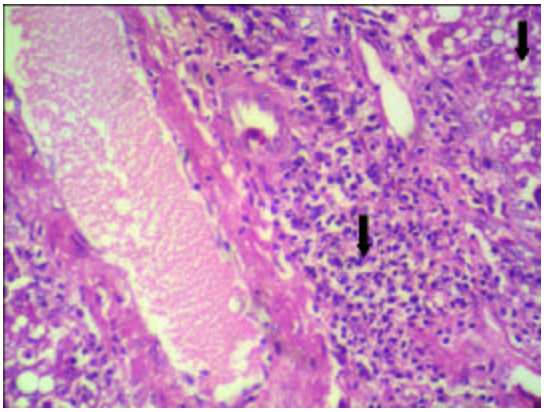

Fig. 13: Liver of treatment 5 showing focal aggregation of inflammatory cells around the portal area. H and E X 400

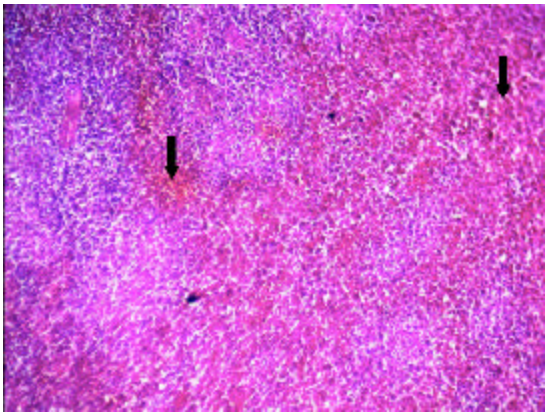

Fig. 14: Spleen of treatment 5 showing congestion of blood vessels in red pulp. H and E X 400

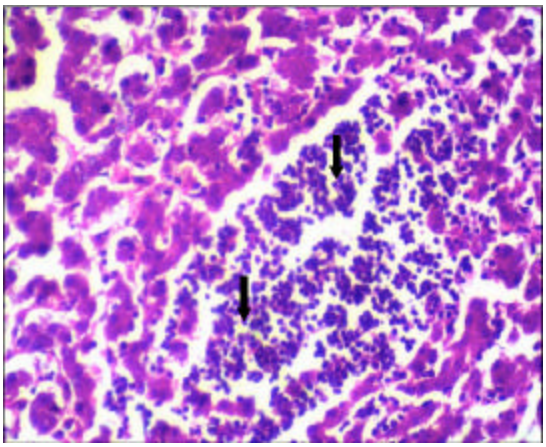

Fig. 15: Liver of treatment 6 showing focal aggregation of inflammatory cells replaced some hepatocytes. H and E X 400

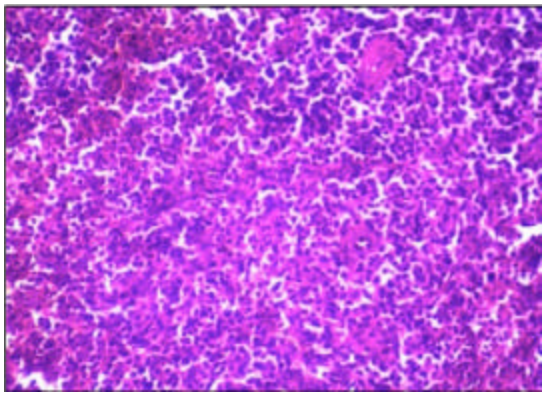

Fig. 16: Spleen of treatment 6 showing normal histological appearance. H and E X 400

and sodium selenite treated groups were fatty liver with focal aggregation of inflammatory cells. While, the spleen showed congestion of blood vessels. These finding were in agreement with previous study, Dehkordi (2014) who reported that Nano-Se and sodium selenite were fed orally to rats with a concentration 0.10 mg/kg BW for 20 days to evaluate the liver histopathology. The results showed that, changes in the hepatocytes and the sinusoids.

The changes in the hepatocytes were principally summarized as hyperemia into sinusoids and inflammatory cells infiltration. The appearance of lymphocytic infiltration with extravasation of red blood cells in the liver tissue may lead that Nano-Se and sodium selenite interfere with the antioxidant defense mechanism, conducting to reactive oxygen species production which in turn, may stimulate an inflammatory response, (Dehkordi, 2014). While, Benko *et al*. (2012) studied the toxicity of Se sources in mice. Those authors used inorganic sodium selenate and sodium hydroselenite, elementary Nano-Se (particle size, 100-500 nm), organic Sel-Plex and Lacto-Micro Selenium were administered for 14 days at concentrations of 0.5, 5 and 50 ppm of Se. High Se concentrations (50 ppm) caused multiple toxic effects in mice. The histopathological examination of liver tissues indicted that selenate is the most toxic Se compound, followed by selenite. The comparison of the Se compounds showed that Nano-Se was more toxic than Sel-Plex and Lacto-Micro Selenium. This discrepancy could be related to the difference in particle size of Nano-Se preparations, indicating that the larger nano particles could be more toxic than the smaller ones. These results showed the importance of using Nano-Se in particle size less than 100 nm. Peng *et al*. (2007) suggested that Nano-Se is a more effective chemopreventive agent at a smaller nano-particle size.

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