



Antifibrotic candidates of Selenium nanoparticles and selenium in the experimental model

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ABSTRACT

This research was designed to compare the efficacy of selenium in nanoscale (SeNPs) with its free form (Se) against liver fibrosis induced by thioacetamide (TAA) in rats. In a completely randomized design, 60 adult female rats were distributed as: Group (1) control (received saline) and other three groups received TAA to induce liver fibrosis (100 mg/kg b.wt of three times a week for 6 weeks). Fifteen rats were termed TAA (Group 2). Rats in group (3) were simultaneously administered SeNPs (0.48 mg/kg/b.wt) orally (TAA+SeNPs). Rats in group (4) were simultaneously administered Se (0.48 mg/kg/b.wt) orally (TAA+Se). TAA injection enhanced liver enzymes activity, oxidative stress markers and inflammatory mediators, while suppressed the activity of the antioxidant enzymes activity versus the control group. SeNPs as well as Se supplementation blunted liver enzymes activity, oxidative stress indicators and inflammatory intermediates, while potentiated the activity of the antioxidant enzymes relative to TAA group. Histological investigation of liver tissue appreciated the biochemical findings. Aforementioned data clearly indicate that the mitigation of oxidative stress and inflammation may be the probable mechanisms by which SeNPs or Se can offer their antifibrotic action. Worth mentioning, SeNPs showed superior effect above Se in its free form in this respect.

INTRODUCTION

Liver fibrosis, an exacerbated wound-healing response to a variety chronic stimuli, is manifested by the immoderate production of extracellular matrix (ECM) proteins particularly type I collagen. It is a character of the most of chronic liver diseases involving non-alcoholic steatohepatitis (NASH), chronic viral hepatitis and alcohol abuse (Scott *et al.*, 2015). This chronic process distorts hepatic architecture, disturbs normal function, and may lead to the formation of cirrhosis associated with morbidity and mortality (Xiaoling *et al.*, 2015). The risk of hepatic fibrosis and complications associated with liver cirrhosis; including ascites, portal hypertension, encephalopathy, liver

failure, and hepatocellular carcinoma, evolve a substantial burden on individual, society, and health care system (He *et al.*, 2015). Activated hepatic stellate cell (HSC) is a key effector cell in the hepatic fibrosis progression. HSCs are activated from quiescent cells to myofibroblast-like cells, which is accompanied with obvious phenotypic alterations, including increased cell proliferation, expression of α -smooth muscle actin (α -SMA) and overproduction of extracellular matrix (ECM) (Novo *et al.*, 2014). Oxidative stress reflects an imbalance between generation and clearance of free radicals, such as reactive oxygen species (ROS), nitric oxide (NO) and lipid peroxidation end products (He *et al.*, 2015). Increasing evidence supports the opinion that oxidative stress is a crucial promotor in HSC activation and hepatic fibrogenesis (Rosenbloom *et al.*, 2013). Inflammation can also cause HSC activation and result in liver fibrosis. Products of the inflammatory cascade like: TNF- α , IL-6, IL-1 β and COX-2 are elevated, independently of the etiology, in liver diseases (Amara *et al.*, 2015).

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The pro-inflammatory cytokines; TNF α is a potent cytokine that exerts pleiotropic inflammatory function *via* triggering downstream signaling cascade leading to hepatic fibrosis (Osawa *et al.*, 2013). Levels of circulating TNF α are increased in patients with liver fibrosis and are associated with poor prognosis (Amara *et al.*, 2015). Thus, any approaches that attenuate its production, receptor activation, or downstream signal transduction should inhibit HSC activation and its subsidiary events (Wu and Zern, 2000). Selenium (Se) is an essential micronutrient with powerful antioxidant properties as it is an essential ingredient of some oxido-reductase enzymes (Ding *et al.*, 2010). Most notable is Se-dependent glutathione peroxidase enzyme (Se-GSH-P $_x$) which catalyses the reduction of hydrogen peroxide (H $_2$ O $_2$) to water (H $_2$ O) by transformation of reduced glutathione (GSH) to its oxidized form (GSSG). Se and Se-GSH-P $_x$ deficiency are associated with higher levels of ROS and lipid peroxidation, which can be reversed by Se supplementation (Ding *et al.*, 2010). Noteworthy, the Se, like all biologically essential trace elements, might be toxic when provided at excess levels of Se might be toxic, and the maximum safe levels for selenium have been set at 0.5 mg/Kg in the European Union (2004) and China (Ministry of Agriculture, 2010) and 2.0 mg/kg for the United States (AAFCO, 2011).

Selenium nanoparticles (SeNPs) have attracted widespread attention because of nanometer particulates display novel features as large surface area, high surface activity, high catalytic potential, powerful adsorbing ability, and low toxicity (Zhang *et al.*, 2008) paralleled by strong antioxidant activity (Zheng *et al.*, 2012). It has been mentioned that SeNPs possess physiological properties similar to selenite and Se-methylselenocysteine in activation but with minimal toxicity (Zheng *et al.*, 2012). Therefore, the objective of this research study was to estimate the antifibrotic activity of SeNPs in comparison with free form of Se in an experimental model of liver fibrosis.

MATERIALS AND METHODS

Preparation of SeNPs

SeNPs was prepared according to Zhang *et al.* (2001) and Wang *et al.* (2007). By using bovine albumin protein as a disperser, elemental selenium ions were produced *via* reducing 25 mM sodium selenite by 25 mM glutathione (GSH). This leads to an aggregation of Se into particles 88.23-92.00 nm in size (SeNPs), forming nano red elemental Se and oxidized glutathione (GSSG). The red solution was dialyzed to separate GSSG from the SeNPs. The final solution containing SeNPs was lyophilized and stored at room temperature.

Characterization of SeNPs

The produced SeNPs were characterized by TEM. According to Chen *et al.* (2008), TEM samples were prepared by dispersing the powder particles onto holey carbon film on copper grids. Then, the micrographs were obtained on TEM (Philips CM-10, FEI Inc., Hillsboro, OR, USA) as shown in Fig.(1).

Rats

Sixty adult female albino rats of Wistar strain (150 \pm 10 g) were obtained from a breeding stock preserved in the Animal House of the National Research Centre, Egypt. They were kept in a group of 5 in polypropylene cage, housed in animal facility in an environmentally conditioned room with respect to light, temperature and air humidity and fed with standard rodent chow *ad libitum* and water. All rats were accommodated with these laboratory conditions for at least two weeks before the commencement of the experiment and they were maintained under the same conditions all over the experiment. All procedures were done with proper approval of Animal care and Ethics Committee of Medical Research of the National Research Centre, Giza, Egypt.

Experimental Setting

Rats were randomly classified into four groups (15 rats for each). The first group received 0.5 ml saline solution intraperitoneally three times a week for 6 weeks and served as normal control group (control). The rats in the second group were injected intraperitoneally (i.p) with 100mg/Kg b.wt of TAA dissolved in saline three times a week for 6 weeks (Strand *et al.*, 2008) (TAA group). The rats in the third group were injected i.p with TAA and simultaneously administered orally with SeNPs 3 mg/Kg b.wt three times/week for 6 weeks (TAA+ SeNPs group). The rats in the fourth group were injected i.p with TAA and simultaneously administered orally with 3 mg/ Kg b.wt Se three times/week for 6 weeks (Heikal *et al.*, 2012) (TAA + Se group).

After completion of the round, the diets were withheld from the experimental rats for 12 hours and then blood samples were immediately withdrawn from the retro-orbital venous plexus under diethyl ether anesthesia, left for 15 min., centrifuged at 1899 xg for separation of serum. After that, the rats were sacrificed by cervical dislocation and the liver was dissected carefully weighed and blotted dry. Then, each liver was divided sagittally into two portions; the first one was immediately homogenized in phosphate buffer (pH 7.4), centrifuged at 1800 xg and the supernatant was obtained for biochemical analysis. The second portion was used for histopathological examination and stained by hematoxylin & eosin stain for examination through the light microscope (Banchroft *et al.*, 1996).

Biochemical analyses

Determination of liver enzymes

Serum aspartate transaminase (AST) and alanine transaminase (ALT) activities were determined colorimetrically using kits purchased from Quimica Clinica Aplicada S.A., Spain according to the method described by Reitman and Frankel (1957). Serum alkaline phosphatase activity (ALP) was measured colorimetrically according to the method of Bowers and Mc Comb (1966) using kit obtained from Stanbio Laboratory Kit, USA. Serum gamma-glutamyl transferase (GGT) activity was measured according to the method of Tietz (1995) using kit obtained from Reactivos GPL Co. Ltd (Barcelona).

Determination of oxidative stress parameters and glutathione content

Homogenate liver was prepared in 50 mM Tris-HCl and 300 mM sucrose, pH 7.4 using homogenizer to give 10% homogenate. This homogenate was used for determination of malondialdehyde (MDA) by reaction with thiobarbituric acid (Ohkawa *et al.*, 1979), nitric oxide (NO) by optimized acid reduction method (Green *et al.*, 1982) and glutathione contents by the reduction of Elman's reagent (5,5'-dithiobis (2-nitrobenzoic acid) "DTNB") (Ellman, 1959).

Determination of enzymatic antioxidants

Homogenate liver was used for assessment of superoxide dismutase (SOD) by inhibiting phenazine methosulphate-mediated reduction of nitroblue tetrazolium (NBT) dye (Nishikimi *et al.*, 1972), catalase (CAT) by reaction with known quantity of H₂O₂ (Aebi, 1984), glutathione peroxidase (GPx) by the recycling of oxidized glutathione (GSSG) to its reduced state (Paglia and Valentine, 1967) and glutathione reductase (GR) by catalyzing the reduction of glutathione in the presence of NADPH (Factor *et al.*, 1998).

Determination of cytokines

Prostaglandin E2 (PGE2), prostaglandin F2 α (PGF2 α), tumor necrosis factor-alpha (TNF- α) and angiogenin (Ang) were quantified in liver homogenate using ELISA kits obtained from Abcam Company (Cambridge, UK) according to the manufacturer's instructions.

Histopathological method

Autopsy samples from the second portion of liver of each rat were taken in the different groups and fixed in 10% formalin saline for 24 h. Washing was performed by tap water, then serial dilutions of alcohol (methyl, ethyl and absolute ethyl) were used for dehydration. Specimens were cleared in xylene and embedded in paraffin at 56° in hot air for 24 h. Paraffin bees wax tissue blocks were prepared for sectioning at 4 microns thickness by sledge microtome. The obtained tissue sections were collected on glass slides, deparaffinized, stained by hematoxylin & eosin stain then examination was done through the light microscope (Banchroft *et al.*, 1996).

Statistical analysis

All data were subjected to a one-way analysis of variance ANOVA, and the significance of the differences between means was tested using Tukey's honestly significant difference test ($p < 0.05$). The software used was SAS, version 9.1 (Statsoft Inc., Tulsa, OK). Values are expressed as means \pm standard error.

RESULT AND DISCUSSION

Biochemical results

The current data show significant elevation in serum AST, ALT, ALP and GGT activities in rats upon TAA administration versus the controls. Whereas, the enzyme activities of AST, ALT, ALP and GGT revealed significant reduction as a result of the treatment with SeNPs (-27.74%, -43.13%, -31.96% and -30.18%, respectively) and Se (-23.66%, -38.63%, -28.55% and -22.86%, respectively) in comparison with TAA-challenged group (Table 1). The tabulated results in Table (2) show significant rise in hepatic MDA and NO levels paralleled by significant drop in hepatic GSH level in TAA administered group relative to the control group. Meanwhile, hepatic MDA and NO levels revealed significant decline in the group treated with SeNPs (-13.70% and -49.71% respectively) and that treated with Se (-7.31% and -43.88% respectively) as compared with TAA-challenged group. Treatment with SeNPs or Se also revealed significant elevation in hepatic GSH level (77.02% and 59.39% respectively) as compared to TAA-challenged group. In the present study, TAA administration evoked significant reduction in liver antioxidant enzymes (CAT, SOD, GRd and GPx) activity versus the control group. Whereas, the activities of the antioxidant enzymes revealed significant up regulation in the group treated with SeNPs (63.41%, 13.43%, 42.26% and 37.56% respectively) and that treated with Se (56.1%, 11.81%, 24.62% and 21.09% respectively) as compared with TAA-challenged group (Table 3). The existing results show significant increase in liver prostaglandin F2 α , prostaglandin E2, angiogenin (Ang) and TNF- α levels in TAA administered group in respect to the corresponding values in the control group. However, treatment with SeNPs or Se evoked significant downregulation in liver prostaglandin F2 α (-7.96% and -2.78% respectively), prostaglandin E2 (-15.13% and -7.94% respectively), Ang (-42.26% and -32.7% respectively) and TNF- α (-29.78% and -23.8% respectively) relative to the corresponding values in TAA-challenged group (Table 4).

Table 1: Influence of treatment with SeNPs and Se on TAA-induced alterations in liver enzymes of rats.

Groups	ALT (U/L)	AST (U/L)	ALP (U/L)	GGT (U/L)
Control	64.60 \pm 3.36	85.50 \pm 2.33	129.70 \pm 4.23	3.75 \pm 0.34
TAA	139.03 \pm 5.87 ^a	127.46 \pm 5.04 ^a	199.58 \pm 3.33 ^a	5.60 \pm 0.45 ^a
TAA + SeNPs	79.07 \pm 4.00 ^b	92.10 \pm 2.06 ^b	135.80 \pm 5.10 ^b	3.91 \pm 0.22 ^b
TAA + Se	85.32 \pm 3.62 ^b	97.30 \pm 4.76 ^b	142.60 \pm 3.10 ^b	4.32 \pm 0.40 ^b

Table 2: Influence of treatment with SeNPs and Se on TAA-induced devastating effect on the oxidant/antioxidant markers in liver tissue of rats.

Groups	GSH (mmol/g tissue)	MDA (nmol/g tissue)	NO (μ mol/g tissue)
Control	0.0957 \pm 0.02	275.11 \pm 13.32	205.89 \pm 5.22
TAA	0.0692 \pm 0.01 ^a	321.20 \pm 12.73 ^a	495.08 \pm 11.00 ^a
TAA + SeNPs	0.1225 \pm 0.04 ^b	277.18 \pm 4.73 ^b	248.96 \pm 9.51 ^b
TAA + Se	0.1103 \pm 0.02 ^b	297.72 \pm 3.93 ^b	277.84 \pm 12.55 ^b

Table 3: Influence of treatment with SeNPs and Se on TAA-induced detrimental impact on hepatic antioxidant enzymes activity of rats.

Groups	SOD (U/g tissue)	CAT (U/g tissue)	GPx (U/g tissue)	GRd (μ mol/g tissue)
Control	2213.54 \pm 35.81	0.52 \pm 0.008	980.15 \pm 13.19	2089.88 \pm 71.89
TAA	1913.21 \pm 7.38 ^a	0.41 \pm 0.004 ^a	870.78 \pm 29.96 ^a	1488.52 \pm 74.43 ^a
TAA + SeNPs	2170.10 \pm 17.82 ^b	0.67 \pm 0.004 ^b	1238.74 \pm 50.36 ^b	2047.65 \pm 54.34 ^b
TAA + Se	2139.26 \pm 2.30 ^b	0.64 \pm 0.008 ^b	1085.18 \pm 33.02 ^b	1802.46 \pm 81.52 ^b

GRd : glutathione reductase; GPx : glutathione peroxidase

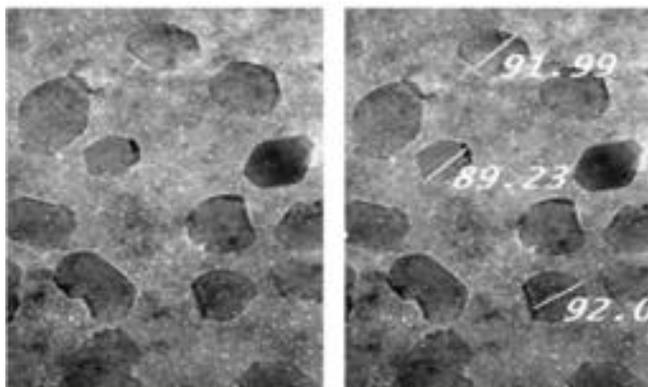
Table 4: Influence of treatment with SeNPs and Se on TAA-induced overshooting of liver inflammatory mediators of rats.

Groups	Prostaglandin F2 α (pg/g tissue)	PGE2 (pg/g tissue)	Ang (pg/g tissue)	TNF α (pg/g tissue)
Control	57.12 \pm 1.75	719.53 \pm 16.19	20.59 \pm 0.98	74.28 \pm 3.24
TAA	70.39 \pm 1.25 ^a	1018.15 \pm 23.88 ^a	38.90 \pm 1.53 ^a	282.27 \pm 12.29 ^a
TAA + SeNPs	64.79 \pm 1.85 ^b	864.11 \pm 17.83 ^b	22.46 \pm 0.49 ^b	198.21 \pm 7.10 ^b
TAA + Se	68.45 \pm 1.47 ^b	937.33 \pm 19.22 ^b	26.18 \pm 1.21 ^b	215.10 \pm 22.76 ^b

Ang : Angiogenin

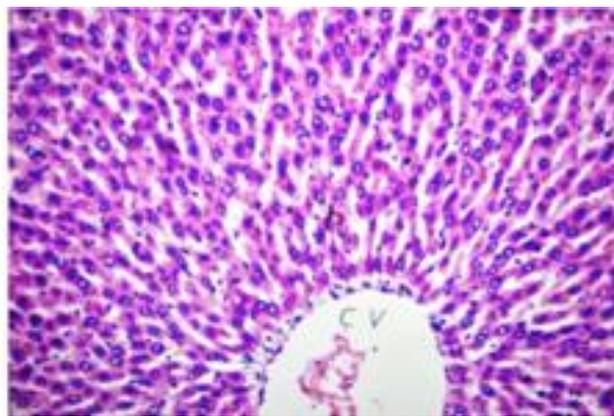
Histological findings

Microscopic examination of liver tissue section of control rat shows normal histological structure of the central vein and the surrounding hepatocytes in the parenchyma as well (Fig-1). Fig. (2) represents photomicrograph of liver tissue section of TAA-administered rat showing fibrosis with inflammatory cells infiltration in between the multiple numbers of newly formed bile duct in portal area that extended between the hepatocytes to form lobules with congesting portal vein. Also, microscopic investigation of liver tissue section of rat administered TAA shows oval cells hyperplasia (Fig. 3). Fig.(4) records photomicrograph of liver tissue section of rat treated with SeNPs showing inflammatory cells infiltration in portal area. Fig.(5) illustrates the photomicrograph of liver tissue section of rat treated with Se showing inflammatory cells infiltration in portal area and between the hepatocytes with congestion of central and portal veins.

**Fig. 1:** High-resolution transmission electron microscopy (TEM) image of SeNPs.

TAA experimental model represents liver fibrosis mimic to human nonbiliary liver diseases. From this pre-clinical animal model, various essential pathological processes and the contributed mechanisms of liver fibrosis can be cleared and participated in the development of novel diagnostic and therapeutic strategies for this disease. TAA is metabolically transformed into thioacetamide-S-oxide (TASO), acetamide and sulfate through microsomal oxidase

system. TASO has been found to cause centrilobular hepatic necrosis. A further metabolism causes biotransformation of TASO to thioacetamide-S, S-dioxide (TASO₂), which covalently binds to proteins forming acetylimidolysine derivatives that act as hepatotoxic compounds (Chen *et al.*, 2015). Metabolism of TAA generates reactive compounds that increase the oxidative stress which is responsible for the damage of liver cells, in parallel with the leakage of AST, ALT, ALP and GGT from the destroyed liver cells (Czechowska *et al.*, 2015). The amount of these cellular enzymes present in the blood reflects the alteration in hepatic cells plasma membrane integrity and/or permeability (Chen *et al.*, 2008).

**Fig. 2:** Optical micrograph of a cross-sectioned liver tissue of rat in the control group showing normal histological structure of the central vein and the surrounding hepatocytes in the parenchyma (H. E x40).

Reddy *et al.* (2004) cited that the hepatotoxic compound yields from the binding of TASO₂ with tissue macromolecules is responsible for the excessive production of oxidative stress in association with considerable reduction in the glutathione (GSH) pool in the liver. The reduction of intracellular GSH due to TAA administration as shown in the present study has been explained by Fontana *et al.* (1996) as they reported that the active metabolite of TAA can combine with the sulphahydryl groups of proteins resulting in rapid depletion of intracellular GSH concentration. Therefore, the decline of GSH becomes one of the most important

toxic effects of TAA which motivates excessive generation of ROS at the mitochondrial level leading to damage of cellular components.

Depletion of endogenous GSH leads to overproduction of H₂O₂ which is very toxic compound and in the presence of transition metal such as iron; it can generate a highly toxic hydroxyl ions that induce lipid peroxidation. This mechanism explains the burden of TAA on hepatic cells following its metabolism into highly reactive metabolites which elicit the denaturation of cellular biomolecules like lipids resulting in lipid peroxidation and its by product (Cheng-Haung *et al.*, 2004). Overproduction of lipid peroxidation product leads to destabilization in cellular lipid substances and motivation of oxidative damage especially of membrane structures.

Several lines of evidences indicated that exposure of cells to H₂O₂ upregulates eNOS expression and NO production (Drummond *et al.*, 2000). This represents the underlying mechanism by which TAA could elevate hepatic NO level in the present work. The major components of the antioxidant protective system in the mammalian cells are the following enzymes; CAT, SOD, GPx and GRd. These enzymes act as a mutually supportive team for defense against ROS (Salama *et al.*, 2013). The suppressed activity of SOD and CAT in liver upon TAA administration might be due to the enhanced lipid peroxidation and/or structural and functional inactivation of these antioxidant enzymes due to overproduction of free radicals (Georgieva *et al.*, 2004). The observed drop in hepatic GSH-Px and GRd activity in TAA administered rats could be attributed to the decreased availability of intracellular GSH. The detectable inhibition in the activity of hepatic SOD, CAT, GPx and GRd indicate hepatic damage in rats following administration of TAA.

NF- κ B acts as a key candidate of fibrosis in HSCs and/or hepatic myofibroblasts (HMF). Luedde and Schwabe (2011) suggested that NF- κ B regulates three key aspects of HSC and/or hepatic myofibroblast biology; activation, survival and inflammatory responses. NF- κ B potentiates hepatic fibrosis due to several actions; direct fibrogenic action, antiapoptotic action and the secretion of macrophage-recruiting chemokines. The intensified activation of NF- κ B in HSC/HMF may also implicate to a tumor-friendly microenvironment in the fibrotic liver (Appel *et al.*, 2015). A wide range of proinflammatory mediators can promote NF- κ B in HSC/HMF including LPS, TNF- α , IL-1 β , angiotensin II and CD40L (Seki *et al.*, 2007). Also, NF- κ B can be activated by the generation of ROS in the liver (Hyoudou *et al.*, 2007). The induction of NF κ B in association with the activation of HSC often relates to liver damage because it imposes a constraint on HSC apoptosis, further leading to aggregating hepatic fibrosis (Czechowska *et al.*, 2015). Wen *et al.* (2014) stated that tissue damage occurred after TAA intoxication is accompanied by significant rise in the proinflammatory modulators. These investigators suggested that such changes are due to activation of NF- κ B. Therefore, the current data of increasing PGF2 α , PGE₂, ang and TNF- α in liver tissue upon TAA administration could be derived from the motivation of NF- κ B signaling. Selenium is an

essential trace element that is presented in the body as Se-containing proteins (selenoproteins), which contain a selenocysteine group instead of the sulfur-containing cysteine. The well-characterized selenoproteins are GPx and thioredoxin reductase (Min-Chang *et al.*, 2014). Selenoproteins perform variety of important physiological tasks. The current biochemical analysis and histological examination indicated that selenium in its two forms could suppress hepatic fibrogenesis and restore liver functions. These findings are in harmony with those of He *et al.* (2004) who stated that selenium has powerful influences on hepatic fibrosis in rats by improving immunity and inhibiting NF- κ B and TGF- β ₁ expression. Se posses anti-inflammatory effects through regression of the proinflammatory mediators, likes TNF- α and IL-1 β and retraction of NF- κ B which has a positive correlation with the other proinflammatory mediators (Min-Chang *et al.*, 2014). In addition, selenium is able to protect hepatic cells from oxidative damage via its free radical scavenging activity (Shafik and El Batsh, 2016) plus its ability to enhance the antioxidant protective system (Newairy *et al.*, 2007; Jihen *et al.*, 2009). Through this way, selenium could reverse liver enzymes activity (AST, ALT, ALP and GGT) in serum (Messarah *et al.*, 2012). The inhibition of the liver enzymes activity in the blood confirms that selenium can maintain the normal structural and architectural integrity of hepatocytes by restricting the leakage of these enzymes. This evidenced the membrane-stabilizing property of selenium.

Nanotechnology is the field of science that deciphers the properties of materials at the nanoscale level. It has proved to be a great boon for modern day science and can be applied to obtain efficacious physicochemical, mechanical, and biological properties of various elements (Sarkar *et al.*, 2015). Pelyhe and Mézes. (2013) and Wang *et al.* (2013) mentioned that selenium in nanoscale has a reduced toxic impact. Moreover, these investigators proved that the preparation of selenium in the form of nanoselenium enhances selenium bioavailability in the body. The present study demonstrated that SeNPs have superior effect in mitigating liver injury and fibrosis comparative to the free form of Se. This was documented by the markedly blunted levels of liver enzymes in serum of SeNPs-treated rats. The present observation was in keeping with the previous finding of Wang *et al.* (2007). SeNPs with small their size and large surface area had more atoms exposed to free radicals for the electron exchanger, with a high potential for scavenging multiple free radicals relative to that in the larger sized antioxidant enzymes specially SOD and GPx (Wang *et al.*, 2013). Thus, SeNPs could strongly repress the oxidative stress markers and restore the non-enzymatic and enzymatic antioxidants in the liver of the treated rats. This is also the second evidence for the powerful activity of SeNPs against liver fibrosis. In addition, SeNPs possess a potent anti-inflammatory capacity which enables them to modulate pro-/anti-inflammatory cytokine profile. The mechanism behind this property depends partially on the inhibition of NF- κ B activation (Wang *et al.*, 2014). This explains the ability of SeNPs to potentially abrogate hepatic proinflammatory mediators and to

recover the liver structural organization of the treated rats as shown in biochemical and histological findings of the current study. This is the third document for the strong ability of SeNPs to mitigate liver fibrosis. On the histological point of view TAA administration showed necrosis with inflammatory cells infiltration and fibrosis (Fig. 2&3). These findings agree with the previous report of Anbarasu *et al.* (2012) who observed that TAA supplementation caused liver toxicity distinguished by centrilobular necrosis along with various gradations of fatty changes comprising of tiny to large sized vacuoles.

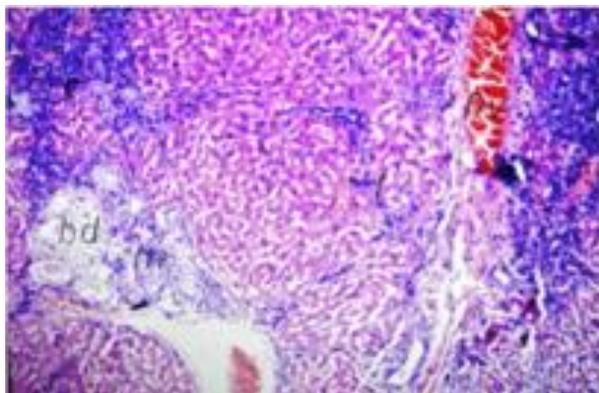


Fig. 3: Optical micrograph of a cross-sectioned liver tissue of rat in TAA group showing fibrosis (f) with inflammatory cells infiltration (m) in between the multiple numbers of newly formed bile duct (bd) in portal area and extended between the hepatocytes forming a lobules with congestion in portal vein (pv) (H. E x10).

Treatment with SeNPs resulted in the disappearance of liver fibrosis but the inflammatory cells infiltration in portal area as shown in Fig. (4). Examination of liver tissue sections of rats Se-treated group are still present showed inflammatory cells infiltration in portal area and between the hepatocytes with congestion of central and portal veins (Fig. 5). Bhattacharjee *et al.* (2014) confirmed these findings as they mentioned that SeNPs and Se can effectively decrease the degree of hepatic fibrosis and support the recovery process (Ding *et al.*, 2010).

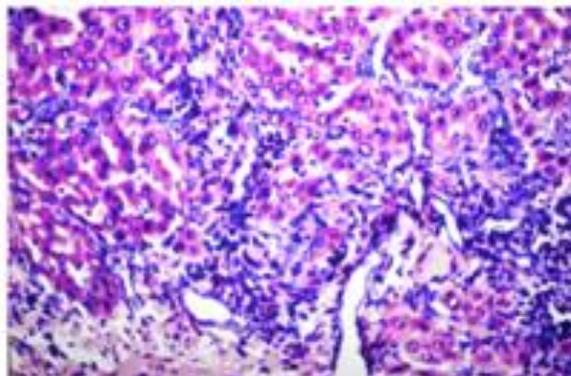


Fig. 4: The magnification of (Figure 3.) showing oval cells hyperplasia (c) (H. E x40).

In conclusion, the outcomes of the current research study encourage the use of selenium either in free form or in

nanoformulation as antifibrotic candidate. The underlying mechanisms for this effect include: 1) membrane stabilizing capacity, 2) free radical scavenging activity, 3) antioxidative potential and anti-inflammatory action. Notably, SeNPs showed superior potency than Se in our study and the main cause could be attributed to the small sized particles, large surface area and increased bioavailability.

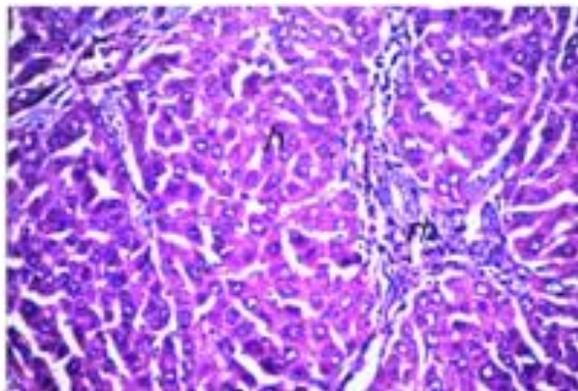


Fig. 5: Optical micrograph of a cross-sectioned liver tissue of rat in TAA + SeNPs group showing inflammatory cells infiltration (m) in portal area (H. E x40).

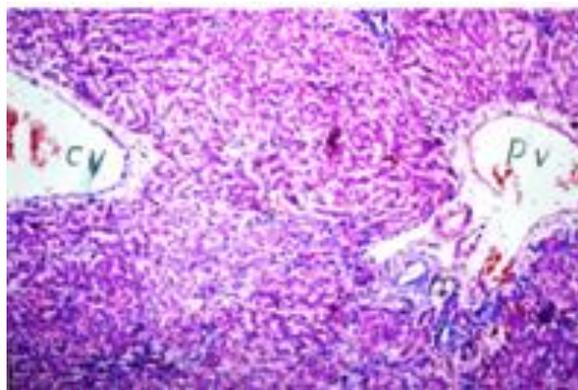


Fig. 6: Optical micrograph of a cross-sectioned liver tissue of rat in TAA + Se group showing inflammatory cells infiltration (m) in portal area and between the hepatocytes with congestion in central (cv) and portal veins (pv) (H. E x10).

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