2. AIM OF THE WORK

The aim of this study is to evaluate the role of selenium supplementation in the pathogenesis of type 2 diabetes in rats.

3. MATERIALS AND METHODS

Animals

The study was conducted on 90 adult male Wister rats weight (150-200 grams). Ninety local adult wistar male rats weighting approximately 150-200 grams obtained from animal house of Medical Research Institute were used in the present study .All experiments were conducted in accordance with the guide for the care and use .Animals were housed 10 per cage at 25° and provided a commercial diet and tap water. All animals were kept under observation for three months to study for acclimation in the laboratory environment .The rats were divided into 3 groups:

- **Group I:** Control group, included 10 healthy male rats.
- **Group II:** sodium selenate treated group. This group was divided into 4 subgroups (10 rats each) each group was daily orally by gavage administered with different concentrations of Sodium selenate (5, 10, 50 and 100 µg/ kg), for three months .
- **Group III:** Selenocysteine treated group. This group was divided into 4 subgroups (10 rats each) each group was daily orally by gavage administered with different concentrations of Selenocysteine (5, 10, 50 and 100 µg / kg), for three months .

Dose preparation:

1- **Stock solution:** 100 mg of selenium compounds (sodium selenate and selenocysteine [Cornel Lab. Company]) was dissolved in one liter of distilled water and this solution are given to the group of 100 ug/kg (Bwt) of rats . (This solution named A) .
2- From solution A we take 250 ml and 250 ml from distilled water forming solution B which administrated by gavage to rats of group of 50 ug/kg (Bwt).
3- From solution B we take 100 ml and diluted it with 400 ml distilled water forming solution C which giving to rats of group 10 ug/kg(Bwt) .
4- Finally we dilute 100 ml of solution C in 100 ml of distilled water to form the solution of 5 ug/kg giving to group of rats at low dose.
All doses administrated according to 1 ul of solution to 1 gram weight of rats.

Methods

After three months the overnight fast animals were scarified under the effect of anesthetize according to the ethical committee of Medical Research Institute and the blood samples were collected and the liver, muscle and adipose tissues were processed for measurements.

Serum was separated from the blood for measurement of :-

A -Laboratory investigations:

1- Lipid profile (cholesterol ,triglycerides ,HDL-chol.LDL-chol.
2- Fasting blood glucose
3- Insulin level
4- Insulin resistant by HOMA-IR
5- Kidney function (Urea and creatinine)

-Tissue homogenate Preparation :-

0.5 gm of each tissue was homogenized in PBS then centrifugated at 4000 rpm for 15 min , the supernatant was taken in aliquot and frozen at -80c tell use for measurement of :-

B – Biochemical assay:

- Ttissue level of phosphor insulin receptor β subunit

-Tissue level of glucose transporter 4 .

- Tissue activity of glutathione peroxidase

- Tissue level of selenoprotein P

- Determination of total protein

Principle:

A modification of the method of Lowry *et al.* was used for the determination of protein in the samples. The colour produced is thought to be due to a complex between the alkaline copper phenol reagent and tyrosine and tryptophan residues of the protein in the sample. The protein concentration in each sample was estimated by referring to a standard curve (Figure 5) which was constructed using bovine serum albumin[(125).]

Reagents:

- Sodium hydroxide 0. 1M.
- Sodium carbonate (anhydrous) 2% in 0.1M NaOH.
- K/Na tartarate 2%.
- Copper sulphate 1%.
- Lowry C reagent: prepared immediately before use by mixing volumes of sodium carbonate, K/Na tartarate and copper sulphate reagent in a ratio: 100: 1: 1.
- Folin Ciocalteau reagent. The working reagent was prepared by diluting the stock reagent 1: 1 (V/V) with distilled water immediately before use.
- Standard bovine serum albumin (200µg/ml)

Procedure:

The homogenized tissues was diluted in distilled water (1: 10). Aliquots of 10µl of diluted samples were mixed with 2.5ml of Lowry C reagent. After incubation for 10 minutes at room temperature, 0.25ml of working Folin Ciocalteau's reagent was added. The tubes were then mixed and incubated in a dark place for one hour at room temperature, after which the absorbance was read at 695nm using spectronic 21 spectrophotometer. A blank containing phosphate buffer saline instead of the sample was treated similarly.

The total protein amount was computed with reference to the protein standard curve (Figure 9) (2,4,6,8,10,20,30,40,50,60,70,80,90,100 µg protein).

Calculation:

The protein concentration of each sample was detected from the standard curve.

$$\text{The total protein concentration (mg/ml)} = \frac{\text{The total protein amount (mg)}}{\text{The sample volume (ml)}}$$

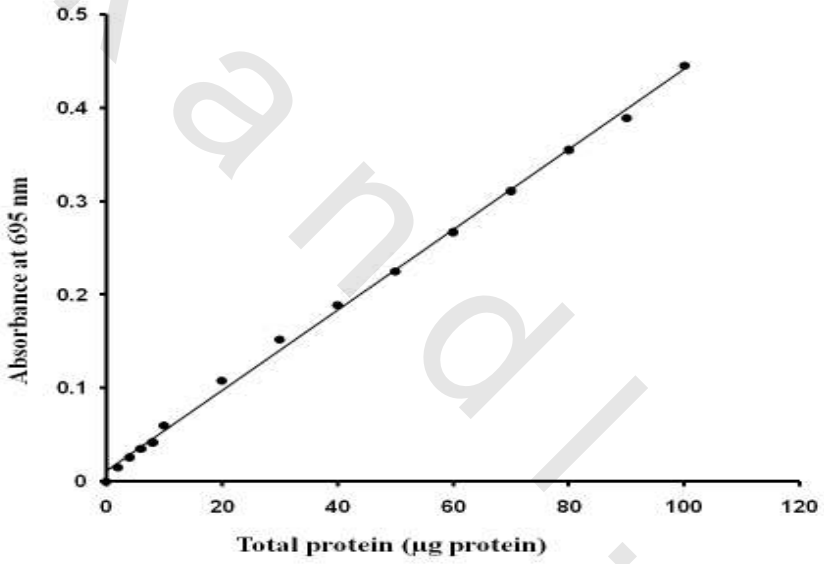

Figure (9): Standard curve of total protein

-Determination of Phospho-Insulin Receptor-β (Tyr1150/1151).

Rat phospho-insulin receptor-β (Tyr1150/1151) ELISA kit (Uscan life science) is used for the non radioactive quantitative of Phospho-Insulin Receptor-β (Tyr1150/1151) in rat tissues.[126]

Principle:

This assay is a solid phase sandwich ELISA based on detected transfected Phospho-Insulin Receptor-β (Tyr1150/1151) protein. An insulin receptor β mouse mAb has been coated onto the microwells. After incubation with sample, both phospho- and non phospho-insulin receptor proteins are captured by the coated antibody. Following extensive washing, phospho-IGF-1 Receptor -β (Tyr1135/1136)/ Insulin Receptor-β (Tyr1150/1151)

rabbit mAb is added to detect the captured Phospho-Insulin Receptor-β (Tyr1150/1151). Anti-rabbit IgG, HRP-Linked Antibody then used to recognize the bound detection antibody. HRP Substrate, TMB, is added to develop color. The optical density for this developed color is proportional to the quantity of Phospho-Insulin Receptor-β (Tyr1150/1151).

Reagents:

- An insulin receptor β mouse mAb coated microwells.
- Phospho-Insulin Receptor-β (Tyr1150/1151) detection Ab.
- Anti-rabbit IgG, HRP-Linked Ab.
- TMB Substrate.
- Stop solution.
- Sealing Tape.
- wash buffer.
- Sample diluent.
- HRP substrate

Procedure:

- 100 µl of sample diluent were added to microcentrifuge tube.
- 100 µl of sample were added to tube and vortex for a few seconds.
- 100 µl of diluted sample were added to the appropriate well then microwells were sealed with tape and pressed firmly.
- Microplate was incubated for 2 hours at 37 ^{o}C.
- Microplate was washed four times with 200 µl of wash buffer and 100 µl of detection antibody was added to each well then micro-wells were sealed with tape and the plate was incubated for one hour at 37 ^{o}C .
- Microplate was washed four times with 200 µl of wash buffer and 100 µl of HRP-linked secondary antibody was added to each well then microwells were sealed with tape and the plate was incubated for 30 minutes at 37 ^{o}C.
- Microplate was washed four times with 200 µl wash buffer then 100 µl of TMB substrate was added and incubated for 10 minutes at 37 ^{o}C .
- 100 µl of stop solution was added to each well.
- The absorbance was read on a microplate reader at a wavelength of 450 nm.

Calculation:

Concentration of P-IR protein was calculated from difference absorbance unit/ mg protein content of the sample (AU/mg protein) at 450 nm.

-Determination of Glucose Transporter-4 (Glut-4):

Rat glucose transporter-4 (Glut-4) ELISA kit (Uscn Life Science) is used for in vitro quantitative measurement of Glut-4 in rat tissues homogenates[127].

Principle:

The microtiter plate provided in this kit has been pre-coated with an antibody specific to Glut-4. Standard or sample are then added to the appropriate microtiter plate wells with a biotin-conjugated antibody preparation specific for Glut-4. Next, Avidin conjugated to horseradish peroxidase (HRP) is added to each microtiter plate wells and incubated. After TMB substrate solution is added, only those wells that contain Glut-4, biotin-conjugated antibody and enzyme-conjugated avidin will exhibit a change in color. The enzyme-substrate reaction is terminated by the addition of a sulphoric acid solution and the color change is measured at 450 nm. The optical density for this developed color is proportional to the quantity of Glut-4.

Reagents:

- Pre-coated, ready to use 96-well strip plate.
- Standard (lyophilized): 40 ng/ml.
- Standard diluent.
- Detection reagent A.
- Assay diluent A.
- Detection reagent B.
- Assay diluent B.
- TMB Substrate.
- Stop solution.
- Wash buffer solution.

Procedure:

- 100 µl of rat Glut-4 standards were added after diluting with standard dilution in duplicate in the order of descending concentrations (40,20,10,5,2.5,1.25,0.625 ng/ml).
- 100 µl of standard diluent was added to blank wells which was set separately.
- 100 µl of homogenized supernatant were added to the remaining sample wells, then plate was incubated for 2 hours at 37 ^{o}C after closing with closure plate membrane.
- 100 µl of prepared detection reagent A were added then plate was incubated for 1 hour at 37 ^{o}C after closing with closure plate membrane.
- Solutions were decanted from the plate and the plate was washed three times with 200 µl diluted wash buffer.
- 100 µl of prepared detection reagent B were added then plate was covered and incubated for 30 minutes at 37 ^{o}C . Microplate was washed five times with 200 µl of wash buffer and 90 µl of TMB substrate solution was added to each well and incubated for 25 minutes at 37 ^{o}C .
- 50 µl of stop solution were added to each well and mixed well then the absorbance was read at 450 nm in a plate reader immediately.

Calculation:

The Standard curve (Figure **10**) was constructed by plotting the difference absorbance unit at 450 nm against the concentration of rat Glut-4 standards.

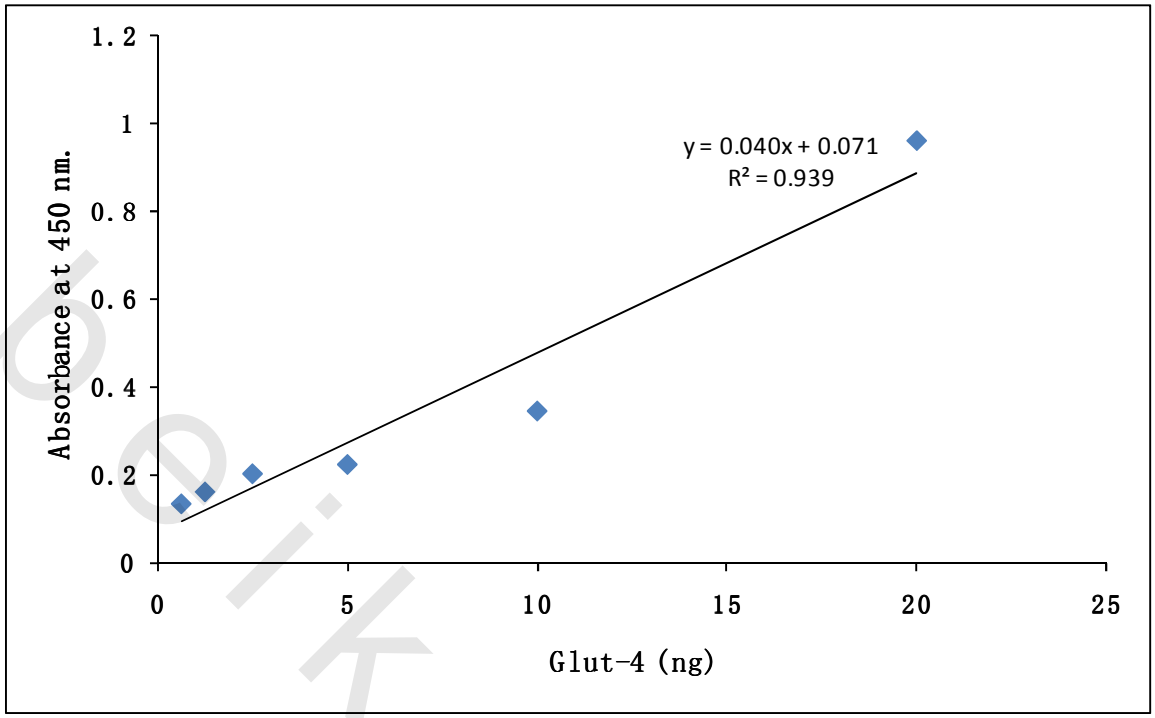

Figure (10): Standards Curve of Glut-4

-Determination of glutathione peroxidase (GPx) activity

There are two major types of GPx; one is selenium-dependent (sGPx) and is active with both organic hydroperoxides and H_2O_2. The other is non-selenium-dependent (nsGPx) and has a negligible activity with H_2O_2. The total and sGPx activities were determined by the method of Flohe and Gunzler.[128] (sigma company)

Principle:

This method is based on monitoring the generation of GSH from GSSG by the action of glutathione reductase (GR) in presence of NADPH. Total GPx activity was measured using cumene hydroperoxide as a substrate while sGPx activity was measured with H_2O_2 as a substrate. The nsGPx activity was obtained as the difference between the total activity and the activity of sGPx.

One unit of enzyme activity was defined as the amount of enzyme required to oxidize 1 µmol of NADPH per minute.

Reagents:

- Potassium phosphate buffer 50 mM , pH 7.
- EDTA azide solution was prepared by dissolving 5mM EDTA and 1mM azide in the phosphate buffer.
- 2 mM GSH in the phosphate buffer.
- 0.25mM H_2O_2.
- 1.5mM cumene hydroperoxide in ethanol.
- 50 U/ml glutathione reductase.
- 0.2mM NADPH in phosphate buffer pH 7.

Procedure:

In a spectrophotometer cuvette, the following reagents were added in sequence: 1 ml of EDTA azide solution, 10 µl NADPH, 10 µl GSH, 2 µl glutathione reductase, and 100 µl tissue homogenate.

The contents of the cuvette were mixed thoroughly and the cuvette was incubated at 37°C for 5 minutes. Absorbance of the contents of the cuvette was then measured at 340 nm by spectronic 21 spectrophotometer. An aliquot of 10 µl cumene hydroperoxide was added to the contents of the cuvette. After thorough mixing, the cuvette was reincubated under the same conditions. The absorbance was measured again after one minute. A blank was run through the same procedures containing phosphate buffer pH 7 instead of the sample.

Calculation:

$$\text{Enzyme activity mU /ml (nmol/min/ml)} = \frac{\Delta\ \text{A/minx1000 x 1000}}{\text{sample volume (µL) x 6.22}}$$

Where ΔA/min = absorbance change/min of the sample - absorbance change/min of the blank, 6.22 is the molar extinction coefficient of NADPH at 340 nm, 1000 is to convert sample volume from µl to ml, and 1000 is to convert the enzyme activity from U to mU.

Results were expressed as mU /mg protein by dividing the enzyme activity per ml of the sample by the protein concentration in the same sample.

- **Rat selenoprotein**

Rat selenoprotein P (SE-P) ELISA kit (WEKA MED SUPPLIES) allows the determination of concentrations of selenoprotein P in Rat serum or tissue[129].

Principle

The kit assay Rat selenoprotein P level in the sample, were purified Rat selenoprotein P antibody was used to coat microtiter plate wells, make solid-phase antibody, then add SE-P to wells, combined SE-P antibody which with enzyme labeled, become antibody-antigen-enzyme-antibody complex, after washing completely, add substrate solution, TMB (A) substrate becomes blue color at HRP (B) enzyme-catalyzed, reaction is terminated by the addition of a sulphoric acid solution and the color change is measured at 450 nm. The optical density for this developed color is proportional to the quantity of Rat selenoprotein P.

Reagents

- Standard: 27 ng/ ml
- Standard diluent
- Enzyme conjugate
- Sample diluent
- Substrates A and B
- Stop solution
- Wash solution

Procedure

- 50 µl of rat selenoprotein standards were added after diluting with standard dilution in duplicate in the order of descending concentrations (18, 12, 6, 3,1.5 ng/ml).
- Blank wells was set separately and all steps were the same but the sample and enzyme conjugate didn't add .
- 40 µl of sample diluent were added to the remaining sample wells, then 10 µl of homogenized supernatant sample was added and mix gently.
- Plate was incubated for 30 minutes at 37 °C after closing with closure plate membrane.
- Solutions were decanted from the plate and the plate was washed 5 times with 200 µl diluted wash buffer.
- 50 µl of enzyme conjugate were added to all wells, except blank well and incubated for 30 minutes at 37 °C after closing with closure plate membrane.
- Solutions were decanted from the plate and the plate was washed 5 times with 200 µl diluted wash buffer.
- 50 µl of substrate A and substrate B were added to all wells, covered and incubated for 15 minutes at 37 °C.
- 50 µl of stop solution were added to each well and mixed well then the absorbance was read at 450 nm in a plate reader within 15 minutes.

Calculation:

The reference curve (Figure **11**) was constructed by plotting the difference absorbance unit at 450 nm against the concentration of rat selenoprotein standards.

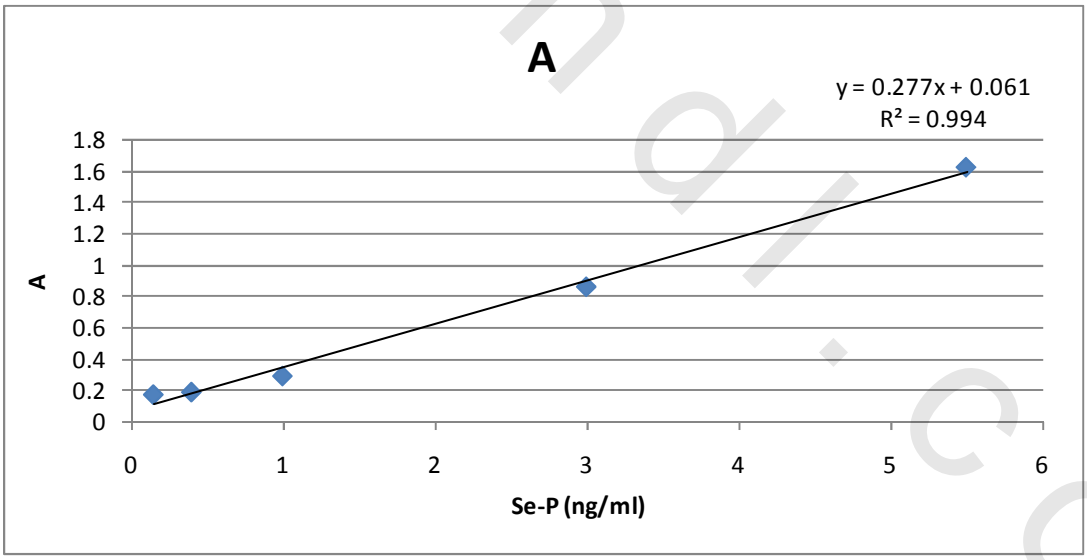

Figure (11): Standards Curve of Se-P

Laboratory Investigations:

Lipid profile[130]

-Determination of triglycerides

The triglycerides level was determined by the enzymatic colorimetric method (Human Kit).

Principle:

Glycerol and fatty acids are first formed by the action of lipase on the triglycerides. Glycerol is then phosphorelated by adenosine triphosphate (ATP) to produce glycerol -3-phosphate and ADP in a reaction catalyzed by glycerol kinase. Glycerol- 3 - phosphate is oxidized by glycerol phosphate oxidase producing dihydroxy acetate phosphate (DAP) and hydrogen peroxide. The latter reacts with 4 aminoantipyrine and 4- chlorophenol under the catalytic influence of peroxidase to form quinoneimine.

$$\text{Triglycerides} \xrightarrow{\text{Lipase}} \text{glycerol + fatty acids.}$$

$$\text{Glycerol + ATP} \xrightarrow{\text{GlycerolKinase}} \text{glycerol-3 phosphate + ADP.}$$

$$\text{Glycerol-3phosphate + } O_2 \xrightarrow{\text{Glycerolphosphate}} \text{DAP + } H_2O_2.$$

$$H_2O_2 + \text{4-aminoantipyrine + 4-chlorophenol} \rightarrow \text{quinnoneimine+HCl + } H_2O.$$

Reagents:

Enzyme /buffer reagent:

- Piperazine-N,N′-bis(2-ethanesulfonic acid) buffer(pH 7.5) 50 mmol/L
- 4 - chloro phenol 5 mmol/L
- 4 - Aminoantipyrine 0.25 mmol /L
- Magnesium ions 4.5 mmol/L
- ATP 2 mmol/L
- Lipase ≥ 1.3 U/mL
- Peroxidase ≥ 0.5 U/mL
- Glycerol kinase ≥ 0.4 U/mL
- Glycerol -3 - phosphate oxidase ≥ 1.5 U/mL

  Standard: Triglycerides 200 mg/dl

Procedure:

- 1 ml of enzyme reagent was mixed with 10 µl of serum sample or triglycerides standard in test tubes.
- Reagent blank was run through the same procedure.
- All tubes were incubated at 37 C for 5 minutes.
- The absorbance of standard (ΔA standard) and the sample (ΔA sample) were measured against reagent blank at 546 nm.

Calculation:

Triglycerides concentration (mg/dl) = 200 x $\frac{\Delta A \text{ sample}}{\Delta A \text{ standard}}$

Where, concentration of standard was 200 mg/dL

-Determination of total cholesterol[130]

Serum total cholesterol level was determined on the basis of an enzymatic calorimetric method (Human Kit).

Principle

Cholesterol esterase (CHE) hydrolyzes esters and H_2O_2 is formed in the subsequent enzymatic oxidation of cholesterol by cholesterol oxidase (CHO) according to the following reaction:

$$\text{Cholesterol ester} + H_2O \xrightarrow{\text{CHA}} \text{Cholesterol} + \text{fatty acids}$$

$$\text{Cholesterol} + O_2 \xrightarrow{\text{CHO}} \text{Cholestene –3–one} + H_2O_2$$

$$2H_2O_2 + \text{4-aminophenazone} + \text{phenol} \xrightarrow{\text{Peroxidase}} \text{Quinoneimine} + HCl + H_2O$$

Reagents:

Enzyme / buffer reagent

- Phosphate buffer (pH 6.5) 100 mmol/L
- 4 – Amino phenazone 0.3 mmol/L
- Phenol 5 mmol/L
- Peroxidase >5 KU/L
- Cholesterol esterase > 150 U/L
- Cholesterol oxidase > 100 U/L
- Standard: Cholesterol 200 mg/dl

Procedure:

1 ml of working reagent was mixed with 10 µl of serum sample or cholesterol standard in test tubes. Reagent blank was run through the same procedure. All the tubes were incubated at 37 °C for 5 minutes. The absorbance of standard (A standard) and the sample (A sample) were measured against reagent blank at 546 nm.

Calculation:

Cholesterol concentration (mg/dl) = 200 x$\frac{\Delta A \text{ sample}}{\Delta A \text{ standard}}$

Where, concentration of standard was 200 mg/dl.

-Determination of HDL- Cholesterol (HDL-C)[130]

High density lipoprotein (HDL–cholesterol) level was determined by precipitation method (Human Kit).

Principle:

When serum is treated with phosphotungustic acid in the presence of magnesium ion, the low density lipoprotein (LDL), very low density lipoprotein (VLDL) and chylomicron are precipitated from serum. The HDL cholesterol remains dissolved in the supernatant. The supernatant then acts as a sample and assayed for cholesterol by an enzymatic method.

Reagents:

Precipitant

Phosphotungstic acid	0.4 mmol/L
Magnesium chloride	20.0 mmol/L
Standard: HDL-cholesterol standard	15 mg/dL

Procedure:

Precipitation:

- 500 µl of precipitant was added to 200 µl of serum sample, mixed well and incubated for 10 minutes at room temperature.
- The mixture was then centrifuged for 10 minutes at 4000 rpm
- After centrifugation the clear supernatant was separated from the precipitate.
- 1 ml of enzyme reagent of Cholesterol determination was mixed in test tubes with 100µl of sample supernatant, cholesterol standard, or distilled water (as blank).
- The mixture was incubated at 37^0C for 10 minutes.
- The absorbance of standard (ΔA standard) and the sample (ΔA sample) were measured against reagent blank at 546 nm.

Calculation:

$$\text{HDL -Cholesterol concentration (mg/dl)} = 52.5 \times \frac{\Delta A \text{ sample}}{\Delta A \text{ standard}}$$

Where 52.5= Standard cholesterol concentration (15) X Sample dilution factor (3.5)

Calculation of LDL- Cholesterol [130]

The low density lipoprotein cholesterol concentration (LDL–C) was calculated from the total cholesterol concentration (TC), the HDL cholesterol concentration (HDL–C) and the triglycerides concentration (TG).

LDL-C = TC − (HDL-C) – TG / 5 mg/dl

- Determination of fasting blood glucose[130]

Plasma glucose levels were determined according to an enzymatic calorimetric method which has been described by Trinder (Human Kit).

Principle:

Glucose is oxidized in the presence of glucose oxidase. The hydrogen peroxide formed reacts under catalysis of peroxidase with phenol and 4–aminophenazone to a red-violet quinoneimine dye. The intensity of the color is proportional to glucose concentration.

$$\text{Glucose} + O_2 + H_2O \xrightarrow{\text{glucose oxidase}} \text{Gluconate} + H_2O_2$$

$$H_2O_2 + \text{phenol} + \text{4-aminophenazone} \xrightarrow{\text{peroxidase}} \text{Quinoneimine} + 4H_2O$$

Reagents:

Working reagent

- Phosphate buffer (pH 7.5) 0.1 mol/L
- 4 – Aminophenazone 0.25 mmol/L
- Phenol 0.75 mmol/L
- Glucose oxidase >15 KU/L
- Peroxidase >1.5 KU/L
- Standard**:** Glucose 100 mg/dL

Procedure:

- 1 ml of enzyme reagent was mixed in test tubes with 10 µl of plasma sample or glucose standard.
- The mixture was incubated at 37 °C for 5 minutes.
- Reagent blank was run through the same procedure.
- The absorbance of standard (ΔA standard) and the sample (ΔA sample) were measured against reagent blank at 540 nm.

Calculation:

$$\text{Glucose concentration (mg/dL)} = 100 \times \frac{\Delta A \text{ sample}}{\Delta \text{ A standard}}$$

Where, concentration of standard was 100 mg/dL.

- Determination of insulin in rat serum [(131)]

Rat insulin ELISA kit (Millipore) is used for the non radioactive quantitative of insulin in rat sera .

Principle:

This assay is a sandwich ELISA based on capture of insulin molecules from samples to the wells of a microtitre plate coated by a monoclonal mouse anti-rat insulin antibodies and the binding of biotinylated polyclonal antibodies to captured insulin, binding of horseradish peroxidase (HRP) to the immobilized biotinylated antibodies, and quantification of the immobilized antibody-enzyme conjugates by monitoring horseradish

peroxidase activities in the presence of 3, 3', 5, 5'-tetramethylbenzidine. The enzyme activity is measured spectrophotometrically by the increased absorbance at 450 nm.

Reagents:

- Assay buffer: (0.05 M phosphate buffered saline, pH 7.4, containing 0.025M. EDTA, 0.08 % sodium azide and 1% BSA).
- Wash buffer: (50 mM Tris buffered saline containing Tween- 20).
- Insulin standards in assay buffer: (0.2, 0.5, 1, 2, 5 and 10 ng/ml).
- Matrix solution: (charcoal Stripped pooled mouse serum).
- Insulin detection antibody: (biotinylated anti-insulin antibodies).
- Enzyme solution: (Streptavidin- horseradish peroxidase conjugates in assay buffer).
- Substrate :(3, 3', 5, 5' - tetramethylbenzidine).
- Stop solution: (0.3 M HCl).

Procedure:

- 10 µl of assay buffer were added to each of the sample wells.
- 10 µl of rat insulin standards were added in duplicate in the order of ascending concentrations. 10 µl of negative and positive control were added to the appropriate wells.
- 10 µl of unknown serum were added to the remaining wells.
- 80 µl of detection antibody were added then incubated at room temperature for 2 hours.
- Solutions were decanted from the plate and the plate was washed 3 times with 200 µl diluted wash buffer.
- 100 µl of enzyme solution were added to each well and incubated with moderate shaking at room temperature for 30 minutes.
- The plate was washed 6 times with 200 µl diluted wash buffer.
- 100 µl of substrate solution were added to each well. The plate was covered and shake in the plate shaker for approximately 20 minutes.
- 100 µl of stop solution were added then the absorbance was read at 450 nm and 620 nm in a plate reader within 5 minutes.

Calculation:

The reference curve (Figure **12**) was constructed by plotting the difference absorbance unit at 450 nm and 620 nm against the concentration of rat insulin standards.

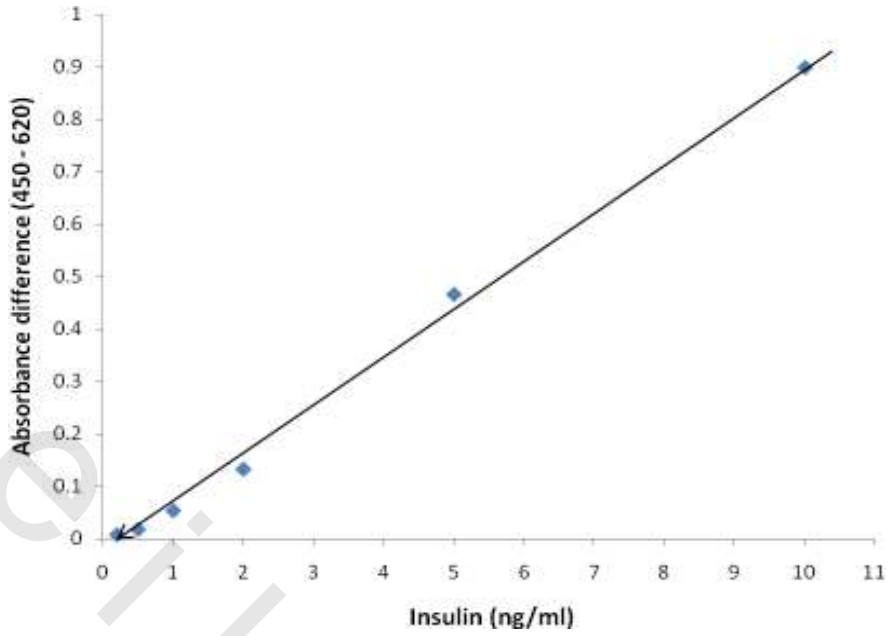

Figure (12): Standard curve of insulin.

- **Insulin resistance by (HOMA)** [132]

The insulin resistance index (IRI) was derived using the homeostasis model assessment (HOMA) as follows :

IRI= fasting insulin (µU/ ml) × fasting glucose (mmol/L) /22.5

Kidney function[130]

- **Determination of Urea**

Principle

Urea in the sample is hydrolyzed enzymatically into ammonia (NH_4^+) and carbon dioxide (CO_2). Ammonia ions formed reacts with salicylate and hydrochlorite (Na ClO), in presence of the catalyst nitroprussid, to form a green indophenols (Human Kit):

$$Urea + H_2O \xrightarrow{Urease} (NH_4^+)_2 + CO_2$$

$$NH_4^+ + salicylate + Na\ ClO \xrightarrow{Nitroprussid} Indophenol$$

The intensity of the color formed is proportional to the urea concentration in the sample

Reagents

R1 Standard	Urea aqueous primary standard	50 mg/dL
R2 Enzymes	Urease	30000 U/ L
R3 Buffer	Phosphate Ph 6.7 EDTA Sodium salicylate Sodium nitroprussid	50 mmol/ L 2 mmol/ L 400 mmol/ L 10 mmol/ L
R4	Na ClO Sodium hydroxide	140 mmol/ L 150 mmol/ L

Procedure:

Reagents	**Sample**	**Standard**	**Blank**
R3	1 ml	1 ml	1 ml
R2	One drop	One drop	One drop
R1	-	10 µl	-
sample	10µl	-	-
The mixture was incubated at 37^0C for 3 minutes then R4 was added			
R4	200 µl	200 µl	200 µl

- The mixture was incubated at 37^0C for 5 minutes
- The absorbance of standard (ΔA standard) and the sample (ΔA sample) were measured against reagent blank at 578 nm.

Calculation:

$$\text{Urea concentration (mg/dl)} = 50x\frac{\Delta A\ sample}{\Delta\ \text{A standard}}$$

Where concentration of the standard = 50 mg/dl

- Determination of Serum Creatinine[(130)]

Principle:

Creatinine forms in alkaline solution an orange-red coloured complex with picric acid. The absorbance of this complex is proportional to the creatinine concentration in the sample (Human Kit).

Creatinine + Picric acid → Creatinine-picrate complex

Reagents:

- Picric acid 26 mmol/l
- sodium hydroxide 1.6 mol /l
- standard 2 mg /dl

Reagent Preparation

- For measurement at 25°C : sodium hydroxide was diluted with Dist. Water in the ratio 1+4.
- Diluted sodium hydroxide and picric acid were mixed for the working reagent in the ratio 1+1.

Procedure:

Reagent	**Blank**	**Standard**	**Test**
Working reagent	2 ml	2 ml	2 ml
standard	—	200µL	—
Serum sample	—	—	200µl

- Were mixed and stopwatch was started
- After 30 second the absorbance of each tube was measured (A1).

- After 2 minutes the absorbance (A2) was measured.
- ΔA_{sample} or $\Delta A_{standard}$= A2 – A1

Calculation:

Concentration of Creatinine in serum (mg/dl) = $2.0 \times \frac{\Delta A\ sample}{\Delta\ A\ standard}$

Where 2.0 is Creatinine standard concentration .

Tissue preparation:-

The rat pancreases were removed and fixed with 10% parraformaldehyd in 0.1 mol/L phosphate buffer solution (pH 7.4) for 24 hrs and embedded in paraffin for making pancreas tissue blocks. Then serial sections of blocks were cut at 5 µm thick in a rotary microtome for heamatoxyline and eosin (H&E).

Heamotoxiline and eosin staining:-

After the paraffin section were dewaxed ,hemotoxylin staining was performed for 3 Sec. followed by eosin staining for 3 min. and then the sections were dehydrated with alcohol , cleared with xyline and sealed. The histopathological abnormalities were investigated under a light microscope .

Statistical analysis of the data

Data were fed to the computer and analyzed using IBM SPSS software package version 20.0. Quantitative data were described using range (minimum and maximum), mean, standard deviation and median. The distributions of quantitative variables were tested for normality using Kolmogorov-Smirnov test, Shapiro-Wilk test and D'Agstino test, also Histogram and QQ plot were used for vision test. If it reveals normal data distribution, parametric tests was applied. If the data were abnormally distributed, non-parametric tests were used. For normally distributed data, comparison between more than two population were analyzed using F-test (ANOVA) and Post Hoc test (Scheffe). For abnormally distributed data, comparison between two independent population were done using Mann Whitney test. Significance of the obtained results was judged at the 5% level.[133]

4. RESULTS

This chapter contain the statistical analysis of glucose homeostasis parameter , lipid profile , kidney functions , selenoprotein P and certain components of insulin signaling in control healthy rats and rats supplemented orally with sodium selenate and selenocysteine at different doses (5,10,50, 100 µg/kg) for three months. The individual data of each parameter are presented in appendix (I).

Glucose homeostasis parameters:-

The results of glucose homeostasis parameters (fasting serum glucose, insulin, and HOMA-insulin resistance index) were summarized in Tables (2 – 4) and Figures (13-15).

It is clear that the supplementation of rats with seleno-compounds showed a dose-dependent increase in fasting serum glucose compared to control (Table 2 and Figure 13). There is no significant change in fasting serum glucose between the rats supplemented with inorganic selenium (sodium selenate) and the rats supplemented with organic selenium (selenocysteine) except the rats supplemented with the dose of 10 µg/kg of sodium selenate showed a significant higher level of fasting serum glucose than the rats supplemented with similar dose of selenocysteine (Table 2, Figure 13) .

The results of fasting insulin level showed dose-dependent decline in rats supplemented with sodium selenate which become significant at dose of 10 µg/kg and higher doses. Selenocysteine supplementation showed a mild non-significant decline in the insulin level compared to control level at all doses with the exception at the highest dose (100µg/kg) which showed significant lower level than control value (Table 3, Figure 14).

The supplementation of rats with the two forms of selenium (selenate and selenocysteine) lead to comparable increase in the insulin resistance index (HOMA) at higher doses (10,50,100 µg/kg) while the lowest dose (5 µg/kg) showed no increase or even decrease in the HOMA index (Table 4 , figure 15) .

Table (2): The level of fasting glucose (mg/dl) in the control rats and rats supplemented with sodium selenate and selenocysteine at different doses

	Control	Sodium selenate (µg/kg)				Selenocysteine (µg/kg)			
		5	10	50	100	5	10	50	100
Min.	71.00	78.00	105.00	108.00	119.00	77.00	100.00	100.00	126.00
Max.	101.00	104.00	152.00	137.00	240.00	103.00	123.00	137.00	156.00
Mean	85.40	87.20	128.40	125.10	169.60	88.50	112.50	122.70	142.70
±SD.	9.95	8.42	11.60	9.39	37.68	8.24	7.11	13.72	10.83
Median	83.50	83.50	129.50	127.00	161.00	90.00	112.50	129.50	144.50
% of change		↑2.11	↑50.4	↑46.5	↑98.6	↑3.63	↑31.7	↑43.7	↑67.1
p_1		0.705	<0.001*	<0.001*	<0.001*	0.545	<0.001*	<0.001*	<0.001*
p_2						0.820	0.003*	0.649	0.053

p_1: p value for Mann Whitney test for comparing between control and each other group
p_2: p value for Mann Whitney test for comparing between sodium selenate and selenocysteine at each concentration
*: Statistically significant at $p \leq 0.05$
↑increase , ↓decrease

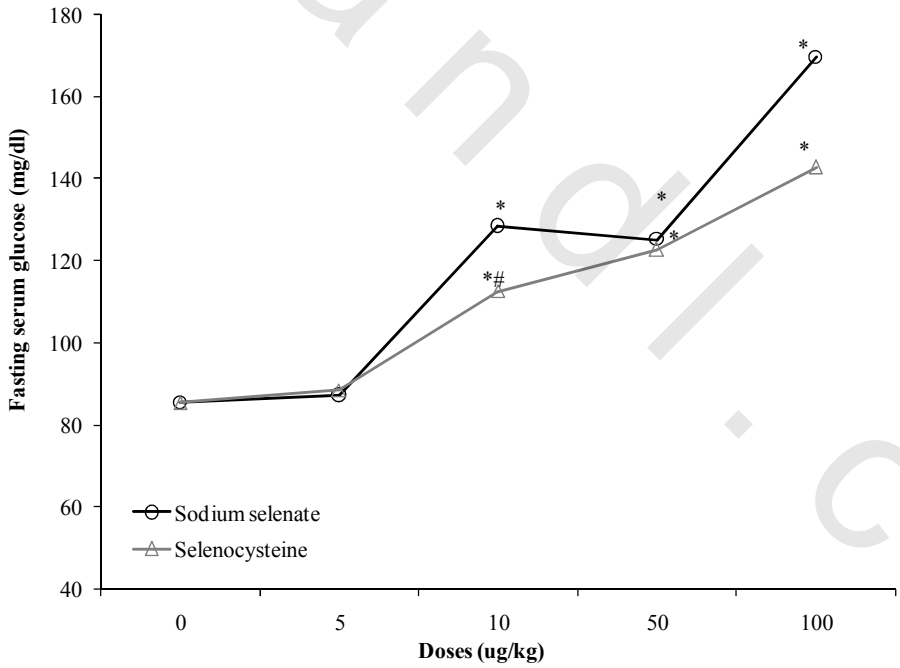

Figure (13): The change in fasting glucose (mg/dl) in the rats supplemented with sodium selenate and selenocysteine at different doses for 3 months
* Significant difference from control (0 dose), # Significant difference from sodium selenate at each dose .

Table (3): The serum concentration of insulin (µg/ml) in the control rats and rats supplemented with sodium selenate and selenocysteine at different doses

	Control	Sodium selenate (µg/kg)				Selenocysteine (µg/kg)			
		5	10	50	100	5	10	50	100
Min.	0.28	0.26	0.22	0.22	0.18	0.27	0.20	0.23	0.22
Max.	0.38	0.37	0.29	0.31	0.24	0.35	0.38	0.33	0.30
Mean	0.33	0.31	0.26	0.26	0.22	0.30	0.28	0.28	0.26
±SD.	0.03	0.03	0.02	0.03	0.02	0.03	0.06	0.03	0.03
Median	0.33	0.32	0.27	0.26	0.22	0.31	0.28	0.27	0.27
% of change		↓6.06	↓21.2	↓21.2	↓33.3	↓9.09	↓15.2	↓15.2	↓21.2
p_1		0.403	0.009*	0.015*	<0.001*	0.119	0.249	0.113	0.010*
p_2						0.594	0.887	0.992	0.331

p_1: p value for Post Hoc test (Scheffe) for comparing between control and each other group
p_2: p value for Post Hoc test (Scheffe) for comparing between sodium selenate and selenocysteine at each concentration
*: Statistically significant at $p \leq 0.05$
↑increase , ↓decrease

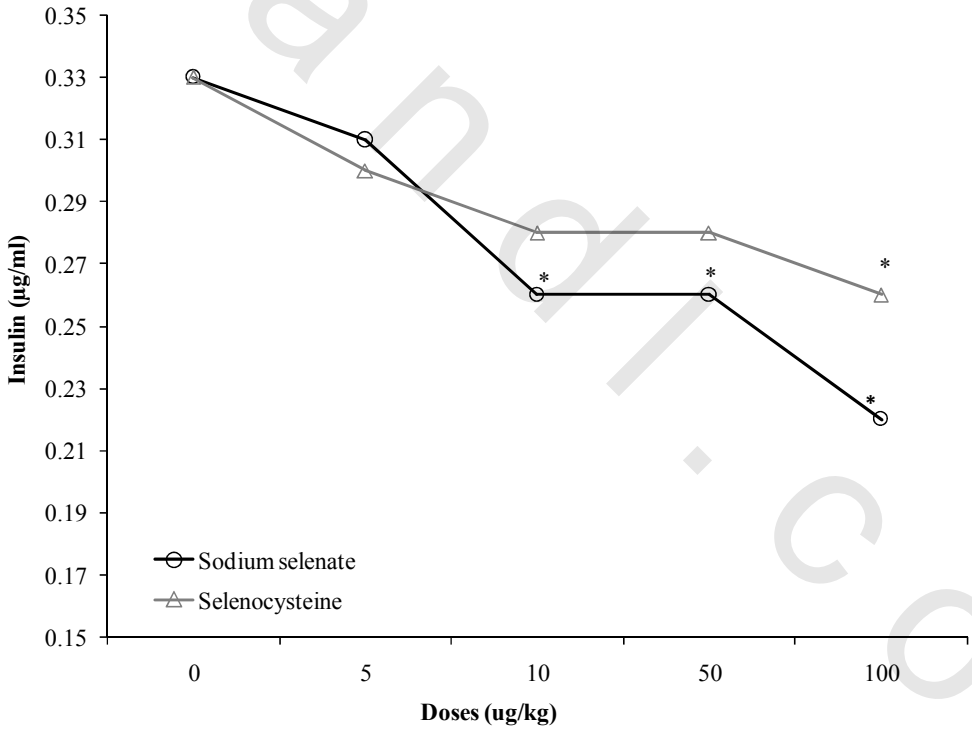

Figure (14): The serum concentration of insulin (µg/ml) in the rats supplemented with sodium selenate and selenocysteine at different doses for 3 months
* Significant difference from control (0 dose)

Table (4): The HOMA-Insulin resistance index calculations in the control rats and rats supplemented with sodium selenate and selenocysteine at different doses

	Control	Sodium selenate (µg/kg)				Selenocysteine (µg/kg)			
		5	10	50	100	5	10	50	100
Min.	1.30	1.30	1.40	1.50	1.70	1.30	1.30	1.50	1.80
Max.	2.00	2.00	2.60	2.50	3.10	2.10	2.70	2.40	2.70
Mean	1.65	1.58	1.97	1.95	2.17	1.54	1.89	2.0	2.19
±SD.	0.21	0.21	0.33	0.33	0.46	0.24	0.44	0.31	0.33
Median	1.60	1.55	2.05	1.95	2.10	1.50	1.90	2.0	2.05
% of change		↓4.24	↑19.4	↑18.2	↑31.5	↓6.67	↑14.5	↑21.2	↑32.7
p_1		0.398	0.016*	0.050*	0.003*	0.136	0.196	0.018*	0.001*
p_2						0.487	0.404	0.732	0.818

p_1: p value for Mann Whitney test for comparing between control and each other group
p_2: p value for Mann Whitney test for comparing between sodium selenate and selenocysteine at each concentration
*: Statistically significant at $p \leq 0.05$
↑increase , ↓decrease

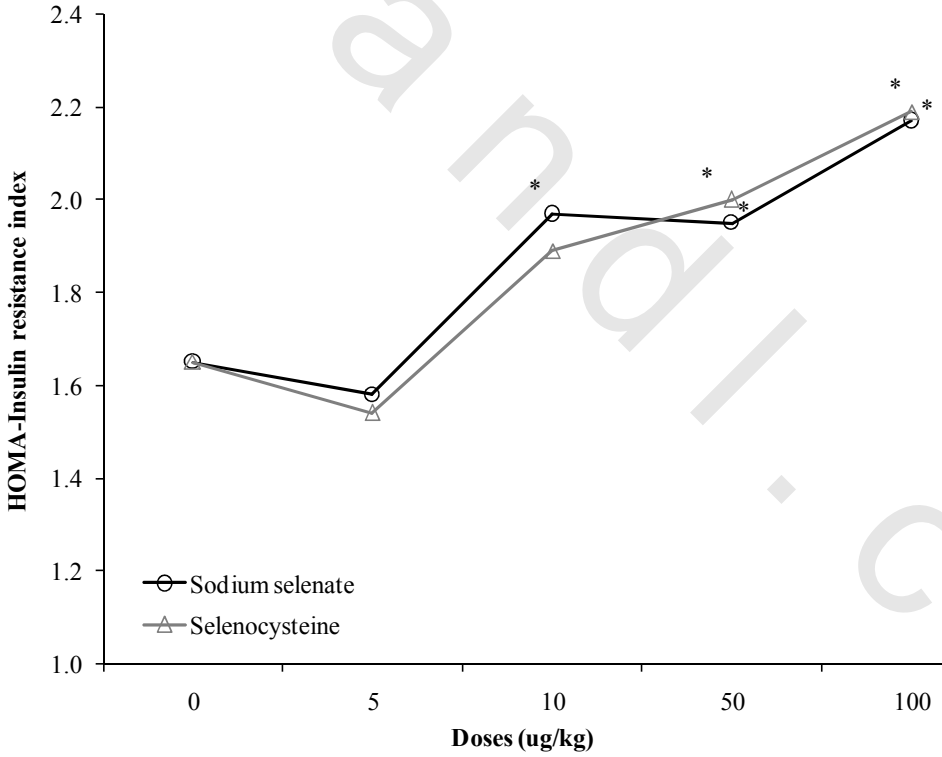

Figure (15): The change in HOMA-insulin resistance index in the rats supplemented with sodium selenate and selenocysteine at different doses for 3 months*
Significant difference form control (0 dose)

Insulin signaling parameters:

The results of phospho-insulin receptor (Ph-IR; represent the active fraction of insulin receptor) in different peripheral tissues are summarized in Tables (5-7) and Figures (16-18). The supplementation of rats with Se compounds cause a significant dose-dependent decline in the liver and adipose tissues contents of P-IR compared to control rats with no difference observed between sodium selenate and selenocysteine (Tables 5,7 and Figures 16,18). In muscle, sodium selenate results in dose-dependent decline of P-IR. Selenocysteine only at dose of 10 µg/kg showed significant decrease in P-IR and the higher doses (50 and 100 µg/kg) showed no significant difference in level P-IR compared to control rats , but significantly higher than sodium selenate treated rats (Table 6, figure 17) .

The results of muscles and adipose tissues contents of GLUT4 are presented in Table (8,9) and Figures (19,20). In muscle, the supplementation of rats with sodium selenate cause a significant dose-dependent decline in Glut4 compared to control. Supplemetation of rats with selenocysteine at low dose (5 and 10 µg/kg) showed no significant changes in the muscles content of Glut4 while at higher doses (50 and 100 µg/kg) showed decline in the muscles Glut4 to similar extend as that observed with sodium selenate (Table 8 and Figure 19) . In adipose tissues, the supplementation with sodium selenate cause a significant dose-dependent decline in the content of Glut4 while selenocysteine supplementation produce no significant effect on the adipose tissue content of Glut4 at all dose except at dose of 50 µg/kg (Table 9, Figure 20)

Table (5): The hepatic content of phospho-insulin receptor-β (ng/mg protein) in the control rats and rats supplemented with sodium selenate and selenocysteine at different doses

	Control	Sodium selenate (µg/kg)				Selenocysteine (µg/kg)			
		5	10	50	100	5	10	50	100
Min.	0.048	0.047	0.038	0.041	0.038	0.047	0.041	0.038	0.038
Max.	0.062	0.062	0.054	0.051	0.051	0.060	0.052	0.052	0.052
Mean	0.054	0.053	0.047	0.045	0.044	0.052	0.047	0.047	0.045
±SD.	0.005	0.005	0.006	0.004	0.004	0.005	0.004	0.005	0.005
Median	0.052	0.051	0.048	0.044	0.042	0.050	0.048	0.048	0.045
% of change		↓1.85	↓13.0	↓16.7	↓18.5	↓3.70	↓13.0	↓13.0	↓16.7
p_1		0.494	0.067	0.001*	<0.001*	0.238	0.006*	0.011*	0.007*
p_2						0.541	0.675	0.286	0.588

p_1: p value for Mann Whitney test for comparing between control and each other group
p_2: p value for Mann Whitney test for comparing between sodium selenate and selenocysteine at each concentration
*: Statistically significant at $p \leq 0.05$
↑increase , ↓decrease

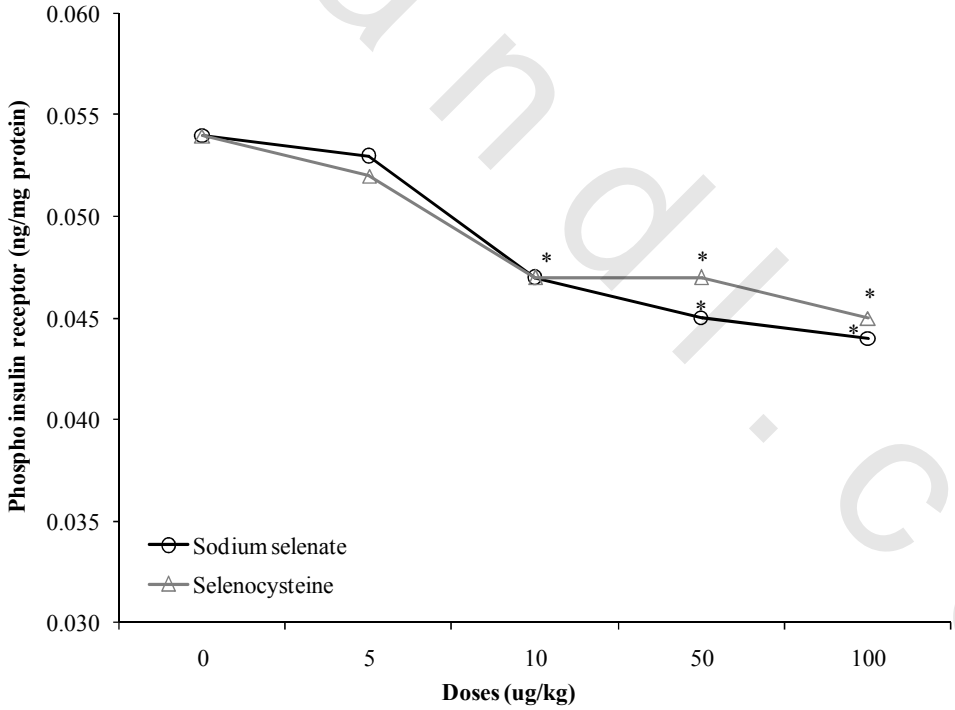

Figure (16): The change in hepatic content of phospho-insulin receptor-β (ng/mg protein) in the rats supplemented with sodium selenate and selenocysteine at different doses for 3 months * Significant difference from control (0 dose)

Table (6): The muscle content of phospho-insulin receptor-β (ng/mg protein) in the control rats and rats supplemented with sodium selenate and selenocysteine at different doses

	Control	Sodium selenate (µg/kg)				Selenocysteine (µg/kg)			
		5	10	50	100	5	10	50	100
Min.	0.049	0.047	0.038	0.038	0.037	0.047	0.041	0.038	0.043
Max.	0.062	0.061	0.052	0.048	0.052	0.060	0.053	0.062	0.061
Mean	0.056	0.055	0.045	0.043	0.043	0.054	0.047	0.051	0.052
±SD.	0.005	0.005	0.005	0.003	0.005	0.005	0.004	0.008	0.005
Median	0.058	0.057	0.044	0.043	0.042	0.057	0.047	0.051	0.051
% of change		↓1.79	↓19.6	↓23.2	↓23.2	↓1.79	↓16.1	↓8.9	↓7.1
p_1		0.543	0.001*	<0.001*	0.001*	0.494	0.001*	0.159	0.169
p_2						0.939	0.517	0.021*	0.005*

p_1: p value for Mann Whitney test for comparing between control and each other group

p_2: p value for Mann Whitney test for comparing between sodium selenate and selenocysteine at each concentration

*: Statistically significant at $p \leq 0.05$

↑increase , ↓decrease

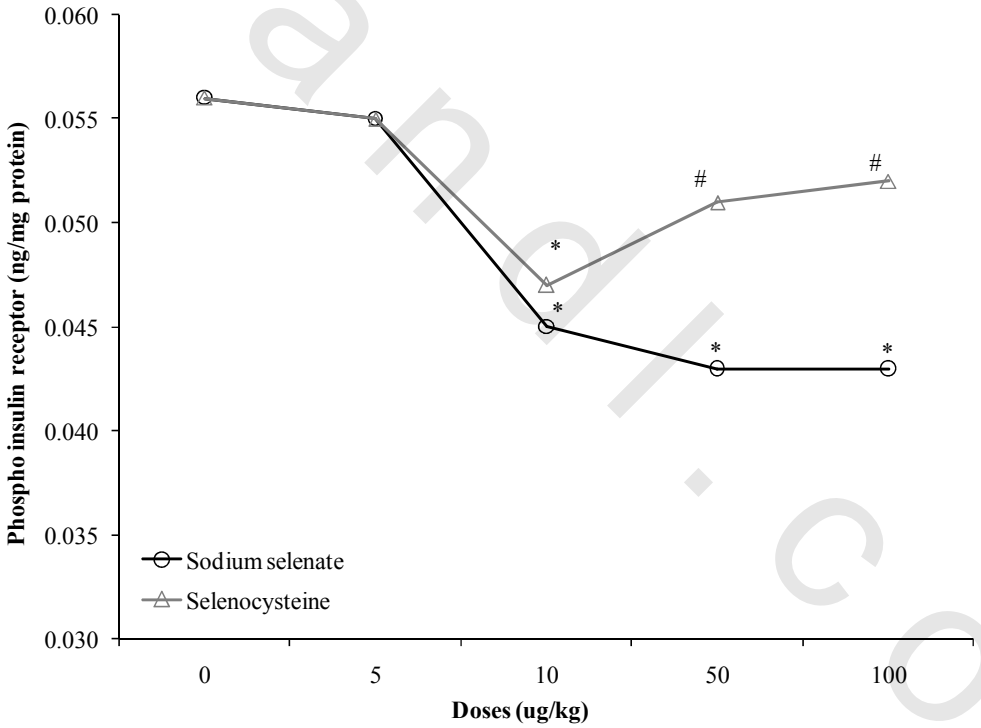

Figure (17): The change in muscles content of phosphor-insulin receptor-β (ng/mg protein) in the rats supplemented with sodium selenate and selenocysteine at different doses for 3 months * Significant difference from control (0 dose), # Significant difference from sodium selenate at each dose

Table (7): White adipose tissues content of phospho-insulin receptor-β (ng/mg protein) in control rats and rats supplemented with sodium selenate and selenocysteine at different doses

	Control	Sodium selenate (µg/kg)				Selenocysteine (µg/kg)			
		5	10	50	100	5	10	50	100
Min.	0.051	0.050	0.037	0.031	0.040	0.049	0.048	0.038	0.038
Max.	0.064	0.063	0.054	0.054	0.052	0.062	0.062	0.054	0.051
Mean	0.055	0.056	0.047	0.043	0.046	0.056	0.053	0.046	0.044
±SD.	0.005	0.005	0.006	0.007	0.005	0.005	0.005	0.006	0.005
Median	0.053	0.057	0.047	0.042	0.045	0.059	0.051	0.045	0.043
% of change		↑1.82	↓14.5	↓21.8	↓16.4	↑1.82	↓3.6	↓16.4	↓20.0
p_1		0.939	0.011*	0.004*	0.001*	0.939	0.176	0.008*	<0.001*
p_2						0.790	0.074	0.425	0.593

p_1: p value for Mann Whitney test for comparing between control and each other group

p_2: p value for Mann Whitney test for comparing between sodium selenate and selenocysteine at each concentration

*: Statistically significant at $p \leq 0.05$

↑increase , ↓decrease

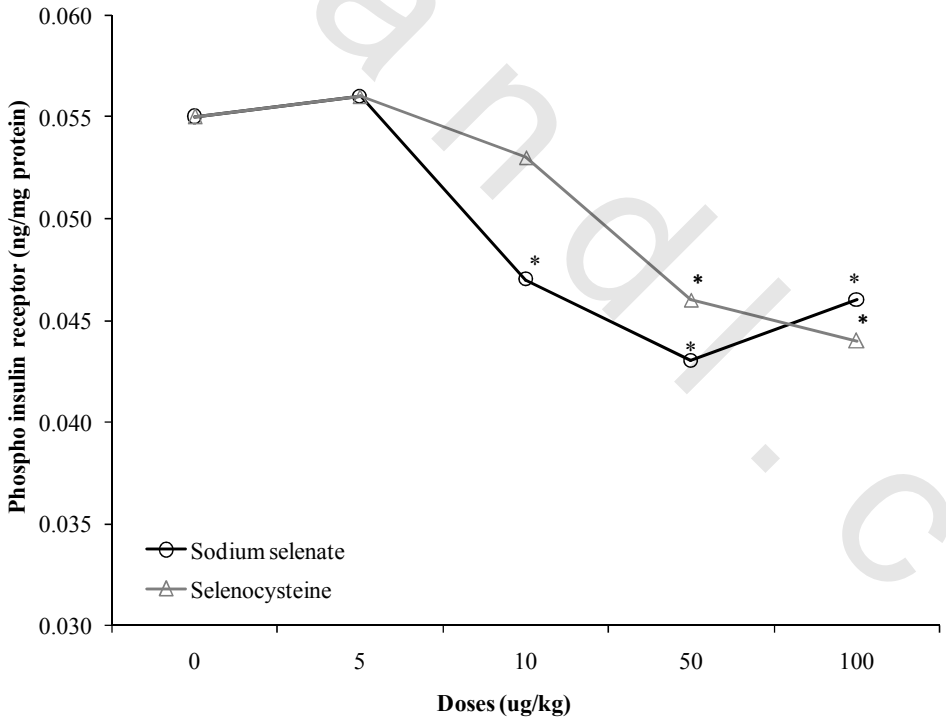

Figure (18): The change in white adipose tissue content of phospho-insulin receptor-β (ng/mg protein) in the rats supplemented with sodium selenate and selenocysteine at different doses for 3 months* Significant difference from control (0 dose)

Table (8): The muscles content of glucose transporter 4 (ng /mg protein) in the control rats and rats supplemented with sodium selenate and selenocysteine at different doses

	Control	Sodium selenate (µg/kg)				Selenocysteine (µg/kg)			
		5	10	50	100	5	10	50	100
Min.	2.20	2.00	1.90	1.70	1.40	2.40	2.70	1.50	1.30
Max.	4.30	4.20	3.10	2.30	1.70	4.40	3.90	2.90	1.80
Mean	3.19	3.06	2.37	2.02	1.55	3.38	3.29	2.07	1.54
±SD.	0.75	0.78	0.41	0.19	0.11	0.78	0.44	0.56	0.16
Median	3.05	2.95	2.30	2.00	1.55	3.35	3.30	1.85	1.55
% of change		↓4.08	↓25.7	↓36.7	↓51.4	↑5.96	↑3.1	↓35.1	↓51.7
p_1		0.677	0.012*	<0.001*	<0.001*	0.849	1.000	<0.001*	<0.001*
p_2						1.000	0.003*	1.000	1.000

p_1: p value for Post Hoc test (Scheffe) for comparing between control and each other group
p_2: p value for Post Hoc test (Scheffe) for comparing between sodium selenate and selenocysteine at each concentration
*: Statistically significant at $p \leq 0.05$
↑increase , ↓decrease

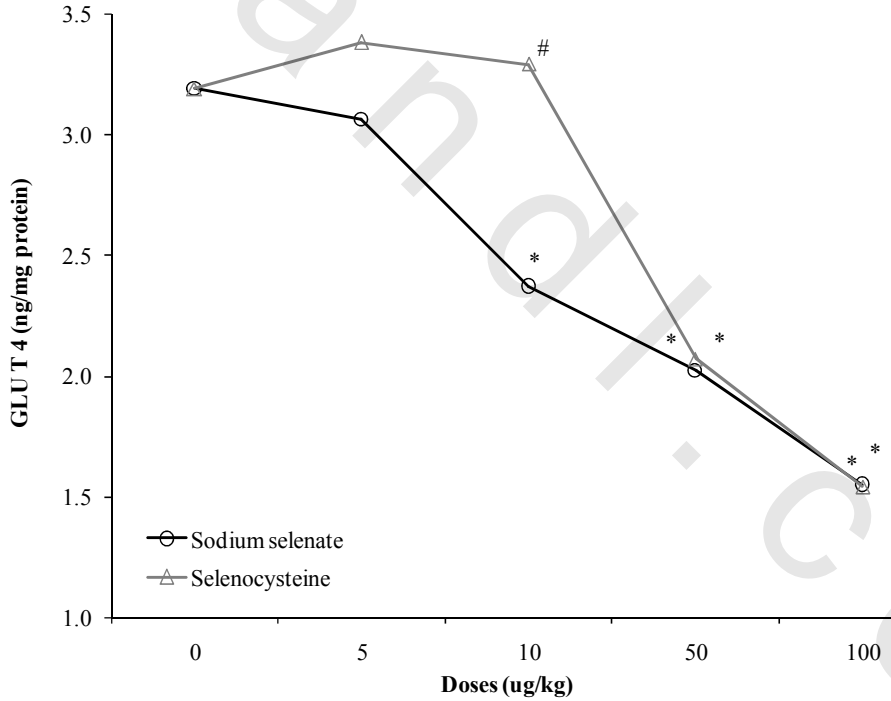

Figure (19): The change in muscle content of glucose transporter 4 (ng /mg protein) in the rats supplemented with sodium selenate and selenocysteine at different doses for 3 months * Significant difference from control rats (0 dose), # Significant difference from sodium selenate at each dose

Table (9): White adipose tissue content of glucose transporter 4 (ng/mg protein) in control rats and rats supplemented with sodium selenate and selenocysteine at different doses

	Control	Sodium selenate (µg/kg)				Selenocysteine (µg/kg)			
		5	10	50	100	5	10	50	100
Min.	2.50	2.30	1.90	1.30	1.20	2.40	2.00	1.90	2.20
Max.	3.10	3.10	2.50	2.20	2.40	3.10	3.30	2.60	3.10
Mean	2.76	2.66	2.17	1.81	1.80	2.75	2.67	2.29	2.89
±SD.	0.25	0.28	0.20	0.27	0.38	0.25	0.47	0.22	0.28
Median	2.70	2.60	2.20	1.85	1.85	2.55	2.75	2.35	3.0
% of change		↓3.62	↓21.4	↓34.4	↓34.8	↓0.36	↓3.3	↓17.0	↑4.7
p_1		0.250	<0.001*	<0.001*	<0.001*	0.195	0.879	<0.001*	0.217
p_2						0.939	0.019*	0.001*	<0.001*

p_1: p value for Mann Whitney test for comparing between control and each other group

p_2: p value for Mann Whitney test for comparing between sodium selenate and selenocysteine at each concentration

*: Statistically significant at $p \leq 0.05$

↑increase , ↓decrease

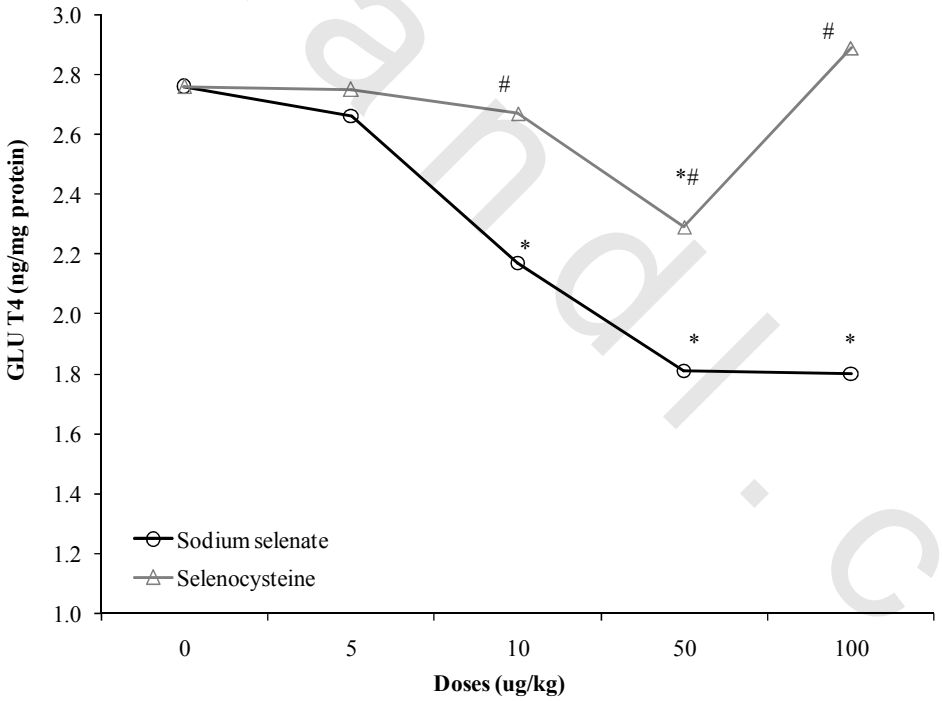

Figure (20): The change in white adipose tissue content of glucose transporter 4 (ng/mg protein) in the rats supplemented with sodium selenate and selenocysteine at different doses for 3 months* Significant difference from control rats (0 dose), # Significant difference from sodium selenate at each dose.

Lipid profile parameters:

The results of lipid profile parameters (Cholesterol , Triglycerides , High density lipoprotein cholesterol (HDL-C) , and Low density lipoprotein cholesterol (LDL-C) were summarized in Tables (10 – 13),and Figures (21 – 24).

The rats supplemented with sodium selenate showed mild dose dependent increase in total cholesterol level compared to control rats , the increase was not more than 12.2% of the control value. Selenocysteine supplementation cause similar extend of change to sodium selenate however the dose of 50µg/kg have no significant effect on the cholesterol level (Table 10, Figure 21)

In contrast to cholesterol which showed mild dose-dependent increase with sodium selenate supplementation, triglycerides showed more dramatic and biphasic change with sodium selenate supplementation. At the low doses of sodium selenate (5 and 10µg/kg) the serum triglycerides level was significantly decreased compared to control while the high dose (100µg/kg) significantly increase triglycerides level by about 45.8%. Selenocysteine supplementation showed no significant effect on the triglycerides level compared to control rats (Table 11, Figure 22) .

The serum level of high density lipoprotein-cholesterol (HDL-C) showed a dose-dependent increase by supplementation with both Se compounds. No significant differences observed between sodium selenate and selenocysteine at any doses (Table 12, Figure 23) . The supplementation of rats with different doses of sodium selenate and selenocysteine showed no significant effects on the serum level of low-density lipoprotein-cholesterol level (LDL-C) (Table 13, Figure 24)

Table (10): The serum cholesterol (mg/dl) in the control rats and rats supplemented with sodium selenate and selenocysteine at different doses

	Control	Sodium selenate (µg/kg)				Selenocysteine (µg/kg)			
		5	10	50	100	5	10	50	100
Min.	96.0	99.00	97.0	100.0	102.0	97.00	100.0	100.0	95.0
Max.	112.0	113.00	143.0	130.0	124.0	113.00	118.0	111.0	121.0
Mean	103.0	104.20	109.70	115.60	114.80	103.90	109.80	105.90	113.70
±SD.	4.97	4.44	13.0	10.21	6.89	4.95	5.37	4.56	8.63
Median	103.0	104.00	109.0	119.50	116.50	104.50	110.50	106.0	117.0
% of change		↑1.17	↑6.5	↑12.2	↑11.5	↑0.87	↑6.6	↑2.8	↑10.4
p_1		0.494	0.121	0.006*	0.001*	0.677	0.012*	0.198	0.009*
p_2						0.879	0.404	0.053	1.000

p_1: p value for Mann Whitney test for comparing between control and each other group
p_2: p value for Mann Whitney test for comparing between sodium selenate and selenocysteine at each concentration
*: Statistically significant at $p \leq 0.05$
↑increase , ↓decrease

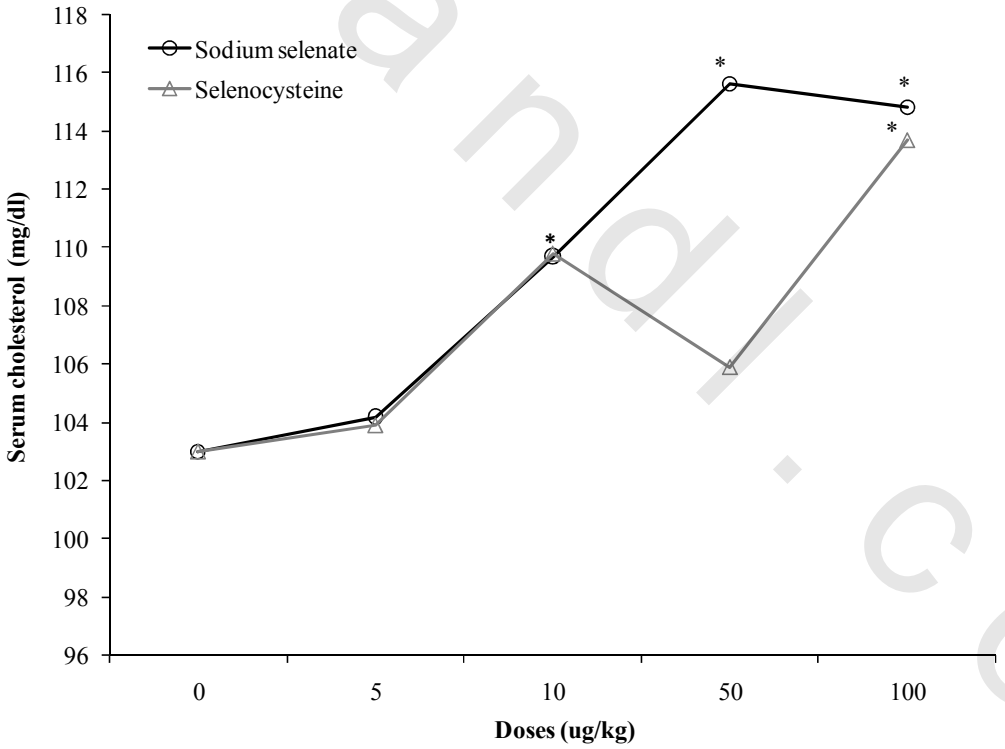

Figure (21): The change in serum cholesterol (mg/dl) in the rats supplemented with sodium selenate and selenocysteine at different doses for 3 months* Significant difference from control rats (0 dose)

Table (11): Serum concentration of triglycerides (mg/dl) in the control rats and rats supplemented with sodium selenate an selenocysteine at different doses

	Control	Sodium selenate (µg/kg)				Selenocysteine (µg/kg)			
		5	10	50	100	5	10	50	100
Min.	46.0	49.00	35.0	35.0	83.0	41.00	49.0	44.0	42.0
Max.	77.0	78.00	66.0	78.0	99.0	71.00	84.0	70.0	82.0
Mean	62.60	55.70	52.0	59.20	91.30	60.40	69.80	60.0	65.90
±SD.	10.94	9.98	12.45	13.27	5.01	11.32	11.68	8.86	18.11
Median	66.0	68.00	57.50	61.0	90.50	64.50	72.50	59.50	78.0
% of change		↓11.02	↓16.9	↓5.4	↑45.8	↓3.51	↑11.5	↓4.2	↑5.3
p_1		0.149	0.045*	0.520	<0.001*	0.791	0.130	0.622	0.384
p_2						0.307	0.008*	0.940	<0.001*

p_1: p value for Mann Whitney test for comparing between control and each other group
p_2: p value for Mann Whitney test for comparing between sodium selenate and selenocysteine at each concentration
*: Statistically significant at $p \leq 0.05$
↑increase , ↓decrease

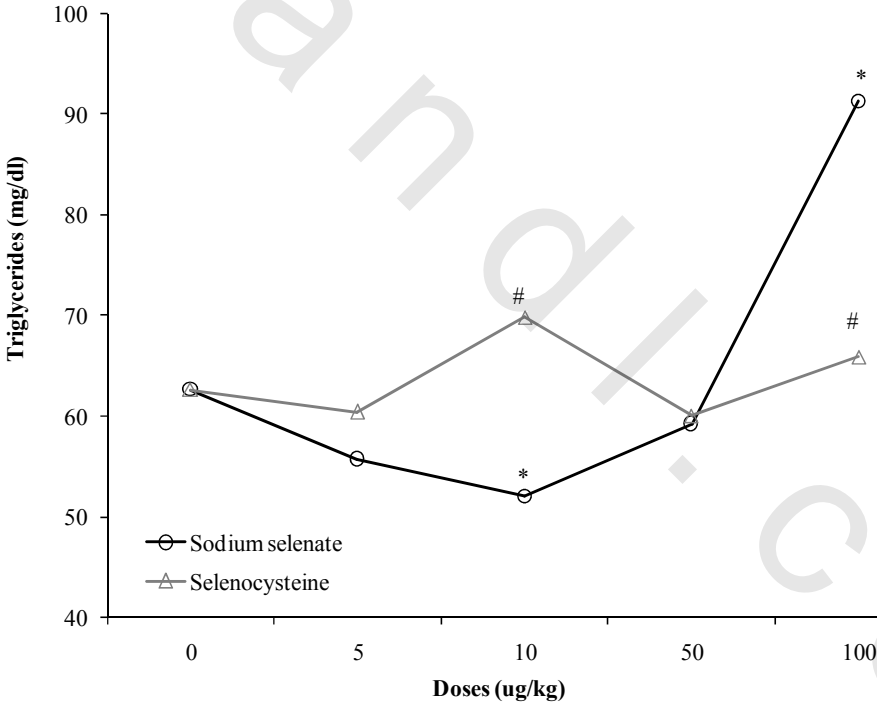

Figure (22): The change in serum concentration of triglycerides (mg/dl) in the rats supplemented with sodium selenate and selenocysteine at different doses for 3 months* Significant difference from control rats (0 dose), # Significant difference from sodium selenate at each dose

Table (12): Serum concentration of high density lipoprotein cholesterol (mg/dl) in the control rats and rats supplemented with sodium selenate and selenocysteine at different doses

	Control	Sodium selenate (µg/kg)				Selenocysteine (µg/kg)			
		5	10	50	100	5	10	50	100
Min.	26.0	28.00	31.0	31.0	37.0	27.00	30.0	30.0	30.0
Max.	35.0	36.00	43.0	43.0	47.0	35.00	40.0	41.0	49.0
Mean	30.80	31.10	36.50	37.90	40.80	30.70	35.30	35.20	41.0
±SD.	2.53	2.42	3.66	3.84	3.12	2.63	3.27	3.65	5.85
Median	31.0	31.00	36.50	39.0	40.0	30.50	34.0	34.50	42.0
% of change		↑0.97	↑18.5	↑23.1	↑32.5	↓0.32	↑14.6	↑14.3	↑33.1
p_1		0.484	0.104	0.016*	<0.001*	0.790	0.343	0.371	<0.001*
p_2						0.674	0.998	0.866	1.000

p_1: p value for Post Hoc test (Scheffe) for comparing between control and each other group
p_2: p value for Post Hoc test (Scheffe) for comparing between sodium selenate and selenocysteine at each concentration
*: Statistically significant at $p \leq 0.05$
↑increase , ↓decrease

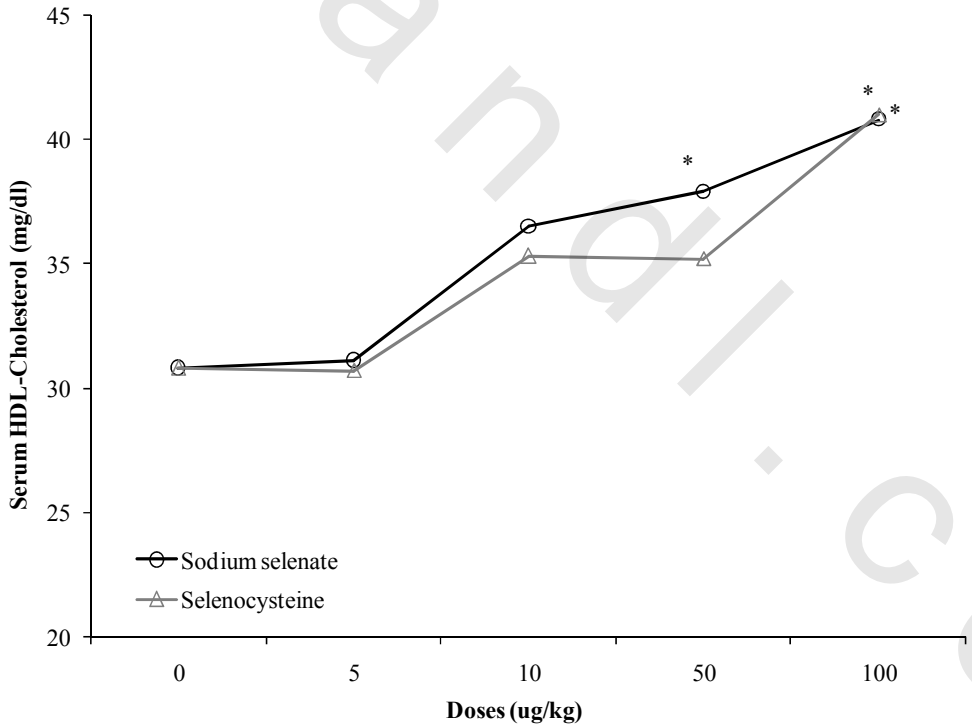

Figure (23): The change in serum concentration of HDL-Cholesterol (mg/dl) in the rats supplemented with sodium selenate and selenocysteine at different doses for 3 months (* Significant difference from control rats (0 dose), # Significant difference from sodium selenate at each dose)

Table (13): The serum concentration of low density lipoprotein cholesterol (mg/dl) in control rats and rats supplemented with sodium selenate and selenocysteine at different doses

	Control	Sodium selenate (µg/kg)				Selenocysteine (µg/kg)			
		5	10	50	100	5	10	50	100
Min.	46.20	50.00	48.20	51.0	46.80	36.70	52.60	51.20	53.0
Max.	71.80	71.70	101.0	77.0	67.40	61.10	69.20	66.40	65.60
Mean	59.68	59.39	62.80	65.86	55.74	57.33	60.54	58.70	59.52
±SD.	7.54	7.41	15.02	9.71	5.89	7.21	6.22	4.60	4.68
Median	58.90	58.20	58.80	68.10	54.80	54.10	60.70	58.70	61.40
% of change		↓0.49	↑5.2	↑10.4	↓6.6	↓3.94	↑1.4	↓1.6	↓0.3
p_1		0.705	0.921	0.186	0.186	0.081	0.791	0.705	0.740
p_2						0.082	0.791	0.082	0.104

p_1: p value for Mann Whitney test for comparing between control and each other group

p_2: p value for Mann Whitney test for comparing between sodium selenate and selenocysteine at each concentration

↑increase , ↓decrease

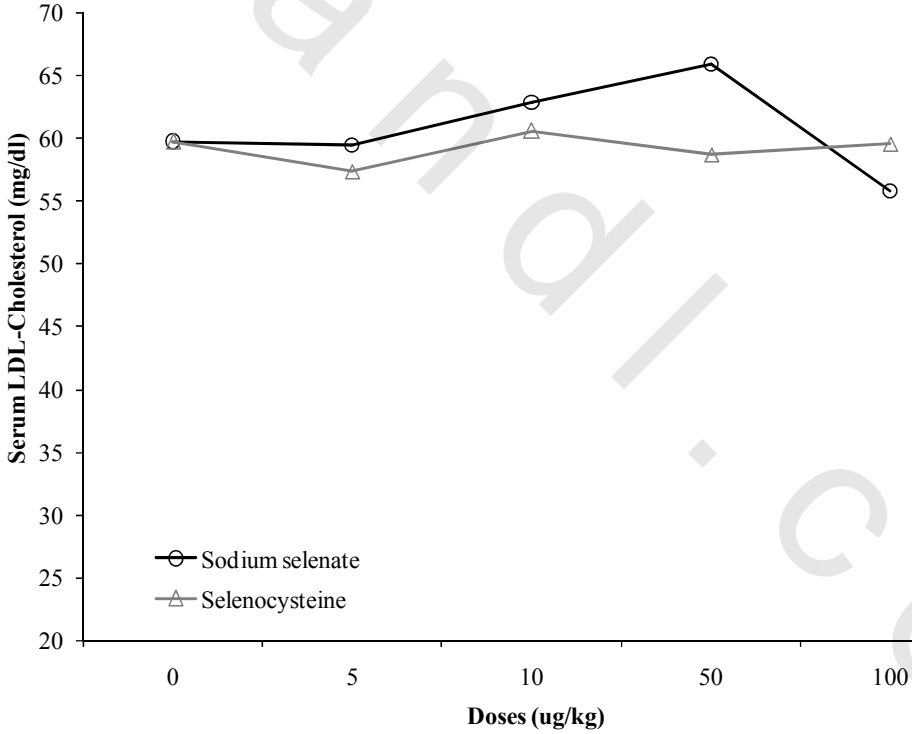

Figure (24): The change in serum concentration of LDL-Cholesterol (mg/dl) in the rats supplemented with sodium selenate and selenocysteine at different doses for 3 months .

Kidney function tests

The results of kidney function tests urea and creatinine were summarized in Tables (14,15) and Figures (25, 26).

The supplementations of rats with Se compounds result in mild increase of urea which significant only with sodium selenate only at dose of 10µg/kg. However the urea level still within the normal range. Selenocysteine supplementation have no significant effect on blood urea level (Table 14, Figure 25)

The serum creatinine level showed a dose-dependent increase by supplementation with Se compounds, however these increases are not significant. The only significant increase was observed in rats supplemented with the highest dose of selenocysteine (100µg/kg) (Table 15, Figure 26).

Table (14): Serum concentration of urea (mg/dl) in the control rats and rats supplemented with sodium selenate and selenocysteine at different doses

	Control	Sodium selenate (µg/kg)				Selenocysteine (µg/kg)			
		5	10	50	100	5	10	50	100
Min.	27	28.00	29	23.5	24	27.00	25.5	25	24
Max.	30.5	33.00	37	31.5	31.5	35.10	30	34	28
Mean	28.95	30.18	33.15	27.9	27.65	31.12	27.5	30.8	25.9
±SD.	1.3	1.78	2.88	3.14	2.51	2.81	1.83	3.33	1.37
Median	29	30.75	33	27.5	28	30.50	27.5	32.5	25.75
% of change		↑4.25	↑14.5	↓3.6	↓4.5	↑7.50	↓5.0	↑6.4	↓10.5
p_1		0.081	0.036*	0.988	0.964	0.088	0.940	0.827	0.279
p_2						0.405	0.001*	0.340	0.862

p_1: p value for Post Hoc test (Scheffe) for comparing between control and each other group
p_2: p value for Post Hoc test (Scheffe) for comparing between sodium selenate and selenocysteine at each concentration
*: Statistically significant at $p \leq 0.05$
↑increase , ↓decrease

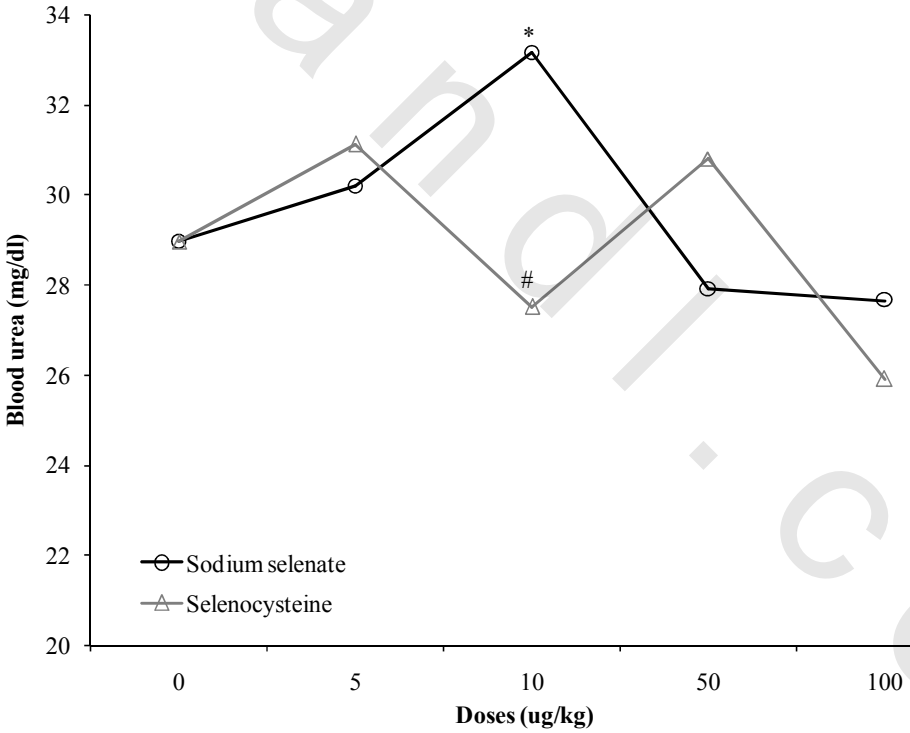

Figure (25): The change in serum urea (mg/dl) in the rats supplemented with sodium selenate and selenocysteine at different doses for 3 months*
Significant difference from control rats (0 dose), # Significant difference from sodium selenate at each dose

Table (15): Serum concentration of creatinine (mg/dl) in the control rats and rats supplemented with sodium selenate and selenocysteine at different doses

	Control	Sodium selenate (µg/kg)				Selenocysteine (µg/kg)			
		5	10	50	100	5	10	50	100
Min.	0.35	0.35	0.3	0.3	0.35	0.38	0.35	0.35	0.4
Max.	0.5	0.49	0.45	0.5	0.75	0.49	0.55	0.7	0.75
Mean	0.43	0.41	0.38	0.40	0.53	0.42	0.43	0.50	0.60
±SD.	0.05	0.05	0.06	0.07	0.13	0.04	0.07	0.14	0.14
Median	0.43	0.40	0.4	0.4	0.525	0.41	0.43	0.43	0.63
% of change		↓4.65	↓11.6	↓7.0	↑23.3	↓2.33	0.0	↑16.3	↑39.5
p_1		0.244	0.971	0.998	0.530	0.591	1.000	0.863	0.042*
p_2						0.540	0.983	0.530	0.900

p_1: p value for Post Hoc test (Scheffe) for comparing between control and each other group
p_2: p value for Post Hoc test (Scheffe) for comparing between sodium selenate and selenocysteine at each concentration
*: Statistically significant at $p \leq 0.05$
↑increase , ↓decrease

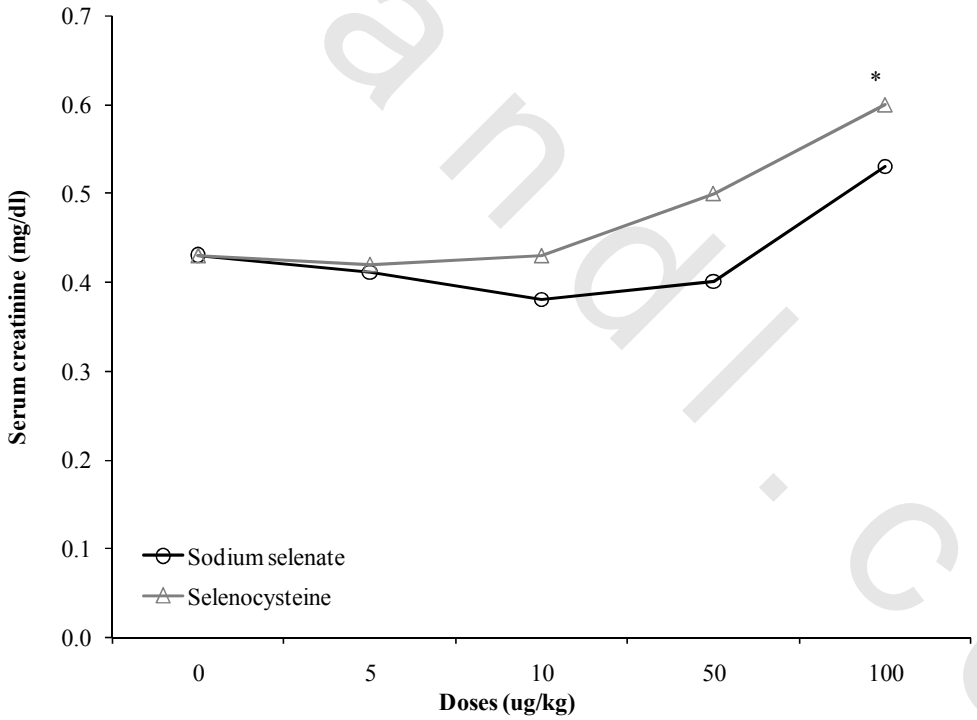

Figure (26): The change in serum concentration of creatinine (mg/dl) in the rats supplemented with sodium selenate and selenocysteine at different doses for 3 months * Significant difference from control rats (0 dose)

Biomarkers of Selenium status

1- Glutathione peroxidase (GPx)

The results of total, Se-dependent (Se-GPx) and non-Se- dependent GPx (n-Se-GPx) were summarized in Tables (16-24) and Figures (27-35).

The hepatic total GPx activity showed no significant dose-dependent increase with Se compounds supplementations at low doses and become significant only at the highest dose of selenocysteine (100µg/kg) which increase the activity by about 23.3% compared to control rats (Table 16, Figure 27).

The activity of total GPx in muscle showed typical dose dependent increase with the supplementation of rats with both Se supplementations; however selenocysteine is more effective in the induction of GPx activity (Table 17, Figure 28).

Also, in the adipose tissues the total GPx activity showed similar pattern of change as that observed in muscle tissues (Table 18, Figure 29).

The activity of Se-GPx in muscles and hepatic tissues showed a significant dose-dependent increase in rats supplemented with Se . It is clear that selenocysteine supplementation is more efficient in the induction of Se-GPx than sodium selenate (Tables 19,20 , and Figures 30,31). In adipose tissues, the supplementation of rats with selenocysteine results in a significant dose-dependent increase in the activity of Se-GPx; however sodium selenate supplementation showed dose – dependent increase in the activity of Se-GPx at low doses (5 , 10 µg/kg) .

In liver there are no significant effect of Se supplementation on the activity of n-Se-GPx (Table 22, Figure 33). In muscle, sodium selenate supplementation; only at the highest dose, significantly activate the n-Se-GPx by about 63% compared to control. The selenocysteine supplementation at the same dose inhibit the activity of n-Se-GPx by about 23.9 % compared to control values (Table 23, Figure 34). In adipose tissues, the activity of n-Se-GPx was significantly inhibited by both Se supplementations; however selenocysteine is more effective in the inhibitory effect on n-Se-GPx. The maximum inhibition observed with the dose of (10µg/kg) (Table 24, Figure 35).

Table (16): Hepatic total glutathione peroxidase activity (mU/mg protein) in control rats and rats supplemented with sodium selenate and selenocysteine at different doses

	Control	Sodium selenate (µg/kg)				Selenocysteine (µg/kg)			
		5	10	50	100	5	10	50	100
Min.	2.0	2.00	2.10	2.20	2.30	2.80	2.30	2.90	3.20
Max.	3.20	3.20	3.40	3.70	4.0	3.30	3.30	3.60	3.90
Mean	2.83	2.84	2.84	2.86	3.34	3.07	2.83	3.20	3.49
±SD.	0.34	0.32	0.46	0.42	0.51	0.18	0.32	0.21	0.23
Median	2.85	2.90	2.85	2.95	3.45	3.10	2.85	3.20	3.40
% of change		↑0.35	↑0.4	↑1.1	↑18.0	↑8.48	0.0	↑13.1	↑23.3
p_1		0.818	1.000	1.000	0.169	0.060	1.000	0.551	0.024*
p_2						0.055	1.000	0.650	0.991

p_1: p value for Post Hoc test (Scheffe) for comparing between control and each other group
p_2: p value for Post Hoc test (Scheffe) for comparing between sodium selenate and selenocysteine at each concentration
*: Statistically significant at $p \leq 0.05$
↑increase , ↓decrease

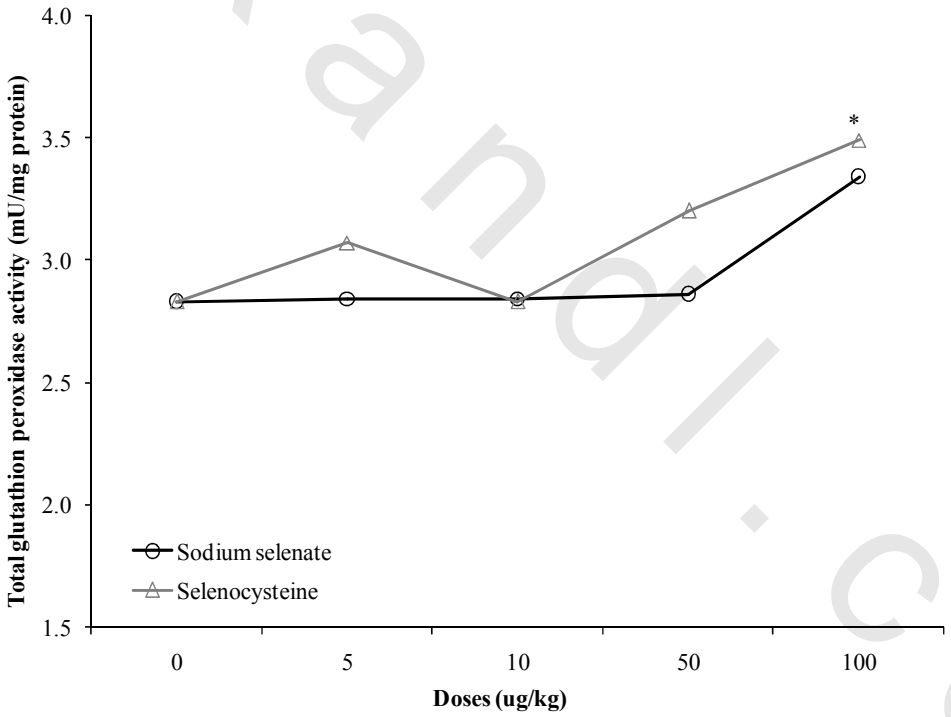

Figure (27): The change in hepatic activity of total glutathione peroxidase in the rats supplemented with sodium selenate and selenocysteine at different doses for 3 months * Significant difference from control rats (0 dose)

Table (17): The muscles total glutathione peroxidase activity (mU/mg protein) in control rats and rats supplemented with sodium selenate and selenocysteine at different doses

	Control	Sodium selenate (µg/kg)				Selenocysteine (µg/kg)			
		5	10	50	100	5	10	50	100
Min.	2.00	2.50	2.10	2.10	3.50	2.50	2.60	2.90	3.30
Max.	3.20	3.30	3.80	3.40	4.30	3.30	3.40	4.40	4.80
Mean	2.60	2.79	2.84	2.91	3.82	2.81	3.10	3.60	4.12
±SD.	0.31	0.25	0.57	0.35	0.28	0.23	0.29	0.47	0.43
Median	2.60	2.90	2.60	2.90	3.75	2.80	3.15	3.55	4.15
% of change		↑7.31	↑9.2	↑11.9	↑46.9	↑8.08	↑19.2	↑38.5	↑58.5
p_1		0.060	0.593	0.014*	<0.001*	0.067	0.003*	<0.001*	<0.001*
p_2						0.402	0.129	0.002*	0.081

p_1: p value for Mann Whitney test for comparing between control and each other group

p_2: p value for Mann Whitney test for comparing between sodium selenate and selenocysteine at each concentration

*: Statistically significant at $p \leq 0.05$

↑increase , ↓decrease

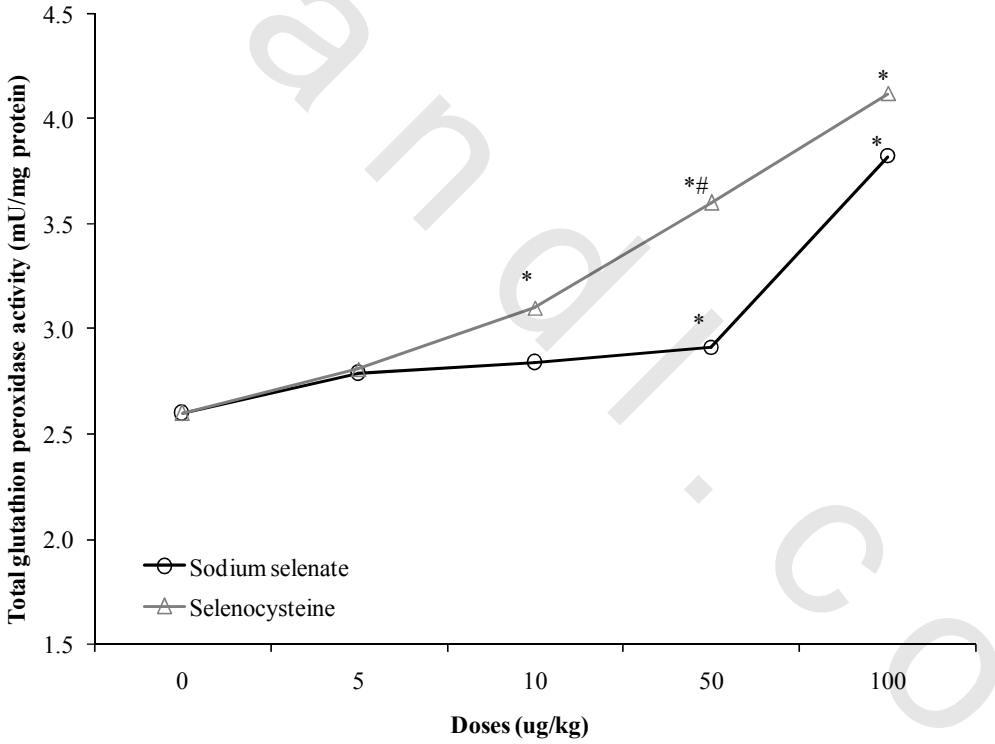

Figure (28): The change in muscle activity in the rats supplemented with sodium selenate and selenocysteine at different doses for 3 months * Significant difference from control rats (0 dose), # Significant difference from sodium selenate at each dose

Table (18): The white adipose tissue total glutathione peroxidase activity (mU/mg protein) in control rats and rats supplemented with sodium selenate and selenocysteine at different doses

	Control	Sodium selenate (µg/kg)				Selenocysteine (µg/kg)			
		5	10	50	100	5	10	50	100
Min.	2.40	2.50	2.80	3.00	3.00	2.50	3.20	3.40	3.60
Max.	3.40	3.50	4.30	4.30	4.50	3.60	4.10	4.30	4.90
Mean	2.93	3.01	3.67	3.66	3.94	3.04	3.75	3.80	4.56
±SD.	0.30	0.29	0.40	0.38	0.39	0.33	0.25	0.35	0.42
Median	2.90	2.95	3.75	3.60	4.00	2.95	3.80	3.60	4.70
% of change		↑2.73	↑25.3	↑24.9	↑34.5	↑3.75	↑28.0	↑29.7	↑55.6
p_1		0.377	0.002*	<0.001*	<0.001*	0.299	<0.001*	<0.001*	<0.001*
p_2						0.847	0.618	0.402	0.004*

p_1: p value for Mann Whitney test for comparing between control and each other group
p_2: p value for Mann Whitney test for comparing between sodium selenate and selenocysteine at each concentration
*: Statistically significant at $p \leq 0.05$
↑increase , ↓decrease

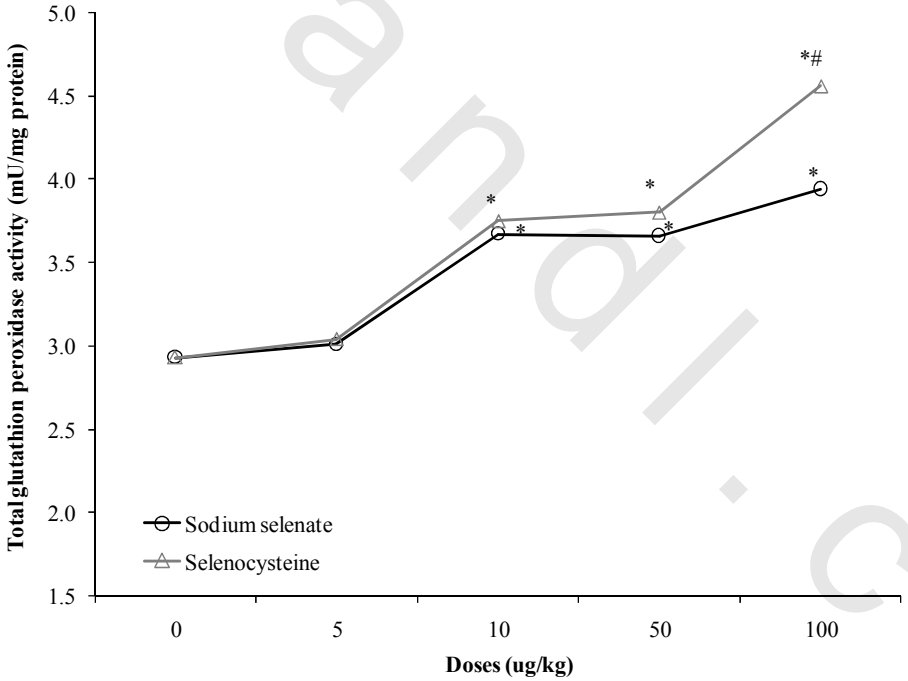

Figure (29): The change in white adipose tissue activity of total glutathione peroxidase in the rats supplemented with sodium selenate and selenocysteine at different doses for 3 months * Significant difference from control rats (0 dose), # Significant difference from sodium selenate at each dose

Table (19): The hepatic activity of selenium dependent glutathione peroxidase (mU/mg protein) in control rats and rats supplemented with sodium selenate and selenocysteine at different doses

	Control	Sodium selenate (µg/kg)				Selenocysteine (µg/kg)			
		5	10	50	100	5	10	50	100
Min.	1.50	1.60	1.40	1.50	1.50	1.60	1.70	1.80	2.00
Max.	2.20	2.40	2.60	2.40	3.10	2.30	2.40	2.80	3.00
Mean	1.77	1.90	1.84	1.88	2.34	1.85	2.02	2.35	2.68
±SD.	0.21	0.23	0.36	0.34	0.52	0.21	0.20	0.31	0.33
Median	1.75	1.90	1.75	1.85	2.30	1.80	2.00	2.45	2.75
% of change		↑7.34	↑4.0	↑6.2	↑32.2	↑4.52	↑14.1	↑32.8	↑51.4
p_1		0.169	1.000	0.997	0.038*	0.356	0.836	0.033*	<0.001*
p_2						0.538	0.962	0.155	0.537

p_1: p value for Post Hoc test (Scheffe) for comparing between control and each other group
p_2: p value for Post Hoc test (Scheffe) for comparing between sodium selenate and selenocysteine at each concentration
*: Statistically significant at $p \leq 0.05$
↑increase , ↓decrease

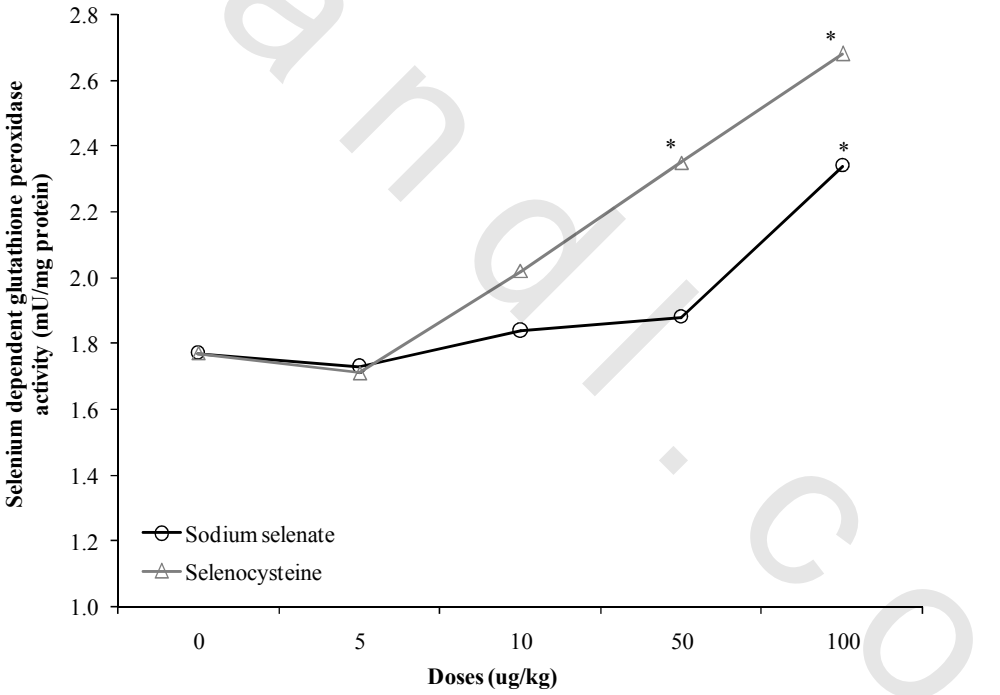

Figure (30): The change in hepatic activity of selenium dependent glutathione peroxidase (Se-GPx) in the rats supplemented with sodium selenate and selenocysteine at different doses for 3 months * Significant difference from control rats (0 dose)

Table (20): The muscle activity of selenium dependent glutathione peroxidase (mU/mg protein) in control rats and rats supplemented with sodium selenate and selenocysteine at different doses

	Control	Sodium selenate (µg/kg)				Selenocysteine (µg/kg)			
		5	10	50	100	5	10	50	100
Min.	1.50	1.60	1.60	1.50	1.80	1.60	1.60	2.20	3.00
Max.	2.20	2.30	2.40	2.40	3.00	2.30	2.80	2.90	4.20
Mean	1.68	1.73	1.86	1.88	2.32	1.71	2.20	2.58	3.42
±SD.	0.23	0.23	0.28	0.28	0.36	0.20	0.41	0.27	0.39
Median	1.60	1.75	1.75	1.85	2.35	1.75	2.15	2.60	3.35
% of change		↑2.98	↑10.7	↑11.9	↑38.1	↑1.79	↑31.0	↑53.6	↑103.6
p_1		0.075	0.057	0.066	0.001*	0.064	0.004*	<0.001*	<0.001*
p_2						1.000	0.062	<0.001*	0.004*

p_1: p value for Mann Whitney test for comparing between control and each other group

p_2: p value for Mann Whitney test for comparing between sodium selenate and selenocysteine at each concentration

*: Statistically significant at $p \leq 0.05$

↑increase , ↓decrease

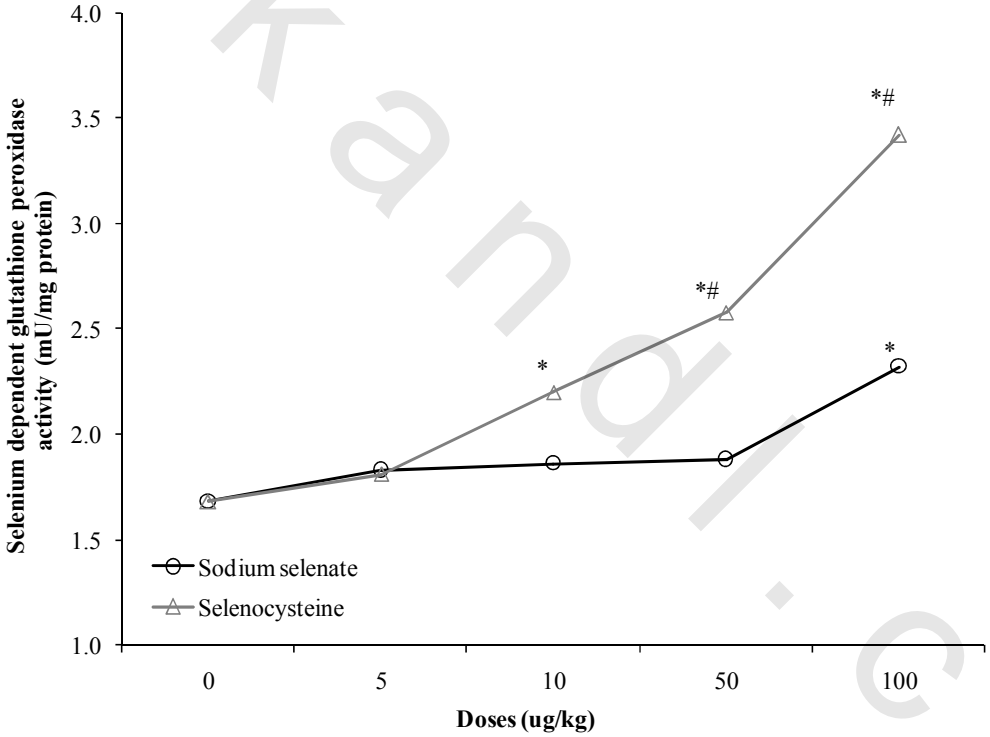

Figure (31): The change in muscle activity of selenium dependent glutathione peroxidase (Se-GPx) in the rats supplemented with sodium selenate and selenocysteine at different doses for 3 months * Significant difference from control rats (0 dose), # Significant difference from sodium selenate at each dose

Table (21): The white adipose tissue activity of selenium dependent glutathione peroxidase (mU/mg protein) in control rats and rats supplemented with sodium selenate and selenocysteine at different doses

	Control	Sodium selenate (µg/kg)				Selenocysteine (µg/kg)			
		5	10	50	100	5	10	50	100
Min.	1.10	1.10	1.70	2.00	2.30	1.30	2.70	2.80	3.10
Max.	1.90	2.00	3.20	3.70	3.50	1.90	3.30	3.70	4.30
Mean	1.52	1.60	2.88	2.83	2.80	1.65	3.13	3.07	3.89
±SD.	0.26	0.29	0.43	0.51	0.39	0.21	0.18	0.25	0.33
Median	1.60	1.65	3.0	2.85	2.75	1.65	3.15	3.0	4.0
% of change		↑5.26	↑89.5	↑86.2	↑84.2	↑8.55	↑105.9	↑102.0	↑155.9
p_1		0.469	<0.001*	<0.001*	<0.001*	0.267	<0.001*	<0.001*	<0.001*
p_2						0.760	0.039*	0.223	<0.001*

p_1: p value for Mann Whitney test for comparing between control and each other group

p_2: p value for Mann Whitney test for comparing between sodium selenate and selenocysteine at each concentration

*: Statistically significant at $p \leq 0.05$

↑increase , ↓decrease

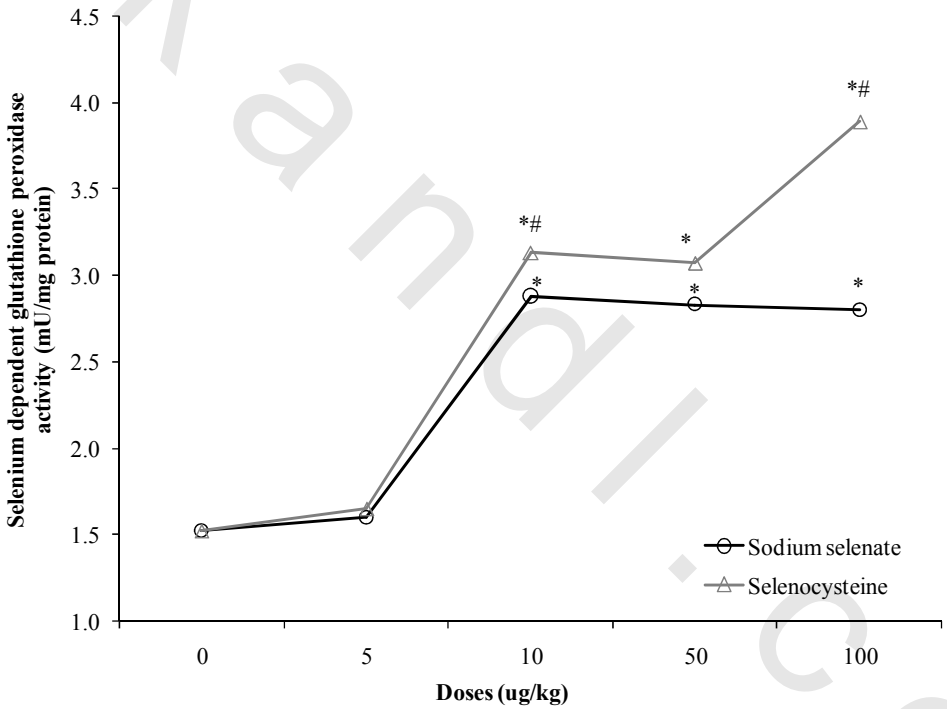

Figure (32): The change in white adipose tissue activity of selenium dependent glutathione peroxidase (Se-GPx) in the rats supplemented with sodium selenate and selenocysteine at different doses for 3 months * Significant difference from control rats (0 dose), # Significant difference from sodium selenate at each dose

Table (22): The hepatic activity of non selenium dependent glutathione peroxidase (mU/mg protein) in control rats and rats supplemented with sodium selenate and selenocysteine at different doses

	Control	Sodium selenate (µg/kg)				Selenocysteine (µg/kg)			
		5	10	50	100	5	10	50	100
Min.	0.50	0.40	0.40	0.20	0.40	0.50	0.50	0.40	0.50
Max.	1.60	1.30	1.70	1.50	1.70	1.60	1.20	1.10	1.30
Mean	1.06	0.94	1.0	0.98	1.0	1.22	0.81	0.85	0.81
±SD.	0.35	0.28	0.44	0.36	0.55	0.34	0.21	0.21	0.28
Median	1.05	0.95	0.90	1.00	1.10	1.30	0.80	0.85	0.80
% of change		↓11.32	↓5.7	↓7.5	↓5.7	↑15.09	↓23.6	↓19.8	↓23.6
p_1		0.447	0.649	0.620	0.789	0.254	0.073	0.094	0.101
p_2						0.051	0.374	0.222	0.647

p_1: p value for Mann Whitney test for comparing between control and each other group

p_2: p value for Mann Whitney test for comparing between sodium selenate and selenocysteine at each concentration

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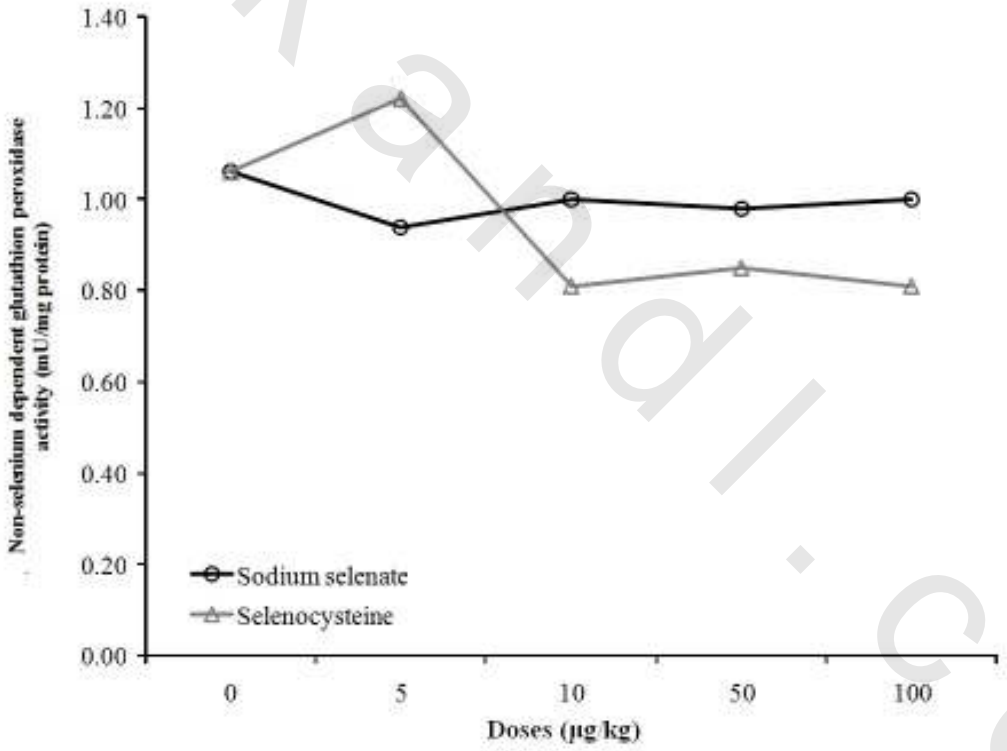

Figure (33): The hepatic activity of non selenium dependent glutathione peroxidase (n-Se-GPx) in the rats supplemented with sodium selenate and selenocysteine at different doses for 3 months

Table (23): Muscles activity of non selenium dependent glutathione peroxidase (mU/mg protein) in control rats and rats supplemented with sodium selenate and selenocysteine at different doses

	Control	Sodium selenate (µg/kg)				Selenocysteine (µg/kg)			
		5	10	50	100	5	10	50	100
Min.	0.50	0.40	0.40	0.40	0.50	0.30	0.50	0.50	0.30
Max.	1.60	1.60	1.60	1.70	2.10	1.30	1.50	1.90	1.10
Mean	0.92	1.06	0.98	1.03	1.50	0.99	0.90	1.02	0.70
±SD.	0.33	0.37	0.48	0.46	0.49	0.30	0.38	0.43	0.22
Median	0.95	1.10	0.95	0.95	1.60	1.00	0.85	0.95	0.70
% of change		↑15.22	↑6.5	↑12.0	↑63.0	↑7.61	↓2.2	↑10.9	↓23.9
p_1		0.321	0.939	0.790	0.013*	0.541	0.848	0.676	0.128
p_2						0.542	0.790	0.940	0.002*

p_1: p value for Mann Whitney test for comparing between control and each other group
p_2: p value for Mann Whitney test for comparing between sodium selenate and selenocysteine at each concentration
*: Statistically significant at $p \leq 0.05$
↑increase , ↓decrease

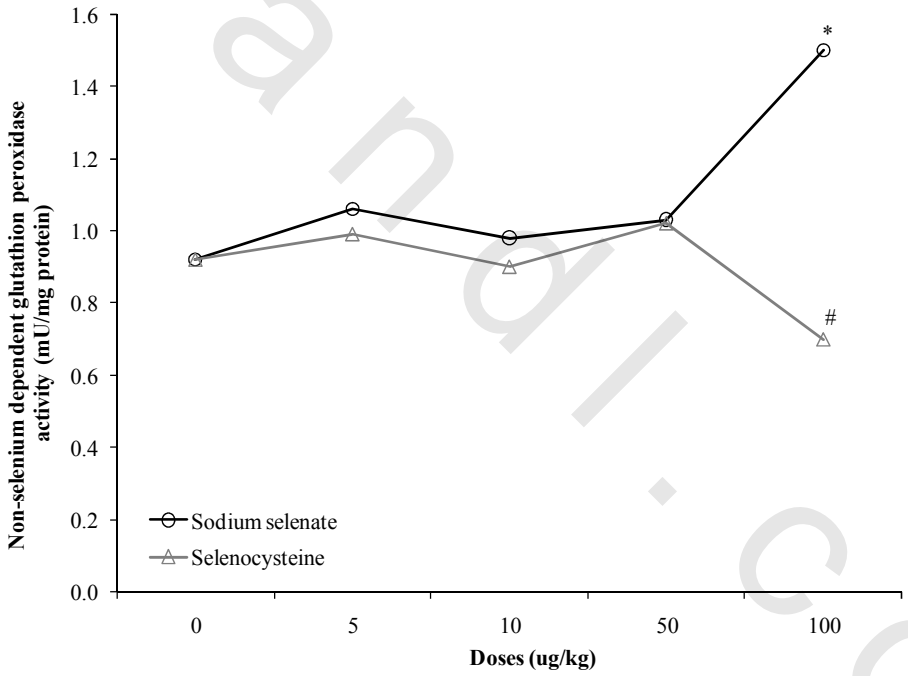

Figure (34): The change in muscle activity of non selenium dependent glutathione peroxidase (n-Se-GPx) in the rats supplemented with sodium selenate and selenocysteine at different doses for 3 months * Significant difference from control rats (0 dose), # Significant difference from sodium selenate at each dose**.**

Table (24): The white adipose tissues activity of non selenium dependent glutathione peroxidase (mU/mg protein) in control rats and rats supplemented with sodium selenate and selenocysteine at different doses

	Control	Sodium selenate (µg/kg)				Selenocysteine (µg/kg)			
		5	10	50	100	5	10	50	100
Min.	0.50	0.60	0.40	0.50	0.50	1.00	0.50	0.40	0.50
Max.	2.0	1.90	1.30	1.20	1.70	2.10	0.80	1.40	0.90
Mean	1.41	1.41	0.79	0.83	1.14	1.35	0.62	0.73	0.67
±SD.	0.42	0.39	0.30	0.30	0.38	0.40	0.13	0.36	0.16
Median	1.45	1.40	0.80	0.85	1.20	1.20	0.60	0.60	0.65
% of change		0.0	↓44.0	↓41.1	↓19.1	↓4.26	↓56.0	↓48.2	↓52.5
p_1		0.969	0.003*	0.004*	0.101	0.380	0.001*	0.004*	0.002*
p_2						0.374	0.242	0.443	0.007*

p_1: p value for Mann Whitney test for comparing between control and each other group

p_2: p value for Mann Whitney test for comparing between sodium selenate and selenocysteine at each concentration

*: Statistically significant at $p \leq 0.05$

↑increase , ↓decrease

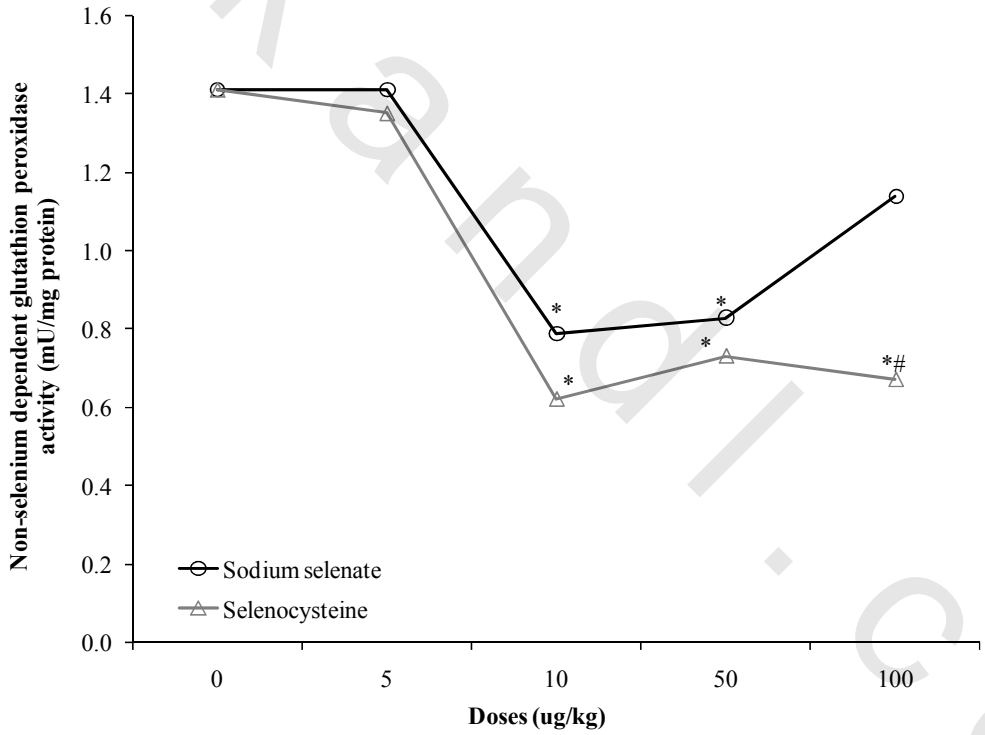

Figure (35): The change in white adipose tissue activity of non selenium dependent glutathione peroxidase (n-Se-GPx) in the rats supplemented with sodium selenate and selenocysteine at different doses for 3 months * Significant difference from control rats (0 dose), # Significant difference from sodium selenate at each dose

1- Selenoprotein P (SeP)

The results of tissues contents of SeP are summarized in Tables (25-27) and Figures (36-38).

In liver and muscle tissues the SeP content showed a significant dose-dependent increase with Se supplementations especially at high doses (50 and 100 µg/kg). The selenocysteine supplementations are more prominent in the induction of SeP than sodium selenate (Table 25,26 and Figure 36,37). In adipose the dose dependent increase of SeP is observed; however the pattern is reversed because the sodium selenate supplementation increases SeP level greater than selenocysteine supplementation (Table 27, Figure 38).

Table (25): The hepatic content of selenoprotein P (ng/mg protein) in control rats and rats supplemented with sodium selenate and selenocysteine at different doses

	Control	Sodium selenate (µg/kg)				Selenocysteine (µg/kg)			
		5	10	50	100	5	10	50	100
Min.	0.21	0.21	0.21	0.22	0.21	0.22	0.21	0.31	0.32
Max.	0.31	0.33	0.33	0.41	0.44	0.33	0.33	0.41	0.51
Mean	0.25	0.26	0.24	0.32	0.36	0.26	0.28	0.35	0.43
±SD.	0.05	0.05	0.03	0.05	0.07	0.05	0.04	0.05	0.07
Median	0.22	0.24	0.24	0.32	0.38	0.24	0.29	0.33	0.43
% of change		↑4.0	↓4.0	↑28.0	↑44.0	↑4.0	↑12.0	↑40.0	↑72.0
p_1		0.261	0.466	0.007*	0.004*	0.191	0.216	0.001*	<0.001*
p_2						0.848	0.062	0.312	0.044*

p_1: p value for Mann Whitney test for comparing between control and each other group
p_2: p value for Mann Whitney test for comparing between sodium selenate and selenocysteine at each concentration
*: Statistically significant at $p \leq 0.05$
↑increase , ↓decrease

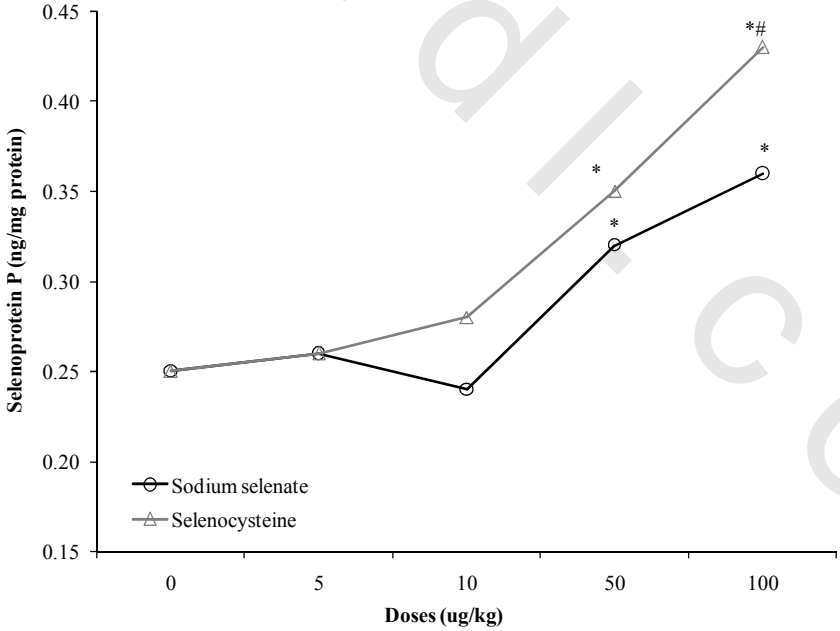

Figure (36): The change in hepatic content of selenoprotein P (ng/mg protein) in the rats supplemented with sodium selenate and selenocysteine at different doses for 3 months * Significant difference from control rats (0 dose), # Significant difference from sodium selenate at each dose

Table (26): The muscle content of selenoprotein P (ng/mg protein) in the control rats and rats supplemented with sodium selenate and selenocysteine at different doses

	Control	Sodium selenate (µg/kg)				Selenocysteine (µg/kg)			
		5	10	50	100	5	10	50	100
Min.	0.11	0.20	0.21	0.21	0.28	0.11	0.22	0.28	0.31
Max.	0.41	0.43	0.39	0.38	0.41	0.43	0.33	0.42	0.51
Mean	0.27	0.28	0.29	0.31	0.33	0.29	0.29	0.36	0.39
±SD.	0.09	0.08	0.07	0.05	0.04	0.09	0.04	0.06	0.07
Median	0.28	0.33	0.31	0.31	0.33	0.29	0.31	0.37	0.41
% of change		↑3.70	↑7.4	↑14.8	↑22.2	↑7.41	↑7.4	↑33.3	↑44.4
p_1		0.885	0.819	0.543	0.119	0.596	0.670	0.003*	0.008*
p_2						0.449	0.876	0.036*	0.056

p_1: p value for Mann Whitney test for comparing between control and each other group
p_2: p value for Mann Whitney test for comparing between sodium selenate and selenocysteine at each concentration
*: Statistically significant at $p \leq 0.05$
↑increase , ↓decrease

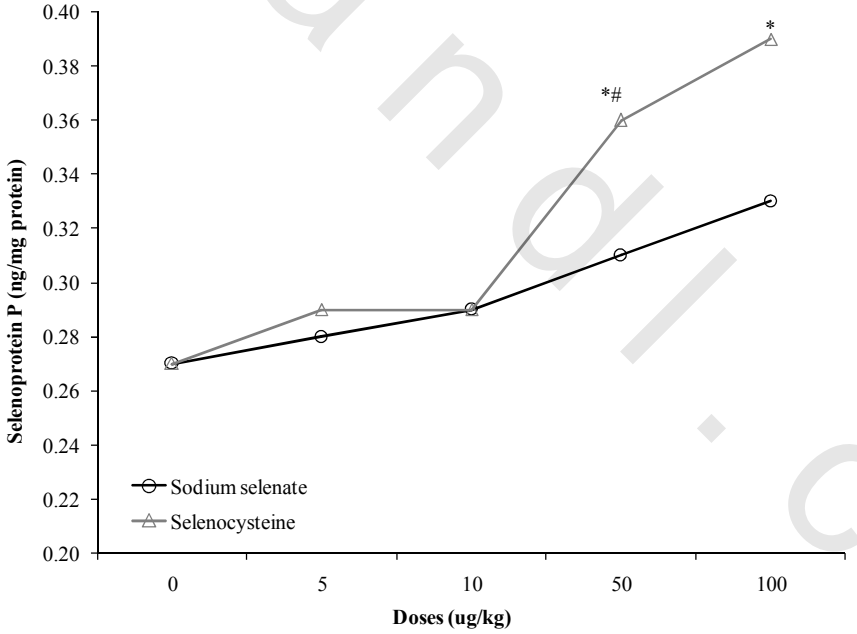

Figure (37): The change in muscle content of selenoprotein P (ng/mg protein) in the rats supplemented with sodium selenate and selenocysteine at different doses for 3 months* Significant difference from control rats (0 dose), # Significant difference from sodium selenate at each dose

Table (27): White adipose tissue content of selenoprotein P (ng/mg protein) in control rats and rats supplemented with sodium selenate and selenocysteine at different doses

	Control	Sodium selenate (µg/kg)				Selenocysteine (µg/kg)			
		5	10	50	100	5	10	50	100
Min.	0.11	0.13	0.22	0.41	0.33	0.11	0.27	0.28	0.35
Max.	0.33	0.35	0.51	0.52	0.62	0.34	0.36	0.45	0.44
Mean	0.28	0.29	0.39	0.46	0.49	0.28	0.31	0.37	0.39
±SD.	0.07	0.07	0.09	0.05	0.10	0.07	0.03	0.06	0.03
Median	0.31	0.31	0.41	0.46	0.51	0.32	0.31	0.40	0.40
% of change		↑3.57	↑39.3	↑64.3	↑75.0	0.0	↑10.7	↑32.1	↑39.3
p_1		0.490	0.012*	<0.001*	<0.001*	0.421	0.446	0.010*	<0.001*
p_2						0.939	0.034*	0.003*	0.030*

p_1: p value for Mann Whitney test for comparing between control and each other group

p_2: p value for Mann Whitney test for comparing between sodium selenate and selenocysteine at each concentration

*: Statistically significant at $p \leq 0.05$

↑increase , ↓decrease

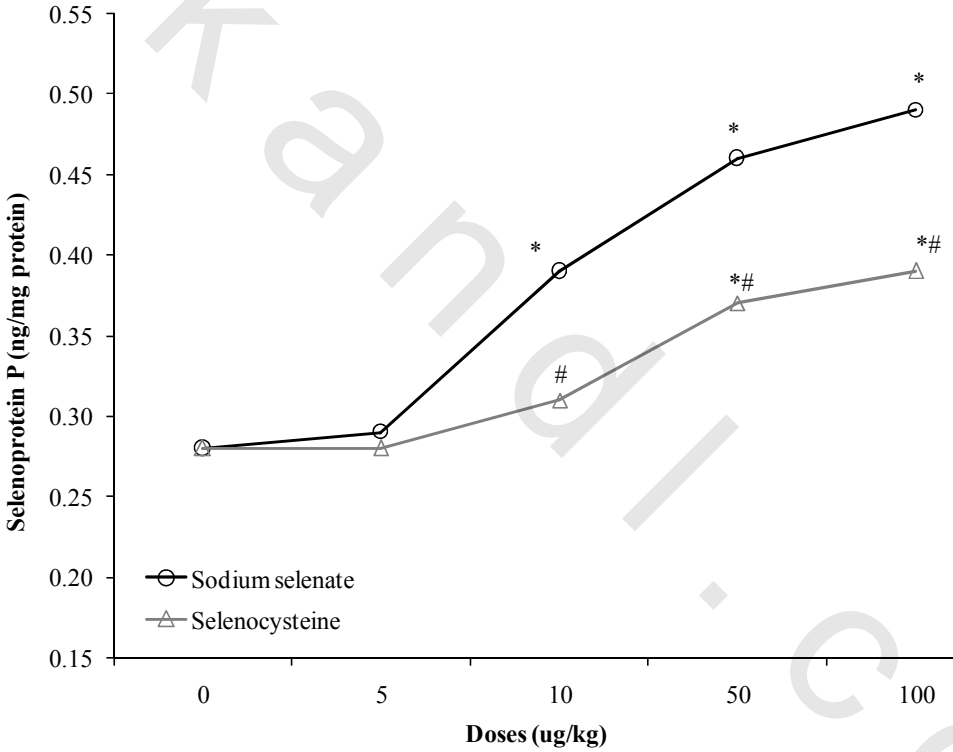

Figure (38): The change in white adipose tissue content of selenoprotein P (ng/mg protein) in the rats supplemented with sodium selenate and selenocysteine at different doses for 3 months * Significant difference from control rats (0 dose), # Significant difference from sodium selenate at each dose .

Correlation

Table (28): Correlation between all parameters

	Parameter	+ve correlation	-ve correlation	Parameter	tissue	group	r	P	No. of fig.
1.	Fasting glucose	+ve		Sel P	liver	selenocysteine	0.548	<0.002	39
2.	Fasting glucose	+ve		Sel P	muscle	Selenocysteine	0.529	<0.003	40
3.	Fasting glucose	+ve		Sel P	Adipose tissue	selenocysteine	0.402	<0.028	41
4.	Fasting glucose	+ve		Se-Gpx	liver	Selenocysteine	0.632	<0.001	42
5.	Fasting glucose	+ve		Se-Gpx	muscle	Selenocysteine & sodium selenate	0.599 0.433	<0.001 <0.017	43
6.	Fasting glucose	+ve		Se-Gpx	Adipose tissue	Sodium selenate	0.463	<0.010	44
7.	Serum insulin		-ve	Se-Gpx	liver	Sodium selenate	-0.489	<0.006	45
8.	Sel P in liver		-ve	GLIUT 4	muscle	Selenocysteine & sodium selenate	-0.742 -0.507	<0.001 <0.004	46
9.	Sel P in muscle		-ve	GLUT 4	muscle	Selenocysteine & Sodium selenate	-0.622 -0.398	<0.001 <0.029	47
10.	Ph-insulin receptor		-ve	GLUT 4	muscle	Selenocysteine	-0.455	<0.011	48
11.	Triglycerides	+ve		Sel P	liver	Sodium selenate	0.483	<0.007	49
12.	Triglycerides	+ve		Se-Gpx	liver	Sodium selenate	0.429	<0.018	50
13.	Triglycerides	+ve		Se-Gpx	muscle	Sodium selenate	0.450	<0.013	51
14.	Se-Gpx	+ve		Sel P	liver	Selenocysteine	0.640	<0.001	52

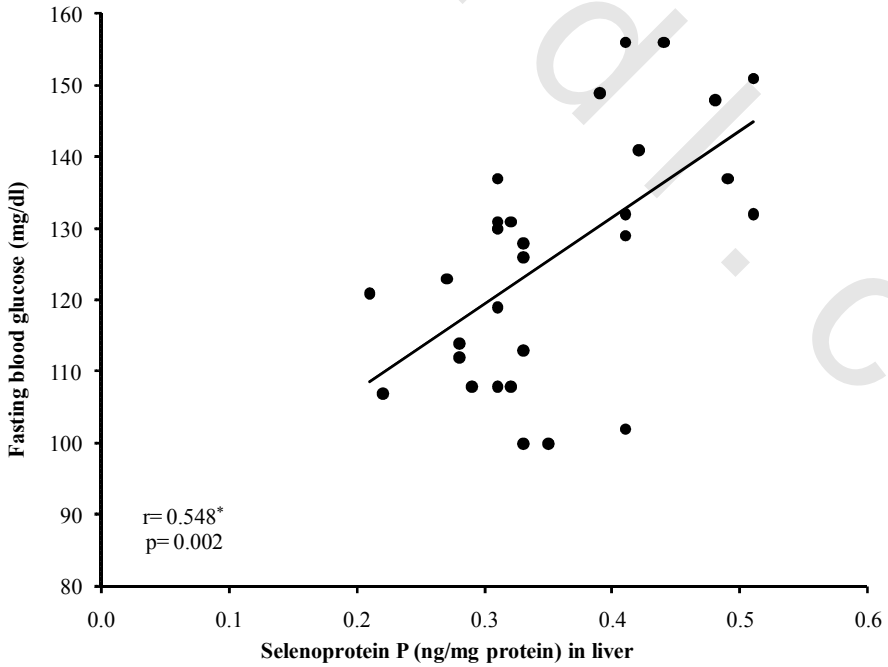

Figure (39): Correlation curve between serum fasting glucose and selenoprotein p in liver in selenocysteine group.

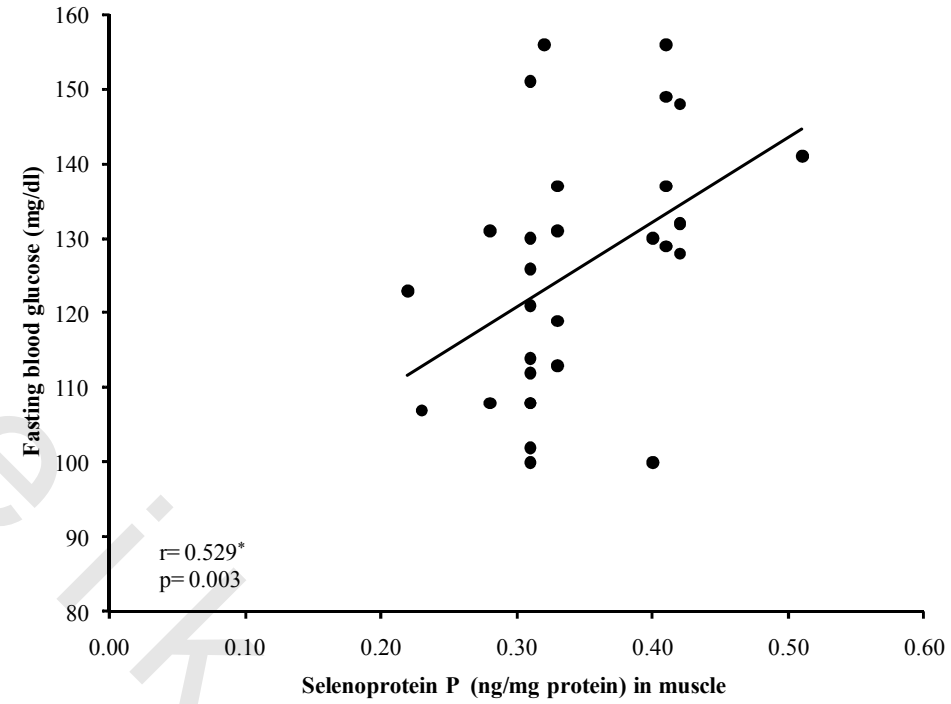

Figure (40): Correlation curve between serum fasting glucose and selenoprotein p in muscle in selenocysteine group.

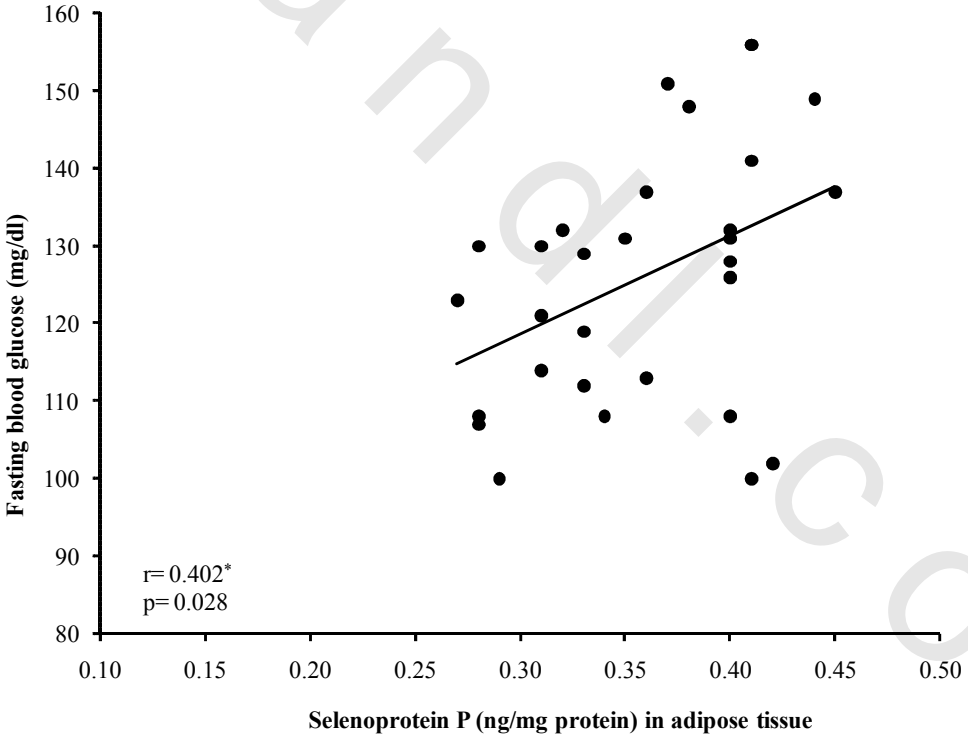

Figure (41): Correlation curve between fasting serum glucose and selenoprotein p in adipose tissue in selenocysteine group .

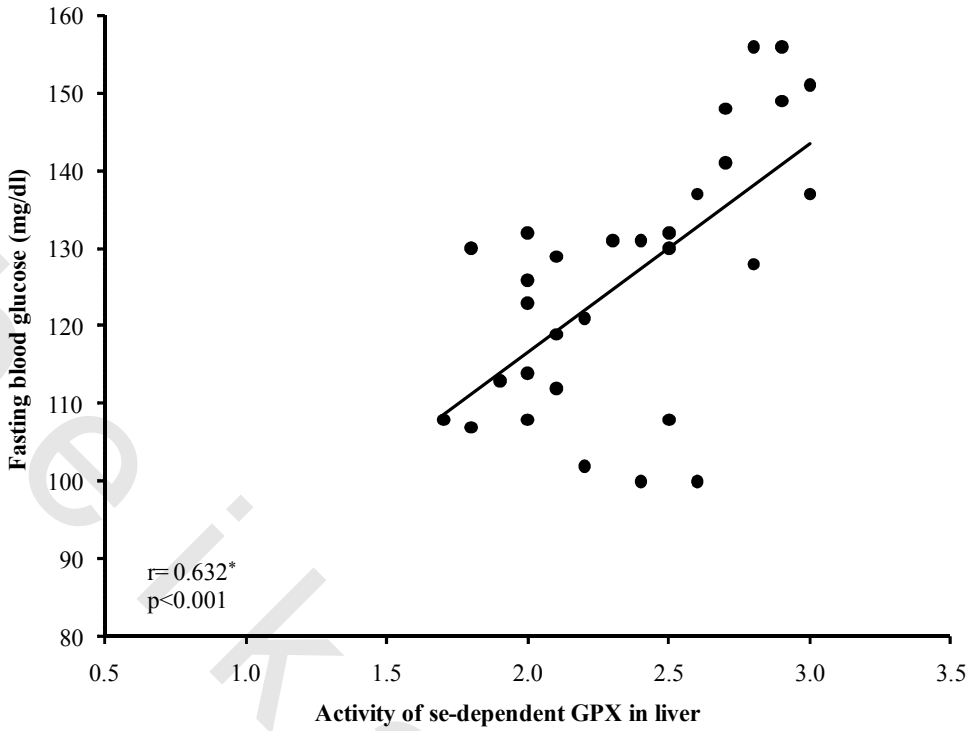

Figure (42): Correlation curve between fasting serum glucose and activity of se-dependent GPX in liver in selenocysteine group.

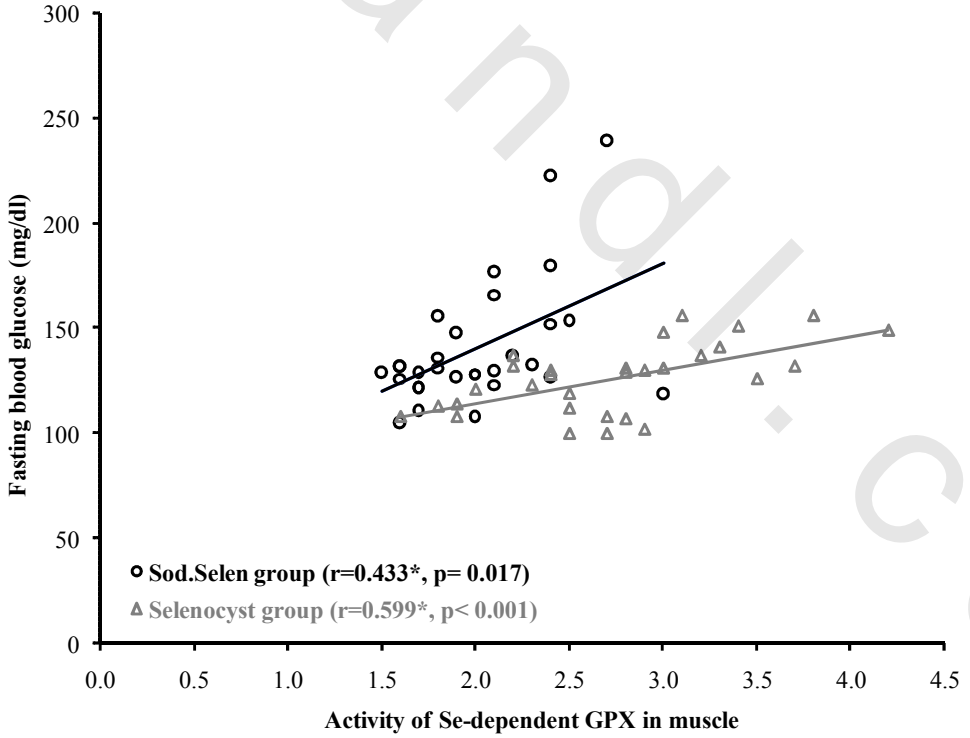

Figure (43): Correlation curve between fasting serum glucose and activity of Se-dependent GPX in muscle in both groups (sodium selenate and selenocysteine)

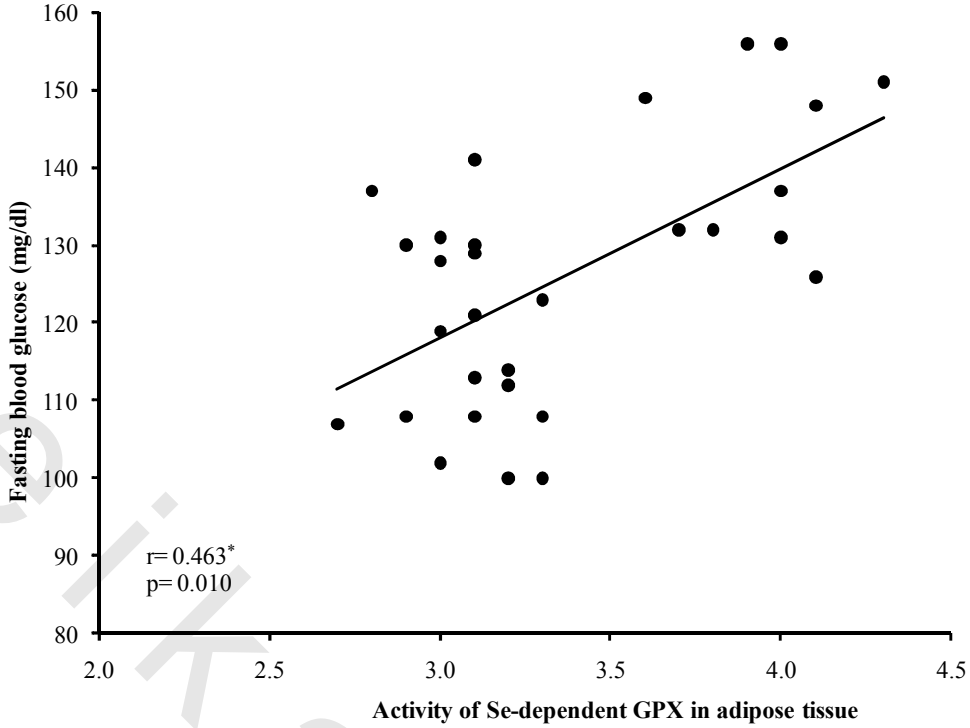

Figure (44): Correlation curve between fasting glucose and activity of Se-dependent GPX in adipose tissue in Sodium selenate group

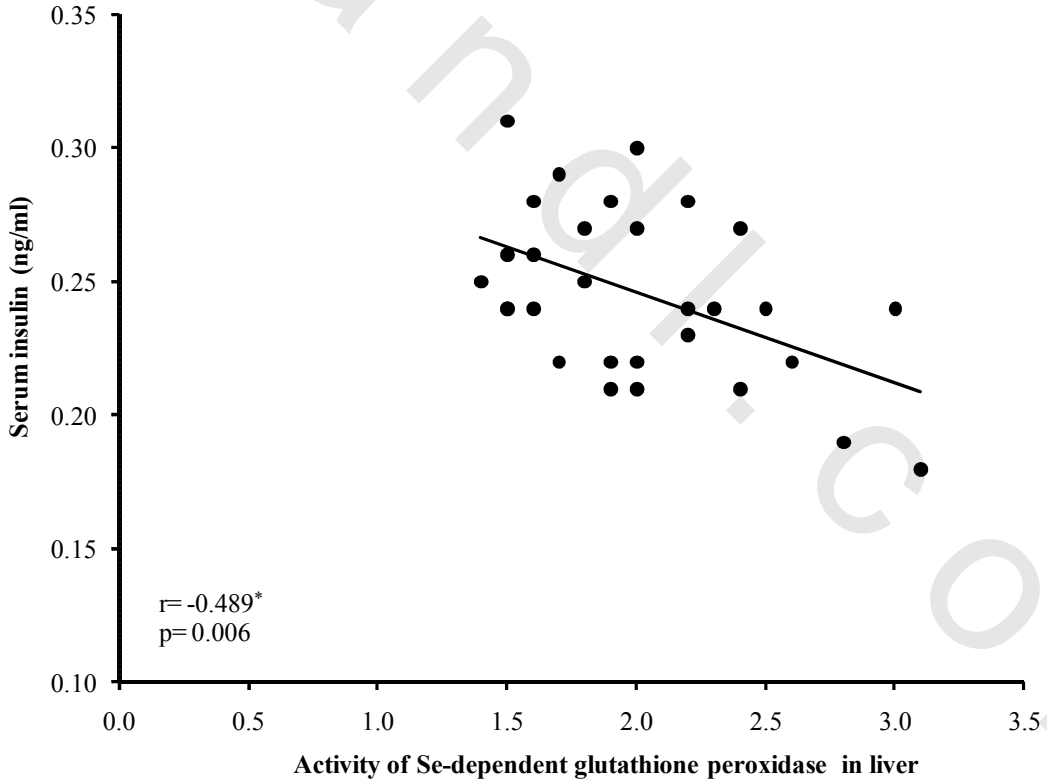

Figure (45): Correlation curve between activity of Se-dependent glutathione peroxidase in liver and serum insulin in sodium selenate group.

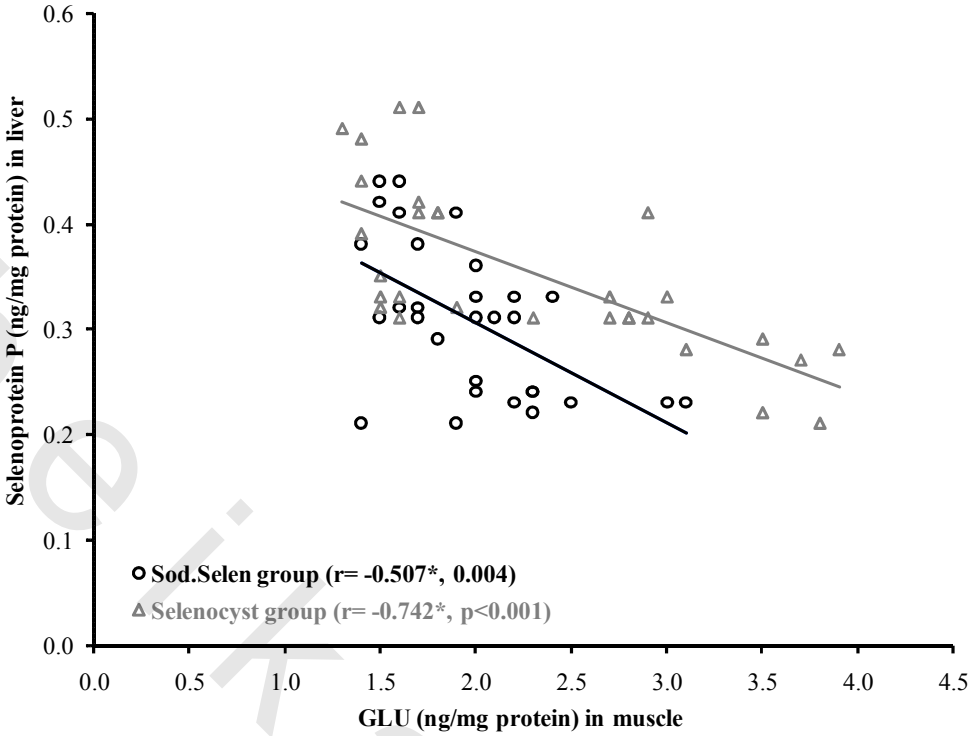

Figure (46): Correlation curve between Selenoprotein P in liver and glucose transporter 4 in muscle in both groups.

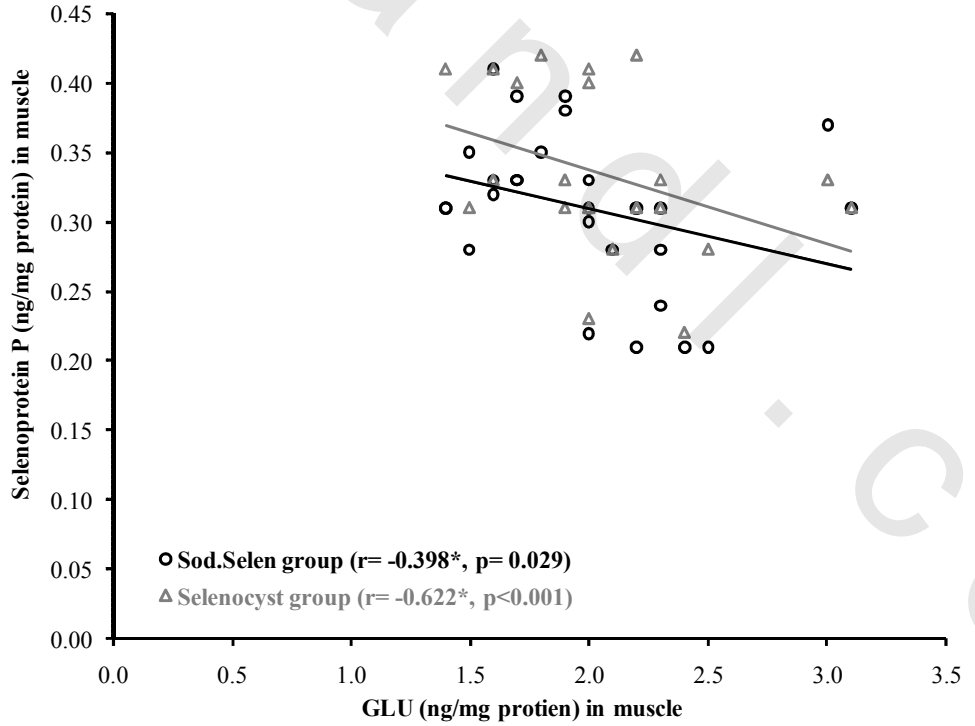

Figure (47): Correlation curve between selenoprotein P and GLUT 4 in muscle in both groups.

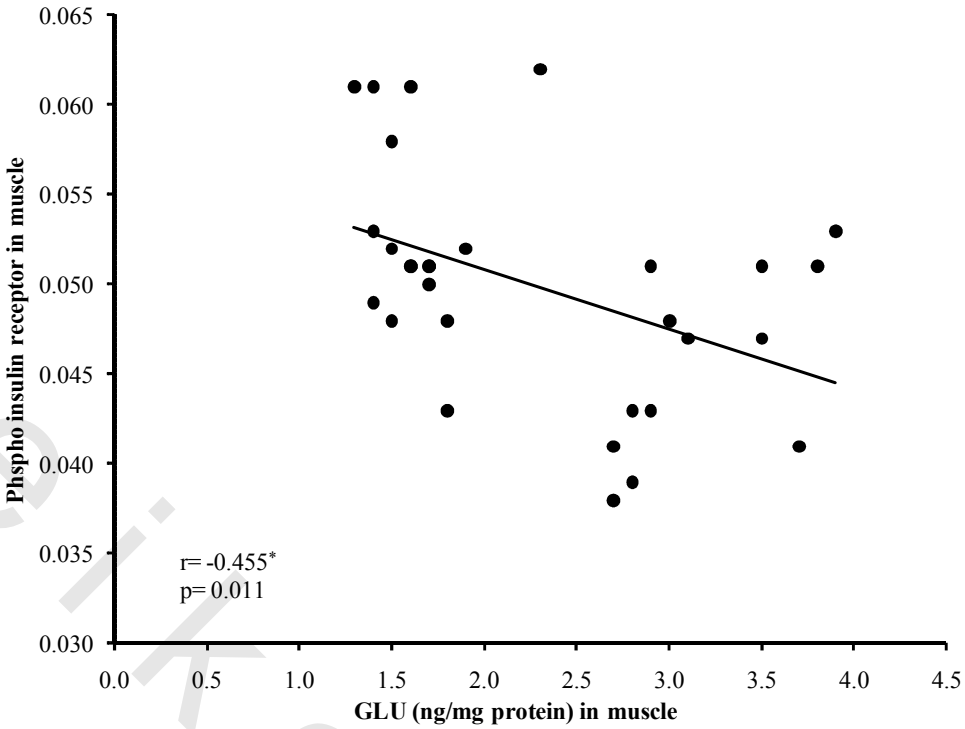

Figure (48): Correlation curve between phspho-insulin receptor and GLUT4 in muscle in selenocysteine treated group.

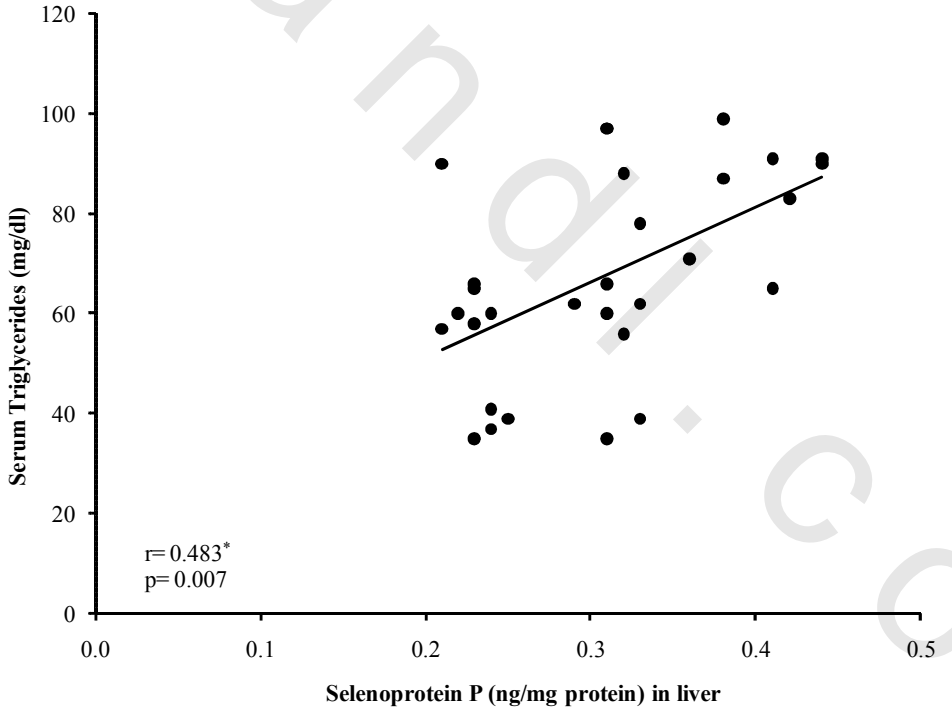

Figure (49): Correlation between serum triglycerides and selenoprotein P in liver in sodium selenate group.

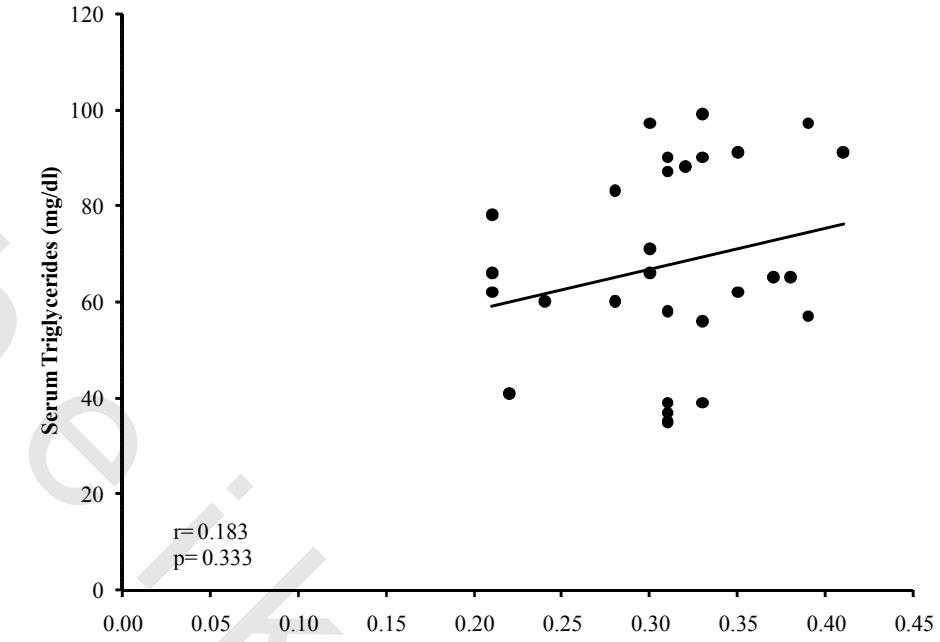

Figure (50): Correlation between serum triglycerides and the activity of Se-dependent GPX in the liver in sodium selenate group.

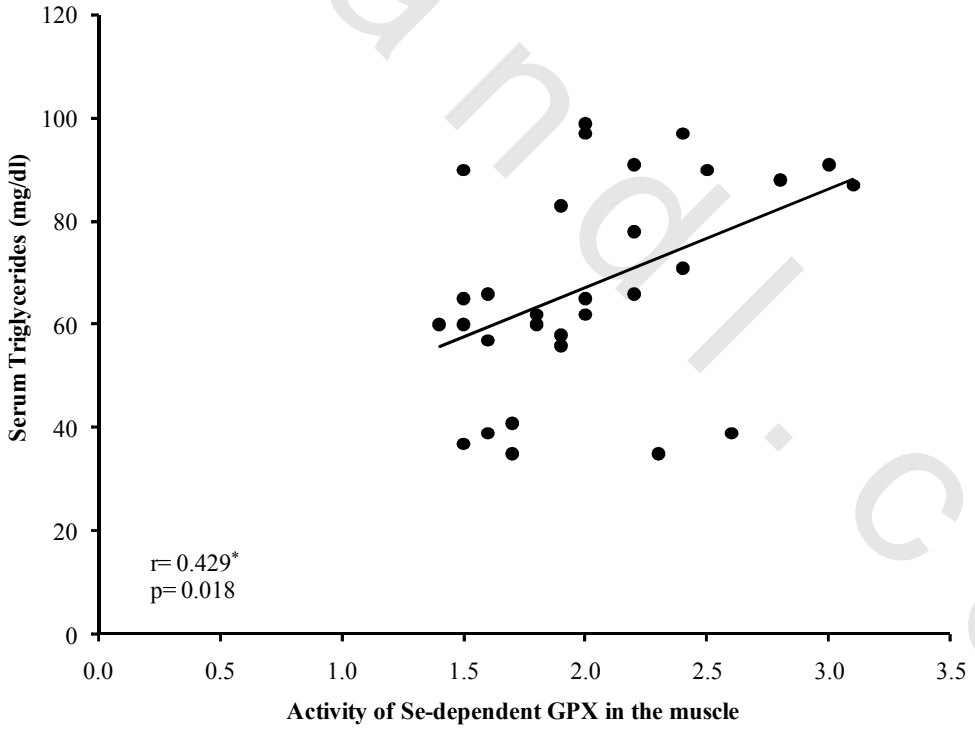

Figure (51): Correlation between serum triglycerides and the activity of Se-dependent GPX in the muscle in sodium selenate group.

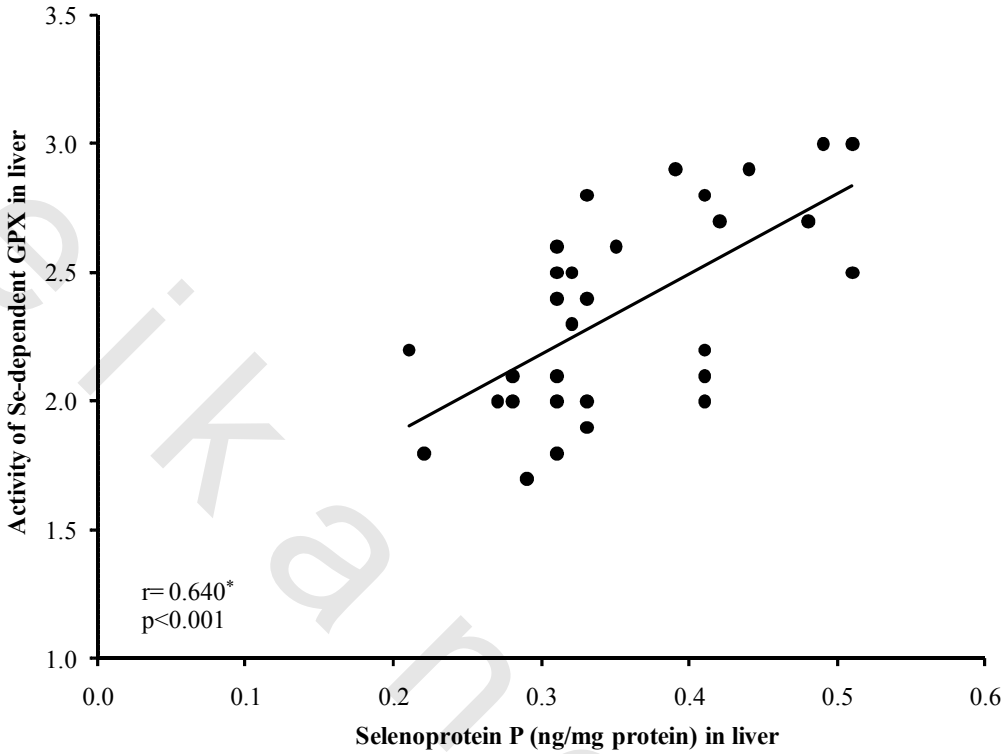

Figure (52): Correlation between the activity of Se-dependent GPX and selenoprotein P in liver in selenocysteine group.

Histopathology of pancreas:

Histopathological examination of H&E stained sections of pancreas revealed that while control rats showed pancreatic islets of normal size, shape and architecture with centrally placed nuclei (Slide 1), the pancreas of rats treated with the highest dose of sodium selenate showed marked inflammation of pancreas and the degenerated islet of irregular shape with cytoplasmic vacuolization and apoptotic cells (indicated by arrow) (Slide 2). Pancreas of rats treated with highest dose of selenocysteine showed very mild change in the islet morphology with no sign of apoptotic cell death (Slide 3).

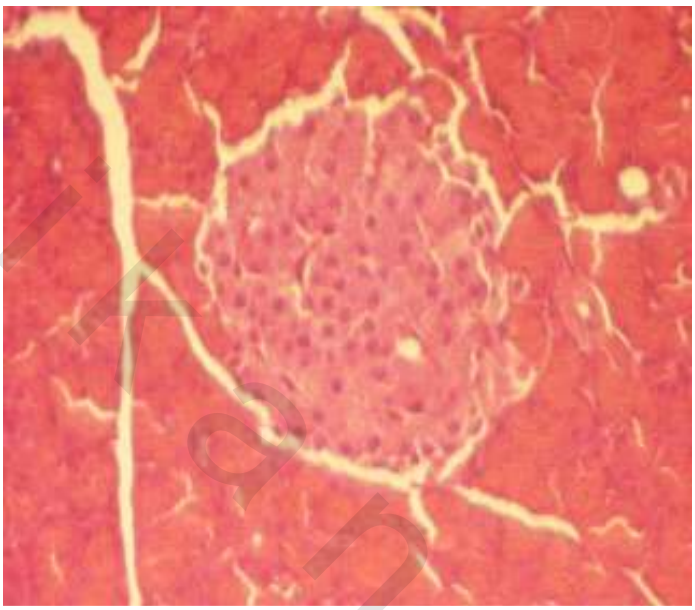

Figure (53) Slide 1 : H&E stain of pancreas section from control rats

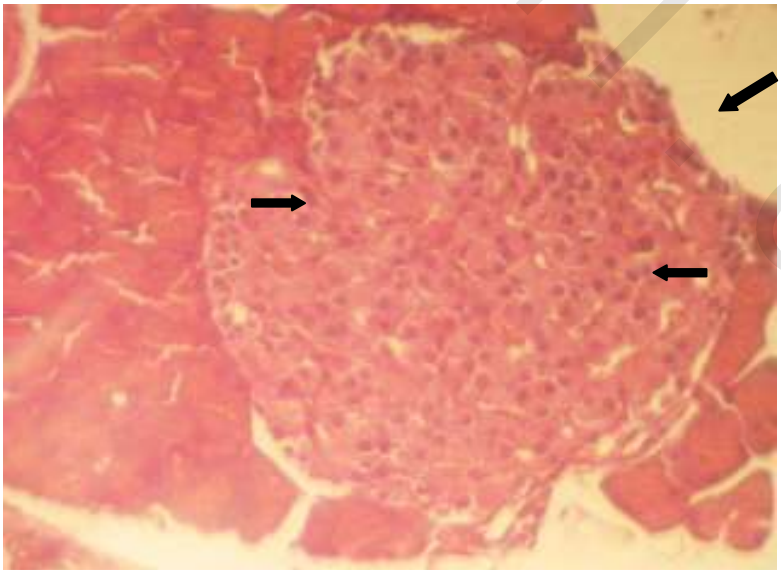

Figure (54) Slide 2 : H&E stain of pancreas section from rats supplemented with 100µg/kg sodium selenate

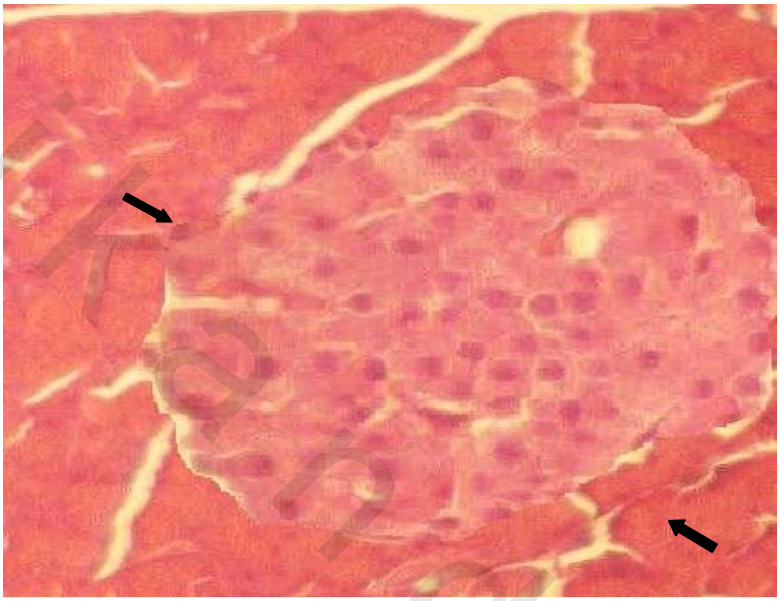

Figure (55) Slide 3: H&E stain of pancreas section from rats supplemented with 100µg/kg selenocysteine