Study of some of Novel Selena-Diazole Derivative activities on Hematological Parameters, Differentiate lymphocytes cells in addition to thyroid hormones levels in Female Rats (One of the series of studies on the impact of the new compound)

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Abstract
A number of organo-selenium compounds have been planned and synthesized for pharmacological properties. A novel selena-diazole derivative compound [4′, 4′- (4, 5, 6, 7-Tetrahydro- [1, 2, 3-] Selena-diazolo [4, 5-e] Pyridine-4, 6-Diyil)] Bis (Benzene-1, 3-Diol)] (SeD) was previously synthesized, identified. The main objective of the current study is the study of some of its pharmacological effects; include hematological profile, T and B lymphocytes count, effects on prothrombin time; also, SeD activity on thyroid hormone.

Two groups of Wister female Rats, Control group (NS) and the second Test (SeD) group received a daily dose of 50 mg/Kg BW for one month. Complete Blood cell count; Deferential Lymphocytes count; Serum Thyroid hormones were evaluated; also, vitro Prothrombin Time (PT) international normalized ratio (INR) and activated partial thromboplastin time (aPTT) were estimated.

The results indicate that a significantly increase in WBC and Lymphocytes%. While Granulocytes and blood platelets count were significantly decreased. a PT and INR values were important differences with the heparin group (H). Total Lymphocytes, T cells and B cells of (SeD) greatly enhanced than control. TSH increased significantly, while T3 and T4 serum concentrations were significantly decline than control. It can be concluded that SeD can increased WBC and lymphocytes cells. SeD at low concentrations decreased were showed significant decline a PT and PT-INR than Heparin treated group. Also, SeD has stimulating effects on TSH and inhibited T4 and T3 thyroid hormones.

Keywords: CBC count, clotting time, INR, aPTT, Total lymphocytes, T cells, B cells, TSH

1. INTRODUCTION
The Selenium (Se) a metalloid, an important element to humans and animals have been confirmed as a chemo-therapeutic and cancer-chemo preventive agent by several pre-clinical and clinical investigations [1]. A novel selena-diazole derivative compound was synthesized by the authors at College of pharmacy, [4′, 4′- (4, 5, 6, 7-Tetrahydro- [1, 2, 3-] Selena-diazolo [4, 5-e] Pyridine-4, 6-Diyil)] Bis (Benzene-1, 3-Diol)] (SeD), identification of the chemical structure as in fig 1 and some of pharmacological effects have been studied like antimicrobial activity, median lethal dose [2]. Furthermore, biochemical profiling [3], the histopathological effects [4] and its effects on reproduction and reproductive hormones were studied [5].

A precise valuation of the coagulation status of the patient is decisive, like in the blood product transfusion to control impaired coagulation and avoid dangerous loss of blood or anticoagulant drugs administration to inhibit thrombotic states, to monitor the transfusion strategy or permit actual anticoagulant dosing, [6]. About 25 selenoproteins were identified in humans, and 24 of them prevail in rodents as Se including proteins, therefore rodent models mainly used in the study of immune responses of Se proteins [7]. Analysis of blood parameters is relevant to the risk evaluation of alterations of the hematological system. Hematological parameters are of diagnostic significance in the clinical evaluation of the state of health. It is an established fact that chronic diseases affect blood cells adversely. Free radicals result in the disruption of membrane fluidity, lipid peroxidation, oxidative DNA and alteration of platelet functions, which have generally been considered to be linked with many chronic health problems such as diabetes,
cancers, inflammation, aging, and atherosclerosis[8]. Platelet indices are platelet activation biomarkers Platelet indices, include plateletcrit (PTC), platelet distribution width (PDW), and mean platelet volume (MPV) which is determined in automatic CBC profiles; they are associated to platelets’ proliferation kinetics and morphology. Platelets stimulation leads to change their shape, increase the surface area and bioactive molecules secretion, like chemokines, cytokines, β-thromboglobulin and growth factors [9]. The Prothrombin Time (PT) test is used to evaluate the ability of blood to clot, PT can be altered by some drugs like the oral contraceptive and it also occurred with some diseases such as liver disease and vitamin K deficiency, also due to high vitamin containing diet like soybeans. The normal range of PT could be more dissimilar among laboratories. Therefore, the results were standardized using International Normalized Ratio (INR), typically normal INR is about 0.9 - 1.1, INR elevates to between 2 and 3.5 on warfarin therapy. PT- INR is used to evaluate bleeding hazard and prognosis in cirrhosis, and to monitor the management of related coagulation disturbances [10]. Activated partial thromboplastin time (aPTT) a test mainly used to evaluate specific coagulation factors. The aPTT mainly used to monitor the heparin therapy response in patients with low clotting time and in excessive bleeding patients such as in hemophilia [11].

In general T cell’s responses are termed cell-mediated immunity; they are primarily produced in the bone marrow and migrate to the thymus for maturation. Which vary from B cells antibodies response; they are produced and develop in the bone marrow. T cells cannot recognize antigen alone, their receptors can recognize only the antigen that bound to proteins of cell membrane, and their responses are excellently antigen-specific, most importantly, unlike B cells, T cells can help eliminate pathogens that reside inside host cells [12]. B cells can produce antibodies by various mechanisms, their antibodies against a proximately unrestricted variety of pathogens. Also, B cells provide co-stimulation and cytokines to T cells, and immunomodulatory effect by secreting cytokines that inhibit disease onset and progression [13]. Flow cytometry able to measure the fluorescence and optical features of a single cell or some other particle-like microorganisms, chromosome and nuclei preparations in a fluid stream when they pass through a light source. The data achieved gives respected information about molecular, biophysical and biochemical aspects of particles[15]. Selenium containing proteins have distinct roles include defense from oxidative stress, removal of signaling peroxides, and decrease of membranes and proteins oxidation in addition to inflammatory response modulation. Furthermore, synthesis of seleno-cysteine for example; Deiodinases of three thyroid hormones are selenium containing proteins named thioredoxin reductases enzymes. The central role of thioredoxin reductases is T4 hormone activation through the removal of iodine [16]. Type I iodothyronine deiodinase, the enzyme responsible in humans for most of the peripheral conversion of thyroxine (T4) to the active form 3,3’,5-triiodothyronine (T3), is a selenoenzyme. While type II deiodinase which responsible for T4 conversion to T3 in the brain, and the type III deiodinase (the inner ring deiodinase responsible for deactivatingT4 and T3) are also selenocysteine enzymes [17].

2. MATERIALS AND METHODS

2.1 Animal preparation

Female Wister rats at age of 30-32days weighted 140-164g; they were divided into two groups, ten rats in each group. Control group (NS) received 2 ml of 5 (DMSO): 95 (normal saline). The second group received daily dose at 50 mg/Kg BW of (SeD) compound dissolved in 5% DMSO: 95% Distilled water (NS) for 30 days. The dose of test compound was determined from previous study by the authors [2], therefor no mortality was reported. Then under the effects of chloroform, the blood collected directly from the heart in EDTA anticoagulant tubes, for complete blood count and different lymphocyte cells count. For Thyroid hormones evaluation blood collected into Gel Containing Tubes, blood centrifuged for 5 min 4000 rpm for plasma separation [14]. In vitro PT and aPTT measurement, blood was drawn directly from the heart from each healthy non-treated female rat into Sodium citrate anticoagulant tubes (VWR Chemical UK), after mixing the tubes, blood centrifuged for 5 min 4000 rpm for plasma separation.

2.2 Complete blood count (CBC)

Blood sample (2mL) kept in EDTA-blood tubes. The hematological tests were done in the Laboratory of Pharmacy College at the University of Basrah by using (Count 60 model Genex. Florida, USA). The instrument can measure and calculate 20 different parameters. The hematologist and analyzer contain solution (HCS-Basolysc) contain cyanide-free lyse reagent (HC- lyse CF) contain cyanide-free lyse reagent,(HCS-Eplode) contain cyanide-free lyse reagent and (HC-cleaner) cleaning solution used to clean fluidics system and the instrument has a printer machine inside with thermal paper. The hematological parameters used in the study were (RBCs, WBCs, Hb, MCV, PCV, MCH, MCHC, Lym %, Mon. %, Gran %) [17].

2.3 In vitro Effects of SeD on rats-Blood coagulation

Blood from healthy non-treated female rats into Sodium citrate anticoagulant tubes (VWR Chemical UK), after mixing the tubes, blood centrifuged for 5 min 4000 rpm for plasma separation[1]. (SeD) was synthesized by authors in a pharmaceutical chemistry lab in Pharmacy College/ University of Basrah, using Automated Coagulation Analyzer [2]. The control groups include normal saline group (NS) and Heparin control group (H), different concentrations from (SeD) compound were prepared; 2.0 (G1), 1.0 (G2), 0.50 (G3), 0.250 (G4), 0.125 (G5) and 0.025 (G6) mg dissolved in 1 mL of 5% DMSO: 95% NS. 450µl of plasma conveyed tube, the tubes incubated in 37°C for 10 min. for each tube 50µl of one concentration of the prepared (SeD) compound was added for one concaved tube, 50 µl of NS added to control plasma and 50 µl of heparin was to heparin control tube, then PT and a PTT were evaluated using Automated Coagulation Analyzer in pharmacology research lab-College of Pharmacy. Prothrombin (PT) and activated partial thromboplastin time (aPTT) were measured in fresh blood plasma with the use of BIOLABs REAGENTS (Maizy, FRANCE) [16]. The responsiveness of the system calibrated relative to the international standard through International sensitivity-index (ISI). The results of the present study of prothrombin time were expressed by INR

INR calculation = (Patient’s time/Normal time) × 100
2.4 Measurements of lymphocytes, T cells and, B cells
Another two groups (10 female rats/each); control non-treated rats and 50mg/kg B.W with SeD for one month. Blood samples were collected into EDTA capped bottles. The measurement was done in Middle East Private Lab/ Basrah. A Navios-Flow Cytometer (Beckman coulter/ Germany) used for T cells, B cells, and Total Lymphocytes counting. The materials used are Lymphocyte separation medium (Capricorn scientific GmbH), Dulbecco phosphate Buffer saline (Stem cell technologies), HLA Total Lymphocyte Enrichment Cocktail (stem cell technologies-Canada). EasySep™ Rat B Cell Isolation Kit and EasySep™ Rat T cell Isolation Kit.

2.5 Evaluation of serum Thyroid hormones levels
Blood sample for hormones evaluation kept in Serum-separating tubes on room temperature for 20-30 minutes. Serum collected using centrifuges for 10minutes at 4000 RPM. Estimation of serum TSH, T4, and T3 concentrations are frequently held as value procedures in identification of thyroid abnormal functions. The Thyroid hormones were evaluated using specific Kits, and i-CHROMA II Immunoassay Reade i-CHROMA II/Belgium.

3. STATISTICAL ANALYSIS
The experimental data were analyzed using Prism statistical analysis software (GraphPad, USA; version 5.0) and all data are presented as mean ± standard error of the mean (S.E.M). Data were analyzed by one-way ANOVA.

4. RESULTS
4.1 Complete blood count
The results of the present study as in Table 1, showed statistically significant increase of White Blood Cells (7.85±0.85), lymphocytes (74.80±1.84) of SeD group than control (4.72±0.54) and (58.91±1.72) respectively. While Granulocytes% of test group decline significantly (20.07±1.57) than in the control group (34.60±1.20). Red Blood Cells count, Hematocrit (HCT), Mean Corpuscular Volume (MCV), Mean corpuscular hemoglobin (MCH), Mean corpuscular hemoglobin concentration (MCHC), and Red Cell Distribution Width(RDW-SD , RDW-CV) the results recorded only a mathematical increase, but not statistically significant. Platelets count significantly decreased in the treated rats (96.60±7.91) than that control (130.71±48.41). In addition, the mean value of Plateletcrit (PCT) significant decline of (0.07±0.003) of the SeD group compared to the control group (0.09±0.01). The mean value of Platelets Larger Cell Ratio (P-LCR) of SeD treated rats was significantly greater (15.08±1.50) than of control group (11.90±2.06).

4.2 In vitro Effects of (SeD) on PT-INR and aPTT
Results of aPTT and PT-INR of all SeD treated groups than the control. Heparin (H) group showed a statistically significant increased than control and all treated groups G1, G2, G3, G4, G5 and G6 treated groups were showed significant decline than Heparin treated group; but there were no significant differences among test compound treated groups, as well as with control group. The results reported in the table (2)

4.3 Measurements of lymphocytes, T cells, and B cells
The results indicated a highly significant increase in numbers of all test parameters include total WBC, total lymphocytes, B cells and T cells as in table 3.

4.4 Effects of SeD on thyroid hormones
TSH plasma level of SeD group (0.017±0.007) was statistically significant decreased than control (0.03±0.007), also T4 concentration of treated group (17.62±7.44) showed important decreased than the non-treated group (58.64±22.28). T3-plasma value (2.97±0.42) in the selenadiazole treated group increased significantly than in control (2.38±0.45).

5. DISCUSSION
5.1 Complete blood count
A CBC count used to determine the number, size, and maturity of the different blood cells. Any abnormalities with blood cell synthesis or elimination also; can be detected. The increment of WBC count thought to be the cause of lymphopoiesis and /or increase of lymphocytes release from the lymph glands myeloid tissue. A reduction in PLT count could be initiated by; reduction in or creation problems of blood platelets, increased aggregation or reduced platelet life span. Adequate Se serum concentrations important for the defective role of neutrophils, Se deficient animals exhibited a reduction in their capability to kill microorganisms; due to a decrease of GPx1 activity[8]. Selenium leads to increased thromboxane A2 and prostacyclin PGI synthesis, an increase in peroxide concentrations led to changes in arachidonic acid metabolism and cyclooxygenase activity. MPV is determined in the megakaryocyte, the platelet volume associated with cytokines (interleukin-6, interleukin-3 and thrombopoietin) that regulate platelet number and cause to produce of the larger platelets. Decreased in the platelet production, undeveloped platelets come to be more active and increased MPV levels. Factors like race, age, smoking status, alcohol consumption, and physical activity, modify blood platelet count and MPV. P-LCR is a pointer of circulating larger platelets (> 12 fl), which is presented as percentage[9].

5.2 In vitro Effects of SeD on rats-Blood coagulation
Estimates of International Sensitivity Index -ISI- for functioning PT measuring systems are correlated as they are determined compared to the identical international standard of the World Health Organization (WHO). As an import, the INR measurement with regulated working systems diminishes system-related variances[6]. The reference ranges of the human for PT and aPTT in the laboratory were 9.8 to 13.5 seconds and 21.0 to 32.4 seconds, respectively[8], a reference value of human INR 0.9-1.3. The results of our study increased in a PTT and INR compare to Control and Heparin groups in a dose-dependent manner, to the best of our knowledge there is no international reference value for rats. The PT-derived INR is used in the pointing of pathology as a display to pro-coagulant treatment ability and bleeding risk valuation. Due to minute half-life of the factor VII, PT-INR is actually sensitive to vitamin-K deficit or antagonism. Prolonged aPTTs are frequently related with unusual bleeding and even as analysts of poor result in the importance of the small PT has not been totally evaluated. Previous studies have furthermore advocated
that abnormal PTTs could be accompanied with an increased hazard of bleeding or thromboembolism. Fast PTTs, mainly if complete on repeated testing; indicate a major risk of consequent death. Careful investigation of low PTTs patients may decrease such associated morbidity and mortality[18].

Se-low level associated with decreased PT which indicates transferal towards hyper-coagulation of the extrinsic system, the responsible factors are III and VII. Both the PT and the aPTT are determined by the transformation of fibrinogen to fibrin, this conversion is depended on the generation of thrombin from prothrombin, which in turn is dependent on activation of factor X in the presence of factor V and calcium. One possible explanation for the disagreement between these two tests might be that the influenced of fibrinogen level or the rate of conversion to fibrin is by the level of thrombin formation, which could be greatest in the prothrombin time, then by nature of the activation of the intrinsic pathway, is more leisure in the aPTT. In animals the aPTT are short in comparison to man, and the sensitivity of this test is significantly reduced[19]. A study revealed that there was a correlation detected between decreased Se levels and intensified platelet aggregation [20].

5.3 Measurements of lymphocytes, T cells and B cells

Selenium is capable to improve the immune status, via its role in neutrophils, and lymphocytes functions. Even with these observations, however the mechanism around such modification is not clear [21]. Se affected adaptive immunity by activation of both T and B cells; high Se levels have a proliferative effect and differentiation of T helper cells. Functions of innate immunity cells also; determined by Se levels. Se exaggerated their capacity of inflammatory signaling and anti-pathogen action, through pathogen-related molecular patterns such as Lipopolysaccharide, also; release of prostaglandins and cytokine mediators [22].

Selenium status is well-known to affect the functioning of all immune system components and its capability to react to infections and cancers; Se is found in significant amounts in immune tissues such as the spleen, lymph nodes, and liver. Consequences of Se deficiency reported to reduce the free radicals production and killing capacity of neutrophils, lymphocyte toxicity, and serum immunoglobulins levels such as IgG and IgM and antibody responses. Also; it was reported that in the neonatal rats nursed by mothers fed low-Se diets result in an impaired responsiveness of thymocytes to Concanavalin A (ConA) [15]. While the stimulation of immunity achieved by Se supplementation is measured by a varied range of parameters such as T-cell proliferation, innate immune cell functions, and many other parameters. A T-cell specific of all selenoproteins was found to decrease mature T cells developing from lymphoid tissues the results, consistent with findings by [23], in current study (SeD) administration leads to increased lymphocyte numbers. In human, there have been reports of a rise in the incidence of autoimmune thyroiditis in regions with low-selenium that are in agreement with studies in mice. An adaptive immune response is affected by Se consumption including the activation and functions of T and B cells. A suggestion from numerous studies confirmed that Se levels and selenoproteins in macrophages control migration and phagocytosis functions [22]. In the present study T-cells, B-
cells and total lymphocytes are significantly increased than the control group which in agreement with a study by [24]. The precise mechanisms by which Se develops immune activity are not completely known. It is probable that Se smears its effect by varying the cell’s redox status or by meeting the increased requirements for selenoproteins of the activated immune cells. It has been previously reported, up-regulation of seleno-phosphate synthetase effects, directed towards seleno-cysteine synthesis, a key constituent of selenoproteins, inactivated T cells and of numerous protein bio-synthesis genes in the lymphocytes after six weeks Se supplementation. Se play an vital role in the growth, activation, and functioning of immune effector cells[24]. B and T lymphocyte both form the main effector cells in the acquired system that mature with contact to immune challenges. Se deficient lymphocytes have minimal ability to proliferate, and in macrophages, leukotriene B4 synthesis is reduced in Se deficiency. These procedures can be enhanced by Se supplementation [26].

Selenium is implicated in the progression and severity of tuberculosis (TB) caused by Mycobacterium tuberculosis [27]. Patients with pulmonary TB have minor selenium statuses in comparison to healthy controls [28]. In animal models, T cell proliferative responses, natural killer (NK) cells activity, lymphokine activated the killer cell, delayed-type hypersensitivity reactions, and responsiveness to vaccines increased as a response to Se supplementation. Supplementation with Se (100mg/day) to subjects with relatively low Se levels (<1.2mmol/L), enhanced plasma Se concentrations and lymphocyte cytotoxic GPX, and the host immune replies increased (such as IFN-g production, T cell proliferation to antigen stimulation and the percentage of total T cells, especially T helper cells) [25]. The effective incorporation into selenoproteins in immunity has been confirmed in cell-culture models. Deficiency in the Se level in the body can produce incompetence immune response. There is a confirmation that Se can modify the pathology of the gut and liver chronic inflammatory diseases as well as in inflammation-associated cancer [22].

5.4 Effects of SeD on thyroid hormones

TSH level elevation indicates hypothyroidism, while decreased TSH plasma level indicates hyperthyroidism. Many factors may influence thyroid activity like; stress, which suppresses the metabolism of thyroid hormones. Stress signs on thyroid cells initiated by limitations in iodine and Se supply of, both elements are important for the thyroid tissue and secretions. Se as a cofactor, it’s an important intra-nuclear and extra-nuclear thyroid enzyme. Glutathione peroxidase (GPX) prevents tissue damage caused by mutagens, as well as oxidative stress and damage, whereas thioredoxin reductase (TrxR) is vital for DNA repair and replication [29].

Impairments of Type2 iodothyronine deiodinase associated with selenium deficiency have adverse effects on the immunity by sub-optimal thymic cells function and development. A study reported that Se affected serumT3 in the contradictory direction, increasing T3 value when Se was limited and reducing when Se was increased. This variance may be due to rats’ serum T3 released mainly is produced from the thyroid, While in humans T3 comes essentially in liver by deiodination of T4[30]. Also, another study described that female rats feed with low selenium and I
supplement developed high TSH levels than in the control group [31].

6. FINANCIAL SUPPORT AND SPONSORSHIP
Nil

7. CONFLICTS OF INTEREST
There are no conflicts of interest.

8. ACKNOWLEDGMENTS
The authors would like to acknowledge the staff of Middle East private Laboratory especially Dr. Ahmed Abd Al-Wahid, technical assistance of Al Sader Teaching Hospital, Basrah, for their technical support.

9. REFERENCES
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Study of some of Novel Selena-Diazole Derivative activities on Hematological Parameters, Differentiate lymphocytes cells in addition to thyroid hormones levels in Female Rats (One of the series of studies on the impact of the new compound)