A Study of the antioxidant and anti-inflammatory activities of 5- selenocyanatouracil compound against paracetamol-induced oxidative stress

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Abstract

Background: glutathione peroxidase, catalase, and superoxide dismutase enzymes are the main antioxidants that serve as the body's first line of defence against oxidative stress.

The aim of the study: To examine 5-selenocyanatouracil anti-inflammatory and antioxidant properties.

Materials and methods: Four groups of rats were employed; the control group received distilled water the paracetamol group received 500 mg/kg body weight of paracetamol, the paracetamol & 5-Selenocyanatouracil groups received 50 mg/kg of 5-selenocyanatouracil after 30 minutes of paracetamol, and the second paracetamol & 5-Selenocyanatouracil group received 100 mg/kg of 5-selenocyanatouracil after 30 minutes of paracetamol administration.

Results: The findings demonstrated that 5-selenocyanatouracil considerably ($p<0.05$) raised levels of superoxide dismutase and glutathione reductase while notably lowering levels of Malondialdehyde and Interleukin-6.

Conclusion: The findings showed that the prepared 5-selenocyanatouracil compound had antioxidant and anti-inflammatory activity.

Keywords: 5-Selenocyanatouracil, Paracetamol, Glutathione reductase, Superoxide dismutase, Interleukin-6, Malondialdehyde.

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Introduction

Selenoproteins are proteins that have selenium (Se) in their polypeptide chain. Selenoproteins have crucial significance for human and animal health because of their antioxidant properties, but some organisms lack the ability to synthesize them. A biological role for selenium is played by selenoproteins. A generation of reactive species of oxygen (ROS) is a chief mechanism by that selenium controls immune cell function (1). The human body contains 3 to 20mg of selenium in total. Poisoning with selenium caused joint stiffness, severe anemia, hair loss, and blindness. Selenium deficiency is linked to the Keshan disease (2). According to numerous studies, selenium derived from organic origins is metabolized significantly and more efficiently than inorganic forms. The majority of Se in the human body comes from dietary sources. Cereals, meat, and seafood are the foods that contribute most to Se intake (3). Twenty-five selenoprotein genes necessary for human health are present in the human genome. Thirteen of the selenoproteins have known uses; at least twelve of them are oxidoreductases, with selenocysteine (Sec) acting as the catalytic redox-active residue in these enzymes. The functions of the remaining twelve selenoproteins are either unknown or only partially understood. Antioxidant selenoproteins are vital for maintaining redox homeostasis and the cell's antioxidant defence. In mammalian cells, glutathione and thioredoxin systems are two important redox antioxidant systems (Minich 2022). Iodothyronine deiodinase (DIO), selenoprotein W, N, and P, as well as glutathione peroxidase (GPXs), and thioredoxin reductase (TRXRs) are the most significant of these selenoproteins.(4) Mammals have eight GPXs, five

of which are selenoproteins (GPX1-4, GPX6). They primarily utilize glutathione or thiol oxidoreductases as reductants. They have a variety of roles, such as detoxifying hydroperoxides, controlling ferroptosis, and signalling with hydrogen peroxide (5). Selenoproteins that reduce thioredoxins as their primary function are called thioredoxin reductases, they also have a wide selectivity that allows them for the reduction of other endogenous and exogenous substrates (6). When ROS are present in low to moderate concentrations, they have favourable effects on immunological response and cellular responses, but when they are present in high concentrations, they create oxidative stress, a detrimental process that can damage all cellular components. Numerous factors, such as immune cell stimulation, cancer, excessive exercise, and ageing, contribute to the endogenous production of free radicals. Exposure to heavy metals, certain drugs (cyclosporine, tacrolimus, and also paracetamol in high doses), and environmental pollutants can result in the production of exogenous free radicals. The cell can be protected from ROS damage by enzymes such as SOD, CAT, GXPs, and GSH (7). paracetamol is one of the most widely used antipyretic and analgesic drugs worldwide. Oxidative stress caused by a paracetamol overdose can cause necrosis, severe liver damage, and kidney issues in both humans and animals. Numerous antioxidants have been investigated to determine whether they can guard against liver and kidney damage brought on by paracetamol(8). Uracil is a well-known pharmacophore in therapeutic chemistry because the pyrimidine-nucleobase is present in many commercial drugs. Though the word "uracil" is typically associated with cancer treatment, a wide range of synthetic compounds based on uracil have therapeutic use. They support the synthesis of nucleic acids and are essential for the metabolism of fats and carbohydrates (9). One chemical element that is present in both natural and man-made settings is cyanide. Although the cyano-group (-C≡N) is found in nature in a variety of forms, a substance's toxicity is still determined by its ability to create free cyanide. One cyanide-containing substance that is found in the biological body is cyanocobalamin (vitamin B12). It is useful in eliminating nitric oxide, and when applied to skin diseases, certain clinical trials have produced positive outcomes (10). The objective of the present study is to examine 5-selenocyanatouracil anti-inflammatory and antioxidant properties.

Materials and methods

Forty male rats were obtained from a pet vet clinic in Erbil, the northern portion of Iraq, in April 2021 for an experiment that will extend from November 2021 to March 2022. The rats were weighed and housed in sawdust-lined polypropylene cages (5 rats per cage). Rats were fed rat pellets and had access to clean water. The rats were first accustomed to the natural lighting and temperature of the laboratory, which was 21±4ºC.The cages had labels and were grouped. Each animal group (n=10 rats) received paracetamol only or with 5-SeU orally through oral gavage for 21 days. The Control group received only 2 mL of distilled water. The rats were divided into four groups, each consisting of ten rats. In Group A, rats were employed as a control; they were given food and (DW) but no additional treatment. Group B: Paracetamol (PA) was dissolved in 2 ml of DW and given orally to rats in this group at 500 mg/kg once per day for three weeks. Paracetamol was ingested by the rats in Group C in the preceding dose,5-SeU which the authors prepared (11), and was given orally to the rats via oral gavage once a day for 21 days, following 30 minutes of PA treatment. The recommended daily dose was 50 mg/kg BW, dissolved in 2 ml of DW. Rats in Group D received paracetamol orally at the prior dose, and 30 minutes later, they received 5-SeU orally once daily for 21 days at a dose of 100mg/kg dissolved in 2ml DW. Following a 21-day course of treatment, the rats were anaesthetized so that blood (about 3 ml) could be drawn directly from the heart. For 20 to 30 minutes, blood samples were kept in gel tubes at room temperature. Serum samples were collected after centrifugation for 10 minutes at 4000 rpm and kept at a temperature of -20°C until biochemical parameters were measured. Serum glutathione reductase (GSH) assay the most common

intracellular thiol, glutathione, is crucial for the detoxification ROS. It is the most crucial defence mechanism for protecting cells from oxidative stress. It was measured by rat glutathione reductase (GSH) ELISA kit (Bioassay Technology Laboratory) in ALBAYAN lab in Basrah/Iraq. An optical density value (OD) of each well was assessed using a microplate reader set to 450nm then to construct a standard curve; on the horizontal axis, an average optical density for each standard was plotted against the concentration. Computer-based curve-fitting software was employed to conduct these calculations, and regression analysis was used to identify the best fit line. Serum superoxide dismutase (SOD) levels assay SOD is the cell's primary detoxification enzyme and highest antioxidant. It is a crucial endogenous antioxidant enzyme that serves as a part of the body's initial line of defence against oxidative stress (15) (Ighodaro and Akinloye 2018). It was measured by rat superoxide dismutase (SOD) ELISA kit (Bioassay Technology Laboratory) in ALBAYAN lab in Basrah/Iraq. The optical density (OD value) of each well was assessed using a microplate reader set to 450nm then to create a standard curve; the average optical density (OD) for each standard was plotted against the concentration on the horizontal axis (X). Then, the best-fitting curve was constructed through the points on the graph. Regression analysis was used to identify the optimum fit line, and computer-based curve fitting software was applied to perform these computations. Serum Malondialdehyde (MDA) levels assay It was measured by rat Malondialdehyde (MDA) ELISA kit (Bioassay Technology Laboratory) in ALBAYAN lab in Basrah/Iraq. The optical density (OD value) of each well was assessed using a microplate reader set to 450nm then to create a standard curve; the average optical density (OD) for each standard was plotted against the concentration on the horizontal axis (X). Then, the best-fitting curve was constructed through the points on the graph. Regression analysis was used to identify the optimum fit line, and computer-based curve-fitting software was applied to perform these computations. Serum interleukin-6 (IL-6) levels assay It was measured by rat Interleukin-6 (IL-6) ELISA kit (Bioassay Technology Laboratory) in ALBAYAN lab in Basrah/Iraq. Using a microplate reader set to 450 nm, the optical density (OD value) of each well was determined. Next, a standard curve was created by plotting the average optical density (OD) for each standard against the concentration of each standard on the horizontal axis. The graph points were subsequently employed to create the best-fitting curve. The most optimal line was determined using regression analysis, and computer-based curve-fitting software was utilized to carry out these calculations. Statistical evaluation was made using SPSS-23-windows (SPSS Inc., Chicago, IL, USA). To decide if there was a statistically significant difference between three or more groups, a one-way analysis of variance (ANOVA) was conducted. To display the data, range, mean, and standard deviation was employed. The results were considered a significant at P value less than 0.05

Results

Glutathione reductase (GSH) According to (Table 1) and (Figure 1), when compared to the A group, the B group had significantly lower serum levels of GSH ($p<0.05$), while the C and D groups significantly outperformed the B group in terms of blood GSH levels ($p<0.05$).

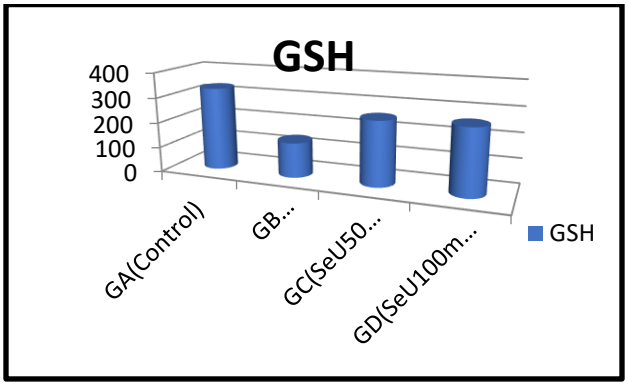

Figure (1) GSH level in serum of rats in A, B, C and D groups

Superoxide dismutase (SOD)

According to (Table 1) and (Figure 2), serum levels of SOD were not considerably decreased ($p>0.05$) in

the B group, in comparison to the A group. While SOD levels, were significantly ($p<0.05$) increased in the C group compared with the B group. When the D group was compared to the B group, serum SOD levels improved significantly ($p<0.05$) as shown in (Figure 2).

Malondialdehyde (MDA)

According to (Table 1) and (Figure 2), the MDA serum level was increased ($p<0.05$) in group B compared with group A. While MDA serum levels were significantly ($p<0.05$) decreased in groups C and D related to groups A and B. Furthermore, there was a significant ($p<0.05$) difference in the MDA concentration between groups D and C.

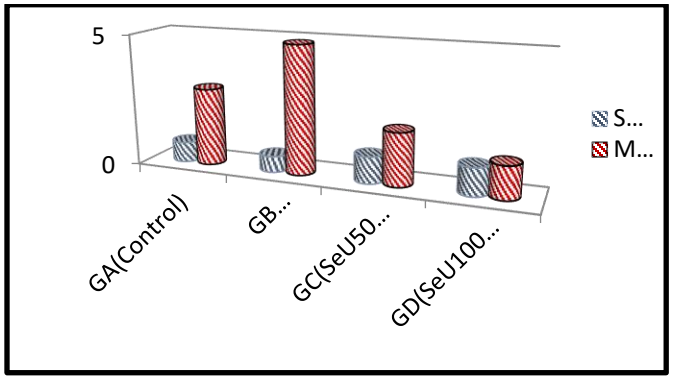

Figure (2) SOD level and MDA level in serum of the rats in A, B, C and D groups

Table (1): Effects of 5-SeU on the activity of antioxidant enzymes of experimental groups

Antioxidant tests / Groups	GSH (ng/L) n=10	SOD (ng/L) n=10	MDA (ng/) n=10
Group A (Control)	331.56±76.559	0.762±0.2	2.93±0.84
Group B Paracetamol(500mg/g)	139.147±101.63 a	0.61±0.358	4.8547±0.6a
Group C 5-SeU(50mg/kg) +PA	256.75±139.65 b	1.00±0.41 b	2.00±0.25 ab
Group D 5-SeU (100mg/kg) +PA	261.65±87.122 b	1.046±0.29 b	1.23±0.67abc
LSD	117.60619	0.39	0 .76845

Values are expressed as mean ± standard deviation. a-refers to a significant difference with the G1, b-refers to a significant difference with the B, ab-refers to a significant difference with A and B groups, abc refers to a significant difference compared with A, B, also to C $p<0.05$.

Interleukin 6 (IL-6)

According to (Table 2) and (Figure 3) below, IL-6 serum levels significantly increased ($p<0.05$) when the B group was compared with the A group. The IL6 level was GD significantly ($p<0.05$) reduced in comparison with GB, with no significant differences with GA. While in Group C: Only 3 samples showed numerical results out of 10 samples in the group prepared for examination, so it is better not to include them in the statistical analysis.

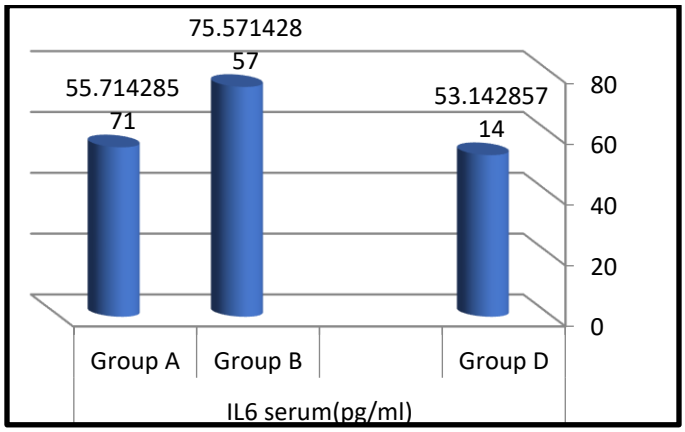

figure (3): IL-6 level in serum of the rats in A, B, and C groups

Table (2): Effect of 5-SeU on interleukin 6 levels of experiment groups.

Groups (N=10)	IL-6 serum(pg/ml)
Group A (Control)	55.71429±8.712334
Group B Paracetamol(500mg/Kg)	75.57143 ±7.253899 a
GroupC5-SeU(50mg/kg) +Paracetamol	53.14286±7.448234 b
Group D 5-SeU (100mg/kg) +Paracetamol	19.85

Values are expressed as mean ± standard deviation. a-refers to significant differences with the control

group, and b-refers to significant differences with the PA group. $p<0.05$

Discussion

The physiological and pathological mechanisms underlying paracetamol-induced liver damage are remarkably complicated, and numerous intracellular and extracellular events, including paracetamol biotransformation, peroxidation stress, apoptotic, aseptic inflammation, microvascular dysfunction, and liver regeneration, are involved. The damage is initiated by these events, which also directly kill hepatocytes, limit the cellular stress response, and encourage liver regeneration and repair. To treat acute liver injury, several different pathways in addition to mitochondrial oxidative stress may be effective therapeutic targets. It should be noted that specific cellular mechanisms may, at different phases of acute liver injury, have paradoxical effects, working both favorably and negatively in the regulation of paracetamol hepatotoxicity (12). Studies have uncovered a wide range of functions for it, including control of immunological responses, leukotriene and prostaglandin metabolism, elimination of electrophilic xenobiotics, cysteine transportation and storage, alteration of redox-regulated signal transmission, regulation of cell division, deoxyribonucleotide synthesis, and antioxidant protection (13). Among the reducing thiols that are most frequently found in cells, reduced glutathione is kept in supply by the enzyme glutathione reductase. In its reduced state, glutathione plays a crucial role in the control of ROS in cells. Optimum conditions for the redox regulation inside a cell or for triggering a cell's planned death are determined by complex interactions between levels of ROS, levels of reduced and oxidized glutathione or other thiols, with an antioxidant like glutathione reductase. ROS function as signalling molecules intracellularly and/or extracellularly (14). The GPx enzyme plays a crucial inhibitory function in lipid peroxidation, safeguards DNA and protein lipids from ROS and RNS, and protects cells from oxidative stress. Additionally, the cyclooxygenase and lipoxygenase sequences have been affected by the anti-inflammatory characteristics of selenium (15). Several researches examined the antioxidant properties of selenium compounds. A different investigation found that diphenyl-diselenide remains active in the liver and kidneys despite increased oxidative stress. The findings demonstrate that the SOD of both 5-SeU & paracetamol groups significantly elevate values than at paracetamol group. The ability to stop lipid peroxidation, protein oxidation, and alter SOD levels were used to evaluate the potential hepatoprotective unique features that caused these outcomes. The data from previous studies indicate that compounds containing selenium are more effective SOD inducers. The enhanced SOD activity has been found in data from numerous sources. In diabetic rats fed a Se-supplemented diet, the plasma levels of GPX and SOD dramatically rose (16). According to a previous study, diphenyl diselenide is active despite elevated oxidative stress in the liver and kidneys (17. According to reports, 1,4-anhydro-4-seleno-d-talitol compound exhibits potent in vitro antiradical effects and is expected as a result to accelerate wound healing in diabetic mice since it increases the generation of SOD and decreases the production of NO(2). The MDA values over all experimental groups were examined in the current investigation, and the findings showed that the 5-SeU & paracetamol groups and paracetamol group showed a significant difference concerning the control group. Also, 5-SeU & paracetamol groups caused a significant decline in MDA levels than in paracetamol group. The findings of a study by (18) revealed that MDA; a lipid peroxidation marker, had a reverse association with selenium serum levels either at normal or hypercholesterolemic patients, definitely with LDL (low-density lipoprotein). Se elevated GSH level and reduced serum C-reactive protein, with a subsequent reduction of plasma MDA. Furthermore (19), confirmed the association of Se with reduced MDA. MDA is a naturally occurring byproduct of lipid peroxidation. MDA is carcinogenic and mutagenic because lipid peroxidation is a recognized mechanism of cellular

damage and a dependable biomarker of oxidation (20). The present research shows a significant decrease in values of IL-6 in 5-SeU & paracetamol group compared with paracetamol treatment alone. Such results were confirmed by(1), who that a human selenium status can be affected by several aspects including the concentrations, and forms of selenium consumed, selenium compounds transformation to metabolites, and genetic character; all influence pathological disorders involving the immune system. In addition to the direct anticancer mechanisms of methylseleninic acid (MSA), this substance also possesses immunomodulatory qualities, which allow it to limit the growth and spread of cancers by influencing the activity of immune cells. It has been demonstrated that MSA alters cytokine levels, histocompatibility protein MHC1 expression, CD8 cytotoxic T-lymphocyte and NK cell functional activity, and macrophage ability to engulf tumor cells. MSA (3 mg/kg body weight) suppressed tumor progress by 61% in comparison to the control group. This suppression was accompanied by a decrease in plasma levels of IL6 and TNF, as well as a rise in GPX levels (21). IL-6 is a pro-inflammatory cytokine that regulates inflammatory processes, bone metabolism, and immunological response, among other physiological processes. On the other hand, IL-6 overproduction may significantly contribute to systemic inflammatory reactions; by stimulating cytokine production. Acute lung injury, hypercoagulability, and the symptoms are generally caused by the production of a considerable number of pro-inflammatory cytokines, resulting in multiple organ failure, inflammation, and elevated ROS generation (22)). Another study includes all the information currently available on the involvement of the most prevalent organic and inorganic selenium-containing substances, besides selenoproteins and Se nanoparticles, in controlling immune-modulatory, antiviral, and anti-inflammatory functions. Most recent information about the role of Se-containing drugs in prevention and COVID-19 cure will also be taken into account, along with the main regulatory systems (23).

Conclusion

The present study showed that the prepared (5SeU) compound had a potential antioxidant and anti-inflammatory activity in a dose-dependent manner. (5SeU) could have a protective effect against paracetamol-induced damage to the liver.

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There aren't any conflicts of interest to report.

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دراسة النشاط المضاد للأكسدة والمضاد للالتهابات لمركب ٥-سيلينوسياناتوراسيل ضد الإجهاد التأكسدي الناجم عن الباراسيتامول

الخلاصة:

المقدمة:انزيمات الكلوتاثيون، بيروكسيديز ،الكاتاليز والسوبوراوكسياد دسميوتيز تعمل كانزيمات مضادة للاكسدة و كخط دفاعي اول ضدالاجهاد التاكسدي .

هدف: المشروع الحالي هو فحص خواص مركب ٥-سلينيوسياناتويوراسيل المضادة للاكسدة والالتهاب .

طريقة العمل: اربع مجاميع اعتمدت ,المجموعة الضابطة اعطيت ماء مقطر, مجموعة الباراسيتول اعطيت ١٠٠ ملغم/كغم من وزن الجسم من الباراسيتول, مجموعة البارسيتامول والسيلينوسياناتويوراسيل اعطيت٥٠ ملغم /كغم من وزن الجسم السيلينوسياناتويوراسيل بعد ٣٠ دقيقة من اعطاء الباراسيتلمول ومجموعة البارسيتامول والسيلينوسياناتويوراسيل الثانية اعطيت١٠٠ ملغم /كغم من وزن الجسم السيلينوسياناتويوراسيل بعد ٣٠ دقيقة من اعطاء الباراسيتامول .

النتائج :اظهرت النتائج ان لسيلينوسياناتويوراسيل رفع مستويات الكلوتاثيون بيروكسيديز ،الكاتاليز والسوبوراوكسياد دسميوتيز, بينما قلل من مستويات مالونداياالديهايد و انترلوكين٦

الخلاصة:اظهرت نتائج البحث ان المركب ٥-سيناتويوراسيل المحضر له فعالية مفيدة مضادة للاكسدة وللالتهاب بطريقة معتمدة على الجرعة.