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Preparation of 5-Selenocyanatouracil by a more convenient method and evaluation of some pharmacological properties

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***Abstract*---**A convenient method for preparation of 5-Selenocyanatouracil (5-SeU), this compound gains importance because it is similar to 5-Fluorouracil which is one of the famous uracil derivatives as an anticancer drug. Some of invitro pharmacological activities such as DPPH antioxidant effects and antimicrobial activity, in addition to median lethal dose (LD50) were estimated. The study aimed to use a convenient synthesis procedure, and evaluation of in vitro pharmacological activity, also to determine the median lethal dose of 5-SeU. 5-SeU synthesis was carried out in very good yield through a direct reaction between 5-Iodouracil and freshly prepared potassium selenocyanate in refluxing aqueous solution to give light-gray crystalline. Antimicrobial measured by disc diffusion method. LD50was determine using Albino rats. 5-SeU compound was branded by FT-IR; ^{13}C- NMR and ^{1}H- NMR spectroscopic data. The results showed no antibacterial effects for most of test bacteria with a low inhibition effect on Staph. Aureus, and Pseudomonas aeruginosa, with moderate inhibition zone at candida culture.

***Keywords*---**uracil, selenium, potassium selenocyanate, DPPH, antimicrobials.

Introduction

Cyanides are classified into inorganic cyanides such as KCN, and NaCN. They are characterized by their high toxicity for several reasons, including the toxic effect on the nervous system and irreversibly interact with iron proteins for example hemoglobin [1]. On the contrary, organic cyanides (Nitriles) are very useful in biological systems and ofhigh importance in the design of drug molecules. A large number of prepared medicinescontain in their composition a cyanide group such as Fadrozol monohydrochloride, Letozole, Nivatinib and Olprinone and many others [2,3].

KSeCN is considered one of the most powerful and important reagent for the insertion of selenium into the organic molecules and an essential source for the preparation of different classes of organic compounds such as diselenides, selenols, and seleninic acids, found in biological systems, especially when associated with a biologically active scaffold [4,5]. KSeCN can be purchased directly from specialized companies or prepared in the laboratory in the insertion reaction of elemental selenium into KCN in aqueous or any suitable organic solvents [6]. In this study, 5-Selenocyanatouracil was prepared in a convenient and cheap method with a good yield from the reaction of KSeCN with 5-Iodouracil in an aqueous medium. The resulting compound was characterized using spectroscopic methods which confirm the proposed structure of the product. Multidrug-resistant pathogens have increased the number of infectious diseases, which have now become the leading cause of death globally. Widely overuses of antimicrobial drugs are the major health concern of antibiotic resistance in bacteria. An infection with multidrug-resistant bacteria could lead to developed death and morbidity rates, intensive care requirements, and economic difficulties.[7]

Acute lethality is a simple and direct, basic indicator of a chemical product's toxicity. As a general rule, the median dose that is expected to kill 50% of a population is calledthe LD50, mainly used to calculate the acute lethality hazard. One of the major influences for both the LD50 and its regulatory integration has already been continuous improvement in animal welfare and cost savings. There are various well-established methods for reducing the number of animals required to obtain the LD50 value that is appropriate for risk communication of acute toxicity today. Classification and labeling of chemicals according to the Globally Harmonized Classification System (GHS), which incorporates LD50 data, is increasing the practicality of these animal testing protocols [8]. Considering; the oxidative stress role in the pathology of several diseases and the use of antioxidants as treatment and/or adjuvants in these conditions. As opposed to other in vitro antioxidant tests, DPPH (2,2 diphenyl 1 picrylhydrazil) is considered the easiest and cheapest way to measure effective antioxidants. An assessment of DPPH's potential for scavenging free radicals and producing hydrogen is used to determine whether a compound might be able to serve as a free radical scavenger or hydrogen source. It is a dark crystalline molecule containing stable free radicals [9]. The DPPH is a method for evaluating antioxidant activity by measuring the ability of antioxidants to limit lipids oxidation, resulting in radical scavenging and determining radical scavenging potential.It is widely used because it takes only a few minutes to complete the analysis. As a free radical, DPPH is a

very stable chemical containing hydrogen reacted with it and exhibit UV- vis absorption up to 517 nm. Following the reduction process, DPPH-DMSO begins to decolorize due to the removal of DPPH by antioxidants. In solution, DPPH radicals have a deep purple hue when reduced to DPPHH, and DPPH radicals have a bright purple hue when reduced to DPPH-H[10].

Materials and Methods

Synthesis of 5-Selenocyanatouracil (5-SeU): All chemicals used in this study were obtained from commercial sources and in the purest available grades used when necessary. All reactions were carried out in distilled water, the melting point was measured by electrothermal melting point apparatus which is heated electrically and is uncorrected. Chromatography (TLC) was carried out using Merck- Alufolien –Kieselgel- 60 F_{254} aluminum sheets; the visualization is under UV- absorption 254nm. The spectra of ^{1}H-NME and ^{13}C-NMR were measured by Bruker AVANCE NEO- 400 run at 400MHz at the college of education- Chemistry Department / Basrah University with TMS ($SiMe_4$) as an internal standard in δ units at 295K. The chemical shifts were expressed in δ- scale (ppm). Carbon, hydrogen, and nitrogen Microanalysis were achieved by an Elemental Analyzerinstrument- Carlo Erba EA 1108. The IR spectra were recorded as Potassium bromide discs on the Jasco FTIR spectrophotometer.

Preparation of 5-Selenocyanatouracil

A solution of potassium selenocyanate (1.5 mmol) was freshly prepared by mixing potassium cyanide (0.98 gm, 1.5 mmol) with selenium powder (1.18 gm, 1.5 mmol) in distilled water 50ml, stirring the mixture at room temperature until disappeared of selenium (ca~ 30 minutes). To this solution (3.57 gm, 1.5 mmol) of 5-Iodouracil disintegrated at 50 ml of distilled water. The resultant mixture was stirred with reflux for 5hr, and then left at room temperature overnight. The reaction was monitored with TLC. Pale-grey crystalline was formed. Filtered & recrystallized using distilled water to afford pure light-gray crystals of the titled compound (2.5 gm, Yield = 77%), MP. 271- 273C°. R_f value= 0.85 using ethanol/benzene (1:9). Anal. Calc. for $C_5H_3N_3O_2Se$: C: 27.79; H: 1.40; N: 19.45; Found: C: 27.63; H: 1.41; N: 19.49. IR(KBr) (υ_{max}.cm^{-1}): 3288, 3138, 3109, 3034, 2104, 1745, 1653, 1606, 1465, 1431, 1215, 1138, 732, 549; ^{1}H-NMR: δ:7.90 (1H-H4), 11.32 (2H br. NH-1, NH-3); ^{13}C-NMR: δ:95.79 (CN), 105.11 (C-5), 147.46 (C-6), 151.72 (C-2), 161.99 (C-4).

Bacteria Strains Preparation

Gramm negative bacteria; E. coli, P. aeruginosa, K. pneumoniae and Gramm positive bacteria S.aurous were obtained from the central Laboratory/College of Pharmacy University of Basrah. All bacterial strains were cultured in Mueller Hinton agar (MHB)(Merck, Germany) at **37 °C,** for 24 hrs.

Disk -Diffusion Method for bacterial sensitivity test

The antibacterial effects of 5-SeU were investigated using the Kirby Bauer, Disk Diffusion Test method against tested pathogens. A sterile cotton swab was used;

the bacterial strains were disseminated on Mueller-Hinton agar (MHA) (Merck, Germany).In the test, a sterilized antibacterial susceptibility disk was employed as blank. Disks were loaded with 5-SeU at 100, and 150µg/ml of DMSO concentrations. Subsequently, the disks were placed on the agar plate and then incubated at 37°C for about 24 hrs. Next to incubation, the inhibitory zone was estimated [11].

Median Lethal Dose (LD50)

Healthy male rats were obtained from Pet Vet Clinic Irbil. Male rats were housed in sawdust-lined polypropylene cages. Rats were fed rat pellets and had access to tap water. Initially, the rats were acclimatized to the laboratory environment, as well as the normal day and light. Room temperature was regulated at about 20±2°C. The oral dose toxicity test was carried out according to the "Organization for EconomicCo-operation and Development" OECD-423 recommendations. At the beginning a pilot trial applied using the up-down staircase approach using little number of rats to assess the starting dose. Male rats were used, the rats randomly divided into six investigation groups, including sex rats /each group. The rats weighting about 120-150 g[12]. All groups were administered (5-SeU) compound orally in one of the following doses:250, 500, 1000, 1500, 2000 or 2500 mg/Kg BW, the dose dissolved in 2 mL of distilled water at 37ºC, and recording of the responses were assessed by death percentage after 48 hrs in each group. Calculated as a percentage of mortality by

$$\% \text{ Mortality} = (\text{number of dead animals/Total number of animals}) \text{ X } 100.$$

The association between the 5-SeU dose, which was administered orally to the rats and the rate of mortality, was depicted in a diagram. X-axis represents the dose that yields any response at Y-axis; then be computed using an equation calculated by the Excel system based on the deviation in the results line. As a result, LD50 is calculated rather than estimated[13].

DPPH scavenging activity assay

DPPH 0.2mM solution was obtained via adding 7.89 mg of DPPH into 100 mL of DMSO and currently keeping it at room temperature (23-25c) and keep within the dark by wrapping aluminum foil around test tubes and flasks for 2 hrs. After adding 3 mL ofDMSO to 1mL of DPPH, the absorbance was measured at 517nm and recorded as control. Various concentrations; 30, 40, 50, and 75 µg/ml of 5-SeU and AA were obtained. The spectrophotometric method to determine 5-SeU and AA antioxidant capacity is principally determined by the absorbance of DPPH radical's being reduced. Each tube of 5-SeU and AA received 1 mL of 0.2mM DPPH. The solution was stirred and sat for 10 min at room temperature. Using a spectrophotometer, the absorbance was measured at 517 nm. As AA was used as a standard material, the equation was used to calculate the suppression of absorbance and DPPH scavenging activity.

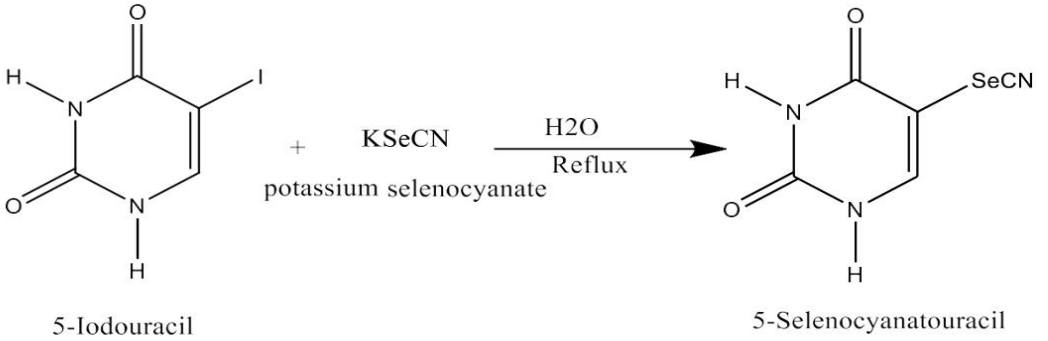
$$(A^0 - A1/A^0)*100 = \text{percent inhibition [14].}$$

A^0 is the Absorbance of the control reaction, while the 5-SeU, or AA Absorbance is A1.Concentration and inhibiting percentage curve have been used to calculate the test sample's IC50 rate. The IC50 of the test samples were measured from the concentration and inhibiting percentage curve [15].

Results and Discussion

Synthesis of 5-Selenocyanatouracil

5-Selenocyanatouracil was first synthesized from the reaction of selenocyanogen chloride (ClSeCN) which is used as a selenocyanating agent obtained from the reaction of potassium selenocyanate (KSeCN) with chlorine gas under dry conditions, this reagent reacted with uracil in a molar ratio of 1:9 [16]. In this study, 5-Iodouracil was reacting with KSeCN in an equimolar ratio in an aqueous solvent to give lighted graycrystals in good yield % ca~ 77%. As shown in Scheme (1):



5-Iodouracil 5-Selenocyanatouracil

Scheme (1): Synthesis of 5-Selenocyanatouracil

The IR spectrum of the prepared compound showed a medium band at 3288 and 3138cm^{-1} refer to ט (N-H1, N-H3) respectively, medium band on 3109 cm^{-1} referred to=C-Se[17]. The weak band on 3034 cm^{-1} refers to C_4 H; Fig. (1). The medium band appeared clearly at 2104 cm^{-1} belonging to the –CN group. Two strong bands on 1745, 1653 cm^{-1} referred to carbonyl groups at C_2O and C_6O respectively [18]. A strong bandat 1606 cm^{-1} is affiliated with N-H bending[19].

Potent two bands appear on 1465, and 1431 cm^{-1} referred on symmetrical and irregularC_4=C_5. Tow strong bands seemed on 1215 and 1138 cm^{-1} might attribute to vibrationalstretching of C_2-N_1 and C_4-N_3 respectively. The potent band referred to 732 cm^{-1} attributed to N-H deformation. A medium band clearly appeared on 549 cm^{-1} which may appear on Se-C [19]. Fig: (1).

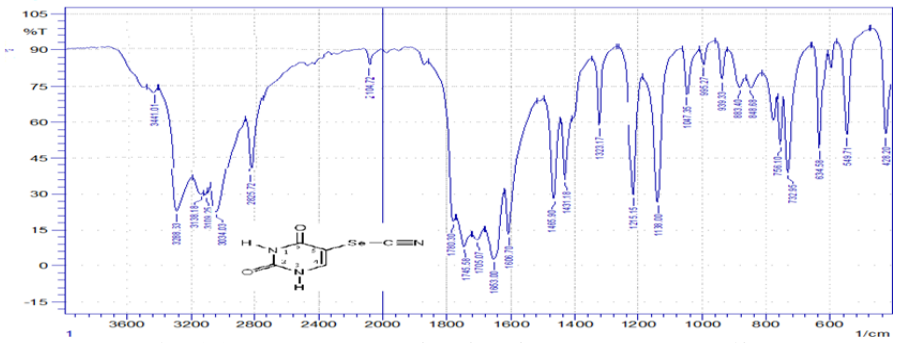


Fig. (1): IR spectrum for 5-Selenocyanatouracil.

The ^{1}H-NMR spectrum shows two interesting singlet signals one at δ= 7.90 attributed toone proton of C_4; i.e. (1H-C_4) while the other broad singlet signal at δ=11.32 belong to two NH protons i.e. (2H, br, NH-1, NH-3). Fig: (2).

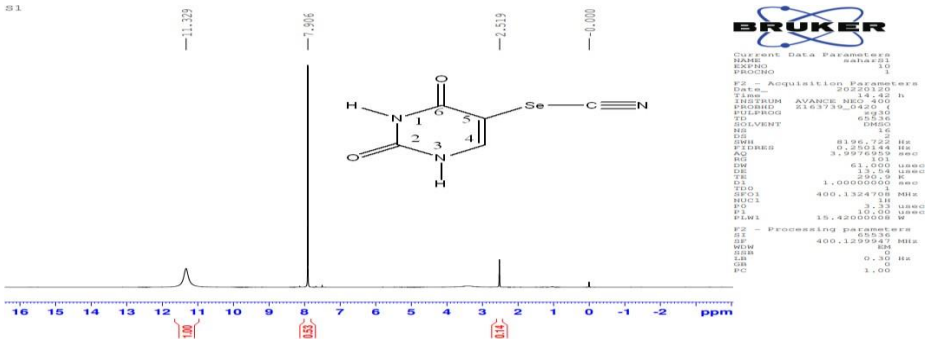


Fig:(2) ^{1}H-NMR spectrum for 5-Selenocyanatouracil in DMSO-d_6

The ^{13}C-NMR spectrum for 5-Selenocyanatouracil was represented in Fig: (3), the spectrum shows five resonances due to four carbon atoms of the uracil ring and the five one belong to the carbon of cyanide group. Thus the signal in the spectrum of the prepared compound at 105.11, 147.46, 151.72, and 161.79 ppm could be assigned to C5, C6, C2 and C4, respectively. The signal at 95.79 ppm belongs to SeCN [16].

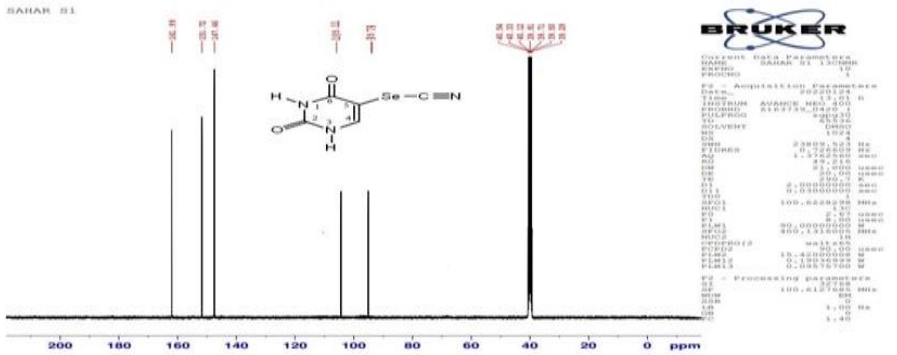


Fig (3) ^{13}C-NMR spectrum for 5-Selenocyanatouracil in DMSO-d_6

Antimicrobial activity

Assessment of antibacterial result of 5-SeU compound on *P aeruginosa*, *E Coli, K pneumonia,* and *S aureus* cultures, data from table (1). Concentration-dependent inhibition of SeU can be observed against some bacteria tests. As concentration increased, effects of 5-SeU generally improved, and the effective conclusions were found at 150µg/mL. 5-SeU has a moderately high inhibitory effect on P. aeruginosa; theresult is 18 ± 1.62 mm, but it has a lower inhibitory zone than *E coli* culture with a highconcentration the consequence of only 14 mm. The control group (DMSO) shows no inhibition zone.

Table (1): human pathogenic bacteria sensitivity to 5-SeU compound, via the filter paper disk method

Bacteria species	5-SeU 100µg/ml	5-SeU 150µg/ml	DMSO ascontrol
S.aureus	7.95±1.666	15.7±1.568	0.0±0.0
E. coli	9±1.585	14.121±2.087	0.0±0.0
Pseudo. Aeruginosa	13.20±2.94	18.0±1.632	0.0±0.0
K. Pneumoniae	0.0±0.0	0.0±0.0	0.0±0.0

5-SeU in two concentrations were evaluated for their antibacterial activity against three types of G- bacteria, including E Coli, Klebsiella pneumoniae and Pseudo aeruginosa. Resistance to existing drugs is gradually reducing their value in the treatment of bacterial infections. Consequently, to resolve this issue, new therapies act by a novel objective by the encoding of multiple genes of a single protein.

The restricted mechanism is stated by limited studies about the antibacterial activity of selenium compounds like synthesized bio-SeNPs(Se nanoparticles) by *Bacillus* sp., which induce fragmentation of DNA *Leishmania major* result in apoptosis. Also, observed they can effectively stimulate reactive oxygen species (ROS) production, butthere is still a need to discover the mechanism of action. On the other hand, the antimicrobial action of nanoparticles was usually attained via impairment ofmitochondria and cell membranes [20].

Antioxidant action using DPPH assay

The significant antiradical activity results were presented in Table (2). Different concentrations of test samples were measured for antioxidant activity. Non-significantly change was observed up to 71.2237 µg/mL ±4.925137 for (5-SeU) compound, and 68.43148 µg/mL ±5.340965 for standard control AA. Also at a low dose (30 µg/mL), there were no significant differences between 5-SeU (26.92529±4.31178), and AA (57.1962 ±10.18565 µg/mL). The antioxidant effects of both test materials are directly related to concentration. Result directs that the line of regression (Y = aX + b) as in the figure (4) ; y = 16.332x + 6.2829 for 5-SeU, and for AA (y = 15.419x + 11.372)

Then median antiradical inhibition rate was designed, IC50 of AA is (49.6153), and (49.26246) of 5-SeU compound. Antioxidant results indicated no significant differencesbetween synthesized compound (5-SeU) and ascorbic acid. The results were illustrated in Table (2) and figure (4)

Table (2): Antiradical activity of (5-SeU) compound directly associated with the concentration. No significant differences in comparison with standard antiradical AA werepresented.

Treated groups	concentration of AA and 5-SeU µg/mL			
	30(µg/mL)	40(µg/mL)	50(µg/mL)	75(µg/mL)
5-SeU	26.925±4.311	40.11671±6.999	61.41±3.356	71.223±4.925
AA	21.694±12.259	37.6092±9.944	60.721±2.562	68.431±5.340
LSD	NS	NS	NS	NS

NS: non significant differences

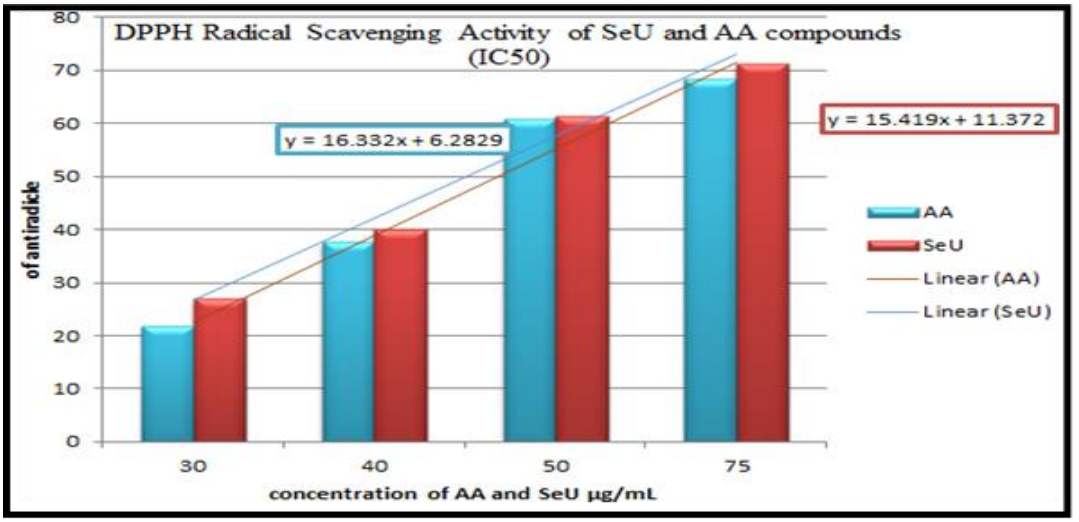


Fig.(4): From the histogram(IC50) calculated for both test 5-SeU and A

DPPH, that's extensively used to estimate the test compound's capability to perform as a scavenger of free-radical and supplier of hydrogen. It is a quick, simple, and cheaper approach to checking out antiradicals capabilities. The DPPH form is constructed at theelimination of DPPH (become stable), DPPH is certainly darkish in color, a crystalline compound made the debris of free-radical which can be stable. In specific, distant a prominent radical and a prominent antioxidant check. When, decreased it converted to DPPH- H, the DPPH radical darkish pink hue in solution, however, while decreased in addition to converting to DPPH-H, it converts to colorless or mild yellow [20].

Numerous studies showed antioxidant activity of organic and inorganic selenium compounds, like Na2SeO3 (10 g / ml) led to an improvement within the DPPH removalcapacity [21]. Another review reported that the selenium antioxidant effect

has a role in treatment of autoimmune disease together with the scientific indication that addition of Se can reduce the progression of the disease through the management the clinical symptoms [22].

Median Lethal Dose

Mortality down to 48 hrs demonstrated that the LD50 of 5-SeU in male rats was 1700 mg/kg, dose calculated from dose-response curve slope equation, results are recorded in the table: 3 , no death were recorded in the first group that received 250 mg/ kg BW of SeU, however when the dose is increased the response percentage 16.66% to both doses 500and 1000 mg/kg, response 33.33% result from administration of 1500 mg/kg, while administration of 2000 and 2500 mg/kg BW to the fifth and sixth groups, the response was 66.66% and 83.33% respectively. The results presented that LD50 between concentrations of 1500-2000 mg/ Kg, according to- Hodge and Sterner scale- that used to evaluate toxicity after oral administration to rats, 5-SeU compound is considered to be of low toxicity[23].

Percentage of graphic design, dead compared to concentration, the LD50 is measured directly from the data equation row. Substances with lower LD50 are more toxic than those with higher LD50. Acute toxicity testing provides evidence of the dose range thatis toxic to animals, and also can be used to measure the therapeutic-index (LD50 / ED50) of any drug or xenobiotics. The results indicated that 50% of responses were obtained at concentration1700 mg/Kg[24].

Table (3): Percentage of rats mortality related to dose of 5-SeU administered

No. ofRats	Dose mg/kg	No. of dead rats	mortality percentage
6	250	0	0
6	500	1	16.66666667
6	1000	1	16.66666667
6	1500	2	33.33333333
6	2000	4	66.66666667
6	2500	5	83.33333333

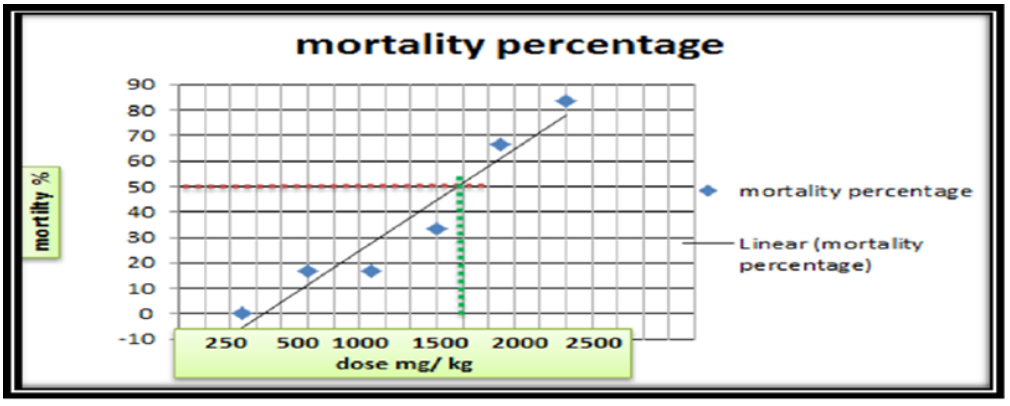


Fig.(5): The relation between 5-SeU dose and response (mortality%)

LD50 test determines the correlation between the dose of test material and the maximum response (death), LD50 is declared as mg/kg BW. It provides evidence of health risks possible to occur from immediate exposure [25]. An element with a decreased LD50 is extra poisonous than one that has a more LD50. LD50 rate offers evidences at the type of doses that might be toxic, additionally, it may support the calculation of healing index (LD50/ED50) of xenobiotics and drugs. Also, it`s vital for the potency of the healing dose of any compound. Many factors are affected LD50 such as age, sexual category, and fasting period [26]. Gender differences show a vital role in the effect of dose-response. Females are more sensitive to compound than males. It is suggested to include females in broad-spectrum acute toxicity studies [27].

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