

Role of Selenium Selenate in Correction the Effects Induced by Gemcitabine in Male Rats

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Abstract

The current study was designed to evaluate the efficiency of selenium to limit the harmful effects of gemcitabine. Twenty four rats divided to four groups. The first considered as a control group, The second injected with gemcitabine, Third group drenched with selenium while the fourth group injected with gemcitabine and drenched with selenium. After 9 days blood samples collected and measurements carried out to determination the levels of T4, LH, Testosterone, glutathione, malondialdehyde, alanine transaminase, aspartate transaminase, alkaline phosphatase, urea, creatinine and lipid profile. Results showed significant decrease in T4, LH, Testosterone, GSH and HDL in rats treated with gemcitabine, while selenium administration with gemcitabine caused significant increase in T4, GSH and HDL compared with group treated with gemcitabine only. On the other hand, gemcitabine caused significant increase in MDA, ALP, ALT, urea, creatinine, LDL, TC and TG, while those parameters decreased significantly in group treated with selenium and gemcitabine. It was concluded that selenium has protective effect against negative effects induced by gemcitabine.

Keywords: selenium, gemcitabine, hormones(T4,LH), biochemical parameters, rats

1. Introduction

Gemcitabine is substance has antitumor activity against malignancies, particularly pancreatic carcinoma (Zhang *et al.*, 2017). Gemcitabine used for treatment breast cancer, lung cancer and other biliary tract cancers (Yi-long *et al.*, 2014; Yang *et al.*, 2015; Jian *et al.*, 2016). Shaib *et al.*, (2008) indicated that gemcitabine is the accepted standard for adjuvant and metastatic treatment of pancreatic cancer. Zinzani *et al.*, (2000) found that gemcitabine is considered as single active agent in patient with cutaneous T cell lymphoma. On the other hand, gemcitabine is used to prevent heart and Kidney rejection (Jesek *et al.*, 2003). The treatment with gemcitabine may develop to dyspnea, acute respiratory distress syndrome and interstitial pneumonitis (Barlesi *et al.*, 2004). Gemcitabine is cytotoxic drug cause serious side effects including nausea, vomiting, dyspepsia, weight loss and cachexia (Jiang *et al.*, 2012). Selenium is essential dietary trace element which play an important role in number of biological process in human and other species (Ognjanovic *et al.*, 2008). Ruiz *et al.* (1998) indicated that selenium content was lower in patients with diabetes mellitus in compared with healthy subjects and there was negative correlation between selenium and HbA1c. High dietary intake of selenium reduce prostate cancer risk (Legg *et al.*, 2008). Xia *et al.* (2005) found relation between selenium deficiency and one type of heart disease which called Keshan disease, also it provide neuro-protection against neural diseases, so it was found relation between selenium and Alzheimer disease (Battin and Brumaghim, 2009). Selenium play an important role in reducing tumorigenesis (Goyal *et al.*, 2006), In addition it's essential element for male fertility (Hawkes and Turek, 2001).

Selenium has effective and important role as antioxidant and removal the effects of some toxic materials (Pavlik *et al.*, 2012), therefore the current study aimed to evaluate the protective role of selenium as antioxidant to reduce effects induced by gemcitabine.

2. Materials and Methods

2.1. Animals

In the current study twenty four white male rats with age (3 – 4) months weighing (110 – 150) gm housed under standard conditions of temperature (22 ± 2) °C and light / dark (12 / 12) hr. Rats were given diet and water *ad libitum*. Gemcitabine HCl was dissolved in normal saline for preparation used dose (50 mg / Kg) (Jiang *et al.*, 2012), gemcitabine injected intraperitoneally at single dose on days 3, 6 and 9. Selenium was used as sodium selenate Na₂SeO₃ at concentration 0.5 mg / Kg diet (Ognjanovic *et al.*, 2008) and given to rats for 9 days.

2.2. Experimental design

twenty four rats were randomly divided into four groups (6 animal for each group) as following :

- 1- control group (C) : rats were injected intraperitoneally with normal saline at days 3, 6 and 9.
- 2-First treatment group (T1): rats were injected ip with gemcitabine at dose 50 mg / Kg at days 3, 6 and 9.
- 3- Second treatment group (T2) : rats were given selenium orally at concentration 0.5 mg / Kg diet.
- 4- Third treatment group (T3) :rats were given selenium at concentration 0.5 mg / Kg diet and also injected with gemcitabine at dose (50 mg / Kg) intraperitoneally at days 3, 6 and 9.

2.3. Blood sample collection

After 24 hours of the end of treatment, blood samples taken by heart puncture and serum isolated by centrifugation. Serum stored at -20°C until the conducting the following investigations:

- a - Determination of Glutathione (GSH) according to the method followed by (AL- Zamey *et al.*, 2001)
- b - Estimation of Malondialdehyde (MDA). MDA was estimated in serum according to (Muslish *et al.*, 2002)
- c- Determination of ALT, AST and ALP. The estimation of ALT and AST carried out depending on method followed by (Reitman and Frankel, 1957) While ALP was measured according to method of Duncan *et al.* (1994).
- d- Urea level was determined according to method (Wills and Savory, 1981) and use kit supplemented by Biomerix (France)
- e- Creatinine was estimated according to (Burtis and Ashwood, 1999)
- f- Lipid profile: Total serum Cholesterol measurement depended on enzymatic colorimetric method (Face, 1982) and using kit supplemented by (Biolabo, France). Method followed by Burtis and Ashwood (1999) was depended to estimation level of HDL. LDL level was calculated according to equation followed by Friedewald *et al.* (1972). Triglycerides were measured by use kit produced by Biomerix (France). according to method (Young and Pestaner, 1975)

3. Results

Results showed significant decrease ($P < 0.05$) in T4 level in group treated with gemcitabine compared with other groups. On the other hand, selenium increase the level of T4 significantly ($P < 0.05$) in comparison with other groups, while administration of selenium with gemcitabine did not differ significantly compared with gemcitabine treated group. LH and testosterone levels decreased significantly ($P < 0.05$) in gemcitabine treated group (table, 1). GSH decreased significantly ($P < 0.05$) in group treated with gemcitabine compared with other groups, while selenium caused significant increase in GSH level compared with other groups, also the administration of the selenium with gemcitabine increase GSH level compared with group treated with gemcitabine only.

MDA increased significantly ($P < 0.05$) in group treated with gemcitabine compared with other groups while selenium caused significant decrease ($P < 0.05$) in MDA compared with other groups. Selenium caused significant decrease ($P < 0.05$) in MDA in group treated with Se and Gemcitabine compared with group treated with Gemcitabine only. Results revealed significant increase ($P < 0.05$) in level of ALP, ALT, AST, Urea and creatinine in group injected with gemcitabine, while selenium caused significant decrease ($P < 0.05$) in urea and creatinine, as well as selenium administration with gemcitabine decrease significantly levels of ALP, ALT, AST, urea and creatinine (table 2, 3). Injection of gemcitabine caused significant ($P < 0.05$) increase in total Cholesterol, LDL and TG, while there was significant decrease ($P < 0.05$) in HDL. Total cholesterol, LDL decreased significantly ($P < 0.05$) in group treated with selenium only, while HDL was increased significantly ($P < 0.05$). TC, LDL and TG decreased significantly while HDL increased significantly ($P < 0.05$) in group treated with selenium and gemcitabine compared with group treated with gemcitabine only (table, 4).

4. Discussion:

Gemcitabine caused significant decrease in levels of LH, T4 and testosterone. On the other hand, administration of selenium to group injected with gemcitabine corrected effects induced by gemcitabine in these hormones. reactive oxygen species (ROS) formation is one of mechanisms of gemcitabine as antitumor agent (Donadelli *et al.*, 2007). Gemcitabine enhances significantly intracellular reactive oxygen species (Donadelli *et al.*, 2011). Kohrle and Gartner (2009) noticed that selenium deficiency led to reduction in GPx activity that in turn caused oxidative damage of thyrocytes, the oxidative damage caused reduction in the biosynthesis of thyroid hormones, and this may be main cause of decreased T4 level in the current study. Dunats *et al.*, (2003) reported improvement in the function of thyroid gland after selenium administration. Decreased levels of LH and testosterone may be caused by oxidative stress induced by gemcitabine. Kumar *et al.* (2009) reported that oxidative stress inhibit hypothalamus function resulting in reduction in gonadotropins production, which in turn, decreased testosterone level.

Selenium encourages the activity of GSH- Px and thus reduce reactive oxygen species production (Klein, 2004), in addition selenium is important constitute of Se-dependent GSH - Px, thus selenium protects cell by inhibiting free radicles production (Yalcin *et al.*, 2004). Maehara *et al.* (2004) mentioned that selenoproteins suppressed intracellular ROS levels induced by gemcitabine. Selenium was significant recover activities of super oxide dismutase and GPx in liver and kidney of melathion treated rats (AL - Othman *et al.*, 2011).

Erkekoglu *et al.* (2014) indicated that Se protects liver and kidney against oxidative stress caused by increased free radicles. Se decrease lipid peroxidation ameliorate antioxidant defense system in liver and Kidney of rats (Ognjanovic *et al.*, 2008). Oxidative damage induced by gemcitabine may caused renal dysfunction resulting the elevation of urea and creatinine levels in blood.

Mysliwiec *et al.* (2002) mentioned that Se caused improvement in lipid profile, therefore Se decreased TC, TG and LDL in animals, also these findings agreed with the results of current study. Oxidative stress caused negative effects in lipid profile in male rabbits (Khudaier (2010)). On the other hand, selenium reduces oxidative stress resulting in modulation changes in lipid profile induced by oxidative stress. Senol *et al.*, (2014) indicated that selenium decreases lipid peroxidation and supports antioxidant defense system by increasing GSH, vitamin E and vitamin C. Selenium reduces oxidative stress by increasing GSH and GSH-Px (Kose and Naziroglu, 2014). Selenium has a protective effect from oxidative damage by increasing mRNA levels of SOD1, CAT and GPx (Zhao *et al.*, 2013).

5. Conclusion

Selenium has a protective role against harmful effects caused by gemcitabine, selenium reduces oxidative stress induced by gemcitabine which, in turn, causes negative effects in hormone level and some biochemical parameters.

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Table(1):Effect of selenium and gemcitabine in some hormone levels in male rats.

groups	parameters		
	T4(mg/L)	LH(mIU/ml)	T(ng/ml)
control	8.3±0.15 ^a	10.4±0.5 ^a	1.44±0.18 ^a
T1	6.74±0.18 ^b	8.6±0.29 ^b	0.7±0.27 ^b
T2	9.48±0.23 ^c	10.8±0.66 ^a	1.82±0.37 ^a
T3	7.28±0.16 ^b	9.6±0.11 ^{ab}	1.1±0.13 ^{ab}

- The similar letters indicate non-significant differences while different letters indicate significant differences at p<0.05

Table (2):Effect of selenium and gemcitabine in(GSH,MDA,ALP,ASTand ALT) in male rats .

groups	parameters				
	GSH (µmol/L)	MDA (µmol/L)	ALP(U/L)	AST(U/L)	ALT(U/L)
control	3.4±0.17 ^a	1.44±0.06 ^a	362.8±33.92 ^a	39.14±2.62 ^a	46.8±3.3 ^{ac}
T1	2.72±0.1 ^b	1.96±0.1 ^b	658±28.99 ^b	55.42±3.98 ^b	64.8±3.48 ^b
T2	4.4±0.19 ^c	0.9±0.1 ^c	309.4±16.28 ^a	33.86±2.68 ^a	38±2.86 ^c
T3	3.1±0.19 ^a	1.2±0.12 ^a	505±37.55 ^c	47.6±2.29 ^b	54.8±5.09 ^{ab}

- The similar letters indicate non-significant differences while different letters indicate significant differences at p<0.05.

Table (3) Effect of selenium and gemcitabine on (urea and creatinine)levels in male rats.

groups	parameters	
	urea(mg/dl)	creatinine(mg/dl)
control	25.22±1.17 ^a	0.58±0.06 ^a
T1	28.76±1.13 ^c	1.67±0.19 ^c
T2	18.25±1 ^b	0.2±0.06 ^b
T3	24.82±1.04 ^a	0.89±0.09 ^a

- The similar letters indicate non-significant differences while different letters denote to the significant differences at p<0.05.

Table (4) Effect of selenium and gemcitabine on lipid profile in male rats.

groups	Parameters			
	TC(mg/dl)	TG(mg/dl)	HDL(mg/dl)	LDL(mg/dl)
control	252.2±3.85 ^a	47.4±0.5 ^a	19.24±0.98 ^a	185.2±2.87 ^a
T1	336.2±3.21 ^b	71.6±0.67 ^b	16.48±0.56 ^b	247.4±2.8 ^b
T2	195±5.01 ^c	39.6±0.67 ^a	26.94±0.65 ^c	127.8±5.56 ^c
T3	284.6±3.38 ^d	49.4±1.12 ^a	20.7±0.85 ^a	209.8±4.42 ^d

- The similar letters indicate non-significant differences while different letters denote to the significant differences at p<0.05.