Effect of experimental selenium toxicity on some cytogenetic parameters in adult Awasiy ewes

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Abstract
The present study was conducted to investigate the effect of selenium toxicity on some blood, cellular and biochemical parameters in adult Awasiy ewes. The first group (DW) included ten ewes pumped with distilled water daily for ten weeks, the second group (SS+RX) included ten ewes pumped with salt sodium selenite (1.2 mg / kg BW) daily and orally for eight weeks and then treated with N-Acetyl L-cysteine (70 mg / kg BW) daily and orally from week 9 to week 10. The third group (SS) also included ten ewes that were injected with sodium selenite (1.2 mg / kg BW) orally daily for 10 weeks. The results of this study also showed a significant increase (P<0.05) in the second group (SS+RX) and SS (SS), which was injected with sodium selenite on serum selenium concentration starting from the first week to At the end of the experiment, selenium concentration showed a decrease starting from the ninth week in the second group (SS+RX) after treatment with N-Acetyl L-cysteine until the end of the experiment compared with the control group. The results of these study also showed a significant (P<0.05) increase of glutathione peroxidase concentration in groups (G2, G3) start in week one (W1) till the fifth week which reach the peak, then its start to decline and continue until the end of experiment in (G3), while it was returned to normal level in the ninth week and remain steady until the end of the experiment in (G2) compared with control group. The cellular tests performed on the blood lymphocytes included chromosomal aberration, which showed significant results of P<0.05 in the two groups that were injected with sodium selenite, the second group (SS+RX) and SS compared to the control group (DW). These abnormalities began from the end of the first week of the experiment until the end of the tenth week in both groups even after treatment with N-Acetyl L-cysteine for the second group (RX + SS) which starts from the ninth week until the end of the tenth week. The second cytological examination included micronucleus, which showed a significant change (P<0.05) in both the (SS+RX) and SS and the (SS) compared to the control group (DW), which began from the end of the third week until the end (N-Acetyl L-cysteine) in the second group (RX + SS), starting from the ninth week and ending with the experiment. The current study indicates that changes in the genetic level of the cells remain constant despite the given treatment and the return of cases to normal bed and laboratory, which confirms that the poisoning of the element of selenium is dangerous and permanent effects.

Keywords: cytogenetic, selenium, N-Acetyl L-cysteine

1. Introduction
Selenium (Se) is a microelement that shows a significant part in the health and all function of animals [1, 2]. The decrease in embryonic death in pregnant animal in the first months is due to selenium supplementation. In immunity, selenium plays a part in the development and the action of all type of lymphocyte [3]. Selenium is microelement that shows a part in the defenses touching the increase of hydro peroxides from cellular breakdown [4]. This natural function processes by selenoprotein, such as the glutathione peroxidase type (GPx), the iodothyronine deiodinases and the thioredoxin reductases, the selenium is chief part in the structural of this enzyme [5]. These enzyme have selenocysteine amino acids in critical locations of the active center. There are multiple functions of selenium in body [6].

There is a relation between vitamin E and selenium in their function as antioxidant also when the dose is insufficient in one of these selenium must be increase the other one to prevent of many anomalies [7]. There is another relation with Sulfur (S) existing in the methionine and cysteine is replaced with selenium to produce selenomthionine and selenocysteine. The two type of selenium common in plant [8].

If toxicity occurs, the selenium must be calculate to avoid the endanger to life of animal and consumer [9]. There are two type of selenium toxicity, acute and chronic poisoning [10]. Glutathione peroxidase-5 (GPx-5) is present in the embryo and the olfactory epithelium, its
role remains unknown [11, 12] reported selenium is correlated to blood cells size therefore well effect on all blood parameter. The object of this study is to estimation of the levels of selenium in ewes serum in normal and toxic conditions. Creation of sub-acute selenium toxicities in Awasiy ewes to study their effects on some oxidative biomarker and DNA and Treatment of sub-acute selenium toxicity.

2. Materials and Methods

2.1 Experimental animals care and Preparation period
(from 1/10/2016 to 11/1/2016)
The animal’s room was prepared for reception ewes by supplying it with lights, air pumps and vaporization with formalin and potassium permanganate. The experimental animals were stayed in the farm (outdoor breeding) and stayed until the end of the experimental study which extend from 1/11/2016 to 14/1/2017. Thirty female crossbreed Awasiy ewes were bought from the local markets, their age ranged between (2-3) years and their weight ranged between (30~40) Kg. These ewes were prepared for experiment by vaccination with sheepfold polivac® vaccine (ovine and caprine clostridial vaccine) (VETAL / TURKEY), and injected with ivermectin at a dose of (1ml/50kg BW), this dose repeated again after (2) weeks, then frequency repeated every month during experiment period to control internal and external parasites. As well as ewes were dosed with Levozane 3% (VAPCO/Jordon) at a dose of (1ml/10kg BW) to prevent pulmonary nematode and tapeworms (Monizia), and oxyclozanid 6% (VAPCO/Jordon) at a dose of (5ml/10kg BW) to treat and control of ruminal & intestinal parasites, Ascaris, and pulmonary nematode infections, chronic fascioliasis and for the removal of tapeworm segment.

2.2 Experimental design for this study
After acclimatization for one month, the ewes were divided into three group equally, First group was a control which injected distill water daily to the end of experiment, the second group was subacut with treatment which dosed with sodium selenite at a dose of (1.2) mg/kg BW for (8) weeks after that these group treated by N acetyl L Systine for two weeks and the third group was sub-acute dosed by the same dose of the sodium selenite without treatment.

2.3 Preparation of toxicity dose and the Toxicity period
(from 1/11/2016 to 1/1/2017)
Ewes of the 2nd & 3rd groups were dosed with sodium selenium at a dose of (1.2) mg/kg BW (Clarke and Clarke, 1970), using a working solution prepared by melting (10) gm of sodium selenium in one litter of distilled water, these doses were given daily orally for (8) weeks. The dose for each ewe was calculated by multiplying the ewes' weight by the determined dose and measuring how much it equal of working solution. On the other hand, ewes of the 1st group were left in a separate room as a control group for comparison. The blood sample taken every week to make the cytological and serum biochemical test.

2.4 Preparation of treatment dose and the treatment period (1/1/2017 to 14/1/2017)
Ewes of the 1st group treated by N-acetyl L-cystine at a rate of (70) mg /kg BW (Osweiler, 1996) four times daily orally for (2) weeks by working solution prepared by melting (20) g of N-acetyl L-cystine in one litter of distilled water.

2.5 Blood samples collection
Every week blood samples were collected from the jugular vein from all thirty ewes of this experiment in two sterile disposable test tubes, one with anticoagulant (EDTA) and the other without anticoagulant, in which (5) ml of blood was collected for serum examinations [13].

2.6 Determination of parameters of the experiment
Glutathione peroxidase activity (GPX) was measured using a special kit (Bio Assay Systems) by the quantitative colorimetric glutathione peroxidase determination [14]. Lymphocyte culture for Chromosomal Aberrations was prepared according to Cytogenetic analysis of human and animal blood lymphocyte [15] and then the percentage of Chromosomal aberrations was estimated [16]. Selenium serum concentration was measured by using flameless atomic absorption [17].

2.7 Statistical Analysis
The Statistical Analysis System- SAS (2012) program was used to show the effect of different factors in study parameters. Least significant difference –LSD test (ANOVA) was used to significant comparison between means in this study [18].

3. Results and Discussion
3.1 Effect of experimental selenium toxicity on glutathione peroxidase in Awasy ewes
Fig.1 represent the concentration of glutathione peroxidation, there were no significant (P≤0.05) differences in glutathione peroxidase (Gpx) concentration in zero among all groups of experiment. There were a significant (P≤0.05) differences of glutathione peroxidase concentration in groups (G2, G3) started in the 1st week (W1) to the end of the experiment. The increase in Gpx is due to increase the selenium intake which considerable a significant structural part from many numbers enzymes for example, glutathione peroxidase, thioredoxin reductase what's more deoxidinases [6]. Glutathione peroxidase is antioxidant enzyme directly proportional to selenium intake; therefore, the role of selenium is very important to performance of the function of the glutathione peroxidase in the body as antioxidant against oxidative stress [19].

In normal condition, selenium have important role in protection the cell membrane of red blood cell and there development due to the role of proteins link to selenium in body (selenoprotine) [20]. These enzymes have an important role in antioxidant, reproduction, and muscle capacity and tumors prevention[6]. At the beginning of the week 6th until the end of the experiment except in the last two weeks in group two (SS+RX), Gpx begin to decline and that coincided with the selenium levels in the body which reached the toxic levels due to the continuous uptake of selenium in the diet in this experiment. When the selenium reach the toxic level it act oppositely as a prooxidant lead to release free radicals that cause decrease in Gpx because of its work as neutralizing the free radical [21].

Therefore the increase in glutathione peroxidase is due to increase of the availability of its substrate (selenium) especially in first five week of experiment. Moreover, it declined when the levels of selenium reached to the accumulative toxic level when it acts as prooxidant in this state.
3.2 Effect of experimental selenium toxicity on chromosomal aberration

Fig. 2 represents the effect of experimental selenium toxicity on chromosomal aberration (chromosomal break B') in Awasiy ewes. There were no significant (P≥0.05) differences in chromatid break in zero time analysis among all groups of experiment. There was a significant (P≤0.05) differences of chromosomal break in groups (G2, G3) started in 1st week (W1) to the end of the experiment as compared with the control group.

Fig. 3 represents the effect of experimental selenium toxicity on chromosomal aberration (chromosomal gap G') in Awasiy ewes. There were no significant (P≥0.05) differences in chromosomal gap in zero time analysis among all groups of experiment. There were significant (P≤0.05) differences in chromosomal gap in groups (G2, G3) started in 1st week (W1) to the end of the experiment as compared with the control group.

Fig. 4 represents the effect of experimental selenium toxicity on chromosomal aberration (chromatid break B') in Awasiy ewes. There were no significant (P≥0.05) differences in chromatid break in zero time analysis among all groups of experiment. There was a significant (P≤0.05) increase in chromatid break in groups (G2, G3) started from the 1st week (W1) to the end of experiment as compared with control group.

Fig. 5 represents the effect of experimental selenium toxicity on chromosomal aberration (chromatid gap G') in Awasiy ewes. There are no significant (P≥0.05) differences in chromatid gap in and first weeks among all groups of experiment. There was a significant (P≤0.05) differences of chromatid gap in groups (G2, G3) start in week three (W2) until the end of experiment compared with control group.

3.3 Effect of experimental selenium toxicity on micronucleus in Awasy ewes.

Fig. 6 represents the results which revealed the effect of experimental study of selenium toxicity by Sodium Selenite at (1.2 mg/kg B.W) in Awasy ewes treated with N-acetyl L-cysteine(70 mg/Kg. BW). There were no significant (P≥0.05) differences in weight in zero time analysis and first two weeks among all groups of experiment. There was a significant (P≤0.05) difference of micronucleus in groups (G2, G3) started in third week (W3) until the end of experiment as compared with the control group.

Because the selenium in normal levels is a significant structural part from many numbers antioxidant enzymes for example, glutathione peroxidase, thioredoxin reductase what is more deiodinases. These enzymes have an important role in antioxidant, reproduction, and muscle capacity and tumors prevention. On the other hand, in toxic levels, the selenium act oppositely as a prooxidant and lead to release reactive oxygen species or free radicals. Selenium compounds have different abilities to generate superoxide, in vitro Inorganic form of selenium appear to react with tissue thiol, such as glutathione and those are reacting with other thiol to generate oxygen free radicals, such as superoxide anion (O2-) by redox catalysis [20, 22]. Selenite reacts with glutathione endogenously in cells or extracellularly causes toxicity by the formation of superoxide and elemental selenium [22, 23]. High reactive oxygen species (ROS) levels may thus induce DNA damage and results in nuclear DNA fragmentation and increased apoptosis [24]. Supraphysiological free radicals damage both nuclear and DNA and induce mutations, and result in decreased ATP production [25, 26, 27]. The meiotic spindle is essential not only for the maintenance of chromosomal organization but also for the second polar body formation [28]. Disorganization of the meiotic spindles could result in chromosomal dispersion, failure of normal fertilization, and termination of development [29]. Alteration of cellular morphology and cytoskeleton has been demonstrated after exposure to an oxidative stress or free radical in various cells such as mouse ocular cells [30], intestinal epithelia [31] and human cortical neurons [32]. In spite of the fact that, N-Acetyl L-cysteine is made from the amino acid cysteine joined to an acetyl group. Acetyl cysteine (sometimes abbreviated as N-A.C or NAC) is a strong antioxidant. It donates the amino acid cysteine to help formation of antioxidant glutathione. Glutathione is a powerful antioxidant normally found in the body [33]. Although, the antioxidant capability of N-Acetyl L-cysteine should eliminate or decrease the deleterious effect of the ROS that generated by the toxicity of selenium which consider the main factor caused the DNA damage [24], the result of G2 which receive the treatment with N-Acetyl L-cysteine showed no significant improvement in chromosomal aberration during the treatment period in this experiment which extended to two weeks. This reveals the severity of irreversible nuclear damage resulted from selenium toxicity.

3.4 Effect of experimental selenium toxicity on level of selenium in serum of Awasy ewes.

Fig. 7 shows the effect of experimental study of selenium toxicity by Sodium Selenite at (1.2 mg/kg B.W) in Awasy ewes treated with N-acetylcysteine(70 mg/Kg. BW). There were no significant (P≥0.05) differences in level of selenium in serum in zero among all groups of experiment. There were a significant (P≤0.05) differences of level of selenium in serum in groups (G2, G3) started in 1st week (W1) to the end of the experiment as compared with the control group due to the level of selenium in serum concentrations of selenium reflect recent selenium intake [34].

![Fig 1: Effect of Experimental selenium toxicity on glutathione peroxidase in Awasy ewes.](image)

![Fig 2: Effect of experimental selenium toxicity on chromosomal aberration (chromosomal break B’) in Awasy ewes.](image)
5. Conclusion
In the present study we found the effect of selenium toxicity in all body functions and especially in antioxidant system. Its necessary part of Gpx structure and so the increase of selenium levels is benefit against oxidative stress unless it reach the accumulative toxic levels when in transform to prooxidant agent and cause for free radical releasing which it is poisonous to each humans and animals lead to sever cytogenetic damage that cannot be revers even after treatment administration of N-acetylle cysteine that used in this study. Moreover, the level of selenium concentrations in serum can reflect recent selenium intake.

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7. References
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