THE ROLE OF VITAMIN C ON DEFENSE SYSTEM OF DOMESTIC RABBIT *Oryctolagus cuniculus* IN MERCURY INDUCED TOXICITY

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Abstract
The aim of the present work is to investigate the protective role of vitamin C in domestic rabbit Oryctolagus cuniculus intoxicated by mercury. Thus, the status of target organs, glutathione and some immune markers are evaluated by using two exposure periods. Male rabbits Oryctolagus cuniculus were exposed for 10 and 22 days to either Hg alone or combined with vitamin C (Hg-VitC), in order to estimate the possible antioxidant protective role of this vitamin. In the first treatment period, results have showed non-significant variations in the relative weight of liver, kidney, spleen and adrenal in both treated groups compared to the control. Glutathione level in Hg group was significantly increased in liver, but it decreased significantly in spleen and adrenal. However, glutathione in Hg-VitC group was higher in liver and lower in spleen compared to the control. The percentage of immune markers lymphocytes, monocytes and basophils were unchanged in all circumstances, except the observed significant augmentation of basophils in the Hg-VitC group compared to the Hg group. In the second treatment period, the relative weight of liver and adrenal were increased significantly when treated by Hg and that compared to the control. The relative organ weight in the Hg-VitC group, revealed a significant decrease in liver and in adrenal compared to the Hg group. Glutathione level in Hg group was decreased significantly in liver, kidney and spleen. Furthermore, glutathione concentration of the Hg-VitC group was significantly decreased in both liver and adrenal, but it was increased in kidney and that compared to the Hg group. Concerning the immune markers, only basophils were significantly decreased in both treatments compared to the control. When comparing the two treatments period, a remarkable decrease was observed in liver glutathione of Hg group and in the percentage of basophils of the two treated groups after 3 weeks. The protective role of vitamin C, however, seems evident on adrenal glutathione level and basophils during the first treatment period, but during the second period, it protected kidney glutathione level, in addition to liver and adrenal relative organ weights.

Key words: Antioxidant, Glutathione, Immune markers, Mercury, Vitamin C.

EFFET DE VITAMINE C SUR LE SYSTEME DE DEFFENSE CHEZ LE LAPIN DOMESTIQUE INTOXIQUE PAR LE MERCURE

Le but de ce travail est donc d'étudier le rôle protecteur de la vitamine C chez le lapin domestique *Oryctolagus cuniculus* intoxiqué par le mercure. Ainsi, le statut des organes cibles, le glutathion et certains marqueurs immunitaires sont évalués en utilisant deux périodes d'exposition. Des lapins mâles *Oryctolagus cuniculus* ont été exposés pendant 10 et 22 jours au mercure Hg seul, ou à la combinaison avec la vitamine C (Hg-VitC), afin d'estimer le rôle protecteur et antioxydant de cette vitamine. Au cours de la première période de traitement, les résultats ont montré des variations non significatives du poids relatif du foie, des reins, de la rate et des glandes surrénales dans les deux groupes traités par rapport au groupe témoin. Le niveau de glutathion dans le groupe Hg a été significativement élevé dans le foie, mais il a diminué de manière significative dans la r ate et les glandes surrénales. Cependant, le niveau du glutathion chez le groupe Hg-VitC était plus élevé dans le foie et plus bas dans la rate par rapport au témoin. Le pourcentage de marqueurs immunitaires à savoir les lymphocytes, les monocytes et les basophiles est resté inchangé dans toutes les

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circonstances, à l'exception de l'augmentation significative observée au niveau des basophiles chez le groupe Hg-VitC par rapport au groupe Hg. Au cours de la deuxième période de traitement, le poids relatif du foie et des glandes surrénales a augmenté significativement chez le groupe traité par Hg en comparaison avec le groupe témoin. Cependant, le poids relatif des organes dans le groupe Hg-VitC, a révélé une diminution significative du poids relatif du foie et des glandes surrénales par rapport au groupe Hg. Le niveau de glutathion dans le groupe Hg a été significativement diminué dans le foie, les reins et la rate. En outre, la concentration de glutathion du groupe Hg-VitC a été significativement diminuée dans le foie et les glandes surrénales, mais elle a été augmentée dans les reins par rapport au groupe Hg. Concernant les marqueurs immunitaires, seuls les basophiles ont été significativement diminués dans les deux traitements par rapport au témoin. La comparaison entre les deux périodes du traitement a révélé une diminution remarquable du taux de glutathion hépatique chez le groupe Hg et du pourcentage des basophiles des deux groupes traités. Le rôle protecteur de la vitamine C, cependant, semble évident sur le niveau de glutathion des glandes surrénales et les basophiles pendant la première période de traitement, mais pendant la deuxièmepériode, la vitamine C a protégé le niveau de glutathion rénal, en plus du poids relatif du foie et des glandes surrénales.

Mots clés : Antioxydant ; glutathion ; marqueurs immunitaires ; mercure ; vitamine C.

1. Introduction

Mercury is considered as the most toxic heavy metal, and even at low concentration in the organism, it can cause a very big variety of psycho-physiological disruptions [1;2].Whatever, the chemical form of the absorbed Hg, it can be oxidized to Hg ions to be accumulated mainly in soft organs. The organism is constantly under the threat of mercury presents in the surrounding environment. However, in order to fight against such toxicity; the organism is equipped with a defense system to provide a good degree of immunity. In fact, the endogenous anti-oxidant defense system linked to the glutathione is considered as the second mechanism arranged by the organism. The mercurial compounds have exceptionally a big affinity for the sulfhydryls groups found in the glutathione, the cysteine, and proteins [3]. However, a treatment with mercury has resulted in a depletion of the cellular defense mechanism from the oxidative stress. Its toxicity has been associated with the induction of oxidative damage in cells [4; 5; 6]. During the oxidative stress, the GSH plays a role in the detoxification of a variety of electrophilic compounds and peroxides via catalysis by glutathione S-transferases (GST) and glutathione peroxidases (GPx) [7; 8]. These last inhibit the lipid proxidation through the reaction with the H_2O_2 and the fatty acids. Owing to its principal role, the glutathione is very abundant in cells and provides an important system in the detoxification of electrophiles and peroxides, and the neutralization of free radicals, capable to cause the cytotoxic or genotoxic damage [9; 10; 11].

Like the glutathione, which defend against the mercurial compounds, the immune system, which considered as a target system, enters in action when mercury circulates in the blood. It is known that mercury leads to some pathophysiological changes, affecting however, the functions of the immune system [12; 13; 14]. As a result, there were changes of lymphocyte counts, morphological alterations in the immune tissues, reduction in the total number of immune cells, or alterations of the immune functions [15]. Mercury can also compromise the immune system while leading to an increase of the susceptibility

to the infections and to the development of auto-immune disease which occurs especially in kidney. Moreover, many studies have reported the toxic effects of mercury on the immune system, where most rats developed kidney glomerular lesions in parallel of the auto-immune reactions, reflected by lymphoproliferation and associated with an increase in the IgG level [16].

Several studies aimed to find out a natural element in order to detoxify mercury. Thus, vitamin C supplementation is essential to a systemic detoxification of mercury [17] because it interferes with the intestinal absorption of the heavy metals by increasing the urinary excretion or creating synergic effect on the chelator element [18], or it transforms the mercury into soluble salt to make it eliminable. Therefore, vitamin C alleviates mercury toxicity [19]. On the other hand, a lack in vitamin C provoked mercury toxicity in the guinea pigs [20], while the study of Hill, [19] showed that ascorbic acid seemed to have a marginal effect in overcoming mercury toxicity in chicks. However, Blackstone et al., [21] demonstrated that mercury significantly reduced the concentration of ascorbic acid in adrenals and spleen of animals. Contrary, Murray and Hughes [22] reported that ascorbic acid in the drinking water increased the amount of mercury in the liver and kidney of guinea pigs when given orally, but not when the element was injected.

The aim of the present work is, therefore, to investigate the protective role of vitamin C in domestic rabbit *Oryctolagus cuniculus* intoxicated by mercury. Thus, the status of target organs, glutathione and some immune markers are evaluated by using two exposure periods.

2. Materials and methods

Experimental animals and treatment

Twenty four male rabbits of local population of 6-7 months old, with an average weight of 1400±54g, divided into 3 groups; the first group used as a control, the second was treated by mercury (1g $HgCl_2$/Kg food) and the third was given a combined treatment (1g $HgCl_2$/Kg food + 8mg vitamin C/animal). However, vitamin C was dissolved in distilled water, where each animal has received it daily by gavages (half in the morning and half in the afternoon). After 10 days continuous treatments, half of the animals of each group were sacrificed, and the remaining were treated by the same manner, and then sacrificed after other 12 additional days.

Samples' collection

Peripheral blood was collected in heparinised tubes for counting leukocytes using Automatic Coulter Counter Machine (T 540), measurement. Then liver, spleen, left adrenal and left kidney have been removed, weighed and then frozen at -18 °C for glutathione measurement according to the method of Weckbeker and Cory [23]. The relative weight of each organ has also been estimated.

Data analysis

Statistical analysis was carried out by student t-test to compare between paired groups, whereas the one-way analysis of variance (ANOVA) was used to compare between the three groups.

3. Results

The first treatment period

The results are shown in table 1. The obtained results showed a slight increase in relative weight of liver, kidney, spleen and adrenalin Hg group compared to the control. However, the liver glutathione concentration presented a significant increase in both treated groups compared to control.

Contrary, the spleen glutathione level revealed a significant decrease in Hg group and in Hg-VitC group as well and that compared to the control. The adrenal glutathione concentration also was significantly reduced in animals exposed to Hg.

There was no significant difference between the groups, although the percentage of lymphocytes of the treated rabbits was slightly higher than those of the control. Accordingly, monocytes percentage was almost similar in all groups. The only change recorded in basophils was their high elevation in Hg-VitC

Table 1. - Mean (X±SD) organ's relative weights of male rabbit *Oryctolagus cuniculus* after 10 days' treatment.

	G1 (Control)	G2 (Hg)	G3 (Hg+Vit C)
Liver	2.609±0.68	3.063±0.52	2.607±0.24
Kidney	0.63±0.032	0.879±0.22	0.77±0.204
Spleen	0.043±0.014	0.06±0.016	0.054±0.008
Adrenal	0.012±0.003	0.018±0.008	0.021±0.012

Table 2. - Mean (X±SD) glutathione concentrations (nM/mg protein) of male rabbit *Oryctolagus cuniculus* after 10 days' treatment.

	G1 (Control)	G2 (Hg)	G3 (Hg+Vit C)
Liver	18.96±1.29	24.26±1.72^{a*}	22.91±1.84^{b**}
Kidney	22.57±2.62	18.13±4.29	21.42±0.89
Spleen	27.96±2.47^{d**}	20.16±4.11^{a**}	21.68±0.56^{b*}
Adrenal	25.76±1.55	20.35±1.71^{a*}	23.13±5.45

a: Control ***Vs*** Hg;**b:** Control ***Vs*** Hg-VitC; **d:** Control ***Vs*** Hg ***Vs*** Hg-Vit C; ***:** p<0.05 ****:** p<0.01

Table 3. - Mean (X±SD) white blood cells percentage of male rabbit *Oryctolagus cuniculus* after 10 days' treatment.

	G1 (Control)	G2 (Hg)	G3 (Hg+Vit C)
Lymphocytes	42.58±11.94	46.75±15.66	47.55±8.55
Monocytes	9.15±3.89	8.1±1.35	8.48±2.34
Basophils	3.48±0.89	2.98±1.48	4.93±1.26^{b*}

b: Control ***Vs*** Hg-VitC; ***:** p<0.05

The second treatment period

Results are presented in table 2. It has been observed a high increase in liver relative weight of Hg group compared to the control. Concerning adrenal, a significant increase in relative weight of mercury treated rabbits compared to the control. The glutathione concentrations of liver, kidney and spleen of animals exposed to Hg were significantly reduced compared to the control. Interestingly, kidney glutathione level in Hg-VitC group was similar as that of the control.

Concerning immunological markers, slight non-significant variations were observed in the percentages of lymphocytes and monocytes in both treated groups. Contrary, the percentage of basophils was significantly lower in the two treated groups compared to the control.

Table 4. - Mean (X±SD) organ's relative weights of male rabbit *Oryctolagus cuniculus* after 22 days' treatment.

	G1 (Control)	G2 (Hg)	G3 (Hg+Vit C)
Liver	2.372±0.27^{d**}	3.481±0.66	2±0.174
Kidney	0.656±0.18^{d*}	1.052±0.27	0.7±0.166
Spleen	0.046±0.009	0.058±0.014	0.045±0.02
Adrenal	0.013±0.004^{d**}	0.03±0.009^{a*}	0.014±0.002

a: Control ***Vs*** Hg; **d:** Control ***Vs*** Hg ***Vs*** Hg-Vit C; ***:** p<0.05 ****:** p<0.01

Table 5. - Mean (X±SD) glutathione concentrations (nM/mg protein) of male rabbit *Oryctolagus cuniculus* after 22 days' treatment.

	G1 (Control)	G2 (Hg)	G3 (Hg+Vit C)
Liver	21.14±1.078^{d*}	16.48±2.035^{a*}	20.5±2.86
Kidney	22.61±1.5^{d***}	16.52±2.24^{a*}	21.62±1.06
Spleen	24.25±2.55^{d*}	15.80±1.50^{a**}	21.8±5.35
Adrenal	22.42±2.75	19.82±3.83	22.73±3.57

a: Control ***Vs*** Hg; **d:** Control ***Vs*** Hg ***Vs*** Hg-Vit C; ***:** p<0.05 ****:** p<0.01; *****:** p<0.001

Table 6. - Mean (X±SD) white blood cells percentage of male rabbit *Oryctolagus cuniculus* after 22 days' treatment.

	G1 (Control)	G2 (Hg)	G3 (Hg+Vit C)
Lymphocytes	44.05±17.49	50±4.88	53.08±19.81
Monocytes	7.35±1.27	5.7±1.37	7.05±2.19
Basophils	3.38±0.97^{d*}	1.2±0.63^{a*}	1.83±0.41^{b*}

a: Control ***Vs*** Hg;**b:** Control ***Vs*** Hg-VitC; **d:** Control ***Vs*** Hg ***Vs*** Hg-Vit C; ***:** p<0.05

Comparison between the first and the second treatment periods

The comparisons between the first and the second treatment periods are summarized in figure 1, 2 and 3.

The comparison between the organ relative weights of the first and second treatment periods did not reveal any significant difference between groups, except for liver in Hg-VitC group, where it was noted a remarkable decrease in this organ after 3 weeks treatment (Fig 1). However, in the Hg group, liver glutathione concentration was significantly lower in the longer period treatment (Fig 2). Moreover, significant reduction in the percentage of basophils of the two treated groups after 3 weeks compared to the 10 days treatment (Fig 3). The other comparisons have not recorded any significant difference.

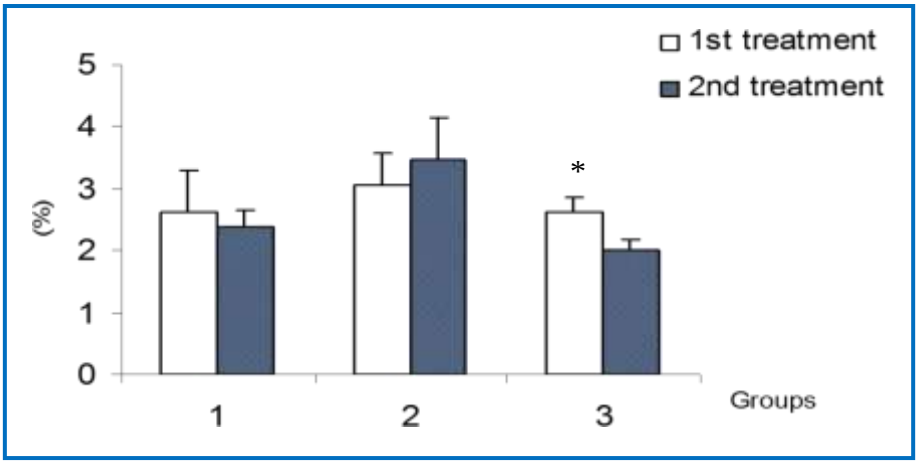

Fig 1. - Comparison of means (X±SD) liver's relative weight (%) of male rabbit *Oryctolagus cuniculus* after 10 and 22 days treatment.

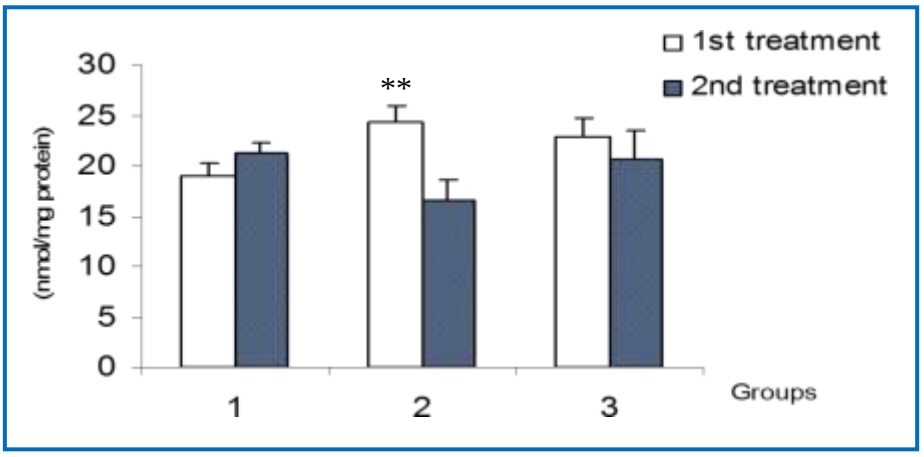

Fig 2. - Comparison of means (X±SD) liver's glutathione concentrations (nmol/mg protein) of male rabbit *Oryctolagus cuniculus* after 10 and 22 days treatment.

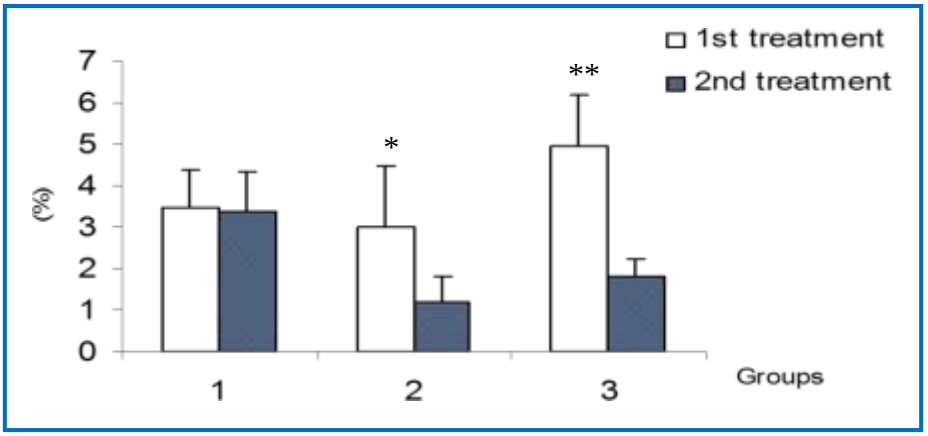

Fig 3. - Comparison of means (X±SD) basophile percentage (%) in male rabbit *Oryctolagus cuniculus*after 10 and 22 days treatment.

4. Discussion

The first treatment period revealed a slight increase in relative weight of all organs in the Hg group; this increase has appeared even in the second treatment period, especially for liver and adrenal. However, the work of Insitotoris et al., [24] has mentioned that after an exposure to Hg chloride, the concentration of Hg in the liver, the kidney, and the spleen has been significantly increased. On the other hand, the combined treatment of the two periods revealed a decrease in the organs relative weight compared to the groups treated by Hg alone, which might indicate the protective action of vitamin C. Such result is in agreement with those of Blackstone et al., [21], who reported that the administration of Hg had altered the kidney and liver growth rate, which have not been affected by a large uptake of ascorbic acid. The presence of thiol groups, however, is probably having a direct role in the conservation and the retention of ascorbic acid in the tissues [25]. Thus, the Hg-induced adrenal hypertrophyin the second treatment period could be prevented by vitamin C supplementation. Jun-Ho Lee et al., [26] revealed that dietary vitamin C had protective effect against Hg accumulation. The actual results have clearly showed a significant increase in the concentration of hepatic glutathione after ten days treatment, but it was remarkably decreased after the second treatment period. Such decrease can be explained by the fact that Hg has exhausted the GSH liver content. In this sense, a study of Bose et al., [27] showed that an administration of a non-lethal dose of Hg to rats has increased the metallothioneins' content of liver and kidney, where hepatic GSH level was decreased up to 51%. In the same order, the investigations of Alexander and Aaseth ; Hirayama, [28; 29] have mentioned that the presence of Hg in the organism leads to the formation of Hg-GSH complex. However, the slight reduction of renal GSH could be attributed to its role in the excretion of the Hg in urines. On the other hand, the work of Bando et al., [30] revealed that after a gavages administration of $HgCl_2$ to rats for three successive days, a significant reduction has been detected in the ratio of hepatic GSH/GSSG, accompanied by an increase in the activity of glutathione peroxidase and glutathione reductase. In parallel, Hussain et al., [31]

showed that the administration of $HgCl_2$ to mice through the digestive tract during 14 days has increased the reduced GSH and the activities of antioxidant enzymes; glutathione peroxydase, glutathione reductase in both liver and kidney.

On the other hand, results have recorded a depletion in spleen and the adrenal GSH levels whether in the first or in the second treatment periods. These results reflect the role of the GSH in Hg detoxification. The GSH concentration comes back nearly to the normal when vitamin C has been supplemented to animals. Gerard-Monnier and Chaudier, [8] showed that there were some synergic interactions between the GSH and other components of the anti-oxidant defense system as the vitamin C and E. Thus, the studies of Rikans et al., ; Sies, ; Well and Xu, [32; 33; 34] showed that the role of ascorbic acid, as a nutritive antioxidant, has only been appreciated lately, where it can neutralize the free radicals, and react directly with the peroxide radicals, in addition to its important antioxidant function of regenerating the reduced GSH.

Mercury supplemented to rabbits has not affected the immune markers after 10 days exposure. However, after 22 days Hg treatment, only basophils were reduced remarkably. It has been reported that the mercurial compounds can modulate the immune system activity by direct or indirect way, leading to subsequent immuno-pathological changes [12; 13]. A study of Wester and Canton, [35], on the freshwater fish exposed to the aqueous methylmercury chloride during three weeks, showed that the basophils have not been differentiated, and sometimes increased in a shrewder way, which probably was due the dysfunction either of the cells or the bone marrow. The direct activation of lymphocytes by Hg has been proposed by several investigations. Thus, the works of Beneko et al., ; Queiroz et al., [36; 37] reported that the exposure to Hg can led evidently to a remarkable stimulation of the humoral immunity. In the same order, Prouvost-Danonet al., ; Queiroz et al., [16; 37] observed an increase in IgG, IgA, and IgM levels in the individuals exposed to Hg. On the other hand, the studies of Gaworski and Sharma, ; Shenker et al., [38; 39] revealed that mercury chloride inhibited the response and reduced the proliferation of T lymphocytes. However, Bernier et al., [40] showed that the administration of high doses of Hg led to a decrease in the profilative activity of spleen cells, leading to reduced rates of lymphocytes and antibodies. Moreover, Lawrence, [41] explained the action of Hg on the lymphocytes by the fact that the Hg reduces the lymphocytes viability up to 50%. The toxicity of Hg on the immune system; T and B cells depends on the species and the strain of the studied animals [42]. On the other hand, the rabbits exposed to the combined treatment of Hg-Vit C presented no significant variations in the percentage of lymphocytes and monocytes in the two treatment periods. Contrary, the supplemented vitamin has increased and then decreased the percentage of basophils in the first and the second treatment periods, respectively. The high elevation of basophils of animals given vitamin C can be explained by the fact that this vitamin has reinforced the immune system.

The non-variations in the monocytes percentage of Hg exposed group was not in parallel of what has been reported by other workers [43; 44] who showed that the exposure of human's monocytes to metals

ions caused a significant reduction in cellular proliferation. In the same way, Noda et al., [45] confirmed that the dental amalgams containing Hg had altered the response of monocyte, while the treatment by vitamin C showed a slight increase in these cells. Thus, monocytes have the capacity to retain a big part of vitamin C, which has an important role in the extension of macrophages life [46]. In fact, the white cells contain approximately 60 times more vitamin C than that of blood plasma; the reserves are quickly exhausted in case of infections. Different investigations were able to demonstrate that the high concentrations of vitamin C allow a good mobilization of white cells, especially neutrophils. It has been noted that Hg exposure releases an excessive amount of vitamin C stored in adrenal, responding however to stress, and reinforcing the immune system [47].

5. Conclusion

Rabbits were given mercury alone or combined with vitamin C for 10 and 22 days. Vitamin C, however, is considered as an antioxidant which acts in collaboration with the glutathione, monocytes and macrophages. Thus, after 22 days, liver and adrenal relative weights have been affected by Hg intoxication on one hand, and responded to vitamin treatment, on the other hand. Moreover, the glutathione levels of adrenal in the first treatment, and of kidney in the second one, have been returned to normal in the presence of vitamin C. Surprisingly, the immune markers seem not to respond to vitamin C supplementation, except the basophils during the 10 days period. The severity of mercury on rabbit is more pronounced in the longer exposure period. To conclude, dietary vitamin C when taken regularly could help to prevent cells from oxidative stress.

6. References

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