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Annals of Biological Research, 2011, 2 (3) :359-367
(http://scholarsresearchlibrary.com/archive.html)

Annals of Biological Research
Scholars Research Library

ISSN 0976-1233
CODEN (USA): ABRNBW

Influence of palm date and vitamin C supplementation on testicular functions of domestic rabbit *Oryctolagus Cuniculus* under mercury exposure

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ABSTRACT

Domestic male rabbits Oryctolagus cuniculus were exposed to either $HgCl_2$ alone (Hg) or combined with vitamin C (Hg-VC), date palm (Hg-DP), vitamin C and date palm (Hg-VC-DP) for a period of 6 weeks, in order to estimate the possible protective roles of these components against mercury intoxication. The epididymal sperm concentration, speed, motility and viability were investigated together with testicular relative weights and plasma testosterone level. Results showed a slight increase of testicular relative weight in the Hg-VC-DP group compared with the control. Testosterone concentration has decreased significantly both in Hg and in VC treated groups. The levels of the hormone in Hg-DP and Hg-VC-DP groups were close to that of the control. Clearly, sperm concentrations in all treated groups were similar to that of the control. Contrary, a decrease in speed, motility and vitality was observed in the Hg group compared to the control. When compared to the control, spermatozoa motility was significantly lower in Hg-DP and in Hg-VC-DP as well. Also, spermatozoa viability was significantly diminished in Hg-VC, Hg-DP and in Hg-VC-DP groups, as that of the Hg treated group. To conclude, the protective role of VC against Hg toxicity seems evident on spermatozoa's speed and motility, while that of DP was noticeable on testosterone and spermatozoa speed. However, the combination of VC and DP together has only ameliorated testosterone level and spermatozoa speed.

Keywords: Mercury, date palm, rabbit, reproduction, sperm, vitamin C.

INTRODUCTION

Mercury represents a dangerous source of environmental contamination **[1]** because of its extreme volatility, rapid transportation in the body and its high capacity to bind functional groups of key molecules. It binds to cell components, modifying their structure or inhibiting their biological activities **[2]**. The toxicity of mercury comes from its strong accumulation in certain tissues, leading to physiological abnormality including the malfunction of the reproductive system, which may affects the health of the progeny. Thus, the disturbance of sperm characteristics in mammals was reported to be affected by mercury **[3]**. Mercury is known to provoke the formation of the toxic free radicals in the organisms. Though, the pro-oxidant activity of mercury in cells is neutralized by antioxidant molecules found mainly in food. An attempt is made to investigate the detoxifying roles of some food components; date palm fruit and vitamin C. The antioxidant protection of vitamin C is particularly important for health. Many studies showed that vitamin C protects organism against external and internal oxidants **[4].** Date fruit has been an important crop in arid and semiarid regions of the world **[5]**. The fruit is well known as a staple food, it is composed of a fleshy pericarp and seed. Dates are an excellent source of simple sugars, minerals and vitamins **[[5**, **6, 7]**. Date is considered as a nutritious fruit as research has indicated the clear contribution of dates to human health when consumed with other food constituents **[7]**. Such fruit has been recommended in folk cure for the treatment of various infectious diseases and cancer **[8]**. The importance of date fruits in human nutrition comes from its rich composition of carbohydrates **[9, 10]**, minerals, dietary fiber, vitamins, fatty acids, amino acids and proteins **[11].** There are at least 15 minerals in dates which are found in various proportions include boron, calcium, cobalt, copper, fluorine, iron, magnesium, manganese, potassium, phosphorous, sodium and zinc **[12].** Selenium, another element believed to be important for immune functions, is also found in dates **[13].** Date fruits contain at least six vitamins including vitamins B1 thiamine, B2 riboflavin, niacin and vitamin A is also present and a small amount of vitamin C **[13].** Date palm fruits in Algeria are widely consumed by general population. It plays economic, cultural and therapeutic roles. Hundreds of varieties are found in local markets holding different names, amongst is the famous Deglet Nour which contains no sucrose, more glutamic acid, glutamine, y-amino-butyric acid, andarginine, and less alanine **[14]**. The aim of this work was to evaluate the impact of mercury on the different reproductive parameters (sperm concentration, speed, motility and vitality), relative weight of testis and testosterone concentration in one hand, and in the another hand, to investigate the protective role of vitamin C and the date palm fruit given to domestic rabbit *Oryctolagus cuniculus* chronically exposed to inorganic mercury.

MATERIALS AND METHODS

Thirty local male rabbits *Oryctolagus cuniculus* aged 6 months old, and weighted 1628.83±121.64 g were divided into equal 5 groups, and they have free access to water and food ad libitum. Animals were put in the animal house (Department of Biology, University of Annaba) under standard conditions of temperature, light and humidity. Fresh mature date palm (Deglet nour variety) was obtained from Biskra region, Algeria and considered as the best date in the country. Vitamin C was purchased from the local pharmacy as tablets of 500mg (SAIDAL Group). The treatment was started by orally administration of mercury (Hg), vitamin C (VC) and the date palm (DP).

The first group used as a control (C) received a daily standard diet. The second group (Hg) was treated by mercury (500mg $HgCl_2$/Kg food); the third (Hg-VC) was given a combined treatment (500mg $HgCl_2$/Kg food + 8mg VC/animal); the fourth group (Hg-DP) was treated by a combination of (500mg $HgCl_2$/Kg food + 100g date/Kg food), while the fifth group (Hg-VC-DP) was given a combination of (500mg $HgCl_2$/Kg food + 8mg VC/animal + 100g date/Kg food). However, vitamin C was freshly dissolved in distilled water, where each animal has received 3 ml daily by gavages (half in the morning and half in the afternoon). After six weeks of exposure, animals have been sacrificed and dissected; where the left testis was taken and weighed to estimate the relative weight (organ weight/body weight). The spermogram has been realized according to the method of WHO **[15]** by making a small incision at the epididymis level in order to obtain a drop of semen. Semen was added to 1ml physiological solution (0.9% NaCl), then the concentration, the speed, the motility and the viability of the spermatozoa were evaluated. At the time of sacrifice, blood samples were immediately collected in labeled polypropylene test tubes containing heparin. Blood was then centrifuged at 4000 rpm/15 minute, and then plasma was obtained and frozen at -18°C. Testosterone concentration has been estimated in plasma by Enzyme-Linked Immunosorbent Assay (ELISA) method.

Statistical analysis has been carried out by Student's *t*-test to determine statistically significant differences between testis relative weights, testosterone level, spermatozoa's concentration, speed, motility and vitality in treated and control animals. The one way analysis of variance (ANOVA) was used to compare between the groups. The significant differences at $p<0.05$ were considered.

RESULTS AND DISCUSSION

The results showed that the treatment by mercury alone revealed a non significant decrease in relative weight of testis of the Hg group compared to the control. However, the groups treated by Hg-VC-DP demonstrated a slight rise in testis relative weight. The observed decrease in testicular weight may be due to the degenerative effect of mercury chloride. Previously **[16]**, orally administrated mercuric chloride to rats has significantly decreased the body weight and testis weight in the high dose group (2 mg/kg), but not in the low dose group (1 mg/kg) after one month continuous exposure. On the other hand, the continuous exposure of Wistar male rats to 50 and 100 ppm mercury chloride in drinking water during 90 days led to an increase in the absolute and relative wet weight of the testis and a decrease in the absolute and relative wet weight of the accessory sex glands **[17]**. Moreover, other study revealed that the subcutaneous injection of Wistar male rats to methylmercuric chloride at a dose of 10 mg/kg per day for 8 days resulted in a 28% testicular weight loss at 14 days after the first injection **[18]**. In addition, the ventral and dorso-lateral prostatic lobes showed a 52-65% decrease at 14 days **[18]**.

Testosterone concentration showed clearly a significant decrease in the Hg group compared to the control. Such decrease could be explained by the fact that Hg has exhausted the testosterone plasma content. In fact, mercury can reacts with the sulfhydryls groups of proteins, inhibits enzymes and disturbs hormones and several cell components. It has been reported that methylmercury suppressed hormone levels, especially testosterone which may be used as an indication of diminished fish reproduction **[19]**. Moreover, the intratesticular testosterone level was reduced by 44%, suggesting that steroidogenesis in these animals was dramatically impaired by mercury **[20]**. The exposure to mercury chloride at 50 and 100 ppm during 90 days through

oral administration in the drinking water caused also a marked perturbation in rat testosterone serum level **[17]**. In this sense, methylmercuric chloride at a dose of 10 mg/kg per day for 8 days **[18]** revealed a reduction in plasma testosterone levels at 6 days after exposure, and remained lower at approximately 20% of control levels during the 14 day observation period. Furthermore, the methylmercury had a dramatic fall in plasma testosterone of the contaminated animals, which seems to be related to a reduction in its secretion. In association with this, the concentration of testosterone in somniferous tubules fluid dropped by 55% in the intoxicated animals **[21]**. Such reduction is owed to the intracellular accumulations of mercury in the Leydig cells as well as in the Sertoli cells of the somniferous tubules **[22]**. On the other hand, the level of plasma testosterone has not been changed significantly in workers chronically exposed to mercury vapor **[23]**.

Mercury group in the present study provoked pronounced reduction of the epididymal sperm speed, motility and viability. Such results are in agreement with a previous reported work **[17, 24].** A non significant decrease in sperm concentration was also observed in this study, especially in the mercury group, which can be explained by hypospermatogenesis induced by an inhibition of spermatogenesis and the preferential loss of maturing and elongated spermatids and the seminiferous tubules were dilated in treated animals **[25].** Furthermore, mercury chloride increased lipid peroxidation, accompanied by significant reduction in antioxidant enzymes activities; superoxide dismutase, catalase and glutathione peroxidase of testes **[25]**. These perturbations suggest an increase in free radical formation after mercury exposure leading to testicular functional inactivation **[17]**. A deleterious effect of mercury on the sperm membrane integrity was interpreted by a significant increase in the lipoperoxidation **[26].** A strong negative correlation between the lipoperoxidation rate and the percentage of viable spermatozoa was reported **[26]**. Thus, mercury is capable of inducing DNA breaks in the sperm nuclei. Almost, 88% of DNA breaks were of double-stranded. Mercury was able to alter the integrity of acrosomal membranes showing an abnormal acrosome reaction. It induced membrane impairments, lowered sperm viability, raised DNA breaks and decreased the acrosome reaction of human spermatozoa leading to sperm dysfunction **[26]**. Other study revealed that the reduced of sperm motility and curvilinear velocity is due to damage to sperm head membranes indicated by the acrosome breakage with formation of various sized microvesicles **[27]**. Oral treatment of adult male monkeys with MeHg for 20 weeks significantly decreased the percentage of sperm motility and speed and increased the percentage of abnormal sperm tail forms **[28]**.

On the other hand, some studies have revealed no effects of mercury on the epididymal sperm counts of contaminated rats **[18, 21]**.

A noticeable improvements in all sperm's parameters were recorded when vitamin C and Date palm were added to rabbit diet, which indicates the protective roles played by these molecules. Supplementation of vitamin C is essential for systemic mercury detoxification **[4]** because it interferes with intestinal absorption of heavy metals by increasing the urinary excretion or by creating a synergistic effect on chelating element **[29]**. However, a lack of vitamin C in the diet causes Hg toxicity to animals **[30].**

It has been reported earlier that the role of vitamin C, 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 **[31]**.

It was mentioned also that supplementation with vitamin E and/or vitamin C reduce ROS generation, prevent loss of motility and the capacity of oocyte penetration in lead exposed rats **[32]**.

Moreover, dates have been identified as having antioxidant and antimutagenic properties, because it contains compounds with potent free-radical-scavenging activity **[13]**. The presence of zinc, manganese, magnesium, or selenium in the diet can protect and change the gastrointestinal absorption of mercury **[33].** Dates have also been considered as a good source of antioxidants, mainly carotenoids and phenolics and vitamins B-complex **[34]**. The mercurial compounds have exceptionally a big affinity for the sulfhydryls groups found in the glutathione, cysteine, and proteins **[35].**

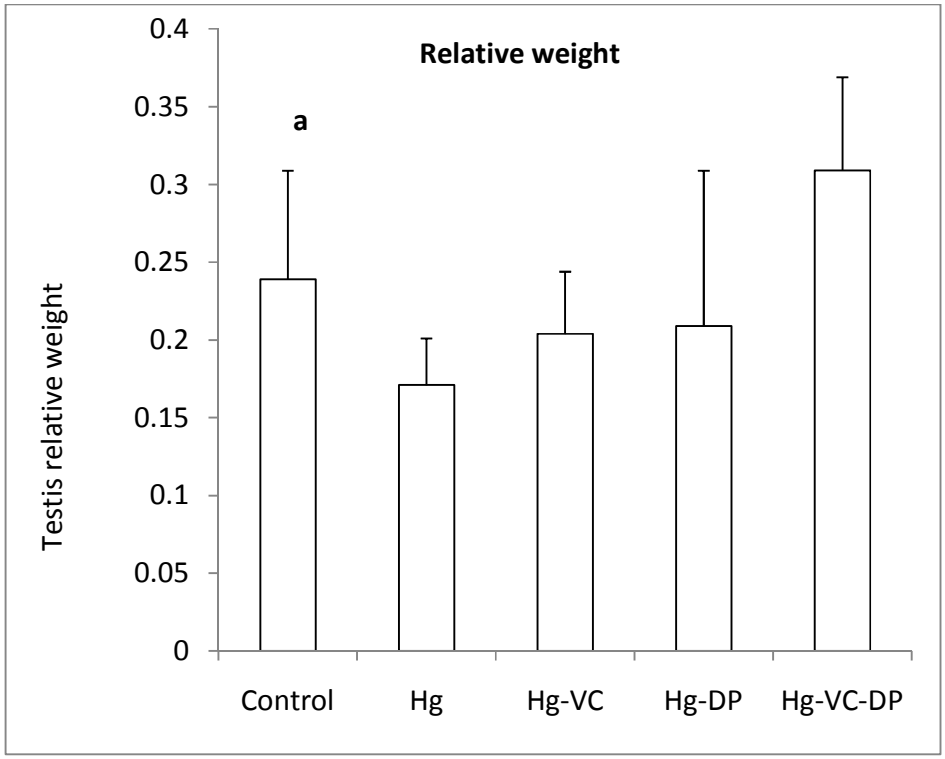

Figure 1: Estimate of mean (X±SD) testis relative weight of male rabbit *O. cuniculus* after six weeks' treatment. *: Significantly different than that of the control (t-student test). a: Significant difference between groups (ANOVA test).

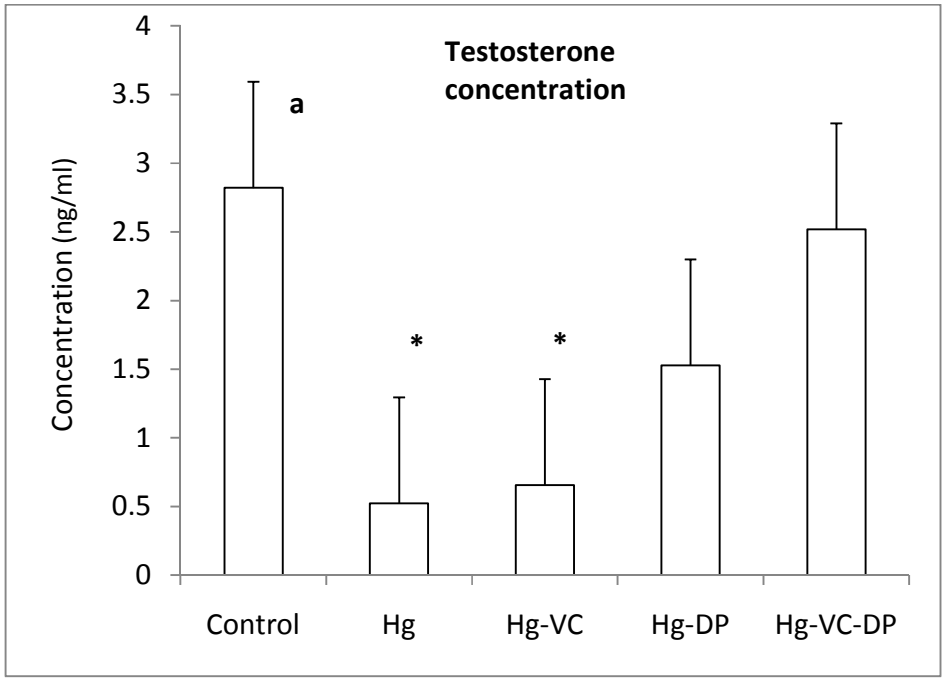

Figure 2: Estimate of mean (X±SD) testosterone concentration (ng/ml) of male rabbit *O. cuniculus* after six weeks' treatment. Statistics as in Fig 1.

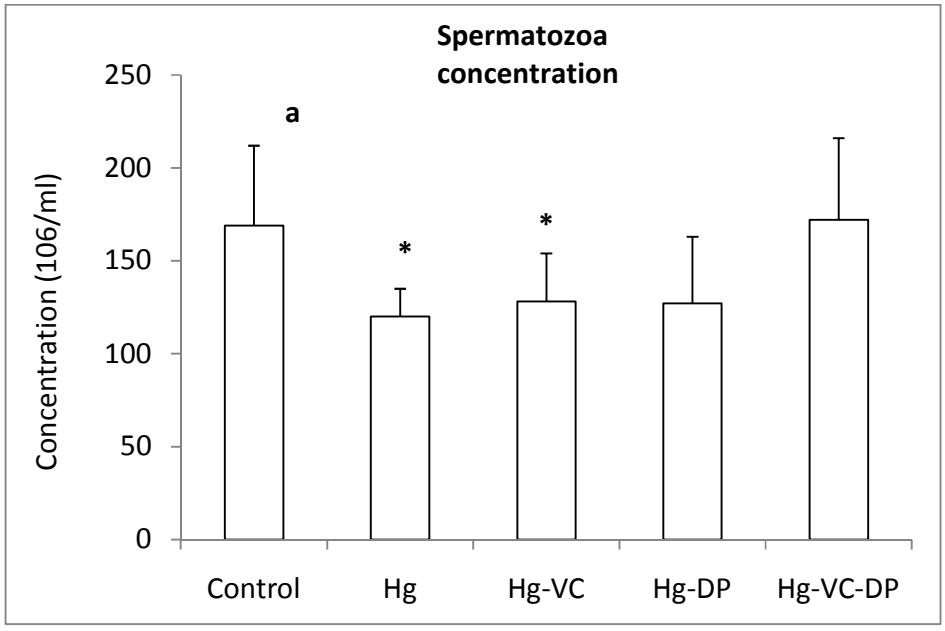

Figure 3: Estimate of mean (X±SD) spermatozoa's concentration (x10^6/ml) in the epididymis of male rabbit *O. cuniculus* after six weeks' treatment. Statistics as in Fig 1.

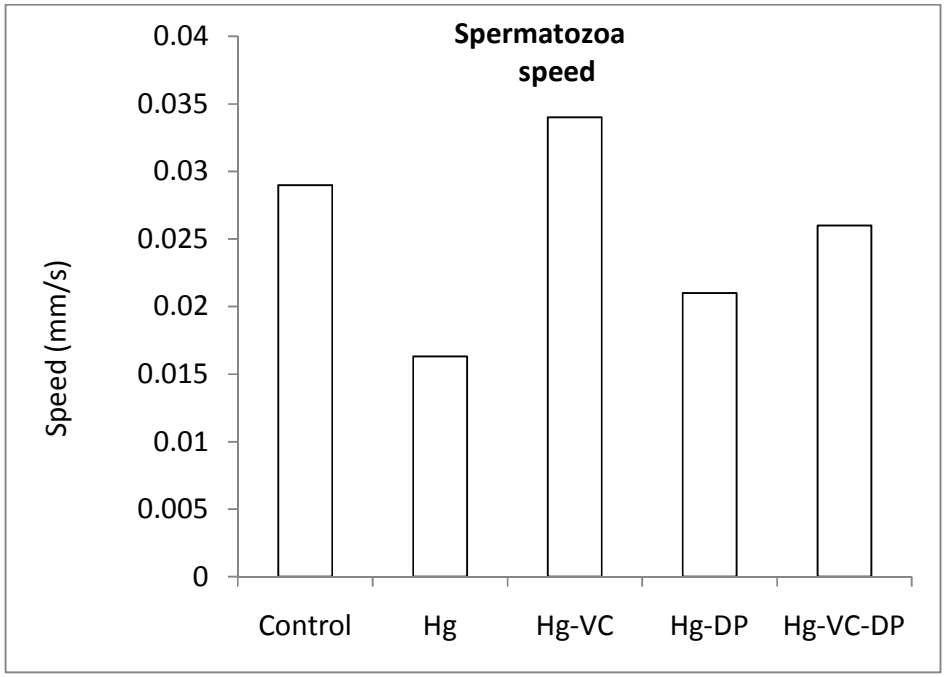

Figure 4 Estimate of mean (X±SD) spermatozoa's speed (mm/s) in the epididymis of male rabbit *O. cuniculus* after six weeks' treatment. Statistics as in Fig 1.

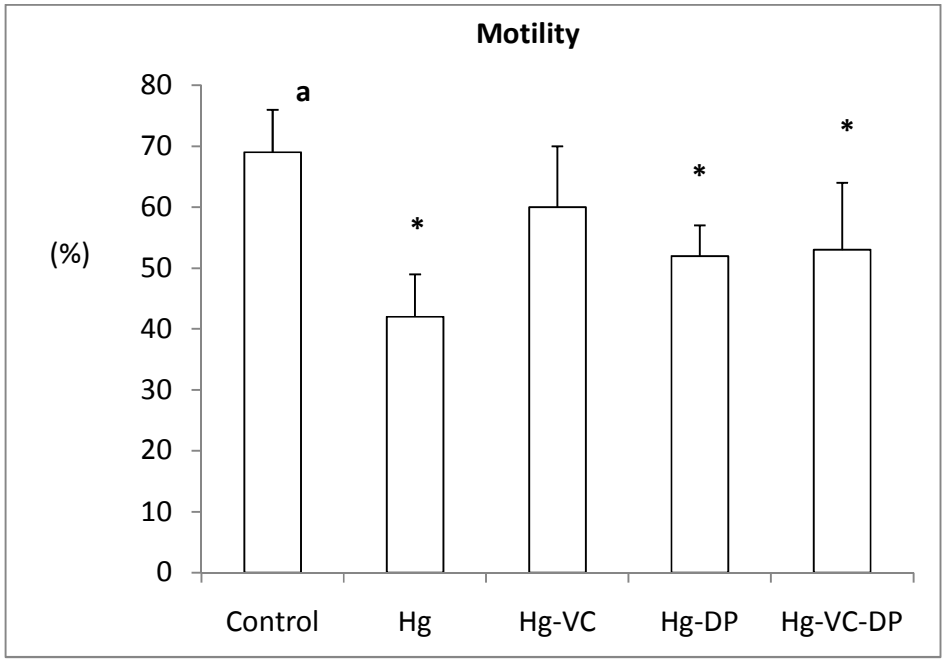

Figure 5: Estimate of mean (X±SD) spermatozoa's motility (%) in the epididymis of male rabbit *O. cuniculus* after six weeks' treatment. Statistics as in Fig 1.

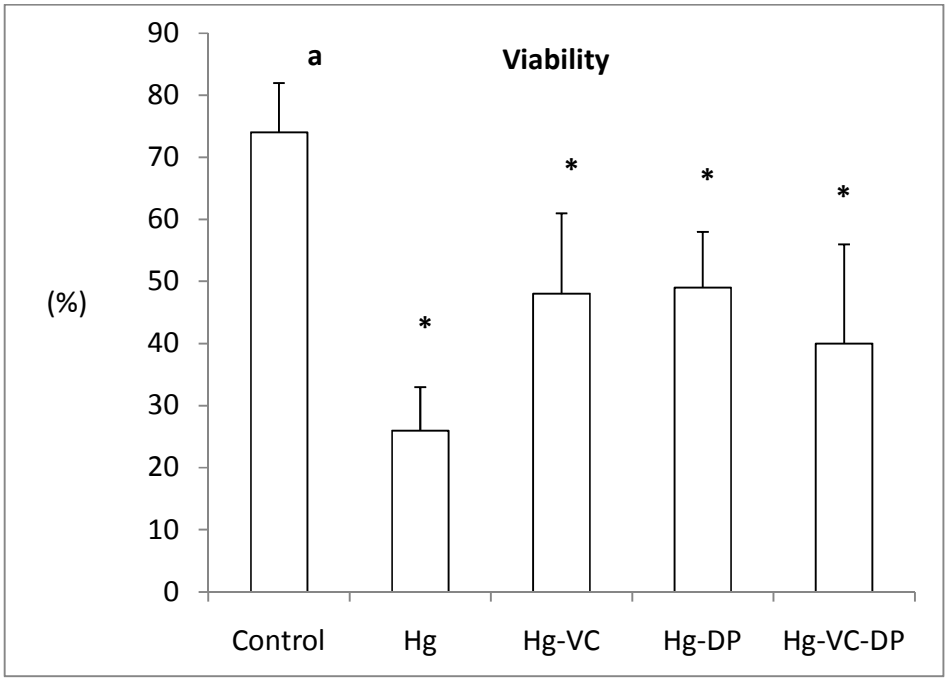

Figure 6: Estimate of mean (X±SD) spermatozoa's viability (%) in the epididymis of male rabbit *O. cuniculus* after six weeks' treatment. Statistics as in Fig 1.

CONCLUSION

It seems evident that Hg has a negative influence on reproduction. However, dietary vitamin C when taken regularly could help to prevent spermatozoa's speed and motility from oxidative stress. On the other hand, the Deglet nour fruit which contains many essential nutrients and antioxidants was able to preserve the testosterone level and the spermatozoa speed. Furthermore, the supplementation of vitamin C and date palm together has maintained a normal level of testosterone and spermatozoa speed against Hg intoxication.

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