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Stereological and histomorphometrical study of kidney following paraquat toxicity and protective effect of vitamin C in male rat

In accordance with the increase in population, the needs of today's societies have led to the widespread use of pesticides and herbicides in order to produce more agricultural products. The remaining of these toxins in agricultural products, water and soil causes them to enter the food cycle of animals and humans. Paraquat is a powerful poison and herbicide used to control weeds in the agricultural industry. Paraquat in the body causes the formation of toxic free radicals that cause serious damage to body tissues, including the kidney. This damage is caused by oxidative stress, so it is necessary to use antioxidants to prevent the harmful effects of oxidation of body compounds. This study tries to evaluate the protective effects of vitamin C in kidney histology by causing oxidative stress caused by paraquat poison in terms of stereology and histomorphometry. In this study, 36 adult rats were used in 6 equal groups with an average weight of 200. Group 1 (healthy control group) received physiological serum three times a week by gavage in the amount of one cc for each mouse. Group 2 (sham control group), paraquat at the rate of 20 mg/kg, Group 3 (positive control group). They took vitamin C in the amount of 200 mg/kg three times a week by gavage. Groups 4, 5, and 6 (treatment groups), in these groups, one hour after the administration of paraquat, vitamin C was given by gavage with doses of 12.5, 50, and 200 mg/kg, respectively. At the end of the 28th day, the mice were weighed and euthanized, and the right kidney of each mouse was transferred to formalin for histomorphometric and stereological studies. After fixation, tissue passage, tissue preparation and performing hematoxylin-eosin staining methods for histology, morphometry, histomorphometry, stereology and pathology studies, Toluidin blue staining to count mast cells, Trichrome-Mason staining to study collagen fibers and PAS staining were used to examine the accumulation of carbohydrate compounds in cells. Histomorphometry was examined by light microscope and invert microscope with the help of dinocapture and NIS software. The resulting data were statistically compared using spss version 25 statistical software, ANOVA statistical method and Tukey's post hoc test in different groups. The results of histomorphometry showed that paraquat in most cases causes a decrease in thickness parameters and an increase in numerical parameters, including the number of tubules or glomeruli and the number of the nuclei of their covering cells. Vitamin C along with paraquat improved the conditions in most of the parameters and in some cases created conditions similar to the healthy control group. The examination of the number of mast cells also showed that in the paraquat group, the amount of mast cells increased due to inflammation, and in the groups treated with paraquat and vitamin C, the number of mast cells decreased with an increase in the dose of vitamin C. Examining the PAS response showed that the paraquat group

had the lowest amount of carbohydrate storage compared to the vitamin C and control groups. The same examination of the dispersion of collagen fibers showed that the dispersion of these fibers in the groups of vitamin C and paraquat showed fibrosis of the kidney tissue, and in the treatment groups, the amount of fibrosis of the kidney tissue was reduced in proportion to the increase in the dose of vitamin C. Also, in the study of stereology, which included the numerical and volumetric density of the studied parameters, it showed that there is no significant difference in most of the parameters among the different groups, but numerically, the results of stereology showed that paraquat has harmful effects on kidney tissue and vitamin C it has a protective role. necrosis in the kidney tissue and this effect of paraquat was determined in the histomorphometry and stereology of the kidney and compared to the healthy control group. It was also shown that paraquat caused kidney tissue fibrosis and increased collagen fibers in kidney tissue. In addition, the present study showed that paraquat increased the average number of mast cells and this indicates the inflammatory role of paraquat in kidney tissue. Against the destructive effects of paraquat, vitamin C, as an antioxidant and repairer of connective tissues, improved the conditions of kidney tissue atrophy and necrosis and also reduced the rate of kidney tissue fibrosis. Vitamin C moderated the inflammatory effects of paraquat by reducing the number of mast cells. In this study, it was found that the protective effects of vitamin C are dose-dependent and the best healing effect was observed in high doses. In addition, it has been determined that vitamin C alone in a high dose had no toxic effects, but it caused kidney fibrosis in the group that did not receive paraquat, while no such effect was observed in the treatment groups.

**Keywords:** stereology, histomorphometry, kidney, paraquat, vitamin C, rat