

Ameliorative Role of Vitamin-C Against *Para-nonylphenol* Toxicity in Reference to Body Weight and Haematological Parameters in Male *Mus musculus*

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Abstract: The aim of this study was to investigate the possible ameliorator role of vitamin-C against the *para-nonylphenol* (*p*-NP) toxicity in male *Mus musculus*. Total 40 male *Mus musculus* were divided in four groups of ten each, the first group received daily dose (0.2ml/day) of vehicle (corn oil) and fed with balanced diet and water *ad libitum*, served as control. Group second received *para-nonylphenol* (250mg/kg body weight/0.2ml/day) dissolved in corn oil, orally through cannula, while group third, received similar dose of *para-nonylphenol* as group second and supplemented with vitamin-C (10 ppm) through drinking water. The animals of fourth group were supplemented with vitamin-C (10 ppm) alone through drinking water for 30 and 60 days. The body weight and haematological parameters i.e. RBCs, WBCs and DLC (Neutrophils, Monocytes, Lymphocytes and Eosinophils) were analyzed after 30 and 60 days. The exposure of *para-nonylphenol* insignificantly decreased the body weight. However, its elevated significantly the WBC counts, lymphocytes and eosinophils numbers after 30 and 60 days treated groups; while the number of RBC counts, Hb percentage, neutrophils and monocytes numbers were significantly decreased after 30 and 60 days as compared to control groups. Apart from this, the co-administration of vitamin-C along with *para-nonylphenol* ameliorated the toxicological effects of *para-nonylphenol* after different duration in *Mus musculus*. All these results showed that *para-nonylphenol* modulate the haematological parameters in male *Mus musculus* and vitamin-C ameliorated the *para-nonylphenol* toxicity.

Key words: *Para-nonylphenol* • Antioxidant • Antagonistic effect • Body Weight • Hematology • *Mus musculus*

INTRODUCTION

Para-nonylphenol is an organic compound of the wider family of alkylphenols and it is a product of industrial synthesis during the alkylation process of phenols. Alkylphenols are widely used as components in chemically synthesized products such as plastics, detergents and other formulated products [1]. Nonylphenol (NP) is widely used as lubricating oil additives, plasticizers and surface-active agents. It has also been found in polyvinyl chloride (PVC) used in the food processing and packaging industries. There are probably diverse routes of human exposure; not only via contaminated foods and drinking water, but also via dermal absorption or inhalation [2]. NP is a xenobiotic

compound have estrogenic in nature known as an endocrine disruptor, also enhances the uterine DNA and protein synthesis in immature female rats [3] and disrupts the gonad development in neonatal rats [4,5]. The majority of reported nonylphenol toxicity has focused on endocrine and reproductive system [6-10]. Apart from this, vitamin-C is an important dietary antioxidant which significantly decreases oxidative damage caused by toxicants [11-15]. Hence, in this study we tried to evaluate the effects of *para-nonylphenol* and the ameliorative role of vitamin-C as an antioxidant against *para-nonylphenol* toxicity in male *Mus musculus* in response to their body weight and hematological parameters (*i.e.* total count of RBC & WBC, differential count of WBC and Hb percentage).

MATERIALS AND METHODS

Experimental Animals: For the present investigation 40 sexually matured male *Mus musculus* (Parke's strain) weighing about 30 ± 5 gms were brought from Govt. Veterinary College, Mahow, Dist. Indore (M.P.) and acclimated to laboratory conditions [$22\pm 3^\circ\text{C}$ room temperature and light and dark photoperiod (14L: 10D h)] in the animal house, Laboratory of Endocrinology, Bioscience Department, Barkatullah University, Bhopal (M.P.). Hygienic conditions were maintained with rice husk bedding in separate polypropylene cages as per ethical rules. Animals were fed on standard mice feed and tap water *ad libitum*.

Chemicals and Reagents: *para*-nonylphenol (Hi-media Analytical Chemicals) and Vitamine-C (ascorbic acid, analytical grade) were obtained from V.K. Traders, M.P. Nagar, Zone-2, Bhopal (M.P.). As *para*-nonylphenol is not soluble in water, so corn oil used as a vehicle.

Experimental Design: Total 40 male *Mus musculus* were divided in four groups of ten each, the first group received daily dose (0.2ml/day) of vehicle i.e. corn oil and fed with balanced diet and water *ad libitum*, served as control. Group second received *para*-nonylphenol (250mg/kg body weight/0.2ml/day) dissolved in corn oil, orally through cannula, while group third, received similar dose of *para*-nonylphenol as group second and supplemented with vitamin-C (10 ppm) through drinking water. The animals of fourth group were supplemented with vitamin-C (10 ppm) alone through drinking water for 30 and 60 days. Five animals from each group were sacrificed by cervical dislocation on day 31st and 61st and immediately blood samples were collected into eppendorff tubes containing EDTA (2mg/ml) from heart through

cardiac puncture and haematological parameters i.e. Hb percentage [adopting the methodology of Sahli's [12], total Red Blood Cell (RBC), White Blood Cell (WBC) Count and Differential Leucocyte Count (DLC) [(Neutrophils, Lymphocytes, Monocytes and Eosinophils) by the [13] methodology were quantified. Along with this, the initial i.e. 0 days and at different intervals i.e. 30 and 60 days of treatments, body weights of all mice were taken out with the help of a laboratory weighing balance and the values were expressed in grams.

Statistical Analysis: The significance values were analyzed by using student "t" test. Values of $P<0.05$ were considered statistically significant.

RESULTS

During the experimental investigation, control and vitamin-C alone treated groups showed a constant increase in their body weight throughout the experiment. While, the animals treated with *para*-nonylphenol showed insignificant decrease in their body weight after 30 days, however this decrease was significantly noticed in 60 days treated group. Besides this, the animals treated with *para*-nonylphenol with supplemented with vitamin-C showed no much variation in their body weight in comparison to control group (Table 1). In hematological parameters, it has been noticed that the total number of RBC, haemoglobin (HB %), neutrophils and monocyte count were significantly lowered in *para*-nonylphenol treated animals after 30 and 60 days as compared to control groups (Figs. 2, 3, 4 and 7). While, the total number of WBC, lymphocytes and eosinophils count were elevated in *para*-nonylphenol exposed group after 30 and 60 days (Figs. 1, 5 and 6). However, the animals treated with *para*-nonylphenol and supplemented with

Table 1: The body weight (gms) of male *Mus musculus* (p) after the exposures of *para*-nonylphenol, *para*-nonylphenol along with vitamins-C supplemented, vitamin-C alone and control after 30 and 60 days.

S.N.	Groups	Duration of Experiment		
		Initial Day	30 Days	60 Days
1.	Control	29.40 ± 1.05	32.55 ± 1.20	35.50 ± 0.85
2.	Para-nonylphenol	30.25 ± 0.56	31.00 ± 1.00	$30.50\pm 1.05^*$
3.	Para-nonylphenol + Vitamin-c	31.55 ± 0.65	33.20 ± 1.46	$35.25\pm 1.50^*$
4.	Vitamin-c	29.25 ± 1.32	32.90 ± 1.06	$36.50\pm 1.18^*$

\pm = SEM of few animals,

*Significant different ($p<0.05$) from the control by students 't' test.

**More significant different ($p<0.01$) from the control by students 't' test.

***Highly significant different ($p<0.001$) from the control by students 't' test

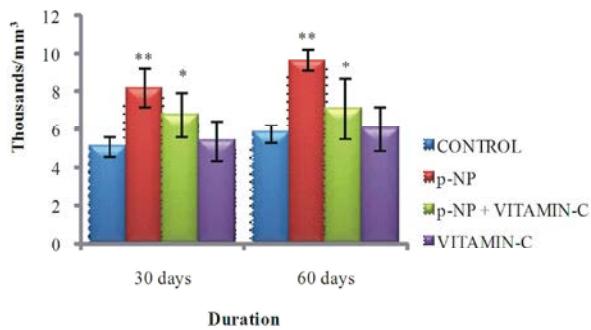


Fig. 1: WBC count of male *Mus musculus* (p) after the exposure of p-NP, p-NP along with vitamins-C supplemented, vitamin-C alone and control after 30 and 60 days.

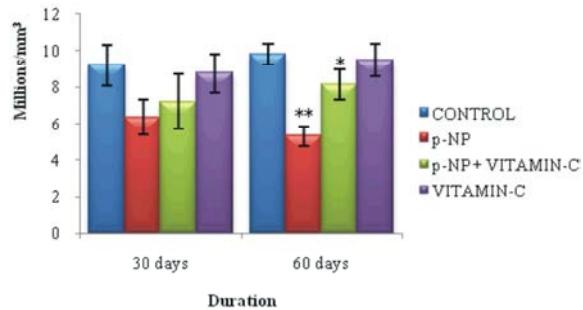


Fig. 2: RBC count of male *Mus musculus* (p) after the exposure of p-NP, p-NP along with vitamins-C supplemented, vitamin-C alone and control after 30 and 60 days.

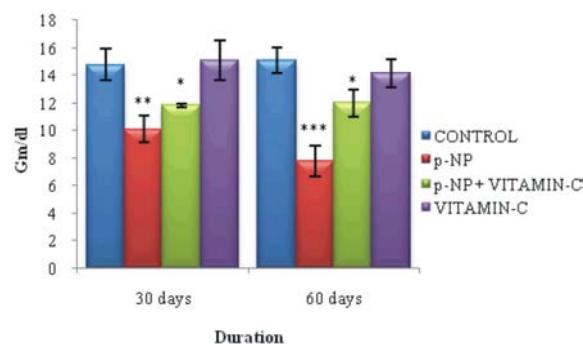


Fig. 3: Haemoglobin of male *Mus musculus* (p) after the exposure of p-NP, p-NP along with vitamins-C supplemented, vitamin-C alone and control after 30 and 60 days.

vitamin-C showed significant recovery in RBC, haemoglobin (HB percentage), neutrophil and monocytes count levels in comparison to *para*-nonylphenol exposed animals. In connection to this, the animals exposed with vitamin-C only also did not show much variations in these parameters in comparison to control group (Figs. 1, 2, 3, 4, 5, 6 and 7).

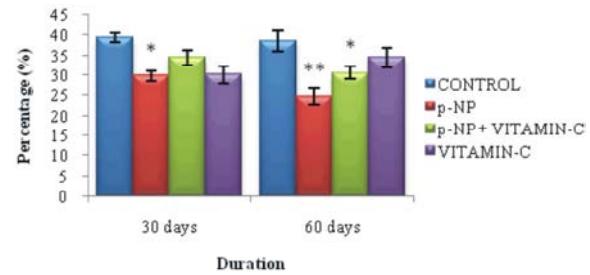


Fig. 4: Neutrophils of male *Mus musculus* (p) after the exposure of p-NP, p-NP along with vitamins-C supplemented, vitamin-C alone and control after 30 and 60 days.

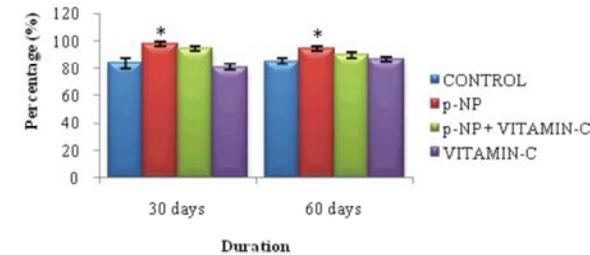


Fig. 5: Lymphocytes of male *Mus musculus* (p) after the exposure of p-NP, p-NP along with vitamins-C supplemented, vitamin-C alone and control after 30 and 60 days.

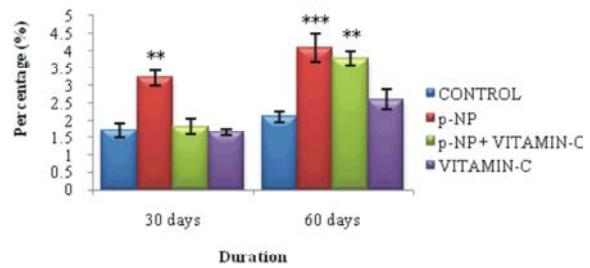


Fig. 6: Eosinophil of male *Mus musculus* (p) after the exposure of p-NP, p-NP along with vitamins-C supplemented, vitamin-C alone and control after 30 and 60 days.

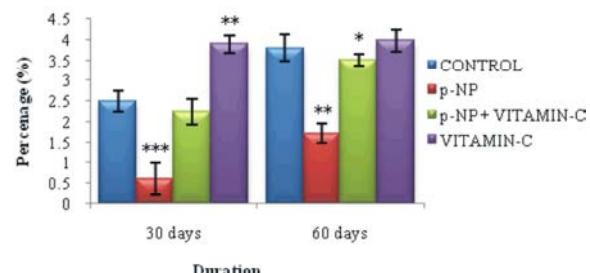


Fig. 7: Monocytes of male *Mus musculus* (p) after the exposure of p-NP, p-NP along with vitamins-C supplemented, vitamin-C alone and control after 30 and 60 days.

DISCUSSION

The toxicity of any compound depends on many factors, such as the chemical and physical form of the compound, route of administration, dose and duration of exposure dietary level of the interacting elements, physiological conditions (pregnancy, lactation etc), nutritional status, age and sex of the exposed individuals [18, 19, 20, 21]. Nonylphenol is a xenobiotic compound have estrogenic in nature known as an endocrine disruptor, also enhances the uterine DNA and protein synthesis in immature female rats and fishes [3, 8]. The *para*-nonylphenol contaminated diet decreased in body weight in rats and mice fed [22, 12]. This reduction in body weight may explained by the ability of *para*-nonylphenol to generate free radicals [23], which may lead to DNA breakage, inhibition of protein biosynthesis and gluconeogenesis, lipid peroxidation, disruption of oxidative phosphorylation in mitochondria, inhibition of blood clotting and apoptosis [24]. In our study, body weights were significantly decreased after 60 days after *para*-nonylphenol exposures and this decrease was ameliorated by vitamin-C when co-administered with *para*-nonylphenol. This may suggest that *para*-nonylphenol directly or indirectly inhibits protein synthesis and gluconeogenesis within the animals may resulted in decreased in their body weight and this effects were ameliorated by vitamin-C supplementation, because vitamin-C is well known antioxidant and it is having the capacity to reduce oxidative damage caused by toxicants [11-15].

As we know that the haemoglobin is the red pigment of the blood present inside the erythrocytes facilitate the loading and unloading of a large amount of oxygen over a physiological critical range of partial pressure of oxygen. It is synthesized in the bone marrow and sources like protoporphyrin, iron and globin [25]. Our study showed that the hemoglobin percentage was decreased significantly in mice after *para*-nonylphenol exposure at different intervals i.e. 30 and 60 days as compared to control group. It is inferred that the decrease in hemoglobin percentage may be due to anemia caused by *para*-nonylphenol or may be due to decreased rate of production of red blood cells or increased loss of these cells or impaired erythropoietin due to a direct or indirect effect.

The alterations in haematological parameters of male mice after *para*-nonylphenol exposures may be modulate immune system through positive and negative feedback mechanism. Our results also corroborated with the works of other authors that *para*-nonylphenol effects on hematological indicators were reflected in changes of the

red blood cell picture and packed cell volume [12]. However, in changes of the white blood picture were repeatedly observed as well - an increase in the leucocyte, lymphocyte and granulocyte numbers and a decline in the neutrophil and monocyte numbers. These findings agreed with the other reports that explain the suppressive effects of *para*-nonylphenol on haematopoiesis and immune responses [12]. In connection to this, the reduction of erythrocyte RBCs count, haemoglobin count, neutrophil and monocyte in response to *para*-nonylphenol toxicity may be attributed to destruction of mature RBCs and inhibition of erythrocyte production due to reduction of haem synthesis by *para*-nonylphenol toxicities. The decrease in the RBCs and Hb may be related to the elimination of RBCs from circulation as a result of *para*-nonylphenol induced extravasation of the blood. The decrease in RBC counts observed with *para*-nonylphenol treatment could be due to haemolysis which causes haemorrhages and reduced erythropoiesis. Various authors have reported similar results with the treatment of various toxicants in rats [26], mice [13], sheep [27], rabbits [28, 29] and goats [30]. It is also reported that vitamin-C alone supplementation increase the viability and keep the normal values of hematological parameters [31]. In connection to this, our study also deals that the animals supplemented with vitamin-C along-with *para*-nonylphenol for the period of 30 and 60 days showed normalcy values of leucocyte, neutrophils, eosinophils and basophils towards control groups. The recovery in these parameters after vitamin-C supplementation along with *para*-nonylphenol exposure suggests that the vitamin-C has protective and beneficial effect on haematological parameters. It may suggest that vitamin-C has antioxidant properties to protect against oxidative damage caused by free radicals.

Lastly, we may also conclude that *para*-nonylphenol induced hematological changes in male *Mus musculus* and vitamin-C ameliorated the toxic effects induced by *para*-nonylphenol due to its antioxidant properties.

ACKNOWLEDGEMENTS

Authors are very grateful to Ministry of Higher Education, Government of Madhya Pradesh for financial support.

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