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Original Research Article

Influence of Ascorbic Acid Supplementation on Hematological Parameters and Free Radical in Adult Male Rabbits

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Abstract

Ascorbic acid (vitamin C) is a water-soluble micronutrient required for multiple biological functions. Ascorbic acid has been found to have a long history of use in traditional systems with antioxidant effects that can control the generation of free radicals. Free radical level was reported to be high in cancer cells. The objective of this study was to observe the effects of ascorbic acid (40mg/kg/BW/day for 12 weeks) on hematological and thiobarbituric acid-reactive substances in adult male rabbits. The effects of ascorbic acid on total erythrocyte counts (RBC), hemoglobin (Hb), packed cell volume (PCV), total leukocyte counts (WBC), mean cell volume (MCV), mean cell hemoglobin (MCH), mean cell hemoglobin concentration (MCHC), plasma glutathione-S-transferease (GST), acetylcholinesterase (AChE) and thiobarbituric acid-reactive substances (TBARS) at end of 12-week of treatment. Treatment with Ascorbic acid caused significant ($P<0.05$) increase in RBC and WBC, while did not cause any changes in Hb, PCV, MCV, MCH, MCHC, the activity of GSH and AChE compared to control. (TBARS) was significantly ($P<0.05$) decreased compared with control group. The study concludes that adding a Vitamin C source with a meal can improve anemia situation and reduce free radicals in adult male rabbits over an extended period of time

Keywords: hematological parameters, free radical, acetylcholinesterase and ascorbic acid.

INTRODUCTION

Ascorbic acid, the accepted name for vitamin C, is available in reduced form (L-ascorbic acid) and oxidized form (L- dehydroascorbic acid) [1, 2]. It is a vitamin found in various foods and sold as a dietary supplement [1]. Vitamin C was discovered in 1912, isolated in 1928, and in 1933 was the first vitamin to be chemically produced [3]. It is on the World Health Organization Model List of Essential Medicines, the most effective and safe medicines needed in a health system [4]. Foods containing vitamin C include citrus fruits, kiwifruit, broccoli, Brussels sprouts, raw bell peppers, and strawberries [5, 6]. Prolonged storage or cooking may reduce vitamin C content in foods [5].

Body requires vitamin C for normal physiological functions. It helps in the metabolism of tyrosine, folic acid and tryptophan [7]. It helps to lower blood cholesterol and contributes to the synthesis of the amino acids carnitine and catecholamine that regulate nervous system [7, 8]. It is needed for tissue growth and wound healing. It helps in the formation of neurotransmitters and increases the absorption of iron in the gut [9]. Being an antioxidant [10], it protects the body from the harmful effects of free radicals and pollutants [6, 11].

Free radicals are produced through biological processes and in response to exogenous stimuli, and controlled by various enzymes and antioxidants in the body. Oxidative stress occurs when free radical formation exceeds the ability to protect against them, resulting in tissue injury following trauma, inflammatory events and chronic conditions, such as atherosclerosis, degenerative disease and cancer [12]. Vitamin E, vitamin C, and β-carotene, often referred to as "antioxidant vitamins", have been suggested to limit oxidative damage in humans, thereby lowering the risk of certain chronic diseases. In epidemiological studies, cardiovascular disease is associated with low plasma concentrations of L-ascorbic acid, tocopherol and β-carotene [12, 13]. The chemical and biological properties of L-ascorbic acid suggest that it can act as an antioxidant *in vivo* [14, 15].

Deficiency of this vitamin is often associated with anemia, infections, bleeding gums, scurvy, poor wound healing, capillary haemorrhage, muscle degeneration, atherosclerotic plaques and neurotic disturbances [6, 7]. For the correction of deficiency, vitamin C is often supplemented in large doses and unlike fat soluble vitamins, toxicity normally does not occur [6, 16]. The current investigation was undertaken to re-examine the effects of dietary vitamin C supplementation on hematological parameters and level of serum free radicals in adult male rabbits over an extended period of time.

MATERIALS AND METHODS

In this study Vitamin C (Ascorbic acid), KEMPOVIT. C 250 oral, solution for oral administration (250 mg/ml) was supplied from Neofarma, Italy (Via Emilia Km 18, n. 1854- 47020 Longiano, Fo, Italy). All other chemicals used in the experiment were of analytical grade. Mature male New Zealand White rabbits (age of 7 months and initial weight of (2.962 ± 0.074 Kg) were used. Ten mature male rabbits were randomly divided into couple equal groups (each five rabbits): Group I: Rabbits were used as control successive weeks. Group II: Rabbits were treated with ascorbic acid. Ascorbic acid was given daily by gavage at a dose of 40 mg/kg BW for 12 successive weeks [17].

At the end of the experimental period, all rabbits were weighed then sacrificed under ether anesthesia. Blood samples were collected in clean dry centrifuge tubes. Plasma was separated by centrifugation at 3000 rpm for 10 minutes and then quickly frozen at -20°c for antioxidant enzymes and free radical analysis.

Blood Enzyme Activities

The blood samples were collected in two tubes: one containing EDTA (anti-coagulant) and the other containing Heparin (anti-coagulant). Noncoagulated blood by EDTA was tested shortly after collection by Particle counter (from ERMA INC.-Tokyo. Model PCE-210) for measuring total leukocyte counts (RBC), total erythrocyte count (WBC), haemoglobin (Hb), packed cells volume (PCV), mean cell volume (MCV), mean cell haemoglobin (MCH) and mean cell haemoglobin concentration (MCHC). The activities of plasma Glutathione S-transferase (GST; EC 2.5.1.18) activity was determined according to [18]. Acetylcholinesterase activity was measured according to [19], Plasma thiobarbituric acid-reactive substances (TBARS) were measured by the method [20].

Statistical Analysis

Where applicable, statistical analysis was carried out in Minitab software; statistical significance was assessed using two samples T- test analysis after detection normal distribution to the data and appropriate $P < 0.05$ consider significant [21].

RESULTS

As shown in Table1 the data were recorded upon the effects of Ascorbic acid on total erythrocyte counts (RBC), hemoglobin (Hb), packed cell volume (PCV), total leukocyte counts (WBC), mean cell volume (MCV), mean cell hemoglobin (MCH), mean cell hemoglobin concentration (MCHC), plasma glutathione-S-transferease (GST), acetylcholinesterase (AChE) and thiobarbituric acid-reactive substances (TBARS) at end of the 12-week of treatment. Treatment with Ascorbic acid caused significant ($P<0.05$) increase in RBC and WBC, while did not cause any changes in Hb, PCV, MCV, MCH, MCHC, the activity of GST and AChE compared to control. TBARS was significantly ($P<0.05$) decreased compared with control group.

Table-1: The overall means (±SEM) of different parameters at end of 12 week of treatment male rabbits with ascorbic acid (40mg/kg BW)

Parameters	Animal Groups	
	Control	Ascorbic acid
Total erythrocyte count (RBC; X 10^6/ mm^3)	5.4± 0.15^b	5.8±0.23^a
Hemoglobin (Hb; g/dl)	13.9± 0.19^a	13.9±0.21^a
Packed cell volume (PCV; %)	44.7±0.47^a	44.6±0.32^a
Total leukocyte count (WBC; x 10^3/mm^3)	7.1± 0.51^b	7.6±0.48^a
Mean cell volume (MCV; fl)	83.2 ± 2.05^a	83.4± 2.44^a
Mean cell hemoglobin (MCH; pg)	26.5± 0.59^a	27.0± 0.59^a
Mean cell hemoglobin concentration (MCHC; dl)	31.1± 0.58^a	31.3± 0.48^a
Glutathione S-transferase (GST; µmol/hr)	0.88 ± 0.00^a	0.90± 0.011^a
Acetylcholinesterase (AChE; µmol substrate hydrolyzed/ min)	3.3 ± 0.09 ^{a}	3.3 ± 0.18^a
Thiobarbituric acid-reactive substances (TBARS)	0.30 ± 0.016^a	0.23 ± 0.011^b

Values are means ± SEM of 5 rabbits in each group. Mean with different letters **(**a- b**)** are significantly difference ($p \leq 0.05$). Mean with the same letters are non-significantly difference ($p \geq 0.05$).

DISCUSSION

In the present study, 40 mg ascorbic acid / kg BW was used for 12 weeks because previous studies showed that 20 or 40 mg ascorbic acid / kg BW provided comparatively more significant amelioration against pesticides, but the lower dose (10 mg/(kg BW day) of ascorbic acid was less effective [22]. Hematological parameters such as RBCs, WBCs, MCV, MCH and MCHC are valuable in monitoring the health status [23]. In current study, treatment with ascorbic acid caused significant ($P<0.05$) increase in RBC and WBC, while did not cause any changes in Hb, PCV, MCV, MCH and MCHC compared to control The results indicated that treatment with ascorbic acid ameliorated its adverse effect on hematological parameters. Similar results have been reported by another study which study the Influence of ascorbic acid supplementation on the hematological parameters in rabbits [24], in common carp (*cyprinus carpio l.*) [25] and in Broiler chicks [26]. And this result refers to the absorption of about 80-90 percent ascorbic acid in the gastrointestinal tract. The absorbed acid circulates freely in plasma, leukocytes and red blood cells and enters all tissues, reaching maximum concentrations of 68-86 µmol / l plasma with oral intakes of 90-150 mg / day. The body uses it in two hours and then usually out of the blood within three to four hours [27].

GST has been reported to play an important role in the detoxification of several chemical compounds [28]. And, AchE is present in innervated tissues, where its function is to terminate nerve impulse transmission. It is also found in the red blood cell membrane [28]. Couple enzymes was not effected by ascorbic treatment compared to control. This results were agree with another study [29]. Free radicals and oxidants play a dual role as both toxic and beneficial compounds, since they can be either harmful or helpful to the body. They are produced either from normal cell metabolisms in situ or from external sources (pollution, cigarette smoke, radiation, medication). When an overload of free radicals cannot gradually be destroyed, their accumulation in the body generates a phenomenon called oxidative stress.

Free radicals and reactive oxygen species (ROS) are continuously produced in the human body [30]. Thiobarbituric acid reactive substances (TBARS) are produced by lipid per oxidation (LPO) and are considered as indicators of oxidative stress [30]. LPO was assessed by measuring the concentrations of TBARS in plasma of male rabbits treated ascorbic acid. The significant decrease in TBARS in rabbits that treatment with ascorbic acid are in agreement with the previous studies [31, 32]. These studies were found the rabbits treated with ascorbic acid (20 or 40 mg/kg B.Wt) showed increase in hematological parameters and decrease in TBARS in plasma.

CONCLUSIONS

Ascorbic acid is an antioxidant that protects the body from the damage caused by free radicals. It is used in many diseases and disorders as a therapeutic agent. The ascorbic acid concentration of 40 mg kg was found to be optimal to increase some hematological parameters and decrease free radical in male rabbits under the current experimental conditions.

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