



The effect of some food additive on the liver function in laboratory animal

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Key words: Rabbits, Phosphoric acid, Calicium

<http://dx.doi.org/10.12692/ijb/13.4.18-23>

Article published on October 14, 2018

Abstract

Phosphorus is an essential element as it represents the second most abundant mineral in the human body. Therefore, this study was designed to investigate the effect of phosphoric acid additive in water on some hematological and biochemical parameters in rabbits. Twelve female adult rabbits were divided randomly into two groups equally. The first group received 10% of phosphoric acid in water while the second group was received tap water as control group. The blood was collected in zero day and in 66 day. The result revealed that the Aspartate Aminotransferase (AST), Alanine Aminotransferase (ALT), Alkaline Phosphatase (ALP) and inorganic phosphorus were significantly increased ($P < 0.05$) in serum of treated group as compared with control group, while the total calcium concentration was significantly decreased ($P < 0.05$) in serum of treated group in comparison with control group. On the other hand the results of the hematological parameters showed a significant decreasing ($P < 0.05$) in the RBC count, Hb and PCV of the treated group, whereas the WBC count was significantly ($P < 0.05$) increased in the treated group. In conclusion, the phosphoric acid additive in water caused several changes in some biochemical and hematological parameters in rabbits.

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Introduction

Phosphorus is an essential element as it represents the second most abundant mineral in the human body (1). As phosphates, phosphorus is essential in numerous bodily functions. Major functions include being the base for all human ribonucleic acid (RNA) and deoxyribonucleic acid (DNA); a component of the major source of energy, adenosine triphosphate (ATP); and present in every cell membrane in the body as a component of phospholipid molecules.

Modern Nutrition in Health and Disease (1999) reports that the current recommendation for daily dietary intake of phosphorus by the Food and Nutrition Board of the Institute of Medicine is 700 mg for adults between the age of 19- 70(2). Protein rich foods and cereal grains are rich sources of phosphorus. In the United States, approximately one half of dietary phosphorus comes from milk and poultry with processed meats and cheese containing more phosphorus than natural products.

Purpose of study is to examine the disadvantages of phosphorus additives in foods and their potential harmful effect on the rabbits. This will be achieved by means of conducting a comprehensive literature review. Recommendations are provided for helping professionals.

Materials and methods

The study was applied on a total number of 12 female rabbits weighing (1 – 1.8) Kg, 4 month old during the period 19/10/20012 to 25/12/20012. The animals were divided into two groups randomly each group contain 6 animals. The first group was treated by 10% of phosphorus and the second group considered as a control without any additive.

The animals were housed in clean plastic cages provided with an easily cleaned floor that contain an opening used for urine and feces drainage. All cages were kept in a conditioned room (28- 32°C) with controlled lightening. All animals were left for 2 weeks for adaptation. Standard pallet diet was provided by metal feeders attached to the front of

cage. Clean and fresh water was supplied daily by pipette watering bottles.

Collection of blood samples

The collection of blood samples were done from heart at (0 day) to (66 day), by using medical syringes. A five ml of blood sample was divided into 2 parts:

- (a) Whole blood for hematological tests.
- (b) Serum for biochemical tests.

Hematological parameters

The total erythrocyte count, TEC (Cell X 10¹²/L) was determined by using haemocytometer with Neuberg slide. Hayem's solution was used for this purpose as described by Sood, (1996). Total leukocyte count, TLC (Cell x 10⁹/L). WBC was estimated by haemocytometer with Neuber improved double slide and using Thoma's SOL. The special pipette was used for WBC (Dacic and Lewis, 1984). Packed cell volume Estimation, PCV (%): Micro-haematocrit method was used to determine the percentage of packed blood cell volume (Coles, 1986).

Biochemical parameters

Alkaline Phosphatase (ALP): ALP was estimated by a colorimetric method Serum Transamination (AST, ALT) (Fischbach, 1999). AST total calcium concentration (mmol/ L) was estimated according to King and Armstrong, (1988). Inorganic Phosphorus concentration (mmol /L) was estimated according to Reitman and Frankel, (1957).

Results and discussion

Table 1 shows a significant decrease ($P < 0.05$) in Ca⁺² concentration in treated group (2.54 ± 0.35) as compared with the control group (4.69 ± 0.04) in 66 day, whereas P concentration showed a significant increase ($P < 0.05$) in treated group (1.52 ± 0.04) as compared with the control group (0.38 ± 0.01) in 66 day.

The relative ratio of calcium to phosphorus can vary markedly under different nutritional conditions the Ca/P ratio on a weight basis varying between 1.3 to 2.0 (Guyton and Hall, (2000).

Increased blood phosphate concentration following phosphoric acid ingestion has been associated with hypocalcaemia and hypophosphatemia. Hypocalcaemia occurs via inhibition 1-hydroxylase, with diminished 1, 25-dihydroxy vitamin D₃ formation (Murphy-Guntekunsand Uribarri, 2005).

Increase serum phosphate concentration also inhibit bone resorption with reduced calcium release hydrogen ion loads produce a metabolic acidosis which further exacerbate hypocalcaemia (Frisch and Wyshak, 1994; Calvo, 2013).

Table 1. The effect of phosphoric acid on serum Ca⁺² and Pi concentration in adult rabbits.

| Parameter | Time | Control | Treated |
|---------------|----------|------------------|------------------|
| Ca m.mol/L | Zero day | A 4.58 ± 0.03 | A 4.52 ± 0.03 |
| | 66 day | A 4.69 ± 0.03 | B 2.54 ± 0.35 |
| Pi m.mol/L | Zero day | A 0.40 ± 0.02 | A 0.39 ± 0.02 |
| | 66 day | A 0.38 ± 0.01 | B 1.52 ± 0.04 |

Increased Pi concentration in the serum may causes excessive excretion of Ca⁺² from the kidney to equilibrium the ratio and could lead to lower of bone density and this is clearly seen in the Fig.1. Phosphoric

acid is used to produce water softener removes Ca⁺² and Mg⁺² ion from hard water the function remains the same by removing Ca⁺² from bone causing osteoporosis (porous bone) (Caravate, 2007).

Table 2. Effect of phosphoric acid on ALT, AST and AP concentration in adult.

| Parameter | Time | Control | Treated |
|----------------|----------|-------------------|--------------------|
| ALT I.U. /L | Zero day | A 87.50 ± 3.43 | A 89.50 ± 3.42 |
| | 66 day | A 88.50 ± 3.40 | B 122.7 ± 15.50 |
| AST I.U /L | Zero day | A 59.9 ± 9.4 | A 58.8 ± 9.3 |
| | 66 day | A 60.5 ± 9.7 | B 97.9 ± 14.3 |
| AP I.U /L | Zero day | A 49.5 ± 0.80 | A 51.3 ± 0.90 |
| | 66 day | A 50.5 ± 0.85 | B 119.5 ± 1.63 |

The data illustrated in Table 2 indicated that the increase of ALT concentration (P<0.05) in treated group with phosphoric acid (122.7 ± 15.50 IU/L) compared with control group (88.50 ± 3.40 IU/L) in 66 days.

AST concentration showed significant (p< 0.05) elevation in treated group (97 ± 14.3 IU/L) in day 66 compared with control group (60.5± 9.7 IU/L). AST is provided whenever there is a damage in the tissue skeletal muscle, heart and liver (Calvo, 2013). The P

concentration in treatment group was 119.5 ± 1.63 IU/L which was significantly (P<0.05) increase in day 66 as compared with control group (50.5 ± 0.85 IU/L).

ALT is an enzyme frequently used in the diagnosis of damage caused by pollutants in Varian tissue such as liver and muscle the effect of elevation is clear in Fig.1 which related to myopathy (Hayes, 2004).

Table 3. The effect of phosphoric acid on RBC, Hb, PCV and WBC count in adult rabbits.

| Parameter | Time | Control | Treated |
|---|----------|-------------------|-------------------|
| RBC count x 10 ⁶ Cell /ml | Zero day | A 14.52 ± 0.23 | A 14.40 ± 0.09 |
| | 66 day | A 14.50 ± 0.22 | B 7.6 ± 0.57 |
| Hb concentration gm/ dl | Zero day | A 7.5 ± 1.50 | A 7.4 ± 1.50 |
| | 66 day | A 7.6 ± 1.53 | B 3.9 ± 0.3 |
| PCV % | Zero day | A 42.0 ± 2.18 | A 40.0 ± 2.15 |
| | 66 day | A 41.0 ± 2.18 | B 23.0 ± 0.99 |
| WBC count x10 ³ cell / ml | Zero day | A 5.20 ± 0.20 | A 5.10 ± 0.20 |
| | 66 day | A 5.30 ± 0.30 | B 4.50 ± 0.60 |

This enzyme plays a key role in mobilizing L-amino acid for gluconeogenesis and function as links between carbohydrate and protein metabolism under altered physiological, pathological and induced environmental condition (De la Torre *et al.*, 2000).

Elevation of the level of AST and ALT in different tissue can be considered as a response to the stress induced by phosphoric acid to generate ketone bodies. Acids like α Ketoglutarate and Oxaloacetate for contributing to

gluconeogenesis and or energy production necessary to meet the excess energy demand.

Alkaline phosphatase the hydrolysis of monophosphate esters and has a wide substrate specificity the activity of Alkaline phosphate has been significantly elevated in the tissue of liver and kidney and muscle increase of Alkaline phosphate activity may be due to pathological processes such as liver impairment kidney dysfunction and bone disease (Vivtor, 1985).

**Fig. 1.** The effect of phosphoric acid on the muscle which cause myopathy.

Alkaline phosphatase a digestive enzyme found on the surface of the small intestine that attacks organic phosphate (Barse *et al.*, 2006). Hematological values

revealed significant ($P < 0.05$) decrease in erythrocytes count, Hb and PCV in blood of treated group 7.6 ± 0.57 cell/ml, 3.9 ± 0.3 gm/dl, and $23.0 \pm 0.99\%$

respectively compared with control group 14.50 ± 0.22 cell/ml, 7.6 ± 1.53 gm/dl, and $41.0 \pm 2.18\%$ in the day 66.

This may cause by haemotoxicity due to phosphoric acid ingestion which caused degeneration of RBC cell. Champe, (2008) found that phosphoric acid cause increase erythrocyte sedimentation rate, WBC count observed an elevation in the number of WBC but not significant.

This may be due to the moderate neutrophilia caused by phosphoric acid toxicity or decrease in level of vitamin D which is related to immunity of body (Green, 1979).

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