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The Effects of Subacute Exposure of Peracetic Acid on Hematological Indices in Wistar Rats

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Abstract: This study was done to determine the effects of subacute exposure of Peracetic acid on Hematological indices in Wistar rats. In this study 18 male Wistar rats divided to two experimental and one control groups. PAA with 99% purity purchased. Then 0.2 and 2 mL of PAA dissolved in 100 mL drinking water. Animals in Treatment Group 1 and 2 received 0.2% PAA daily for 4 weeks and 2% PAA daily for 4 weeks, respectively. After the animals had been sacrificed hematological parameters examined. Experimental results concerning this study were evaluated using SPSS v.11.5 and expressed as Mean±SD $p < 0.05$ was considered significant. Mean±SD of WBC of groups 1 (2.45±0.77) and 2 (3.63±0.23) decreased significantly as compared with control group (5.3±0.57). RBC in control and 1, 2 groups were 8.34±0.39, 7.48±0.11 and 7.61±0.46, respectively. Also HCT and PLT decreased significantly in groups 1 and 2 as compared with control group. This study showed that oral consumption of Peracetic acid with concentration 0.2, 2% for 4 weeks can cause decrease hematological parameter in animal model.

Key words: Peracetic acid, hematological parameters, exposure

INTRODUCTION

Peracetic acid's primary use in food processing and handling is as a sanitizer for food contact surfaces and as a disinfectant for fruits, vegetables, meat and eggs (Evans, 2000). PAA can also be used to disinfect recirculated flume water (Lokkesmoe and Olson, 1993). Other uses of PAA include removing deposits, suppressing odor and stripping biofilms from food contact surfaces (Block, 1991; Mosteller and Bishop, 1993; Marriot, 1999; Fatemi and Frank, 1999). It is also used to modify food starch by mild oxidation and is used as bleach (National Academy of Sciences, 1996).

It is well identified that water disinfection such as sodium hypochlorite, chlorine dioxide and Peracetic acid through chlorination causes the formation of a mixture of disinfection by-products (DBPs), many of which are genotoxic and carcinogenic (Marabini *et al.*, 2006).

The use of chlorinated disinfectants (sodium hypochlorite (NaClO), chlorine dioxide (ClO₂) or Peracetic acid (CH₃COO₂H, PAA) during drinking-water production has been shown to generate halogenated compounds as a result of interactions of humic acids with chlorine. Such chlorinated by-products have been shown to induce genotoxic effects and consumption of chlorinated drinking-water has been correlated with increased risk for cancer induction in human populations (Gustavino *et al.*, 2005). Conti *et al.* (2005) evaluated the genotoxicity of two widely used drinking water disinfectants, 0.1 to 0.5 mg L⁻¹ sodium hypochlorite (NaClO) - chlorine dioxide (ClO₂) and Peracetic acid (PAA, CH₃-CO-COOH₂).

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In general, the highest levels of genotoxicity were observed under acid conditions; at acid pH, significant effects were induced by low concentrations of ClO₂ and PAA.

The potential risks of all high-level disinfectants are serious (Kennedy *et al.*, 2005).

There are limited researches about the health effects for administration of PAA and vast administration of PAA in human contact material and disinfection of drinking water and foods. Therefore this study with focusing on hematological effects of PAA in rats designed.

MATERIALS AND METHODS

Chemicals

Peracetic acid (PAA) is purchased from Behban Chemistry Company Gorgan, Iran. All other chemical such as analytical grade obtained from Merck (Germany).

Animals

The study was performed in 2007 on 18 albino male Wistar rats of 10-12 postnatal weeks (provided from the Iranian Pasteur Institute) in the Faculty of Medicine, Gorgan University of Medical Sciences. The rats were randomly divided into three equal case groups on the basis of the differences between the periods of exposure to PAA.

Treatment group (Group 1) received 0.2% PAA daily for 4 weeks.

Treatment group (Group 2) received 2% PAA daily for 4 weeks.

There was also a control group that only received drinking water daily for 4 weeks. All animals had freely access to solution as drinking water.

Approval for this study was gained from the Gorgan University of Medical Sciences animal Care and Ethics Committee. It was found by means of a digital scale that the mean weights for each group were 198.83 g (1), 155.83 g (2) and 202.50 g (control group).

Preparation of PAA Solutions

PAA with 99% purity purchased. Then 0.2 and 2 mL of PAA dissolved in 100 mL drinking water. Approximately 1000 mL PAA solutions of each dosage of solution prepared.

Hematological Examination

When the experiments had expired, each of the rats of the two experiments and the control group were anaesthetized with ether. Five milliliter blood were taken from heart (right ventricle) and collected in tube containing EDTA for hematological investigations. Then blood parameters such as White Blood Cells (WBC), Red Blood Cells (RBC) count, Hemoglobin (Hb %), HCT: Hematocrit, Platelets (PLT) count, mean corpuscular volume (MCV), Mean Corpuscular Hemoglobin (MCH) and Mean Corpuscular Hemoglobin Concentration (MCHC) examined by cell counter (Sysmex KX-21N Automated Hematology Analyzer, Germany). Several evaluations of hematological parameters determination by the Sysmex KX-21N have been published. (Lindenblattl *et al.*, 2006; Chaturvedia *et al.*, 2001; Malin *et al.*, 1999).

Statistical Analysis

Experimental results concerning this study were evaluated using SPSS v.11.5 (ANOVA test) and expressed as Mean±SD. Also Post huc (Tukey test) performed for determine difference between groups. $p < 0.05$ was considered significant.

RESULTS AND DISCUSSION

Mean±SD of body weight in the beginning and end of study shown in Table 1. Findings of blood parameters such as WBC, RBC, HGB, HCT, MCV, MCH, MCHC and PLT shown in

Table 1: Mean±SD of body weight in control and treatment groups

| Group | First body weight | Week 2* | Week 3 | End body weight* |
|---------|-------------------|--------------|--------------|------------------|
| Control | 202.50±18.51 | 203.50±15.59 | 209.33±15.53 | 203.17±15.56 |
| Group 1 | 198.83±39.12 | 189.17±37.21 | 192.00±38.98 | 184.17±21.00 |
| Group 2 | 155.83±18.50 | 152.50±18.77 | 167.67±19.50 | 154.20±7.79 |

Values are means±SD. Calculated from n = 6 in each group, *: p < 0.05 compared to control (ANOVA). (1): Treatment Group received 0.2% PAA daily for 4 weeks. (2) Treatment Group received 2% PAA daily for 4 weeks

Table 2: Effect of Peracetic acid on blood parameters (WBC, RBC, HGB, HCT, MCV, MCH, MCHC, PLT)

| Group | WBC* | RBC* | HGB (g dL ⁻¹) | PLT* | MCHC | MCH | HCT* (%) | MCV |
|---------|-----------|----------|---------------------------|---------------|------------|------------|------------|-------------|
| Control | 5.30±0.57 | 8.34±.39 | 14.28±0.81 | 982.16±207.41 | 30.04±0.38 | 17.78±1.22 | 48.90±0.62 | 59.38±3.54 |
| Group 1 | 2.45±0.77 | 7.48±.11 | 12.85±0.07 | 384.00±514.77 | 29.00±0.14 | 17.20±0.14 | 44.30±0.00 | 59.25±0.919 |
| Group 2 | 3.63±0.23 | 7.61±.46 | 13.76±0.49 | 468.00±189.79 | 32.02±1.97 | 18.00±0.0 | 43.53±1.19 | 58.87±1.16 |

White blood cells (WBC). Red blood cells (RBC) count. Hemoglobin (Hb%). HCT: Hematocrit. Platelets (PLT) count. Mean corpuscular volume (MCV). Mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC). Values are means±SD. Calculated from n = 6 in each group. *p < 0.05 compared to control (ANOVA). (1): Treatment Group received 0.2% PAA daily for 4 weeks. (2) Treatment Group received 2% PAA daily for 4 weeks

Table 2. Mean±SD of body weight, WBC, RBC, HCT and PLT decreased significantly in groups 1 and 2 as compared with control group. Mean±SD of WBC of groups 1 (2.45±0.77) and 2 (3.63±0.23) decreased significantly as compared with control group (5.3±0.57). RBC of control group and groups 1, 2 were 8.34±0.39, 7.48±0.11 and 7.61±0.46, respectively. Also HCT and PLT decreased significantly in groups 1 and 2 as compared with control group. Post hoc test did not shown any significant differences between treatment groups.

Finding of this study revealed that hematological variables such as WBC, RBC, HCT and PLT decreased significantly in Peracetic acid treatment groups when compared with control group. Decreasing leukocyte counts were observed in our study is comparable with finding of Heinze *et al.* (1984) study. He revealed Bronchopneumonia and liver granuloma in Mice and guinea pigs were exposed to Peracetic acid aerosol (186 or 280 mg mL⁻¹) 30 min twice daily for 90 days also, Increased incidence of lung tumors and decreased leukocyte counts were observed in mice.

There is limited information about toxicity of PAA on hematological parameters. However some investigators have studied the effects of PAA on various organs. Muller *et al.* (1988) investigated chronic Peracetic acid (wofasteril) administration on the rabbit oral mucosa, vaginal mucosa and skin. In mentioned study mucosal tissues showed no inflammations after administration of Peracetic acid. But study of Laub *et al.* (1990) indicated damaging to Langerhans cell population after application of Peracetic acid. Also the results of Wutzler *et al.* (1987) showed that higher concentrations of Peracetic acid lead to considerable changes of the epithelium of the urinary bladder in form of partly focal partly diffuse haemorrhagic necrotizing urocystitides. Moreover Peracetic acid at 3% caused dermatitis on guinea pig skin.

It is known that Peracetic acid through chlorination causes the formation of a mixture of disinfection by-products (DBPs), many of which are genotoxic and carcinogenic (Marabini *et al.*, 2006).

The application of chlorinated disinfectants such as Peracetic acid in during drinking-water production has been shown to generate halogenated compounds, that induce genotoxic effects consumption of chlorinated drinking-water has been correlated with increased risk for cancer induction in human populations (Gustavino *et al.*, 2005).

According to US National Library of Medicine's (2007) Peracetic acid caused oxidative stress and subsequent disruption of their cell membrane, via the hydroxyl radical (OH•). As diffusion of chemical is slower than the half-life of the radical, it will react with any oxidizable compound in its vicinity. It can damage virtually all types of macromolecules associated with a microorganism: carbohydrates,

nucleic acids (mutations), lipids (lipid peroxidation) and amino acids (e.g., conversion of Phe to m-Tyr and o-Tyr). This ultimately leads to cell lysis and true microbial death (US National Library of Medicine's, 2007).

Hematocrit is a blood test that measures the number of red blood cells and the size of red blood cells. It gives a percentage of red blood cells found in whole blood. Low Hematocrit may be due to: Blood loss (hemorrhage), Bone marrow failure, Destruction of red blood cells. In our study the number of red blood cells decreased, subsequently percentage of Hematocrit decreased.

Despite there are limited studies about effects of PAA on human system specially on hematological parameters. We extrapolate from those works that could effect on hematological parameters for these reasons:

- Peracetic acid's mechanism of action is hypothesized to be the denaturation of proteins and enzymes and increased cell wall permeability by breaking sulphhydryl and disulfide bonds (Baldry, 1983; Block, 2001). These effects could results decrease of cell viability of red blood cells, with blood cells.
- The primary mode of PAA action is oxidation. PAA disinfects by oxidizing of the outer cell membrane of cells. The mechanism of oxidation is the transfer of electrons, therefore the stronger the oxidizer, the faster electrons are transferred to cells and the faster the cells is inactivated or killed. Therefore PAA has a higher oxidation potential than chlorine sanitizers. PAA also inactivates enzymes that are responsible for discoloration and degradation, such as peroxidase (Greenspan and Margulies, 1950).
- With regarding to the instability of peaacetic acid to exist as itself, the primary degradates are acetic acid, oxygen and water (US Environmental Protection Agency, 2000).

In conclusion, the results of this study showed that Peracetic acid can cause decrease of WBC, RBC and decrease of Hematocrit and platelet.

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