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MODIFICATION OF HEMATOLOGICAL INDICATORS IN PARACETAMOL INTOXATION IN ALBINO NMRI MICE AND EVIDENCE OF PROTECTIVE EFFECT OF SILYBUM MARIANUM VEGETAL EXTRACT

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Abstract

The medicine is either a substance or a combination of several substances used to diagnose, prevent, improve or cure a disease. The use of drugs provides many benefits, but it also involves risks because, following drug-body interaction, adverse effects or reactions occur. These undesirable reactions are favored by polymedication, long-term treatment and particular physiological states (pregnancy, breastfeeding, elderly) or pathological conditions (kidney failure). In daily practice, Paracetamol is a commonly used medicine, covering almost all specialties and age groups. It is part of non-opioid analgesics and antipyretics, often used to treat pains with different localizations and intensities. The purpose of this paper is to investigate the effects of experimental intoxication with Paracetamol, highlighting the beneficial effect of the Silybum marianum vegetal extract on the haematological index in NMRI Albino mice. We used paracetamol in the form of injection, known as perfgalgan (Bristol-Myers Squibb), at the dose of 400 mg/kg/body of substance, and the plant extract from Silibum marianum was given by oral gavage after Paracetamol intoxication. In order to accomplish the purpose of this paper, the number of hematiocytes, leucocytes and the amount of hemoglobin was determined in the four experimental variants: the control group, the group in which the mice were experimentally intoxicated with Paracetamol, the group in which, after the experimental intoxication with Paracetamol, the mice were treated with vegetable extracts of Silibum marianum, and the group in which the mice were treated only with Silibum marianum vegetal extract.

Keywords: Experiment, mice, paracetamol, Silybum marianum.

1. INTRODUCTION

Paracetamol is an analgesic and antipyretic medicine widely used in medicine, being used and sold with or without prescription in many countries (Kittisupamangkol, 2009).

Acute poisoning with Paracetamol has 4 stages:

- The initial stage (0-24 hours) is characterized by nausea, vomiting, anorexia, lethargy, sweating;
- Intermediate stage (24-48 hour, characterized by increased transaminases (AST, ALT), bilirubin;
- Hepatic stage (3-4 days), concretized by hepatic encephalopathy, confusion, lethargy, vomiting, spontaneous bleeding, coma;

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- The recovery period, which lasts for up to five days, during which the transaminase values return to normal, and the liver structure recovers within 3 months of intoxication.

Paracetamol induced poisoning model is commonly used to study potential hepatoprotective activity of various plant extracts.

Silybum marianum (SM), known as the armor, is a plant that grows in the form of bushes, reaching the length of 2m, preferring less sandy soils, especially stony ones. The plant has been used to treat various diseases more than 2000 years ago, a plant of Mediterranean origin, being widespread throughout Europe and Central Asia, including Romania.

For therapeutic purposes, crushed seeds are used, which are rich in saponosides, silimarin, fitomelan, fumaric acid, all of which favor the regeneration of liver cells and enhance the liver's property to protect itself from infection.

It has been demonstrated by numerous studies and experiments that silimarin has hepatoprotective properties and is often used to treat different liver disease (Elmowafy M. et al., 2013), especially caused by oxidative stress (Féfer J. and Lengyel G., 2012).

Rasool et al. monitored the ability of silimarine and glycyrrhizin to reduce oxidative stress in mice with hepatic lesions caused by carbon tetrachloride (CCl4). Thus, male Wistar mice were divided into 6 groups as follows: the first group- witness, while groups 2, 3, 4, 5 and 6 received a single dose of CCl4 (50% solution of CCl4 in liquid paraffin, administering peritoneal 2ml / kg) twice a week to induce hepatic injury. For 6 weeks the animals were fed with silimarine and glycyrrhizin in different doses. It has been found that CCl4 determines hepatic injury by significantly elevating alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP) and reactive thiobarbituric acid (TBARS), at the same time as total protein and glutathione reduction activities (GSH), superoxide dismutase (SOD) and catalase (CAT).

Treatment with different doses of silimarine and glycyrrhizin significantly reduced ALT, AST, ALP and TBARS levels, and increased GSH, SOD and CAT levels.

Midia Kamali and Arman Seyed Mostafaei also highlight the hepatoprotective effects of feed mixtures containing *Silybum marianum* in experimental conditions induced by carbon tetrachloride (CCl4) in broiler chickens. In the experiment the chickens were divided into 6 groups: the first group – for control, where the chickens were fed with supplements, the birds in the second group were fed basal diets and received CCl4, the birds in the third group were fed with the diet basal supplemented with 60ppm extract of *Silybum marianum* fruit, birds in groups 4-6 had basal diet supplemented with 40ppm SM and CCl4, 60 ppm S.M. and CCl4, and 80 ppm and CCl4. Blood samples were taken to determine alanine aminotransferase (ALT), aspartate aminotransferase (AST), glutamine oxaloacetic transaminase (SGOT), serum pyruvate glutamate transaminase (SGPT), total protein, albumin, globulin and hepatic cytochrome P450.

In the groups of mice treated with CCl4, the medium values of ALT, AST, SGOT and SGPT serum increased significantly compared to the control group. CCl4 determined the elevation of cytochrome P450 while the administration of various concentrations of *Silybum marianum* determined its reduction. It has been observed that treatment with *Silybum marianum* fruit extract has considerably reduced tissue damage caused by CCL4.

Also in their paper, Abdo M. et al. evaluated the effect of oral administration of silimarine extract. A dose of silimarine extract (20 or 100 mg / kg body weight) was administered via oral gavage. Subsequently, the mice were injected with a single dose of CCI4 (2 ml / kg body weight).

After 24 hours, the ratio between liver weight and body weight, serum transaminase levels and histological analyzes showed significant liver damage that was inhibited by silymarin in a dose-dependent manner. Molecular analysis has shown that silimarin has reduced expression of pro-

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inflammatory MCP-1 chemokine, TGF-beta proinflammatory cytokine, and collagen I in isolated hepatic stellate cells (HSCs), these being the key effector cells of hepatic fibrosis.
As a general conclusion of this experiment, it can be argued that the oral administration of the tested silimarine extract inhibited the hepatocellular lesion, proving that silimarine extract had direct effects on the expression of pro-inflammatory and pro-fibrogenic genes in HSC in vitro.

2. MATERIALS AND METHODS

In the present study were used white Swiss albino mice - NMRI strain, weighing over 30 g, maintained under standard laboratory conditions, in metal cages with special feed and water ad libitum at circadian rhythm controlled 12 hours day / 12 hours of darkness, and an ambient temperature of 22 ± 2°C.
Animals come from SPF Animaleria, Baneasa Station, Cantacuzino Institute - Bucharest.
Four groups were used in the experiment, each group consisting of 10 mice, such as: a witness group, a group of mice injected intraperitoneally with Paracetamol at a dose of 400mg / kg body, a group of mice injected 400mg / kg body Paracetamol and treated with gavage with 0.2ml armor extract for 14 days, given once every 7 days, and a group treated only with a 0.25ml armor extract administered by gavage once every 7 days for two weeks.
The weight of the animals was tracked by daily weighing, using electronic scales.
Vegetable extracts of armor were obtained by two primary and advanced processing, using ethanol as the solvent in various concentrations (Lupuleasa et al, 2005).
Determination of the number of blood and white blood cells was performed by hematocytometric technique, using the Thoma counting chamber (Picoş and Năstăsescu, 1988).
Determination of hemoglobin concentration was achieved using the cyanomethemoglobin method (Jain, 1986).
The experiments were conducted in compliance with the bioethics norms and the European directives on the protection of animals used for scientific purposes.

3. RESULTS AND DISCUSSIONS

As a result of the determination of the number of elements (liver and leucocytes), average values were shown in Figures 1 and 2.
Analyzing the results presented in Figure 1, there is a 19% decrease in the number of red blood cells at 7 days after the administration of Paracetamol at 400 mg / kg / body, compared to the witness group, with a slight increase in 14 days from the administration of the *Silybum marianum* plant extract. Significant reduction ($p < 0.05$) in the number of red blood cells indicates, in addition to damage to mature red blood cells, a decrease in the erythropoiesis process, indicating a possible inhibition of the release of erythropoietin from the kidneys.
Similar results have been obtained by Oyedeji K.O. and Collab (2013) but also Daniel and Clement (2008).
Following the evolution of leukocyte counts in Figure 2, a significant increase ($p<0.05$) was observed, with a 32% number of white blood cells versus the witness variant at 7 days after Paracetamol administration at a concentration of 400mg / kg body weight, after which a slight decrease of the values is observed, which can indicate the beneficial effect of *Silybum marianum* plant extract after 7 days after its administration in the concentration of 0.25 ml.

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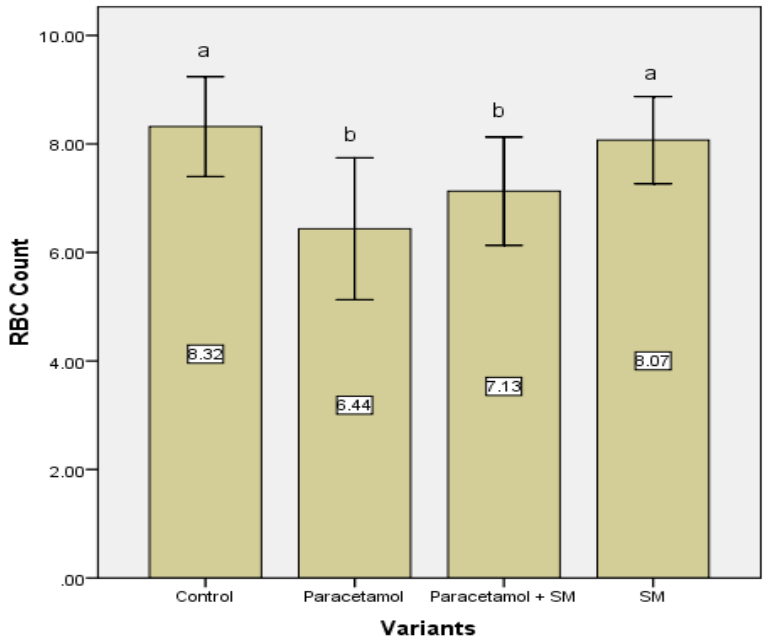

Figure 1. Influence of Silybum marianum extract on the number of red blood cells (Nx106 / µl) in case of paracetamol-induced toxicity (Control = variant control; Paracetamol = paracetamol 400mg / kg body weight group; P + SM = paracetamol group 400mg / kg body treated with Silybum marianum extract SM = group treated with Siybum marinum vegetal extract; the letters represent the Duncan test interpretation: Different letters show significant differences, p <0.05)

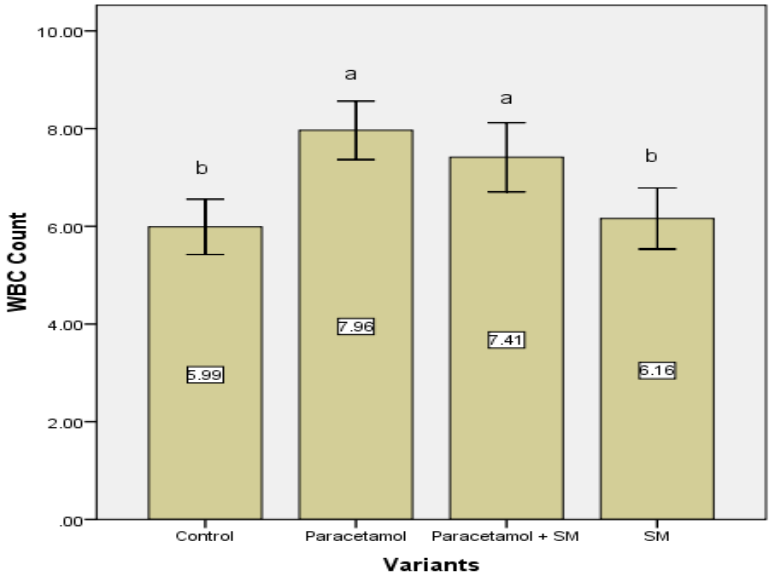

Figure 2. Influence of Silybum marianum extract on the number of Leukocytes (Nx10□ / µl) in case of paracetamol-induced toxicity. (Control = variant control; Paracetamol = group with paracetamol 400mg / kg body weight; P + SM = group with paracetamol 400mg / kg body treated with Silybum marianum extract, SM = group treated with Siybum marinum vegetal extract; the letters represent the Duncan test interpretation: different letters show significant differences, p <0.05)

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At 7 days after the administration of Paracetamol at a concentration of 400 mg / kg body weight, hemoglobin decreased (Figure 3), indicating a reduction in oxygen transport capacity and implicitly a reduction in tissue oxygenation.
This result is consistent with that obtained by Oyedeji K.O. et al. but also by Adedapo et al (2007).

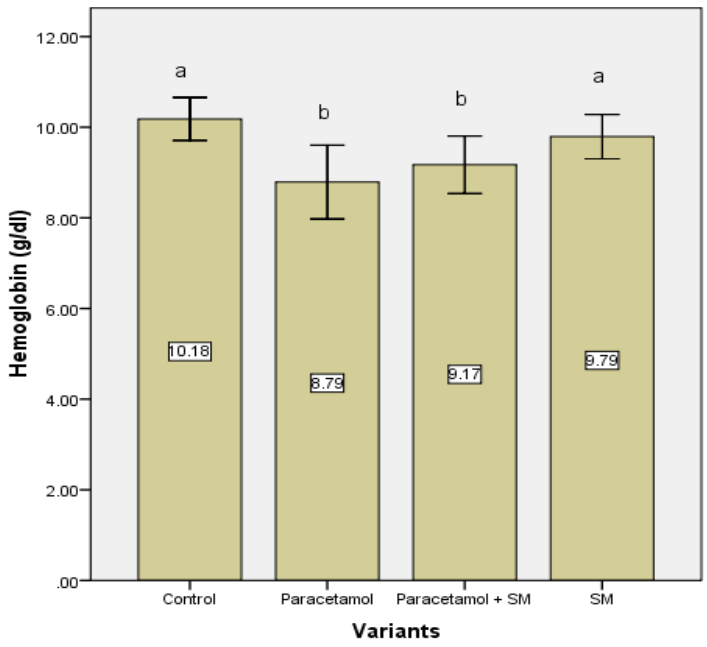

Figure 3. Influence of Silybum marianum extract on hemoglobin in the case of paracetamol-induced toxicity (Control = variant control; Paracetamol = group with Paracetamol 400mg / kg body weight; P + SM = group with Paracetamol 400 mg / kg body treated with Silybum marianum extract, SM = group treated with Siybum marinum plant extract; (the letters represent the interpretation of the Duncan test: different letters show significant differences, p <0.05)

4. CONCLUSIONS

The results obtained showed that there were significant changes in the number of red blood cells and leucocytes after administration of paracetamol at a concentration of 400 mg / kg / body as compared to the witness group, in the sense that it produced a decrease of 19% compared to the witness group of the number of red blood cells at 7 days after administration, with a 32% increase in white blood cell count.
Also, the decrease in hemoglobin was found 7 days after paracetamol administration, indicating a reduction in oxygen transport capacity and implicitly a reduction in tissue oxygenation, as well as a possible occurrence of anemia.
After the *Sylibum marianum* plant extracts, we notice a slight recovery of the number of red blood cells and leucocytes, which can demonstrate the beneficial effect of *Sylibum marianum* extracts.
Paracetamol caused insignificant changes in the body weight of NMRI Albino mice after treatment with paracetamol.

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