EXPERIMENTAL EVALUATION OF THE ACTION OF ETHEPHON AND SODIUM SELENITE ON SOME HEMATOLOGICAL PARAMETERS IN RATS

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EXPERIMENTAL EVALUATION OF THE ACTION OF ETHEPHON AND SODIUM SELENITE ON SOME HEMATOLOGICAL PARAMETERS IN RATS (Abstract): Ethephon (2-chloroethylphosphonic acid) is one of the most intensely used plant growth regulators. **Aim:** Assessment of hematological parameters during subacute intoxication with ethephon (200 mg/kg bw/day) in rats and evaluation of the potential protective effect of selenium (1 mg/kg bw/day) (administered as sodium selenite) in rats during the experimental intoxication by oral route with high doses of ethephon (200 mg/kg bw/day). **Material and methods:** Male Wistar rats were randomly assigned to four groups: Control group, Ethephon group (200 mg ethephon/kg bw/day, 14 days), Selenium group (1 mg Se/kg bw/day, 14 days), Selenium+ethephon group (200 mg ethephon/kg bw/day and 1 mg Se/kg bw/day). Blood samples were collected on the 15th day and a complete blood count was conducted by using an automated hematology analyzer. **Results:** Mean red blood cell count was 7.06x10⁶/µl in the Ethephon group and 7.18x10⁶/µl in the Selenium group, as compared to 7.16x10⁶/µl in the Control group. Platelet count decreased from 1028.66x10³/µl in the Control group to 999.66x10³/µl in the Ethephon group. Total leukocyte count was 7.82x10³/µl in the Ethephon group and 8.40x10³/µl in the Selenium+ethephon group, as compared to a mean of 8.07x10³/µl in the Control group. **Conclusions:** The modification of some of the hematological parameters in the groups to which ethephon was administered can be suggest-ive of the harmful effect of this substance (which is present as a residue in food products) on consumer health. Orally administered selenium (as sodium selenite) at a dose of 1 mg Se/kg bw, seems to have acted as a toxic and not as a protective agent during the intoxication with ethephon. **Keywords:** ETHEPHON, PLANT GROWTH REGULATOR, SELENIUM, HEMATOLOGICAL PARAMETER.

Ethephon (2-chloroethylphosphonic acid) (fig. 1) is one of the most intensely used plant growth regulators, due to its wide action spectrum (1).

In pomiculture and vegetable growing, ethephon can be used to hurry up flower formation, ripening or coloring of fruits, etc. (2, 3). Ethephon is also used as a treatment for cereals, to increase their mechanical resistance, by shortening and
hardening the straw (4). In some countries, ethephon is extensively used for post-harvest acceleration of ripening of bananas and mango fruits (5).

Fig. 1. Chemical structure of ethephon

In practice, ethephon is mainly formulated as solutions in concentrations between 20 and 720 g/L and is marketed under multiple brands, which correspond to specific uses. Formulations which have ethephon as active substance are registered in more than 60 countries worldwide (6, 7). The mechanism of action is because it is easily absorbed by plants and transformed in ethylene (the main metabolite), which is a natural hormone for plants (8).

In case of oral administration to laboratory animals (rats and mice), ethephon can induce digestive manifestations (gastroenteritis, gastric necrosis), respiratory and renal diseases, cardiac fibrosis, brain damage (mineralization processes) and liver damage (necrotic hepatitis). Ethephon is also teratogenic (9, 10). Other authors (11, 12) reported for ethephon direct cholinergic effects and indirect effects on the contraction of intestinal musculature. El-Okazy (13) demonstrated that ethephon perturbs some hematological and biochemical parameters in laboratory animals.

Selenium is an important biological antioxidant, because it is a constituent of glutathione peroxidase, the selenoenzyme that catalyzes the reduction of lipid peroxides and hydrogen peroxide, thus preventing the harmful effects of lipid peroxidation and protecting erythrocytes from hemolysis; in addition, selenium protects cells and cell membranes from oxidative processes (14, 15, 16).

The aim of this study was: a) to assess the hematological parameters during sub-acute ethephon poisoning (200 mg/kg bw/day) in rats and b) to evaluate the potential protective effect of selenium (1 mg/kg bw/day, administered as sodium selenite) in rats during experimental poisoning by oral route with high ethephon doses (200 mg/kg bw/day).

**MATERIAL AND METHODS**

Sodium selenite (purity 99%; CAS 10102-18-8) and ethephon (purity 96%; CAS 16672-87-0) were purchased from Sigma Aldrich. All other reagents were of analytical grade.

The animal study protocol was approved by the Research ethics committee of “Grigore T. Popa” University of Medicine and Pharmacy Iași, Romania. All procedures were conducted according to the 86/609/EEC directive and internal regulations of the university.

Male Wistar rats purchased from the “Cantacuzino” National Institute for Research and Development in Microbiology and Immunology were used. The animals were housed in collective cages, in relative constant environmental conditions (temperature 18-22°C; light-dark cycle: 12 hours/12 hours) and were given standard feed for rats and water *ad libitum*.

Rats, weighing 180-230 g, were randomly assigned to 4 groups of 6 animals each, to which the following treatments were given by gavage after 12 hours of fasting: Control group (C) - physiological saline; Ethephon group (ETH) - ethephon 200 mg/kg bw/day as aqueous solution; Selenium+ethephon group (SE+ETH) -
ethephon 200 mg/kg bw/day as aqueous solution and selenium 1 mg/kg bw/day as sodium selenite aqueous solution; Selenium group (SE) - selenium 1 mg/kg bw/day as sodium selenite aqueous solution.

All animals were weighed again on the 15th day, after which they were anesthetized with intraperitoneally administered ketamine (100 mg/kg bw, as injectable solution for veterinary use). Blood samples were collected in vacutainers containing anticoagulant (K₂EDTA). A complete blood count was performed by using a Sysmex XT 1800 (Sysmex Corporation, USA) automated hematology analyzer. The experimental data were processed by using Microsoft Excel 2013 with Data analysis add-on. A paired Student’s t-test was used (p < 0.05 was considered significant).

RESULTS AND DISCUSSION

The results obtained for the erythrocyte count (RBC) (fig. 2) show that in animals treated with ethephon the mean RBC was slightly lower (p = 0.398) than in the Control group (C). An insignificant increase in mean RBC was seen in the Selenium group when compared to the Control group (p = 0.882). At the same time, in the Selenium+ethephon group mean RBC was slightly higher than that recorded in the Ethephon group (p = 0.611), but much lower than in the Control group (p = 0.830).

Fig. 2. Mean levels of red blood cell count (RBC), hemoglobin concentration (HBG) and hematocrit (HCT)

Hemoglobin concentration (HBG) and hematocrit (HCT) are parameters on which the investigation related to the quality of erythropoiesis is based on. Thus, mean HBG level (fig. 2) for the group treated with ethephon was almost like that for the control group (p=0.923). After comparing the levels recorded for the group treated with selenium and ethephon, for which the mean value was 13.84 g/dL, with those obtained for the group treated with selenium alone (mean value 14.36 g/dL) a slight decrease in mean HBG was noticed (p= 0.451). The results obtained from this experimental study agree
with those reported by Al-Fartosi (16), who highlighted a decrease in HBG in the animal groups treated with ethephon. For the group treated with selenium, the decrease of mean HBG and of other parameters that characterize the process of erythropoiesis is in total agreement with the results obtained by other authors (17, 18), who assessed the action of sodium selenite on hematological parameters in rats. El-Okazy (13) reported that no significant variations in HBG (p>0.05) were observed at low doses of ethephon; the decrease was significant when the doses were increased (p<0.01). Also, the RBC decreased, in correlation with the dose of ethephon ingested by the animals.

Regarding HCT, parameter which expresses the volume percentage of red blood cells in blood (fig. 2), the mean levels were in accordance with mean HBG concentrations; thus, mean HCT for the Ethephon group was slightly decreased when compared with the Control group, but without statistical significance (p>0.05). In the case of the group to which ethephon was administered together with sodium selenite, mean HBG was insignificantly lower when compared to the Selenium group (p=0.508) and Control group (p= 0.161). This evolution, in direct correlation with the variation of mean HGB levels suggests a reduced interference of ethephon and/or selenium with the erythropoiesis process in rats.

After analyzing the data obtained for erythrocyte indices (tab. I), very low group variations have been recorded, in accordance with the other erythrocyte indices; thus, mean corpuscular volume (MCV) and mean corpuscular hemoglobin (MCH) for the groups treated with an association of selenium and ethephon were lower than those for the Ethephon and Selenium group, respectively (p<0.05), and lower than those obtained in the Control group (p>0.05). Mean corpuscular hemoglobin concentration (MCHC) and red cell distribution width (RDW-SD) did not show statistically significant differences between groups (p>0.05).

The data obtained by us are generally in agreement with those reported in the scientific literature. Thus, Al-Fartosi (16) reported that sodium selenite reduces erythrocyte levels and hemoglobin concentrations in a dose dependent manner. This evolution of erythrocyte count and hemoglobin concentration was confirmed by other authors (18). In addition, Jia et al (19) confirmed that sodium selenite administered to rats in a sub chronic experiment led to a dose dependent decrease in red blood cell counts and hemoglobin concentration. The mechanism by which selenite reduces the RBC may be hemolysis, which leads to depletion of circulating red blood cells (18).

<table>
<thead>
<tr>
<th>Group</th>
<th>MCV (fL)</th>
<th>MCH (pg)</th>
<th>MCHC (g/dL)</th>
<th>RDW-SD (fL)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>min</td>
<td>max</td>
<td>mean</td>
<td>min</td>
</tr>
<tr>
<td>Control</td>
<td>57.30</td>
<td>58.10</td>
<td>57.63</td>
<td>20.10</td>
</tr>
<tr>
<td>Ethephon</td>
<td>56.30</td>
<td>61.00</td>
<td>58.10</td>
<td>19.50</td>
</tr>
<tr>
<td>Selenium</td>
<td>56.00</td>
<td>59.50</td>
<td>58.46</td>
<td>19.10</td>
</tr>
<tr>
<td>SE+ETH</td>
<td>53.20</td>
<td>56.80</td>
<td>55.32</td>
<td>18.80</td>
</tr>
</tbody>
</table>

MCV = mean corpuscular volume; MCH = mean corpuscular hemoglobin; MCHC = mean corpuscular hemoglobin concentration; RDW = red cell distribution width;
Platelets are anucleate cytoplasmic fragments, rich in granules, involved in hemostasis and in the initiation of tissue repair processes and vasoconstriction after vascular lesions and during inflammatory processes. Our research showed that mean platelet count (PLT) for the group treated with ethephon (tab. II) was significantly decreased when compared to the Control group (p=0.050). For the group treated with selenium, mean PLT was decreased in comparison with both the Control group (p>0.05) and Ethephon group, but the decrease was not statistically significant. Platelet count for the group treated simultaneously with Ethephon and sodium selenite was higher compared with the Selenium group, without approaching the levels recorded in the Control and Ethephon groups, respectively; in both cases, the drop in mean PLT was statistically insignificant (p>0.05).

Platelet indices, mean platelet volume (MPV), respectively, which indicates the variations in platelet volume, and platelet distribution width (PDW), which indicates the uniformity in size of platelets, are very useful for the differential diagnostic of thrombocytopenia (tab. II). The Control group had a mean MPV value of 6.96 fL and a dispersion of values given by a standard deviation of 0.294. For the other three groups, the mean values are higher than those observed for the Control group; for the association ethephon-selenium the value was significantly decreased when compared to the Selenium group (p=0.003), but increased in comparison with the Control group (p=0.023). These results are in accordance with the data published by El-Okazy (13), who reported that ethephon altered the hematopoiesis process, depending on the dose, exposure period and animal species. The results obtained for PDW, which reflects the variation in platelet size, correlated with the distribution of PLT values and with the percentage of macro thrombocytes (P-LCR), respectively (tab. II). The increase in mean PDW values was statistically significant when compared to the Control group, both in the ethephon group and in the Selenium group (p=0.029 and p=0.004, respectively). In the group treated with an association of selenium and ethephon, PDW decreased significantly (p=0.021), when compared to the group treated with selenium alone, but it did not reach the levels in the control group.

<table>
<thead>
<tr>
<th>Group</th>
<th>PLT (x10^9/µL)</th>
<th>MPV (fL)</th>
<th>PDW (fL)</th>
<th>P-LCR (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>min</td>
<td>max</td>
<td>mean</td>
<td>min</td>
</tr>
<tr>
<td>Control</td>
<td>939</td>
<td>1103</td>
<td>1028.66</td>
<td>6.50</td>
</tr>
<tr>
<td>Ethephon</td>
<td>847</td>
<td>1118</td>
<td>999.16</td>
<td>7.10</td>
</tr>
<tr>
<td>Selenium</td>
<td>697</td>
<td>1006</td>
<td>930.83</td>
<td>7.40</td>
</tr>
<tr>
<td>SE+ETH</td>
<td>848</td>
<td>1146</td>
<td>964.85</td>
<td>7.00</td>
</tr>
</tbody>
</table>

PLT = platelet count; MPV = mean platelet volume; PDW = platelet distribution width; P-LCR = platelet large cell ratio;

An increase in the percentage of macrothrombocytes was observed in all groups, in comparison to the Control group, but especially in the group treated with selenium; the increase was statistically significant for all groups, but especially for the group
Experimental evaluation of the action of ethephon and sodium selenite on some hematological parameters in rats

treated with selenium (p >0.05). These results would suggest an influence of selenium at the doses administered to rats on the process of hemostasis, by increasing the percentage of macrotrombocytes.

White blood cells (WBC) are involved in the immune defense processes of the body. Depending on their structure, leukocytes are classified in granulocytes or polymorphonuclear leukocytes (neutrophils, eosinophils and basophils) and agranulocytes or mononuclear leukocytes (lymphocytes and monocytes). Their role is complex and different, depending on their type. In our research, the mean values of total WBC count (tab. III) for the Ethephon and Selenium groups are very near, but slightly decreased in comparison to the Control group (p>0.05 for both groups). Conversely, a statistically insignificant increase was observed in WBC for the Selenium-ethephon group (p>0.05) when compared to the Control, Ethephon and Selenium groups. The mean values for this parameter are suggestive for the intervention of ethephon, but also of selenium (administered as sodium selenite) as xenobiotics, intervention manifest through the alteration of the leukocytic formula.

The results obtained in this experimental study are in contradiction with those reported by El-Okazy (13), who found that WBC showed a significant, dose dependent increase (p <0.01) in the groups treated with ethephon, when compared to the Control group. Pisek et al. (20) confirmed the dose dependent reduction of WBC in laboratory animals after administering sodium selenite, a phenomenon which was also observed during our research. Sadeghian et al. (21) suggested that when administering selenium in supranutritional, but subtoxic doses in sheep, the WBC does not vary or barely increases; the explanation could be the antioxidant properties of selenium and its protective effects towards oxidative stress. Other authors (22) reported that selenium at oral doses of 1 mg/kg bw, administered to mice for 10 days, slightly increased WBC.

Basophils were detected only in the Selenium and in the Selenium+ethephon groups (tab. III), suggesting that their occurrence is due exclusively to selenium. For the groups treated with ethephon, respectively selenium, the mean values are lower than those observed for the Control group (p<0.05). On the other hand, in case of the Selenium+ethephon group, the mean value is approximately 70% higher when compared to the Ethephon group and 64.5% higher when compared to the Selenium group. In both cases, the increase is not significant (p>0.05). These results would suggest that the association of selenium with ethephon tends to normalize the decrease in eosinophils determined by the two substances, administered individually. Our results are in accordance with those reported by Aghwan (23), who highlights the role of selenium, in low doses, in normalizing the hematological parameters in laboratory animals.

Lymphocytes are mononucleate cells with role in antibody systems and immunological reactions. The number of lymphocytes is an easily accessible parameter for examining the potential immunotoxic chemical substances. Within our current research, the mean percentage of lymphocytes (fig. 3) for the groups treated with ethephon, respectively with selenium, are higher than those observed for the Control group. In both cases, the increase is statistically significant (p<0.05). The percentage of lymphocytes for the group treated with ethephon and selenium is lower than that for the Selenium group.
According to a study conducted on mice, Wang et al (24) reported that for the group treated with a dose of 47.7 mg ethephon/kg bw/day, the percentage of lymphocytes from peripheral blood rose by 37% and in the group treated with 143 mg ethephon/kg bw/day, the increase was 54%.

Surprisingly, the percentage of lymphocytes from the total number of white blood cells from blood did not show any difference between the Control group and the groups treated with ethephon. This means that not only the lymphocyte count rose, but also the number of granulocytes. It could be suggested that ethephon may also influence all nucleate cells from blood.

### TABLE III

<table>
<thead>
<tr>
<th>Group</th>
<th>WBC (10^3/µL)</th>
<th>W-LCC (x10^3/µL)</th>
<th>Baso (%)</th>
<th>EO (%)</th>
<th>W-LCR (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Min</td>
<td>max</td>
<td>Mean</td>
<td>min</td>
<td>max</td>
</tr>
<tr>
<td>Control</td>
<td>8.03</td>
<td>8.13</td>
<td>8.07</td>
<td>1.12</td>
<td>1.63</td>
</tr>
<tr>
<td>Ethephon</td>
<td>6.85</td>
<td>8.91</td>
<td>7.82</td>
<td>0.80</td>
<td>1.76</td>
</tr>
<tr>
<td>Selenium</td>
<td>7.86</td>
<td>8.23</td>
<td>8.01</td>
<td>1.15</td>
<td>1.57</td>
</tr>
<tr>
<td>Selenium+ethephon</td>
<td>5.84</td>
<td>10.61</td>
<td>8.40</td>
<td>1.10</td>
<td>2.75</td>
</tr>
</tbody>
</table>

**WBC** = white blood cell (leukocyte) count; **W-LCC** = white large cell count; **Baso** = basophils; **EO** = eosinophils;

Monocytes are white blood cells with the capacity of phagocytosis; they are formed in the bone marrow, they reach the blood stream and invade various tissues during an infectious process. Mean monocyte counts (fig. 4) are practically identical for the Ethephon and Selenium+ethephon groups and slightly decreased compared with the Control or Selenium group (p>0.05 for all groups).

![Fig. 3. Percentages of lymphocytes in the experimental groups](image-url)
CONCLUSIONS
The results of this study highlighted the effects of orally administered ethephon on hematological parameters in rats; the changes in the values of some complete full blood count parameters in the groups treated with ethephon may be suggestive of the harmful effect on consumer health of this plant growth regulator, present as a residue in food products. Selenium, administered orally as sodium selenite, at a dose of 1 mg/kg bw, seems to have acted as a toxic and not as a protective agent during ethephon poisoning.

REFERENCES


