# THE PROPOLIS EXTRACT PROTECTIVE ROLE ON RED BLOOD CELLS ANTIOXIDANT ENZYMES IN CADMIUM INTOXICATED RATS

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**Summary**

The present work deals with the cadmium effect, administrated in a single dose (20ppm/kg b.w.) on propolis hydroalcoholic extract both preventive and treated rats. The following parameters were determined: malonildialdehide (MDA), glutathione (GSH), catalase (CAT), superoxid dismutase (SOD), glutathione peroxidase (GSHpx) and glutathione reductase (GSHred).

The obtained results revealed that the hydroalcoholic propolis extract significantly decreased the lipid peroxidation. The preventive treatment decreased the red blood cells CAT, SOD, GSHpx and GSHred activities after 24 hours from the Cd intoxication. After a further hydroalcoholic propolis extract treatment, an increasing of the studied enzymes activities was observed.

**Key words**: cadmium, propolis, antioxidant enzymes, rats

Cadmium is a metal with a very toxic effect on the living organism. It induce severe modification both in blood and tissues (7,8). After absorption, Cd is transported by γ globulin, red blood cells and metalothioneine (4). Cd is accumulated in the citoplasmatic soluble fraction in the liver, kidney and pancreas homogenates (4). Cd stimulates both the metalothioneins in blood and tissues and the oxygen free radical species (ROS) production (6). So, Cd led to lipid peroxidation and destabilizes the membranes properties.

Cadmium interferes with zinc from the enzymatic systems. Cadmium is a zinc and cooper antagonist. (2, 4)

Vitamin E, vitamin C, selenium, glutathione and a lot of other antioxidants were used to annihilate the toxic effect of Cd.

In our previous researches we demonstrated that the propolis hydro alcoholic extract has antioxidant properties comparable with vitamin C (3,5).

In the present work we studied the protector effect of an hydro alcoholic propolis extract in cadmium intoxication both in pretreated and treated rats. This effect was studied by measuring the lipid peroxidation level (MDA) as well as the activities of some antioxidant enzymes as catalase (CAT), superoxid dismutase (SOD), glutathione peroxidase (GSHpx) and glutathione reductase (GSHred).

## Materials and methods

The experiment was carried on 35 adult Wistar male rats, with a body weight of 220-240 g, one year old, maintained in good physiological conditions. The experiment took place during four weeks and was carried out in two major stages. In the first stage (the preventive one), rats were divided in three groups. Each group included 15 rats and was treated after the following protocol:

L1- control, received 0.5 ml hydroalcoholic solution (the same concentration as those used for the propolis extract); L2 – received 0.5 ml hydroalcoholic propolis extract by gastric tubing; L 3- received 0.5 ml distilled water by gastric tubing.

After two weeks 20 ppm Cd / kg b.w. (as CdCl2) in single doze was administrated by gastric tubing in the L2 and L3 rats,. After 24 hours from the intoxication, 5 rats from each group, were sacrificed. Blood and tissue samples were collected.

In the second stage the same treatment was followed for the L1 and L3 groups and the L2 group was split in another two subgroups: L2a, which received 0.5 ml hydroalcoholic propolis extract till the end of the experiment (the treated group) and L2b which received 0.5 ml distilled water (the untreated group). After two weeks under general narcosis, blood was collected on heparin, by cardiac punction and than sacrificed; tissues were collected.

Catalase (CAT) was determined in whole blood by Sinha colorimetric method. Malondialdehyde (MDA), glutathione were determined from plasma by colorimetric methods and superoxid dismutase (SOD), glutathione peroxidase (GSH-px), glutathione reductase (GSH-red) activities were determined in red blood cells hemolyzates by colorimetric methods (1).

The data are presented as means ± S.D. values. TTest was used to analyze mean differences between experimental groups for each parameter separately and between groups.

## Results and discussions

The results are presented in table 1 and 2 and figure 1-3.

**Table 1**

**MDA average values and oxidoreductase activities in the first stage of the experiment in Cd intoxicated and propolis preventive treated rats**

| Group | MDA µ mol/g | CAT UI | SOD UI | GSHpx UI | GSH red UI | GSH µ mol/g |
|---|---|---|---|---|---|---|
| L1 | 29.82 ± 1.22 | 43.51± 6.32 | 5.53 ± 0.78 | 39.37 ± 2.22 | 8.50 ± 0.39 | 8.10 ± 0.21 |
| L2 | 26.94 ± 1.34 | 35.2 ± 4.14 | 14.79 ± 1.14 | 24.80 ± 2.72 | 6.74 ±0.84 | 15.18 ± 0.95 |
| L3 | 40.11 ± 3.24 | 46.8 ± 7.32 | 20.95 ± 2.33 | 32.09 ± 3.16 | 10.98 ± 1.05 | 10.22± 0.88 |

L1- control group, L2- propolis extract preventive administration and Cd intoxicated group, L3 distilled water and Cd intoxicated

**Table 2**

**MDA average values and oxidoreductase activities in the second stage of the experiment in Cd intoxicated and propolis preventive treated rats**

| Group | MDA µ mol/g | CAT UI | SOD UI | GSHpx UI | GSH red UI | GSH µ mol/g |
|---|---|---|---|---|---|---|
| L1 | 26.06 ± 1.02 | 44.89± 3.21 | 6.43 ± 0.13 | 33.19 ± 2.16 | 7.61 ± 0.32 | 7.84 ± 0.11 |
| L2a | 23.14 ± 0.64 | 31.89 ± 3.05 | 19.47 ± 1.03 | 20.29 ± 2.43 | 4.67 ±0.49 | 12.12 ± 0.31 |
| L2b | 27.18± 2.10 | 19.09 ±2.04 | 15.41 ± 1.09 | 24.32 ±2.23 | 5.85 ± 0.73 | 13.55± 1.07 |
| L3 | 43.11 ± 1.25 | 26.36 ± 1.18 | 21.98 ± 1.11 | 29.66 ± 2.72 | 8.32 ± 0.85 | 5.55 ± 0.13 |

In the prevention stage, in the L3-untreated and Cd intoxicated group MDA average values registered the highest values as the other two groups as follows: 1.3 times higher as L1, respectively 1.48 times higher as L2 and at L2 (2 weeks propolis pretreated group) were the lowest. This values indicated the increasing of the lipid peroxidation in the Cd subcronic intoxicated group. As at L2 (propolis pretreated group) the MDA values were the smallest, we concluded that the propolis hydroalcoholic extract has antioxidant properties. Similar values were obtained by another authors (2,6).

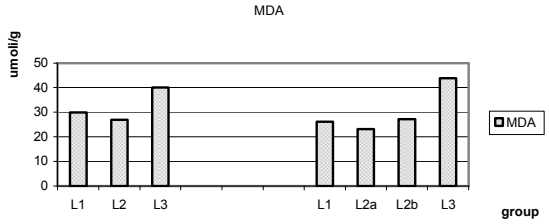


Figure 1. The MDA average values in the first and the second stage of the experiment

The catalase activity (which decompose de hydrogen peroxide) and the SOD activities (which transforms the superoxid anion in hydrogen peroxide) are presented in figure 2.

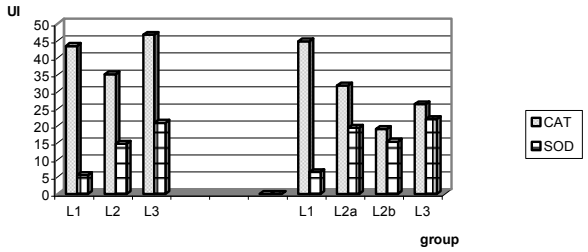


Figure 2. The CAT and SOD activities values in the first and the second stage of the experiment

At the L2 group the CAT and SOD activities values were lower as the ROS were scavenged by the propolis extract. Similar results were obtained by other authors (2,3).

The GSHpx is strongly related with the red blood cells GSH amount. The lowest activities were registered for GSHpx and GSHred at L2 (propolis pretreated) and the higest values were registered at L3 (similar with CAT and SOD activities).

The GSHred activity is dependent on $NADPH+H^+$ amount. $NADPH+H^+$ is a coenzyme synthesized in the pentozophosphates cycle.

In the second stage of the experiment, the MDA average values at L2a (propolis treated group after Cd intoxication) were with 15 % lower as at the untreated group L2b ( after Cd intoxication).

The CAT, SOD, GSHpx and GSHred at L1 and L3 groups registered similar activities values in the second stage of the experiment as in the first one but there were observed some interactions between some of the extract components and the glutathion SH groups. The GSH average values were slightly higher at the untreated group as at the treated one.

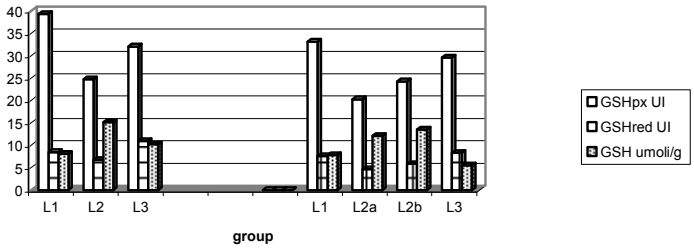


Figure 3. The GSHpx, GSH red activities values and GSH average values in the first and the second stage of the experiment

The enzymes activities registered high variations between the untreated and the treated groups

The GSHred average values were, at the treated group, 1.25 times lower as at the untreated one.

## Conclusions

1. The MDA average values increased in Cd administration; in the same time, the red blood cells antioxidant enzymes activities were influenced by either the Cd or the propolis extract administration. The propolis extract diminished the lipid peroxidation.
2. The propolis extract was more efficient in prevention as in treatment.
3. The CAT and SOD activities were higher at the intoxicated and treated group as the intoxicated and untreated group.
4. The GSH px and GSH red activities were lower at the intoxicated and treated group as the intoxicated and untreated one.

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