# PROTECTIVE EFFECT OF BENTONITE ON OXIDATIVE STRESS INDUCED BY PARAQUAT

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## ABSTRACT

The protective effect of bentonite on oxidative stress induced by paraquat (PQ) in male Wistar rats was studied. There were five groups, each consisting of 8 animals. The substances were administered by gastric gavage. The first group received PQ only. The second and the third ones were given natural and synthetic bentonite, respectively, an hour after the administration of PQ; the fifth group remained untreated. The animals were treated on a daily basis throughout 8 consecutive days. Blood samples were taken on days 0, 1, 4, 6 and 8.

The oxidative stress was assessed via the activity of catalase (CAT) and the quantity of malondialdehyde (MDA). Oxidative stress was significant in the first and 192th hour following the first application of PQ. The protective effects of both natural and synthetic bentonite in oxidative stress were noticeable as soon as 24 h after the first treatment and lasted up to 144 h. Nevertheless, both absorbents developed a significant protective effect (approximately 50 %) 1 h and 192 h after the first application of PQ, indicating that these protectors act as molecular sieves and thus suppress the process of lipid peroxidation and the development of more intensive oxidative stress.

Keywords: bentonite, oxidative stress, paraquat poisoning, molecular sieves

## INTRODUCTION

Paraquat is a highly toxic, non-selective herbicide. Since there are no known pharmacological antagonists of paraquat (PQ), the management of poisoning has been directed towards the modification of its absorption or to decreasing the oxidant-induced cellular damage [1], or by administration of symptomatic antiinflamatory therapy, lysine acetylsalicylate [2]. The high toxicity of PQ is achieved by damaging the cell integrity due to highly reactive free radicals which emerge from redox cycling in the presence of molecular oxygen.

The aim of the current research was to determinate the influence of natural and synthetic bentonite as adsorbents in rats poisoned with PQ. Another aim of this study was the to get insight into the mechanism of toxicity of PQ after prolonged peroral (p.o.) administration of 25mg/kg. It was to be achieved by estimation of parameters of oxidative stress, *id est* the activity of antioxidative enzyme catalase (CAT) and the level of lipid peroxidation via malondialdehyde (MDA). In addition to this, the research on the protective effect of natural and synthetic bentonite in the oxidative stress provoked by PQ was to be done.

## EXPERIMENTAL

The investigation was performed on 32 adult male Wistar rats (220-230 g body weight, bw) divided into 3 experimental and a control group. Animals in experimental groups (I to III) were treated by gastric gavage, with PQ («Gramoxone», Syngenta Crop Protection), in the dose of 25 mg/kg b.w. The first experimental group received PQ only, the second was treated with 40 % water suspension of natural bentonite (ITNMS, Belgrade) in the volume of 10

ml/kg b.w. in the same manner, an hour after the administration of PQ, whilst the third one was treated with the same volume of synthetic bentonite (ITNMS, Belgrade). The fourth group was the control one.

All the three experimental groups were treated once a day, throughout 8 consecutive days. Blood was collected 1, 24, 96, 144 and 192 h after the beginning of the treatment and treated with an anticoagulant (sodium citrate 3.8 % w/v).

The CAT activity was estimated by the method of Beutler [3].

The level of lipid peroxidation (LP) was assayed through the concentration of thiobarbituric acid as the reactive substance (TBARS) in the red blood cells according to Uchiyama and Mihara [4]. The haemoglobin concentration was determined by the cyanmethemoglobin method [5].

## RESULTS AND DISCUSSION

In the current investigations a positive correlation between the changes due to oxidative stress provoked by PQ (i.e. between the activity of CAT, antioxidative defense enzyme, and the intensity of LP estimated through MDA concentration) was observed after 1 hour and 192 hours after the beginning of its application in all the groups treated.

The CAT activity increased, as well as the concentrations of $H_2O_2$ (Table 1).

Table 1. CAT activity in rats poisoned with PQ (mmol $H_2O_2$/min/g Hb)

| Hours after the beginning of intoxications | 1 | 24 | 96 | 144 | 192 |
|---|---|---|---|---|---|
| Paraquat | 68.7 | 46.8 | 65.6 | 43.4 | 68.5 |
| Natural bentonite | 42.2 | 48.6 | 54.2 | 40.2 | 32.7 |
| Synthetic bentonite | 19.0 | 48.8 | 60.8 | 41.3 | 36.4 |
| Control | 26.4 | 28.3 | 27.5 | 28.6 | 28.3 |

A statistically significant increase in CAT activity was observed 1 h, 96 h and 192 h (Table 1) after the beginning the poisoning with PQ, compared with the activity of the enzyme 24 h (31.88 %) and 144 h (36.83 %) after the beginning of the intoxication.

Natural and synthetic bentonite administered p.o. in doses 4 g/kg b.w. significantly decreased the activity of CAT in first and 192th hour after the beginning of poisoning, synthetic bentonite being more efficacious. An hour after the administration of PQ synthetic bentonite reduced the CAT activity by 72.34 % in comparison with the activity in animals treated only with PQ, whilst the efficacy of natural bentonite was approximately half as high (36%, Table 1). Both adsorbents tested decreased the CAT activity with similar efficacy 192 h after the beginning of poisoning, the natural bentonite causing the decrease of 52.40 % and the synthetic 47.02 % (Table 1). However, neither natural nor synthetic bentonite influenced the CAT activity importantly 24, 96 and 144 h after the beginning of PQ application (Table 1).

Paraquat induces LP in erythrocytes producing various concentrations of MDA over time. At the beginning of intoxication (1 and 24 h after the poisoning began) MDA concentrations rose, then declined and finally increased in the terminal phase of intoxication (Table 2).

Table 2. MDA concentrations in rats poisoned with PQ (mmol MDA/g Hb)

| Hours after the beginning of intoxications | 1 | 24 | 96 | 144 | 192 |
|---|---|---|---|---|---|
| Paraquat | 42.3 | 59.2 | 31.4 | 34.7 | 48.6 |
| Natural bentonite | 19.2 | 50.1 | 29.2 | 41.5 | 19.7 |
| Synthetic bentonite | 23.4 | 46.3 | 28.1 | 38.5 | 20.3 |
| Control | 11.3 | 11.0 | 12.4 | 10.8 | 11.1 |

The adsorptive capacity of bentonite towards PQ is proved and is approximately 5 % [6]. However, its adsorptive capacity is not sufficiently protective in PQ poisoning, thus additional therapy including active coal perfusion and/or symptomatic NSAIDs therapy is necessary [2, 7, 8]. According to voluminous research and data it is supposed that the toxic effect of PQ happens on molecular level and is a result of free radical production in redox reactions [9,10]. The aim of the current investigation was the testing of the adsorbent-protective effect of bentonite in oxidative stress due to PQ.

In the current research the activity of CAT in PQ intoxication accompanies the changes of the concentration of hydrogen peroxide. Statistically significant decrease in CAT activity was detected 24h (31.88 %) and 144 h (36.83 %) after the beginning of PQ intoxication. The enzyme was possibly in some way inhibited or its capacity may have been significantly exhausted due to intensive elimination of $H_2O_2$ synthesized as a result of conjoined activity of $PQ^{\cdot +}$ and $O_2^{\cdot -}$, in order to maintain the homeostatic mechanisms in cells after intoxication with PQ. The exhaustion of the enzymes involved in antioxidative protection (SOD, CAT, GR, GPx, etc.) that sequester the reactive products, as well as the decrease in NADPH and glutation (electron donor), disturb the redox state in cells and promotes oxidative stress (OS) [11, 12]. OS due to continuous $O_2^{\cdot -}$ production in redox reactions developed 192 h after the beginning of intoxication, which was confirmed by the increased activity of CAT (68.50 mmol/min/g Hb, Table 1). The processes happening during PQ redox cycling are irreversible and lead to cell death [9].

With/without enzyme interaction, in the presence of transition metals (Fe or Cu), superoxid anion radical $O_2^{\cdot -}$ transforms into hydrogen peroxide ($H_2O_2$), molecule with pronounced oxidative properties. The product of homolytic break of unstable connection in $H_2O_2$ (HO-OH) is the hydroxil radical ($^{\bullet}HO$), the most potent reactive radical, that instantly react with numerous cell macromolecules, including lipids, nuclein acids and proteins [13, 14].

In the present investigations, 1h after the beginning of intoxication with PQ, the process of LP was statistically significant ($p<0.001$). The increase in LP at the beginning of intoxication was an expected indicator of activated aggressive, oxidative mechanisms (15). In the following 96 h the concentration of MDA, the parameter that describes the intensity of lipid peroxidation, decreased insignificantly in comparison with the untreated animals, which was obviously the consequence of the activation of antioxidative system.

In the present research, 1 h after the application, natural and synthetic bentonite decreased the activity of CAT for 36 % and 72.34 %, respectively, while their influence 192 h after administration was quite similar (52.4 % and 47.02 %, respectively). In addition, both bentonites decreased LP in the first hour from the beginning of poisoning for 54.61 % (natural) and 44.68 % (synthetic bentonite). On the other hand, 192 h from the beginning of the intoxication the difference in their adsorbent efficacy was almost invisible (natural bentonite=59.47 % and synthetic=58.23 %).

By comparison of the relations CAT/LP in the group administered PQ only and the control group, it was remarkable that the relation in the former group (CAT/LP=0.78) 24 h after the beginning of intoxication was 3.37 times lower than in the latter (CAT/LP=2.63); this indicates the more intensive process of lipid peroxidation. This proves our hypothesis that PQ induces the synthesis of peroxide that is not degraded by CAT, but that it cooperates with superoxide anionic radical ($O_2 \cdot^-$) leading to intensifying of the process of LP and thus to synthesis of larger quantities of MDA.

## CONCLUSION

Paraquat provokes oxidative stress that is considerable 1 h and 192 h after the beginning of intoxication.

Both adsorbents expressed significant protective effects (approximately 50 %) 1 h and 192 h after the beginning of PQ intoxication, which indicates that both natural and synthetic bentonite acted as molecular sieves and thus suppressed the process LP and the development of more intensive oxidative stress.

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