# Preliminary Data Regarding Decreased Catalase Specific Activity in the Tears as a Result of Environmental Stress

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*Oxidative stress represents the imbalance between the production of reactive oxygen species and the organism's capacity to produce antioxidants. This phenomenon has captured lately a lot of attention, with an additional increased interest being manifested towards the relationship between psychological stress and oxidative stress. In the present study we decided to observe the changes which occur in stress environmental conditions applied to rats subjected to swimming and treadmill exercises, by focusing on a preliminary determination of (CAT) specific activity, an enzyme known to catalyse the decomposition of hydrogen peroxide into water and oxygen, and a valuable antioxidant protector, with possible implications into the dry eye pathology. Our results could suggest a possible dry eye animal model induced through stress and a possible implication of the oxidative stress markers in the occurrence of this ocular pathology, as suggested by the significant decrease in the CAT activity registered in rat tears collected after the application of environmental stressors (e.g. swimming and running) versus the control group.*

*Keywords: catalase; rat animal models; stress; oxidative stress; environmental stress*

Oxidative stress represents the imbalance between the production of reactive oxygen species (ROS) and the organism's capacity to produce antioxidants [1].

This phenomenon has captured lately a lot of attention, with an additional increased interest being manifested towards the relationship between psychological stress and oxidative stress.

Moreover, several researches pointed out a strong link between psychological stress and various pathologies such as carcinogenesis [2], psychiatric diseases [3], atherosclerosis [4], autoimmune disorders [5] and even ophthalmology related pathologies [6].

Even more, aside from the psychological stress, it has been demonstrated that physical exercise practiced in an extended, intense manner can also cause an increase on the levels of oxidative stress [3, 7, 8], although there are other studies that showed the presence of an increase in the antioxidant activity if the organism is able to adapt to the physical exercises it performs [9], thus reducing oxidative stress and inflammation even in old rats [10].

Therefore, this variety of results has led to serious debates in regard to the physical activity that modifies oxidative stress. In this way, studies researching the levels of glutathione (GSH- an important antioxidant) in rats assigned to swimming tasks reported reduced levels [11], whereas others researches analysing a different type of psychical exercise demonstrated an increase in the GSH serum levels [12, 13].

Another representative indicator of oxidative stress is superoxide dismutase (SOD) enzyme being among the first to oppose ROS, which was observed to express either increased activity during physical activity, proof of oxidative stress counteraction, or decreased activity, indicative of oxidative stress development. Glutathione peroxidase (GPX) and catalase (CAT), enzymes involved in counteracting oxidative stress, reacts in an analogous way, sometimes presenting decreased values, sign of oxidative stress, while other times increased levels were reported [9, 14-16].

Lately there is an ascending trend in trying to understand the differences between various types of physical exercises, such as swimming, running on a treadmill and the changes that follow them in oxidative stress rates.

In this context, part our group has also previously researched the effects of short-time exercises in rats running on a treadmill. In this way, it was shown that increased oxidative stress levels were recorded in the rats serum [17]. Also, in another report where untrained human subjects were implicated in bicycle exercise training, it was observed that those who were previously administered vitamin C had reduced oxidative stress, in comparison with those who did not receive this antioxidant [18].

An increased attention was directed towards the connections between oxidative changes and ophthalmological pathology, such as glaucoma [19] or cataracts [20]. Our group previously studied the influence of oxidative stress balance on keratoconus, proving the relevance of the oxidative stress status modifications in various ocular conditions [21].

Another pathological ocular condition where oxidative stress presents alterations is the dry eye syndrome [6]. This affection is defined as an illness of tears and ocular surface of multifactorial provenience, which leads to manifestations of discomfort and visual perturbance associated with instability, increased osmolarity of tear film and inflammation [22]. This could be relevant especially in the context regarding the relationship between oxidative stress, inflammation and dry eye pathology.

In the present study we decided to observe the changes which occur in stress environmental conditions applied to rats subjected to swimming and treadmill exercises, by

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focusing on a preliminary determination of CAT specific activity, an enzyme known to catalyse the decomposition of hydrogen peroxide into water and oxygen, and a valuable antioxidant protector, with possible implications into the dry eye pathology.

## Experimental part

*Materials and methods*

Animals

In this experiment we used 21 adult Wistar rats acquired from the Victor Babes National Institute of Research and Development, Bucharest, Romania. At the beginning of the experiments their weights ranged between 250g and 300g. The animals' weight was monitored from the day of their arrival in the experiment room and at 2 day intervals. The rats were housed in a room with controlled temperature and humidity, daily monitored, at circadian cycle of 12h light/12h darkness (7:00-19.00), with free access to water and food. All the animals involved in the experiment were treated according to the existing guidelines of animal bioethics stated in the Act on Animal Experimentation and Animal Health and Welfare Act from Romania. The procedures applied were in conformity with the European Communities Council Directive of 24 November 1986 (86/609/EEC). Efforts were made to minimize animal suffering and to decrease the number of animals included. The Ethics Committee of Grigore T.Popa University of Medicine and Pharmacy approved the current study.

All animals (n=21) were placed in the described environment 5 days before the experiments, in order the animals to adapt to the environment.

On the 5$^{th}$ day, all rats were tested on the treadmill at a speed of 1km/h for 3 min. After that, we chose those with the highest running potential and labelled them group 1, the others being distributed to groups 2 and 3. The groups were selected as follows: group 1 (7 rats): runners; *group 2* (7 rats): swimmers; group 3 (7 rats): control group.

On the 8$^{th}$ day, the animals were injected subcutaneously with 2 ml/kg pilocarpine 1% for stimulating lacrimal glands secretion. Lacrimal secretion was collected using human tears' Schirmmer tests that were adapted by being cut to ¼ of standard sizes. This method is non-invasive and painless for tears collection (no need of prior anaesthesia).

From day 9, the rats were trained to getting used to the physical activity they had to expand. The training lasted 5 days. Rats from group 1, at the beginning of the experiment were running constantly with a speed of 2 km/h and during the experiment the speed was progressively rised to 4 km/h for 15 min. The second group swam in a custom made rat pool at a constant temperature of 34ºC, for avoiding additional heat stress, at the beginning for 5 min, increasing the time progressively to 45 min. Swimming as exercise was chosen because it has much lower risks of causing damage to the limbs of rats and is considered less traumatic.

At the end of the fifth week, after the physical exercise, the rats were subcutaneously injected with 2mL/kg pilocarpine 1%, for tears secretion stimulation and the tears were collected using the technique described above and immediately frozen at -40°C. Animals were sacrificed by intraperitoneal injection of ketamine and xylazine 1:1 (ketamine 100mg/kg, xylazine 10mg/kg). Euthanasia was practiced in a special necropsy room, one animal at a time, because vocalizing, sometimes in a wide frequency, imperceptible to humans, or the release of certain pheromones, could cause agitation among the other animals.

Biochemical estimations

The catalase (CAT) enzyme activity was measured by employing colorimetric method according to Sinha [23].

Results were statistically analysed using one-way analysis of variance (ANOVA). All of the results are described as mean ± SEM. Statistically significant outcome was considered to be at $p<0.05$.

## Results and discussions

Preliminary measurement procedures of the CAT levels registered in rat tears, collected after the application of environmental stressors (e.g. swimming and running), indicated a statistically significant decrease in CAT enzyme specific activity in rats subjected to both running and swimming tasks ($p<0.05$) (fig. 1).

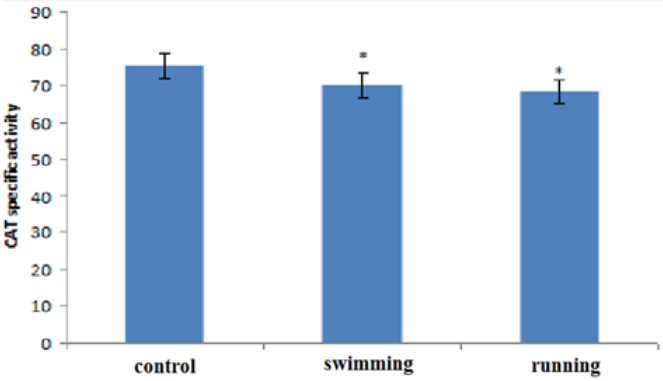


Fig. 1. The effects of physical training vs. control on CAT specific activity from rat tears (n=21). The values are mean ± SEM (n=7 animals per group). *p< 0.05 vs. control group

These results are suggesting an increased oxidative stress rate, considering that CAT is a representative element of the antioxidant system.

Considering our results, presently we confirmed an increase of the oxidative stress levels in stressed rats through running and swimming tasks, indicated by the decreased levels of CAT enzyme found in tears of the stress rats vs. the controls.

As previously mentioned, it is considered that psychological stress along with physical stress either induced through exercises or other methods, increases oxidative stress, although it must not be omitted that oxidative stress is not every time a bad outcome, but a necessary process to occur during physiological function of the body. For instance, it is involved in the destruction of invading pathogens through the production of increased levels of reactive oxygen species, which also react to adjusting effervescent inflammatory responses generated in specific circumstances [24].

However, the damage develops when the balance between the production of reactive oxygen species (ROS) and antioxidant system's actions is altered [25, 26]. Thus, even if ROS production in beneficial in fighting infection, exaggerated presence of ROS contributes to the development of DNA damage causing mutations or gene expression alterations [27] and also affect cell membranes [28] through a cascade of damaging chemical reactions.

In this way, a suspected cause of the imbalance between pro-oxidants and antioxidants among many others is psychological stress [29, 30]. For instance in a mouse animal model of psychological stress generated through cutting the whiskers, a crucial locomotive sensor in mice [31] in the absence of which hyper locomotion anxious behaviour manifests, a significant increase in the tissue thiobarbituric acid reactive substance (TBARS) levels - indirect indicator of lipid peroxidation and protein carbonyl, a marker of protein oxidation- was recorded [32].

Therefore, the results of Wang's study states that high levels of oxidative stress are reported due to a psychological stressor occurrence, accentuating the possible connection between oxidative stress and psychological distress.

Moreover, physical stress is another element that induces oxidative damage, while swimming [33] along with running [34, 35] represent stress factors in rats, as proved by the presence of elevated specific stress indicators, such as plasma glucocorticoids and catecholamines. In this way, Brant and his colleagues also demonstrated in their study that running decreases the plasma rates of vitamin A, a known antioxidant factor [36], which might indicate the presence of oxidative stress. On the contrary, there are reports where an increased antioxidant activity is registered as a mechanism of counterbalance oxidative stress [9, 37], an increased level of CAT being recorded as opposed to our obtained results. However, as earlier remarked, the existing information regarding oxidative stress markers and antioxidants is still extremely controversial in the present literature.

To this extent, as mentioned in the beginning, when following SOD values in physical exercise conditions, it was observed an intensified SOD activity [38, 39]. Nonetheless, following the same parameter in other experiments designed somewhat resembling the findings above, SOD activity differed, being reported as unaffected in rats acute and chronic workout [37]. Even more, GPX presents with a similar situation either encountering high levels in rats restrained to exhaustive treadmill running [40] or recording no difference in serum GPX activity in the context of acute, extended, exercise of mild intensity in human subjects [41].

In this way, a possible explanation behind these variations of results could be the background in physical training of the tested subjects. For instance, Alessio's group indicated an elevated lipid peroxidation level in the sedentary group put to exercise, while no changes were observed in the lipid peroxidation rates in the trained group after subjection to exercise [37].

In the case of ocular disorders, lately oxidative stress has been shown to possibly have an import role. As already remarked, there are literature findings that point oxidative stress as a determinant factor in the occurrence of ophthalmological pathologies such as photokeratitis [42], cataract [43], glaucoma and macular degeneration [44, 45] or dry eye [46].

These data could be relevant in the ophthalmological context knowing that our research group is currently working on new determinations of several other oxidative stress markers in tears of stressed rats, such as SOD, GPX and malondialdehyde (MDA), but also in studying lacrimal glands, which might confirm that this would prove to be a valid dry eye animal model. In this way, these studies could have a future implication in the management of this disease, maybe through administering different types of either antioxidants or anti-inflammatory agents.

On this context we should mention that this is the first time to our best knowledge when CAT and dry eye are conjoined although there is still need of the imuno-histochemical analysis for solid proof in this matter.

In conclusion, we could state that since we observed decreased CAT levels in rat tears following physical training, a possible relevance of this stress animal model in the context of dry eye pathology might be possible.

## Conclusions

This preliminary study of a possible new dry eye animal model induced through stress indicates the existence of a possible implication of the oxidative stress markers in the occurrence of ocular pathology, by a significant decrease in the CAT levels, an important antioxidant enzyme.

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