

## Blood Glutathione Peroxidase Activity in Patients with Osteoarthritis Treated with Oxicams

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**ABSTRACT:** *Purpose.* The main objective of this study was the comparison of the influence for three non-steroidal anti-inflammatory drugs (NSAIDs) belonging to the oxicam class, namely piroxicam and tenoxicam, as non-selective inhibitors of cyclooxygenase (COX), and meloxicam, a selective COX-2 inhibitor, on glutathione peroxidase (GPx) activity in patients with osteoarthritis of the knee. *Material/Methods.* Thirty adult subjects clinically and radiographically diagnosed with knee osteoarthritis, who were not previously subjected to any treatment, were enrolled. They were divided in three groups, each with ten subjects. The serum levels of GPx were assessed at baseline and after twenty days of treatment. The first group received piroxicam at a dose of 20 mg orally daily, the second group was treated with tenoxicam at a dose of 20 mg orally daily, and in the third group meloxicam was administered in a dose of 15 mg orally daily. *Results.* After the treatment, it was observed an increase of the GPx activity in all groups. The group treated with meloxicam presented the highest rise in the GPx level ( $p = 0.052$ ). *Conclusions.* The 20 days study concerning the effects of treatment with NSAIDs belonging to the oxicam class in subjects with knee osteoarthritis revealed that piroxicam, tenoxicam and meloxicam determined a slightly increase in the GPx activity, although this rise had no statistical significance.

**KEYWORDS:** oxicam, NSAIDs, glutathione peroxidase, oxidative stress, osteoarthritis

### Introduction

Osteoarthritis is defined as a heterogeneous group of disorders, being characterized by joint symptoms, associated with alterations in the integrity of the cartilage, subchondral bone and periarticular structures [1]. Osteoarthritis cartilage undergoes changes of normal bone turnover due to an imbalance between anabolic and catabolic processes [2].

Oxidative stress is involved in many diseases, namely: arthritis, atherosclerosis, cancer, diabetes, hypertension, through different mechanisms [3]. The term of “reactive oxygen species” (ROS) is usually used to refer both to free radicals, like superoxide radical anion ( $O_2^{\cdot-}$ ), hydroxyl radical ( $HO^{\cdot}$ ), peroxy radical ( $ROO^{\cdot}$ ), nitric oxide ( $NO^{\cdot}$ ), and to non-radicals, like hydrogen peroxide ( $H_2O_2$ ), peroxynitrite ( $ONOO^-$ ), singlet oxygen ( $^1O_2$ ), hypochlorous acid ( $HOCl$ ), ozone ( $O_3$ ), which are able to generate free radicals by different reactions [4].

ROS are involved into osteoarthritis pathogenesis. The level of oxidative stress markers presents an increase in patients with osteoarthritis, as compared to healthy persons [5,6].

According to OARSI (Osteoarthritis Research Society International)

recommendations, the selective and non-selective inhibitors of cyclooxygenase (COX) are included into pharmacological treatment strategies for osteoarthritis [7]. Non-steroidal anti-inflammatory drugs (NSAIDs) have their influence on the prostaglandins synthesis by inhibiting the COX, COX-1 and/or COX-2 isoforms. The anti-inflammatory action mechanism of NSAIDs is not yet completely understood. Besides the COX inhibitory effect, different NSAIDs have possible other additionally action mechanisms. NSAIDs influence the oxidative stress, some of them decreasing, other increasing the reactive species production [8].

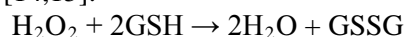
In osteoarthritis, non-steroidal anti-inflammatory drugs are demonstrated to act through inhibition of prostaglandin synthesis by blocking COX activity – both COX-1 and COX-2. Nevertheless, part of their mechanisms of action may be related to reduction of superoxide and free radical generation [9], prevention of the formation and release of free radicals and degradative enzyme of synovial macrophages and polymorphonuclear leukocytes, influencing the mobility, chemotaxis and aggregation of neutrophils and macrophages, but also through direct neutralization of free radicals [10,11].

According to these considerations numerous studies have been conducted and they have shown the ability of NSAIDs to interfere with oxidative stress in various diseases [12,13].

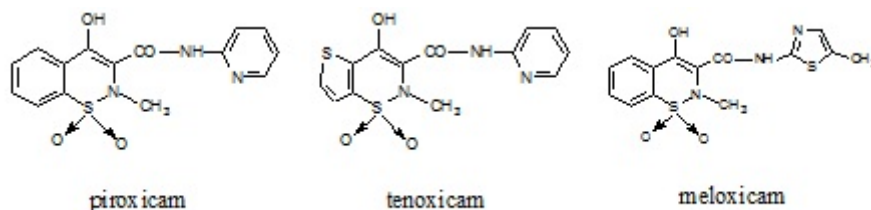
Hydrogen peroxide ( $\text{H}_2\text{O}_2$ ) may interfere with some cellular systems capable to produce energy. For example, it can inactivate glyceraldehyde-3-phosphate dehydrogenase or can form  $\text{HO}^\bullet$  in the presence of transitional metals ions [4].

GPx is a member of the antioxidant systems. It catalyzes the reduction of hydrogen peroxide

and organic hydroperoxides using glutathione (GSH), resulting  $\text{H}_2\text{O}$  and oxidized glutathione (GSSG) [14,15]:



Oxicams are a class of non-steroidal anti-inflammatory drugs. These compounds comprise an enolic acid structure. They can act as non-selective inhibitors of COX, like: piroxicam, tenoxicam, lornoxicam, and as selective inhibitors of COX-2, like meloxicam (Fig1) [16].



**Fig.1. Chemical structures of piroxicam, tenoxicam, and meloxicam.**

## Material and methods

**Subjects and sample collection.** Thirty subjects, having signed the informed consent, with knee osteoarthritis were recruited from the Medical Clinic No. 1, Emergency County Hospital Craiova. Distribution of subjects in groups was realized according to a study protocol, approved by the Ethics Committee of University of Medicine and Pharmacy of Craiova, and to well defined inclusion and exclusion criteria. Ten subjects with a mean age of  $52.8 \pm 4.23$  years were treated with piroxicam, 20 mg p.o. daily. The second group of ten subjects, mean age of  $54.2 \pm 2.15$  years, was treated with tenoxicam, 20 mg p.o. daily. The third group of subjects with the mean age of  $59.9 \pm 9.14$  years was treated with meloxicam, 15 mg p.o. daily. Blood GPx activities were assessed at baseline and after 20 days of treatment. Venous blood samples were collected *à jeun*, processed to obtain plasma and the supernatant was frozen until use for analysis.

**Determination of glutathione peroxidase activity.** GPx activity was determined using a Randox Laboratories kit, and was based on the following principle: GPx catalyzes the oxidation of glutathione by cumene hydroperoxide. In the presence of glutathione reductase and nicotinamide adenine dinucleotide phosphate (NADPH), the oxidized glutathione is

immediately converted to its reduced form with a concomitant oxidation of NADPH to  $\text{NADP}^+$ . The decrease in the absorbance was measured at 340 nm. GPx activity was expressed as U/l. Measurements were performed using a Beckman type UV-VIS spectrophotometer, DU-65 model.

**Statistical analysis.** Statistics Package for Social Sciences (SPSS) was used for the statistical analysis. Results were expressed as mean  $\pm$  SD (standard deviation). Changes observed before and after treatment were assessed by the paired sample *t* test. A *p*-value less than 0.05 was considered statistically significant.

## Results

All subjects completed the study. The baseline GPx activity in piroxicam treated group was slightly higher than in the other two groups (Table 1).

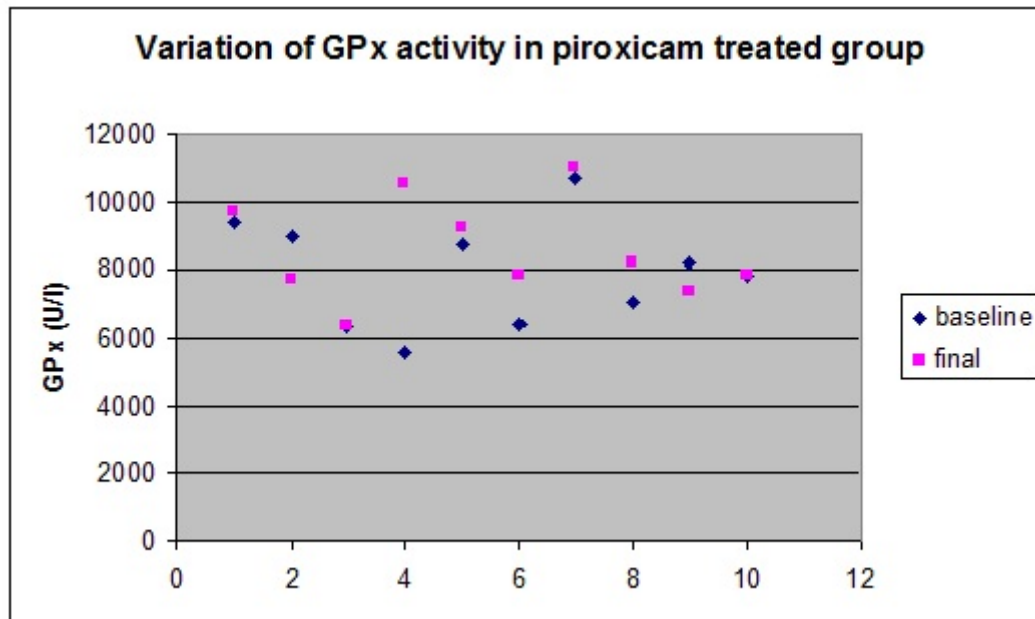
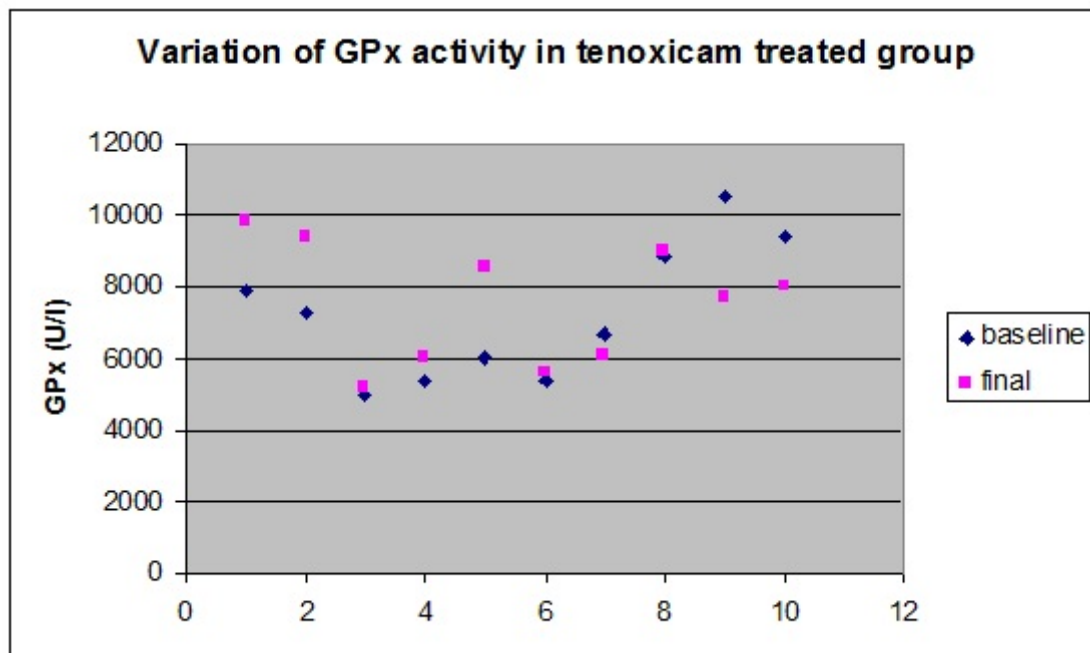
The variation of the GPx activity for each subject at baseline and at the end of treatment with piroxicam, tenoxicam and meloxicam, respectively, were presented in Fig.2, 3 and 4, respectively.

The results showed that GPx activity increased in all three groups of subjects (Fig.5), but none of these three drugs had a statistically significant influence on the GPx activity (Table 1).

**Table 1. Glutathione peroxidase (GPx) activity in the groups of study.**

Oxicam-treated group	Age (mean±SD)	Baseline GPx activity (U/l) (mean ±SD)	Final GPx activity (U/l) (mean ±SD)	<i>p</i> value
Piroxicam	52.8±4.23	7920±1600.3	8588±1494.6	0.25
Tenoxicam	54.2±2.15	7239±1907.7	7532±1683.3	0.58
Meloxicam	59.9±9.14	6662±1772.2	8272±2744.4	0.052

*p* values represent baseline versus final measurements in each group (*paired t test*)

**Fig.2. Variations of the GPx activity in the group of subjects treated with piroxicam.****Fig.3. Variations of the GPx activity in the group of subjects treated with tenoxicam.**

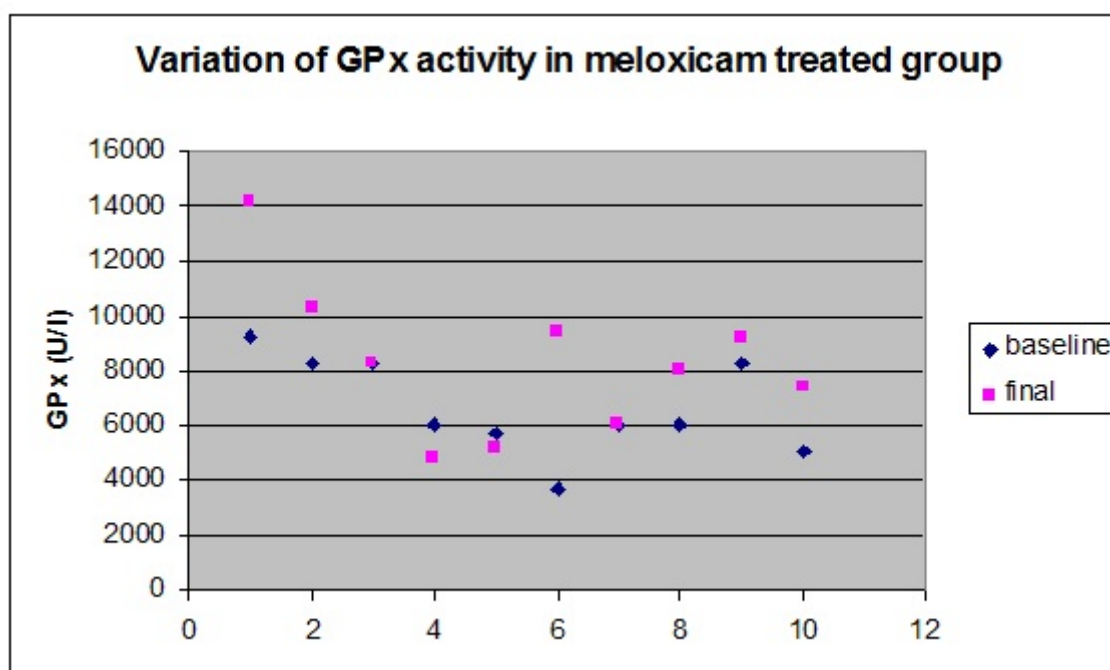


Fig.4. Variations of the GPx activity in the group of subjects treated with meloxicam.

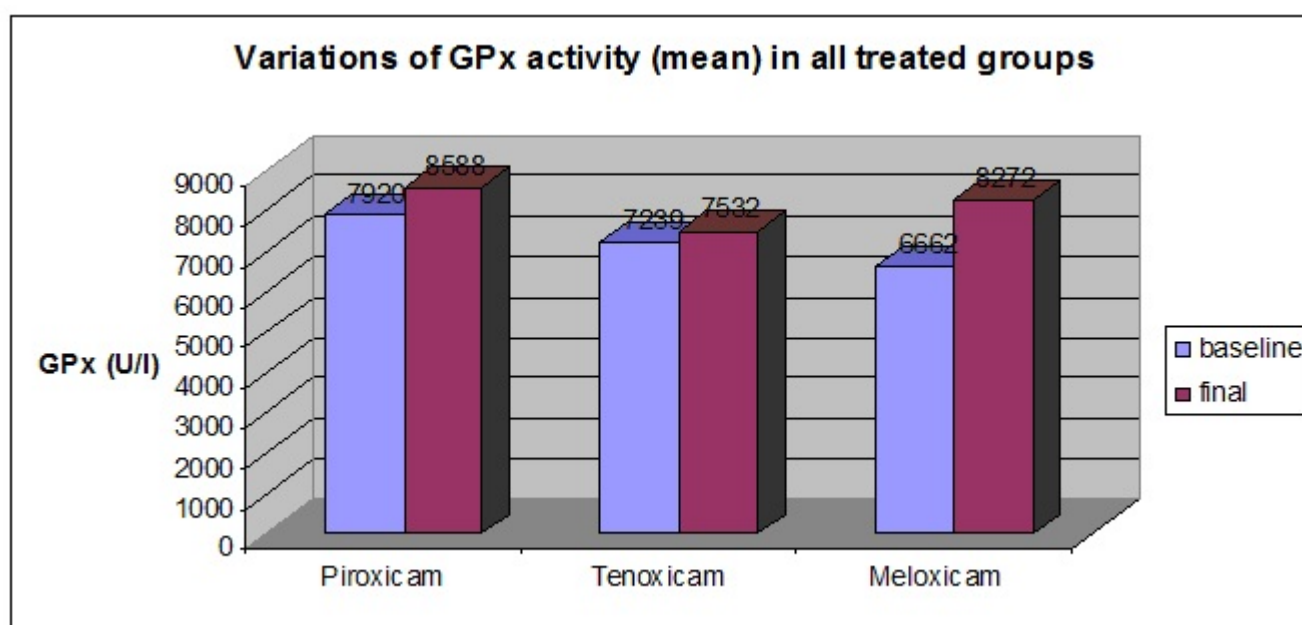


Fig.5. Variations of the GPx activity (mean) in piroxicam, tenoxicam, and meloxicam treated groups.

## Discussion

The conducted studies regarding the influence of NSAIDs on GPx are rather few, and the activity of this enzyme has registered insignificant variations [17,18].

The tenoxicam activity of neutralization for hydroxyl, superoxide, and peroxy radicals, which are involved into inflammatory reactions, was tested *in vitro* in many types of non-cellular systems. This drug has demonstrated its property

of scavenging the hydroxyl radical and the superoxide *in vitro* [19]. Orhan and Sahin found that piroxicam, ketorolac, naproxen and tiaprofenic acid determine a significant inhibition of the glutathione peroxidase from human erythrocytes *in vitro* [20]. Van Antwerpen and Nève have studied *in vitro* scavenging effect of ROS for piroxicam, tenoxicam, lornoxicam, meloxicam, nimesulid and ibuprofen. They discovered that piroxicam has an antioxidant activity higher than

tenoxicam, ibuprofen and nimesulid [21]. Agha et al. observed an increased of the GPx plasmatic activity, in rats with experimental induced arthritis [22]. Bartosiewicz et al. realized that piroxicam produces significant changes of the GPx activity in patients with rheumatoid arthritis [23]. In a clinical study realized on patients with osteoarthritis it was observed that piroxicam determined a significant increase in superoxide dismutase activity [24], celecoxibe induced a decrease in the GPx activity, while tenoxicam determined an increase in the GPx activity, with no significance from the statistical point of view [25].

In this present clinical study, the values of GPx presented a slightly increase in all groups of subjects. From all oxicams, meloxicam had the highest influence ( $p = 0.052$ ), even if the variation was not statistically significant ( $p > 0.05$ ).

## Conclusion

This present clinical study revealed that the activity of glutathione peroxidase, a member of the antioxidant systems, increased after 20 days of treatment with piroxicam, meloxicam and tenoxicam, respectively, as compared to baseline. However, none of these three NSAIDs belonging to oxicam class had a statistically significant influence on the GPx activity in patients with knee osteoarthritis.

COX inhibition alone did not seem to fully explain the antioxidant effects demonstrated by the NSAIDs including oxicams, although free radicals were generated during the prostaglandin synthesis from arachidonic acid under the influence of COX. This fact suggested an additional mechanism of action on the oxidative stress, independent of COX inhibition which might provide new therapeutic ways for osteoarthritis treatment.

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