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# Oxidative stress, hemoglobin content, superoxide dismutase and catalase activity influenced by sulphur baths and mud packs in patients with osteoarthritis

## Uticaj sumpornih kupki i pakovanja blata na oksidativni stres, sadržaj hemoglobina i aktivnost superoksid-dismutaze i katalaze kod bolesnika sa osteoartritisom

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**Abstract**

**Background/Aim.** It is weel-known that sulphur baths and mud paks demonstrate beneficial effects on patients suffering from degenerative knee and hip osteoarthritis (OA) through the increased activity of protective antioxidant enzymes. The aim of this study was to assess lipid peroxidation level, *i.e.* malondialdehyde concetration, in individuals with knee and/or hip osteoarthritis (OA), as well as to determine the influence of sulphur baths and mud packs application on the activity of superoxide dismutase (SOD) and catalase (CAT) in order to minimize or eliminate excessive free radical species production (oxidative stress). **Methods.** Thirty one patiens with knee and/or hip OA of both sexes were included in the study. All OA patients received mud pack and sulphur bath for 20 minutes a day, for 6 consecutive days a week, over 3 weeks. Blood lipid peroxidation, *ie* malondialdehyde concentration, superoxide dismutase and catalase activity were measured spectrophotometrically, before, on day 5 during the treatment and at the end of spa cure. Healthy volunteers (n = 31) were the controls. **Results.** The sulphur baths and mud packs treatment of OA patients caused a significant decrease in plasma malondialdehyde concentration compared to the controls ($p < 0.001$). The mean SOD activity before the terapy was 1 836.24 U/gHb, on day 5 it rose to 1 942.15 U/gHb and after the spa cure dropped to 1 745.98 U/gHb. Catalase activity before the therapy was 20.56 kU/gHb and at the end of the terapy decreased to 16.16 kU/gHb. The difference in catalase activity before and after the therapy was significant ($p < 0.001$), and also significant as compared to control ($p < 0.001$). At the end of the treatment significant increase of hemoglobin level and significant decrease of pain intensity were noticed. **Conclusion.** A combined 3-week treatment by sulphur bath and mud packs led to a significant decrease of lipid peroxidation in plasma, as well as pain intensity in the patients with OA. These changes were associated with changes in plasma activity of SOD and CAT and a significant increase of hemoglobin level suggesting their role in beneficial effect of spa therapy in the patients with OA.

**Key words:**
**osteoarthritis; baths; sulfur; mud therapy; oxidative stress.**

**Apstrakt**

**Uvod/Cilj.** Poznato je da sumporne kupke i pakovanja blata imaju povoljno delovanje kod bolesnika sa osteoartritisom (OA) kolena i kuka zahvaljujući povišenoj aktivnosti antioksidantnih zaštitnih enzima. Cilj ove studije bio je praćenje nivoa lipidne peroksidacije, tj. koncentracije malondialdehida, kod bolesnika sa osteoartritisom (OA) kolena i/ili kuka, kao i utvrđivanje uticaja sumporne kupke i blatnog pakovanja na aktivnost superoksid-dismutaze (SOD) i katalaze (CAT) sa ciljem da ublaže ili eliminiše oksidativni stres. **Metode.** Bolesnici sa OA kolena i/ili kuka oba pola (n = 31) bili su uključeni u studiju. Svi bolesnici dobijali su blatna pakovanja i sumporne kupke 20 minuta dnevno, šest dana u nedelji, ukupno tri nedelje. Nivo lipidne peroksidacije u krvi, tj. koncentracija malondialdehida, aktivnost superoksid-dismutaze i katalaze mereni su spektrofotometrijski, pre tretmana, petog dana lečenja i na kraju tretmana. Kontrolnu grupu činile su 33 zdrave osoba. **Rezultati.** Tretman blatnim pakovanjima i sumpornim kupkama kod bolesnika sa OA izazvao je značajno sniženje koncentracije malondialdehida u plazmi u poređenju sa

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kontrolnom grupom ($p < 0,001$). Prosečna vrednost aktivnosti SOD bila je 1 836,24 IJ/gHb pre početka tretmana, 5. dana porasla je na 1 942,15 IJ/gHb i nakon *spa* tretmana opala na 1 745,98 IJ/gHb. Aktivnost katalaze pre terapija bila je 20,56 kIJ/gHb, a na kraju tretmana 16,16 kIJ/gHb. Razlika u aktivnosti katalaze pre i posle terapije bila je značajna ($p < 0,001$), a takođe je značajna i u poređenju sa kontrolnom grupom ($p < 0,001$). Na kraju lečenja došlo je do značajnog porasta nivoa hemoglobina i značajnog smanjenja bola i povećanje obima pokreta u zahvaćenom zglobu. **Zaključak.** Sumporne kupke u kombinaciji sa pakovanjima blata tokom tronedeljnog tretmana dovele su do značajnog smanjenja stepena lipidne peroksidacije u plazmi i smanjenja intenziteta bola kod obolelih od OA. Ovo je bilo praćeno promenama u aktivnosti antioksidantnih enzima, SOD i CAT i porastom nivoa hemoglobina, što ukazuje na njihovu ulogu u postignutom terapijskom efektu banjskg treatmana.

**Ključne reči:**
**osteoartritis; kupke; sumpor; lečenje blatom; stres, oksidativni.**

## Introduction

Osteoarthritis (OA) has been known to be a common disease of aged people [1–3] almost from time immemorial [4]. In fact, it is as old as mankind. As, the most common form of arthrosis, it is seen not only in human populations but nearly in all vertebrates and even in dinosaurs. In spite of this, the etiology and exact mechanism of OA initiation and development are still not fully understood [5–8]. As a matter of fact, OA etiology is multifactorial [9]. Some of these factors are aging *per se* [10], gender, local biomechanical influences as well as genetic anomalies, injures, focal lesions and metabolic disorders [11]. In relation to this, a few studies have reported involvement of reactive oxygen species (ROS) and reactive nitrogen species (RNS), *ie* oxidative stress, in OA development [12–14]. In addition, one of the possible mechanisms in OA development was thought to be related to excessive free radical production and/or diminished capacity of antioxidative protection [15, 16]. However, Hallivell and Gutteridge's list of the conditions in which ROS/RNS are implicated does not contain degenerative osteoarthritis, but rheumatoid one only[17]. Tiku et al. [13] have found that reactive oxygen species are involved in aging process and in the pathogenesis of osteoarthritis. Henrotin et al. [12] claim that ROS production has been found to increase in patients with OA. On the contrary, Mazzetti et al. [18] reported that chondrocytes from patients with OA produced higher level of nitric oxide (NO) in comparison with rheumatoid arthritis patients. From the above mentioned it is not fully clear whether in patients with OA oxidative stress occurs or not. If it occurs, the question arises whether ROS or RNS play predominant role in causing oxidative stress. Currently, it is considered that mainly hydroxyl radical ($HO^{\cdot}$), superoxide anion radical ($O_2$), hydrogen peroxide ($H_2O_2$), (NO) and peroxynitrite (ONOO-) excessive formation in the body are involved in inflammatory arthritis only or might be in OA. In any case, it should be established which kind of treatment should be applied: drugs, surgery or spa therapy. It seems that Ekmekciogly et al. [19] and Scheidleder et al. [20] were the first or among the first to demonstrate beneficial effects of sulphur baths and mud packs on patients suffering from degenerative knee and hip osteoarthritis in which ROS/RNS are implicated. According to their findings it is achieved by sulphur baths through the increased activity of antioxidant protective enzymes.

Ubiquitous free radical production occurs continuously in all living cells mainly as a byproduct of aerobic metabolism and under physiological circumstances being counterbalanced by cellular antioxidant system. Therefore, under normal conditions free radical level does not exceed defense cells capacity. Excessive free radical formation, originating either from endogenous or exogenous sources, leads to oxidative stress damaging tissues, lipids, carbohydrates, proteins and DNA. The damage is minimized or stopped by antioxidant defense activation in one of the three ways: preventing free radicals ´ production, scavenging excessive radicals and/or accelerating radicals´ decomposition [15].

Thus, the aim of the present study was to assess whether in patients with degenerative knee and/or hip osteoarthritis oxidative stress occurs and to investigate if balneotherapeutic sulphur baths and mud packs combined may influence the patients' oxidative stress status through antioxidant protective enzymes activation.

It is common to assess balneological reaction in the first week of the treatment with sulphur baths [21].

## Methods

Thirty one patients with knee and/or hip osteoarthritis of both sexes participated in the randomized controlled study. The patients were diagnosed according to the American College of Rheumatology criteria [22, 23]. The group comprised 20 women (64.5%) with the mean age of 55.0 years, and 11 men (35.5%) with the mean age of 56.18 years. None of them had inflammatory arthritis, cancer, coronary heart disease, diabetes mellitus or any other disease which could have been associated with the increased oxidative stress. Blood samples were taken by venipuncture before the beginning and at the end of spa therapy as well as on the day 5 during the therapy for the assay of lipid peroxidation (malondialdehyde), Copper-Zinc superoxide dismutase (CuZn-SOD) and catalase (CAT) activity.

The extent of the peroxidative reaction in plasma was determined by measuring malonyldialdehyde (MDA) concentration (prepared in 50 mM Tris- HCl buffer, pH = 7.4 in a ratio of 1:50) by the modified thiobarbituric acid method without stimulation of peroxidative processes with $Fe^{2+}$ and ascorbate [24].

Superoxide dismutase (SOD) activity was determined by the adrenaline method of Misra and Fridovich [25], based on

the spectrophotometrical measurement of the degree of adrenaline auto-oxidation inhibition by SOD contained in the examined samples. Total specific SOD activity and that of MnSOD (after CuZnSOD inhibition with potassium cyanide - KCN) were measured, and then CuZnSOD activity was calculated. Catalase was measured spectrophotometrically by the method of Beutler [26], based on the rate of hydrogen peroxide degradation by the action of CAT contained in the examined samples.

Besides, red blood cells count, hematocrit, hemoglobin content, the mean corpuscular volume index (MCV), the mean corpuscular hemoglobin (MCH) and the mean corpuscular hemoglobin concentration (MCHC) were determined. Hematologic parameters were measured using an Autolyser 808.

The protocol of balneotherapeutic treatment was as follows. All OA patients received mud pack and sulphur bath for 20 minutes a day, 6 consecutive days a week, over 3 weeks. Namely, each patient received mud pack followed by sulphur bath on the same day. The application of hot native mineral mud pack of 42ºC lasted for 20 minutes. After that, with mineral water rinsed patients went into individual bath-like pool containing native thermomineral sulphurous water, the temperature of which was adjusted individually according to patient's wish (32–34ºC).

The study protocol was approved by the Ethics Committee of our institution and each participant signed a consent.

As controls served thirty three healthy volunteers, 19 (61.2%) males with the mean age of 53.31 years and 14 (38.8%) women with the mean age of 52.28 years. They were age-matched with the patients so that the mean age of the patients group was 55.42 years, whereas that of the control was 52.88 years. All blood analyses done in the controls were the same as in the treated patients.

Data values are presented as the mean ± standard deviation. For statistical analysis Student *t*-test and Fisher exact test were used. The values of $p < 0.05$ were considered statistically significant.

## Results

A sulphur baths and mud packs combination produced malondialdechyde concentration decrease in patients with knee and hip OA (Figure 1). This decrease was statistically significant ($p < 0.001$), as compared to the controls. The mean value in patients after the therapy was 8.16 μM/mL, while in controls it was 9.19 μM/mL. Balneotherapeutic treatment affects the antioxidant status of patients with degenerative OA. The activities of superoxide dismutase and catalase, which inhibit lipid peroxidation, were changed characteristically, which was obvious not only at the end of spa therapy but also during the therapy itself. The mean blood SOD activity before the therapy was 1836.47 U/gHb. On day 5 during the therapy SOD activity was increased to 1 942.15 U/gHb. At the end of spa cure SOD activity was lower than before the therapy (Figure 2). The difference in the activity before and after the treatment as compared to the

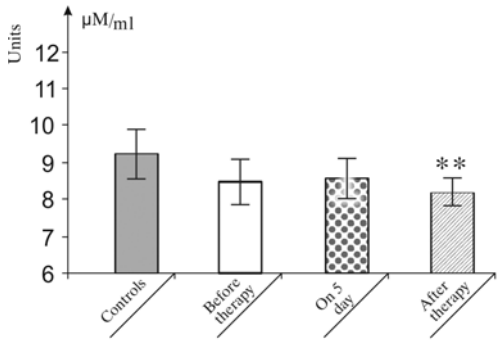


**Fig. 1 – Malondialdehyde concentration ($\bar{x} \pm SD$) in the controls and in the patients group before and after the sulphur baths therapy and on the day 5 during the therapy**

*** $p < 0.001$ *vs* controls (Student's *t*-test)**

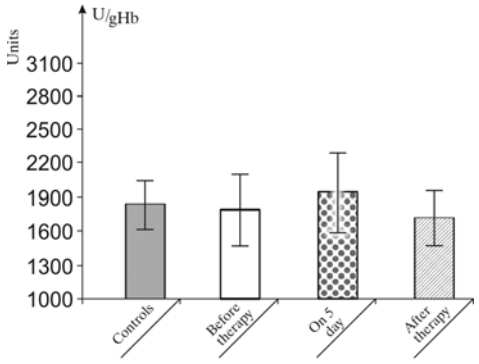


**Fig. 2 – Superoxide dismutase activity ($\bar{x} \pm SD$) in the controls and the patients group before and after the sulphur baths therapy and on the day 5 during the therapy**

controls was at the limit of significance. In contrast to SOD, catalase activity was the highest before the beginning of the therapy. It was higher than in controls but the difference was not significant. Before the therapy catalase activity was 20.56 kU/gHb, whereas in controls it was 18.54 kU/gHb. On the day 5 catalase activity was decreased. At the end of spa therapy it was lowered to 16.16 kU/gHb (Figure 3). This decrease was statistically highly significant ($p < 0.001$) as compared to that before the treatment and the controls. In the controls hemoglobin content was 13.62 g/dL, whereas in the patients before the balneotherapeutic treatment it amounted to 12.97 g/dL, the difference being significant ($p < 0.01$, *t*-test, Figure 4). On the day 5 of sulphur bath Hb concentration rose to 13.19 g/dl and at the end of the therapy it was 13.62 g/dL. The difference before and after the spa cure was significant ($p < 0.001$). In the patients' blood the mean red blood cell count was lower than in controls and the decrease was significant. In the course of spa therapy on the day 5 the number of circulating red blood cells was increased. At the end of the therapy the increase was higher than before the treatment and the difference was significant ($p < 0.001$).

The mean corpuscular hemoglobin (MCH) in the patients was lower than in the controls, but without statistical significance. In the course of the treatment MCH rose and at the end of spa cure it was 29.44 pg. This increase in respect to MCH value before the therapy was statistically significant ($p < 0.001$).

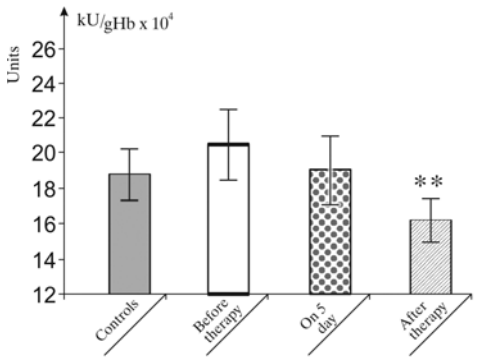


**Fig. 3 – Catalase activity (x̄ ± SD) in the controls and in the patients group before and after the sulphur baths therapy and on the day 5 during the therapy**

*** *p* < 0.001 *vs* before the therapy and controls**

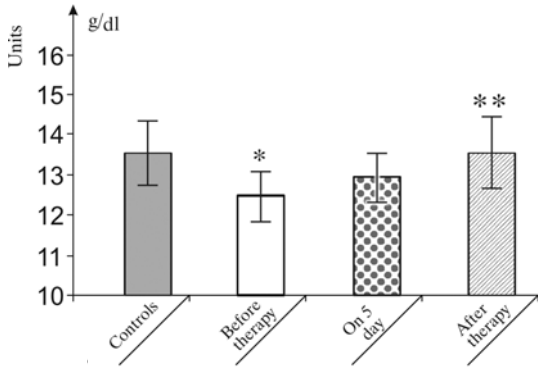


**Fig. 4 – Hemoglobin level (x̄ ± SD) in the controls and in the patients group before and after the sulphur baths therapy and on day 5 during the therapy**

*** *p* < 0.01 *vs* controls**
**** *p* < 0.001 *vs* before the therapy**

The mean corpuscular hemoglobin concentration (MCHC) before the therapy was lower than in the controls. It rose during the therapy and after a 3-week treatment it was statistically significantly higher than before the spa cure ($p < 0.001$) (Figure 5).

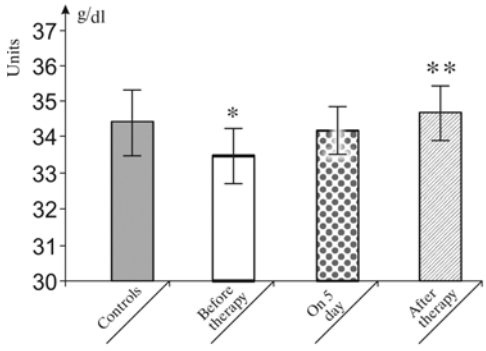


**Fig. 5 –Mean corpuscular hemoglobin concentration (x̄ ± SD) in the controls and in the patients group before and after the sulphur baths therapy and on the day 5 during the therapy**

**** *p* < 0.001 *vs* before the therapy**

At the end of the treatment we noticed a significant decrease of pain intesity ($p < 0.001$) (Figure 6) and increasing a range of motion in the joints.

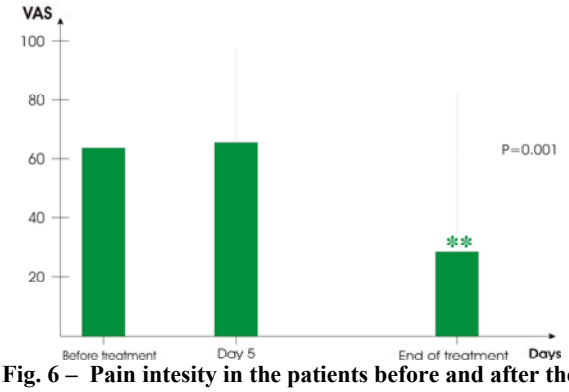


**Fig. 6 – Pain intesity in the patients before and after the sulphur baths therapy and on the day 5 during the therapy.**

*** *p* < 0.001 *vs* before the therapy; VAS – Visual Analogne Scale**

## Discussion

Despite a long balneotherapy use, at least since Roman times [27], in musculoskeletal and other disorders treatment [28–30] the precise mechanism of beneficial effects of spa therapy remains unclarified yet [31]. After it has been found that free radicals may be involved in many diseases, including rheumatoid ones, either as a cause or as a consequence [32, 33] new data on defense mechanisms were obtained thanks to the application of spa therapy [34].

Now it is known that several factors play an important role in the mechanism of favorable effect of spa therapy in patients with OA. They are mineral water temperature *per se* [35], chemical composition, *ie* mineral content of the given water spring [36], mineral water radioactivity [37], resort environment and other known and unknown influences. From the physiological point of view, temperature of spring water affects patients beneficially in several ways. First of all, balneotherapeutic heat application induces vasodilatation [35] and consequently an increased blood flow as a natural physiological response. The increased blood flow ensures a better oxygen supply of injured or inflammated tissues [38]. In this way local tissues hypoxia is diminished or eventually eliminated [39]. Maintaining such a high flow level, the harmful metabolic products, including excessive free radical species, will be removed at a faster rate. Free radical removal and better tissues oxygenation support fast cells repair. Another possible mechanism by which a spa therapy temperature-related beneficial effect(s) is accomplished may be, at least partly, heat-shock proteins production. It is known that heat-shock proteins appear in response to elevated temperature [40]. They are linked to the increased SOD and catalase activities that assist the favorable effect(s) of spa treatment in OA. Otherwise, under certain circumstances or stresses some other molecular chaperones appear. Chaperones are essential in various phases of cellular metabolism, synthesis, maturation, stabilization of an unstable protein conformation, correct refold of proteins and cell survival [40].

Our results showed that a thermal sulphur baths and mud packs combination enhanced the antioxidant function such as the activities of SOD and catalase, which inhibit lipid peroxidation. According to our best knowledge we have not found published data that SOD and CAT activity were changed in patients suffering from knee and hip OA, in the course of combined sulphur baths and mud packs treatment itself (day 5), which we did. Different trend and level of SOD and CAT, observed on the day 5 in our trial, indicate the importance of such a finding. It would be important for two main reasons. Firstly, it might contribute to a better understanding of the mechanisms of antioxidant protection enzymes. Secondly, it might be possible to determine more reliable minimum or optimum spa cure duration, at least for certain cases. It could have socioeconomic aspect.

Free radicals tissue damages may be minimized or even prevented by an optimal function of antioxidant defense system. An essential component of antioxidant enzymes is sulphur that contributes remarkably to their antioxidative potential [19, 20, 36]. In connection with this sulphur and sulphur containing compounds have been used for therapeutic purposes, particularly in the treatment of musculoskeletal disorders for centuries. Hence, Hildebrandt and Gutenbrunner [36] demonstrated sulphide penetration through the skin during sulphur bath therapy but further transdermal sulphide metabolism was not elucidated. In the meantime sulphite importance in antioxidative mechanism has been described by Beinert [41] and Mitsuhashi et al. [42]. Furthermore, Scheidleder et al. [20] pointed out advantageous effects of sulphur drinking cures on the antioxidative defense system and on the decrease of the peroxide level.

Karagülle et al. [43] showed that thermal sulphur baths have an anti-inflammatory effect on the experimental arthritis in rats. Rizzo R et al. [44] have found that sulphur concentration in arthritic tissue is reduced to about one-third with respect to that in normal tissue. Recently, McCartney et al. [45] demonstrated potent scavenging properties of hemoglobin for NO nitrate and nitrite, so affecting the development and severity of arthritis. Not only did hemoglobin deplete endogenous level of NO metabolite but significantly ameliorated the arthritic lesions in experimental animals. The increased hemoglobin level during sulphur bath therapy may have the same function.

According to Ekmekciogly et al. [19] a 3-week sulphur baths therapy reduced oxidative stress, expressed in a 17.2% decline in peroxide concentration and also in a significant decrease in SOD and glutathione peroxidase activity in patients with degenerative OA. Our results are in accordance with those reported by Ekmekciogly et al. [19]. Namely, we found a decrease of lipid peroxidation (MDA plasma level), as well as lowered SOD and catalase activity after a 3-week of combined sulphur baths and mud packs therapy. They interpreted their findings as a consequence of a reduced oxidative stress in the course of sulphur therapy that led to a lower expression of free radicals scavenging enzymes. On the basis of our results, the given explanation is reasonable and probably the most acceptable. Anyway, these data do offer further insight into the mechanism of sulphur bath therapy.

Similar results in decreasing SOD and CAT activity have been reported by Bender et al [46].

The increased number of circulating red blood cells and MCHC in the patients' blood during sulphur baths and mud packs application, as well as at the end of the spa therapy is not easy to explain. Without further investigation it is difficult to say with certainty whether this increase originates from blood redistribution or from erythropoiesis stimulation. One of the strongest stimuli for erythropoietin formation regulating erythropoiesis is oxygen demand or hypoxia. Besides, erythropoietin is influenced by sex hormone, *ie* androgen [40]. Thus, testosterone stimulates erythropoietin formation and release. It may in part account for the higher red blood cells count in adult males. On this basis there are two presumptions. Koviljača Spa, formerly known as a Royal Spa, is located in the western Serbia, at low altitudes of about 126 m above the sea level. At that altitude barometric pressure and atmospheric oxygen partial pressure ($pO_2$) are within normal range, so that the effect of so-called hypoxic hypoxia (altitude hypoxia) as a cause of erythropoiesis stimulus, is ruled out. Therefore, other possible factors and presumptions remain. Considering that osteoarthritis is a chronic disease some degree of local tissue hypoxia may be present. It might be that sulphur, from sulphur baths, after penetrating the skin interacts with antioxidant protection enzymes seemingly potentiating their capacity or it is incorporated in some thiol compounds. It, along with tissue hypoxia, might contribute to hemoglobin increase which is a potent NO scavenger. Notably, the potentiation of mild radioactivity from Koviljača Spa mineral water on erythropoiesis directly or indirectly via sex hormone activity cannot be excluded.

## Conclusion

A combined 3-week treatment by sulphur bath and mud packs led to a significant decrease of lipid peroxidation in plasma, as well as pain intensity in the patients with OA. These changes were associated with changes in plasma activity of SOD and CAT and a significant increase of hemoglobin level suggesting their role in beneficial effect of spa therapy in the patients with OA.

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