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Static magnetic field action on some markers of inflammation in animal model system—in vivo

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Abstract Experimentally induced acute inflammation in rats is a good model system which includes the complexity and dynamics of the processes; moreover there are many defined markers for following and estimating changes in an observed system. In this paper, we discuss an in vivo model of acute inflammation induced by carrageenan. Carrageenan-induced paw edema is a model of non-infectious acute inflammatory reaction to assess the contribution of mediators involved in vascular changes associated with acute inflammation and potential treatments. A complex approach into the investigation of possible effects of static magnetic fields (SMF) action on experimentally induced acute inflammation in rats (by measurements of the levels of specific stress markers) is very important in understanding the possible inflammatory mitigation effects, pain relief, and oxidative stress state of an organism. This approach could help for better understanding of the possible mechanisms of interaction of dynamic processes such as inflammation healing and pain relief with external SMF (25 mT at 10, 20 and 30 min exposure time). Results indicate that SMF with B = 25 mT for 30 min diminished the inflammatory process and decreased the levels of inflammatory markers (fibrinogen) and stress markers (ACTH, Cor) into the blood plasma in rats as when compared with sham exposed animals. The exact mechanism by which SMF contributes to the acceleration of inflammatory healing and decrease of inflammation markers in blood plasma in rats still remains unclear. We assume that two possible mechanisms exist. One is the direct interaction of SMF with free active oxygen forms (free radicals) affected by their membrane processes and related with the physiological functions. Another possible mechanism might be related to dynamic regulation of inflammation healing process.

Keywords Static magnetic field · Inflammation · In vivo

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1 Introduction

Effects of SMF have been studied on different levels of structural organization of biological systems, from subcellular to organism, including clinical applications (Rosch and Markov 2004). In a recent paper, van Rongen et al. (2007) reported that no acute effects, other than transient phenomena such as vertigo and nausea, have been observed upon exposure to static magnetic flux densities up to 8 T. There are no reports of long term or chronic adverse effects following prolonged static magnetic field exposure. There are a number of papers which show that SMF have an impact on dynamic physiological and biophysical processes. The complexity of the living systems and their dynamic regulation plays a crucial role in the occurrence of

biological effects of SMF (Gorczynska and Wegrzynowicz 1986; Feinendegen and Muhlensiepen 1988).

Investigations of the SMF action on inflammation are based mainly on in vivo studies of some diseases such as arthritis (Taniguchi et al. 2004). We have chosen an animal inflammatory model which could provide useful information from both practical and theoretical points of view. It is well accepted that acute inflammation has three main components: (1) Alterations in the vascular caliber that lead to an increase in blood flow, (2) Structural micro-vascular changes, increasing permeability of the blood vessel wall and allowing plasma proteins and leucocytes to leave circulation causing extravasations and edema formation, (3) Emigration of the leukocytes from the microcirculation, their accumulation in the focus of injury, and their activation to eliminate the offending agent (Silbernagl and Lang 2000).

An acute inflammation represents a local reaction associated with the symptoms, known since antiquity, of *pain* (dolor), *swelling* (tumor), *reddening* (rubor), and *warmth* (calor). In addition, there are general inflammatory reactions (*acute-phase response*). Rapid activation of *mast cells* (in tissue) or their counterparts in blood, the *basophil leukocytes*, or *basophils*, is an example of the occurrence of a very strong acute inflammatory reaction (Silbernagl and Lang 2000).

Vasodilation is the cause of the reddening and warming at the site of inflammation and of reduced blood flow velocity which makes it possible for the chemotactically attracted leukocytes to swim to endothelium adjacent regions. Influence of SMF on microcirculations has been widely studied by Gmitrov and Ohkubo (2002), Gmitrov et al. (2002); Mayrovitz and Groseclose (2005), Morris and Skalak (2005), Okano et al. (2005). Microcirculation plays an important role in the processes of reddening, warming and edema formation. The above mentioned papers reported evidence for the effects of SMF on vasodilatation. Endothelium has been stimulated in the inflammatory area among others, IL-4 (from TH2 lymphocytes) led to the redistribution of selectins from their normal intracellular stores in granules to the luminal cell surface. The increased endothelial permeability (loosening of endothelial cell connections) allows the leukocytes to slip into the extravascular space (*diapedesis*). Furthermore, more protein-rich fluid (*inflammatory exudate*) reaches the interstitial spaces and leads to swelling or edema formation, which is a complex process between microcirculation and water saline equilibrium, and blood vessel permeability (Morris and Skalak 2008). Application of a 10- or 70-mT SMF for 15 or 30 min, immediately following histamine-induced edema resulted in a significant, 20–50% reduction in edema formation. In addition, a 2-h, 70-mT field application to carrageenan-induced edema also resulted in significant (33–37%) edema reduction. Field application before injection or at the time of maximal edema did not influence edema formation or resolution, respectively.

In extreme cases even erythrocytes leave the blood vessels (*hemorrhagic inflammation*). Pain arises, which brings the injury into consciousness (changed behavior) and stimulates a reflex action to nurse the inflammed region (e.g., a limb). Several publications (Holcomb et al. 2000; Laszlo et al. 2007; Sandor et al. 2007), have addressed the SMF influence on the inflammatory processes and have shown that SMF can affect pain perception having mild mitigation effect, indirectly affecting nociceptors. The *neutrophils* that have migrated to the site of inflammation and the *macrophages* that are recruited from circulating monocytes now try to phagocytose the pathogens causing the inflammation, digesting them by means of their lysosomes. These, in relation with the activation of the immune response processes could be influenced by SMF (Gorczynska 1986; Gorczynska and Wegrzynowicz 1989). A significant decrease in the counts of monocytes, platelets, peripheral T and B lymphocytes levels was observed for SMF and ELF–EMF exposed groups. The granulocyte percentage was significantly increased. The results indicate that there is a relationship between the exposure to SMF or ELF–EMF and the oxidative stress through distressing redox balance leading to physiological disturbances (Hashish et al. 2007). The process of inflammation is also related to free radical formation. The total antioxidant capacity of the organism showed a significant increase in post-exposures when compared with pre-exposures to the static magnetic field ($P < 0.05$). The oxidative stress index and total oxidant status showed a significant decrease in post-exposures when compared with pre-exposures to a 1.5 T magnetic field (for each, $P < 0.01$). The 1.5 T static magnetic field induces the effect of decreasing oxidative stress in men following short-term exposure (Sirmatel et al. 2007).

This paper is therefore focused on the elucidation of possible beneficial effects of SMF on inflammation healing and pain relief.

2 Materials and methods

2.1 Animals

The experiments were carried out on 240 male Wistar rats (*Rattus norvegicus*) (180–200 g) the animals were acclimated to 22 ± 1°C, kept in a 12:12 h light/dark cycle and given commercial rat food and tap water ad libitum (Fig. 1). Ethical guidelines of the International Association for the Study of Pain in conscious animals were followed. Animals were divided into exposure (at 25 mT) and control (0 mT) groups. On each day (1, 2 and 3) 20 animals of the control group were decapitated and the blood samples were

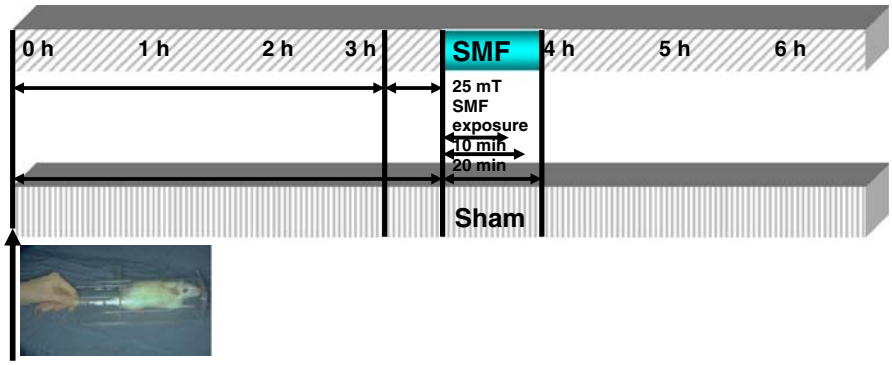

Fig. 1 Design of the experiment- inflammatory model realization after intraplantar injection of carrageenan 20–60 μmol/g, elicited an inflammatory response. SMF/Sham exposure begins after 3.5 h after injection for different time intervals of 10, 20 and 30 min

investigated. All animals of the exposed groups received 25 mT SMF, for 10, 20 or 30 min daily for 3 consecutive days. Each day 20 animals of all exposure groups were decapitated and the blood samples were investigated. The total number of animals was 240.

2.2 Reagents

Standard, radio immune assay analysis (RIA) kits for assessment of the levels of the stress markers (Corticosteroids, and ACTH) were used. RIA requires three type of materials: a radioactively labeled preparation of the substance to be measured, antibody to this material, and a biological fluid (blood plasma), containing an unknown amount of the material.

The rats were decapitated without anesthesia. Blood was collected from the trunk and divided into two sets of tubes containing heparin. Plasma was separated by centrifugation at 3,000*g* for 10 min and frozen at −20°C until it was assayed for hormone levels. Plasma ACTH and CORT were assayed by a double antibody radioimmunoassay (RIA) method specific for rats using commercial kits (BRAHMS ACTH, Germany; CORT-CT2, CIS, Bio International, Paris). (These assays were performed in the laboratory of Prof. S. Milanov in the Hospital of the Ministry of Interior, Sofia and the values expressed as pg ACTH/ml and nmol CORT/l).

Lipid peroxidation was assesed by measuring the quantitative formation of malondialdehyde (MDA) in blood plasma by Spectrophotometry [UV-VIS Spectrophotometer, Shimadzu UV-250 (190–900 nm range)]. SOD Assay Kit (Fluka) was used for luminescent detection of catalase and SOD activity in blood plasma in vivo (Luminometer Shimadzu UV-1050). Fibrinogen Assay Kit (SIGMA–ALDRIDGE) was used for UV–VIS Spectrophotometry of blood plasma samples.

2.3 Statistical method

One-way ANOVA test was employed for comparison of the experimental groups. The values are expressed as mean ± S.E.M. and the level of significance was set at $P < 0.05$. Non-linear regression analysis and Friedman test were applied for testing the correlations between the groups.

3 Results and discussion

In this article, we investigated whether 25 mT SMF with time of exposition 10, 20 and 30 min can induce changes in inflammation induced lipid-peroxidation stress in blood plasma in rats. Accurate markers for lipid peroxidative stress in vivo are direct products of lipid peroxidation such as MDA and a major antioxidant enzyme, superoxide-dismutase (SOD). In parallel with this, we investigate the levels of stress markers such as CORT and ACTH after SMF action. For better understanding of the inflammatory pathology and the process of inflammation healing, during the three consecutive days of investigation we measured fibrinogen plasma levels. Compared with the control group, the product of lipid peroxidation, e.g., MDA level, were significantly increased in blood plasma of rats with concomitant increase of SOD at the early stages (1st and 2nd day after initiation of inflammation). The tendency of decreasing the blood plasma levels of inflammatory markers diminished at the 3rd day after exposure. However, there was no significant alteration in CORT and ACTH among the groups ($P < 0.05$). Levels of Fibrinogen in blood plasma consequently decreased up to the 3rd day and had a maximum at the 2nd day in comparison with the control.

Figure 2 presents the results of development of paw edema characterized by volume changes, as result of different concentrations of carrageenan. Paw edema volume changes became stable at 3-rd hour after carrageenan injection and remain relatively stable up to 6 h of investigation.

Figure 3 presents results of development of paw edema characterized by volume changes, with SMF exposure and at sham exposure conditions. The assessment was performed 3.5 h after carrageenan injection and paw edema

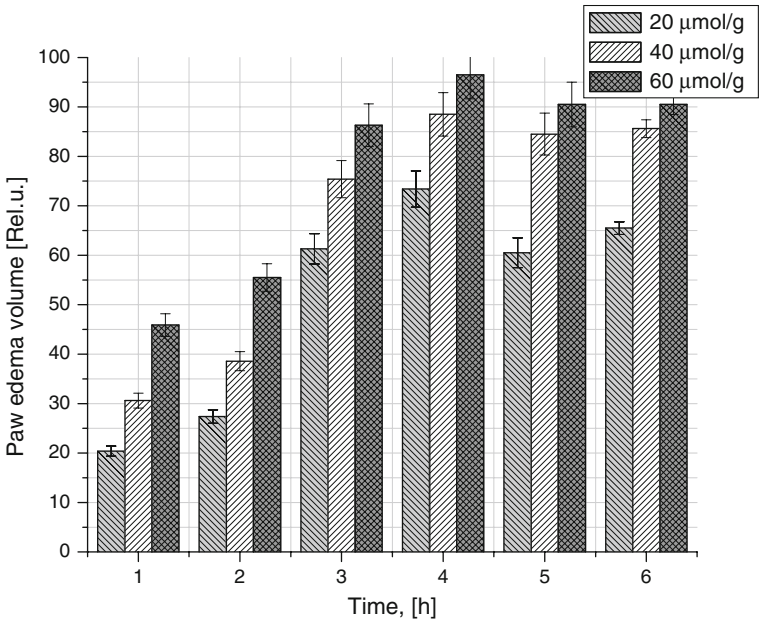

Fig. 2 Time dependence of carrageenan induced paw edema at three different (20, 40, 60 µmol/g) concentrations of carrageenan

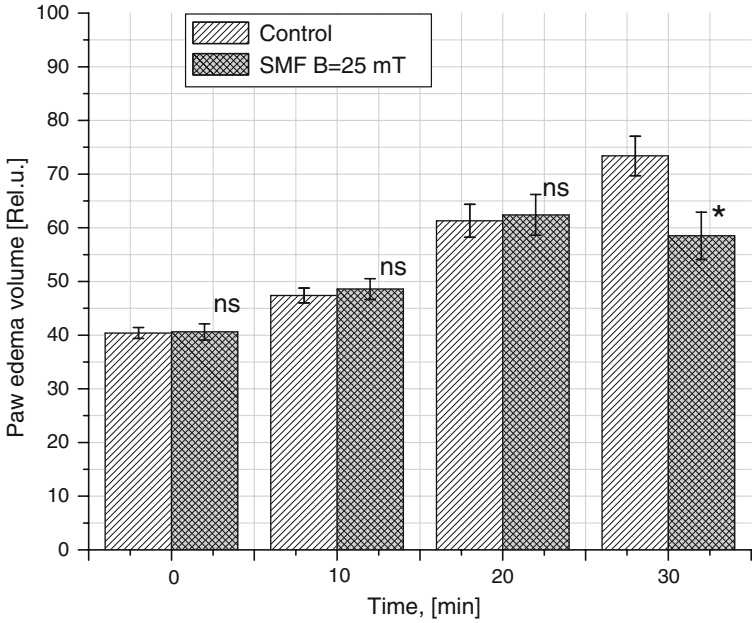

Fig. 3 Time dependence of 40 µmol/g carageenan induced paw edema volume in control and sham groups at different expositions at SMF B = 25 mT for 10, 20, and 30 min

formation. Paw edema volume changes became stable at the 3-rd hour after carrageenan injection and maintained relative stability up to the 6 h period of investigation. We took measurements of paw edema volume every 10 min up to the 4-th hour since inoculation of carrageenan. In Fig. 3 significant changes at 30 min SMF exposure have been observed—significant decrease against control of paw edema volume.

In addition to paw edema formation, we investigated the plasma levels of fibrinogen. In Fig. 4 we can see significant decrease of fibrinogen plasma levels in all SMF exposed groups when compared with the control.

Plasma levels of fibrinogen are not specific but significant parameters related to the process of inflammation blood rheological characteristics. Inflammatory process is always related to oxidative stress in biological systems and is a good marker for inflammation in vivo. Inflammatory process is always related to reactive oxygen substance production (ROS-production). One of the non specific markers for ROS-production is the level of MDA in blood

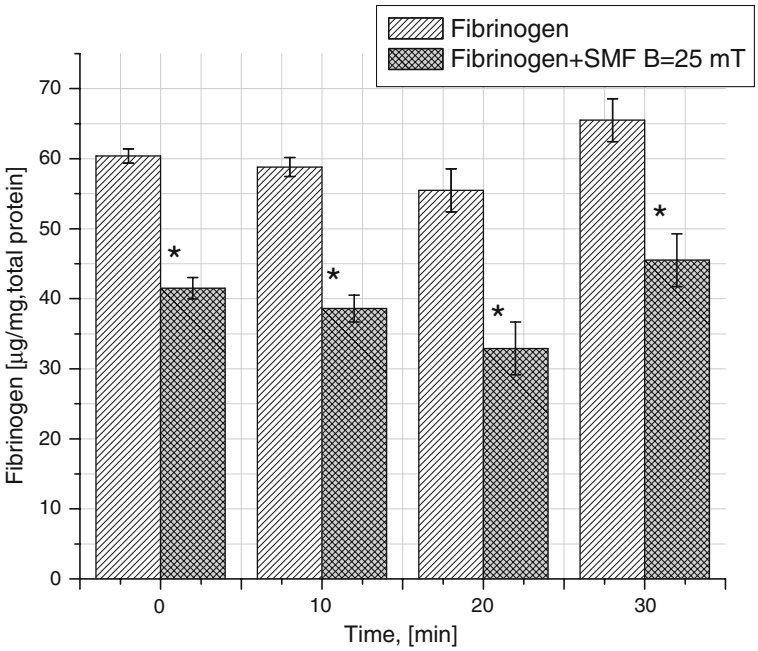

Fig. 4 Time dependence of blood plasma concentrations of fibrinogen in control/sham and different expositions at SMF B = 25 mT for 10, 20, and 30 min after 40 µmol/g carageenan induced inflammation

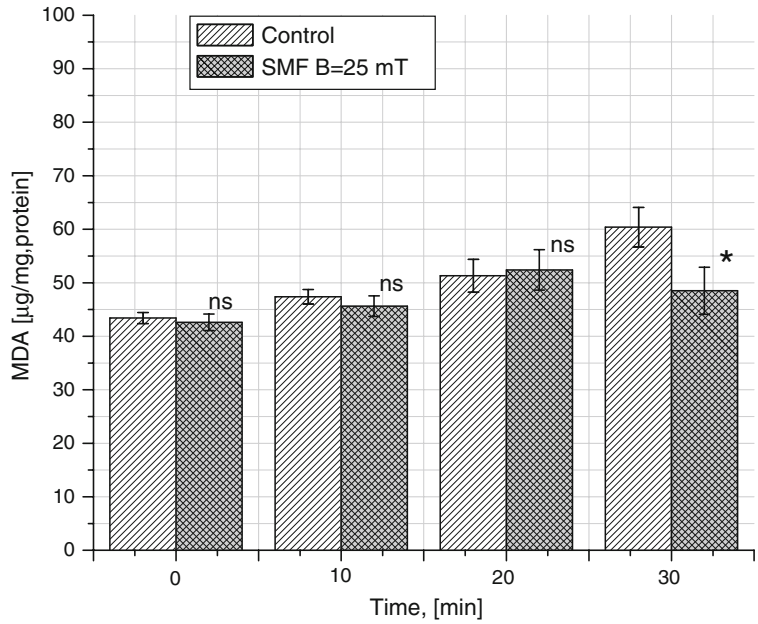

Fig. 5 Time dependence of blood plasma concentrations of MDA at control/sham and different expositions at SMF B = 25 mT for 10, 20, and 30 min, after carageenan induced inflammation

plasma and the results are presented in Fig. 5. An outcome of this investigation is the screening of susceptibility of antioxidant system of living organisms after SMF action. The antioxidant system plays a central role as a mediator in many stress-induced and stress- promoted reactions, mainly during the inflammation.

MDA corresponding to the highest level of lipid peroxidation is seen in this figure: only 30 min exposure with SMF B = 25 mT can lead to significant decrease in the concentration of MDA in blood plasma. In Fig. 6 are presented results of blood plasma levels of some of the most important anti-oxidant enzymes such as SOD and anti-oxidant enzyme catalase (CAT) of living organisms. Because blood plasma level of these enzymes is related to gene expression, this is one possible reason for the lack of significant effect after SMF action at this system. Our experiment ended 3.5 h after inoculation of carrageenan, and possibly this time is too long a period for observation of such relatively fast generated component of anti-oxidant system. As an indicator of SMF action of inflammatory

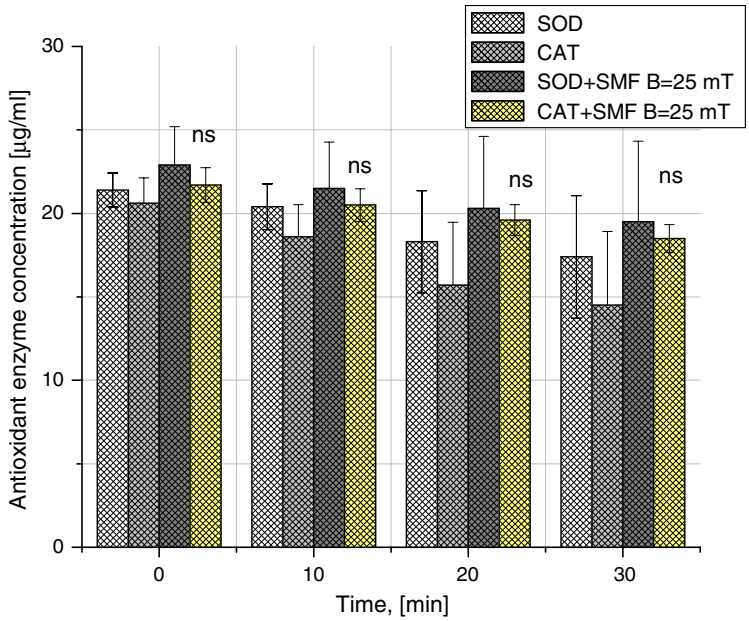

Fig. 6 Time dependence of blood plasma concentrations of SOD and CAT at control/sham and different expositions at SMF B = 25 mT for 10, 20, and 30 min, after carageenan induced inflammation

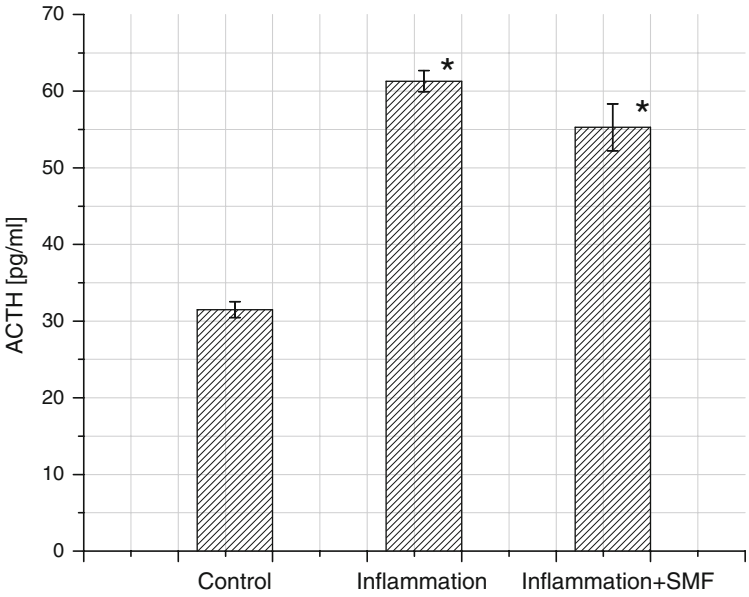

Fig. 7 Dependence of blood plasma concentrations of ACTH at control/sham, control plus inflammation and inflammation plus expositions at SMF B = 25 mT for 30 min

induced stress in organism, we use ACTH and CORT levels in blood plasma. The results showed significant ($P < 0.05$) decrease in the elevation of inflammatory process and levels of ACTH and CORT (Fig. 7). Decreasing of the ACTH and CORT blood plasma levels is probably related with reducing of other inflammatory stress related parameters (Fig. 8).

4 Conclusion

- Exposure to SMF of 25 mT for 30 min resulted in decreasing the severity of the inflammatory process and showed cumulative action in some investigated parameters.
- Static magnetic fields action decreases the levels of MDA, one of the markers for oxidative stress in blood plasma in rats.
- Static magnetic fields action decreases the fibrinogen concentration in blood plasma in parallel with edema volume.
- The exact mechanism by which SMF induces suppression of inflammation processes and decrease of the levels of inflammatory markers in blood plasma in rats still remains unclear.

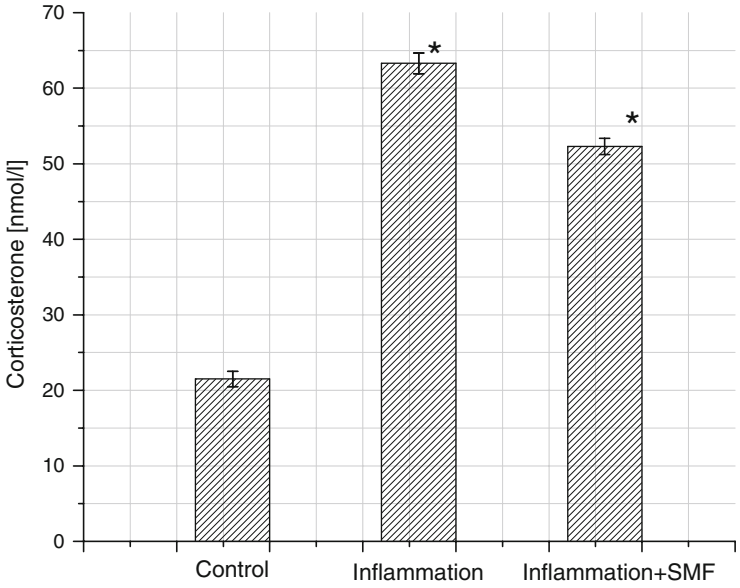

Fig. 8 Dependence of blood plasma concentrations of CORT at control/sham, control plus inflammation and inflammation plus expositions at SMF B = 25 mT for 30 min

- ACTH and CORT blood plasma levels were significantly decreased after 30 min 25 mT SMF exposure.

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