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Original Article

Medicinal benefits of Green Tea (Camellia sinensis) on the Envenomation Pathogenesis

Bienfaits du Thé Vert (Camellia sinensis) dans la Pathogénie de l'Envenimation Scorpionique

Megdad-Lamraoui Amal, Adi-Bessalem Sonia, Laraba-Djebari Fatima*
USTHB, Faculty of Biological Sciences, Laboratory Cellular and Molecular Biology, Department Cellular and Molecular Biology, BP32, EL Alia, Bab Ezzouar 16111, Algiers, Algeria.

ABSTRACT

Introduction: Green tea and its polyphenolic catechins have shown beneficial effects in several inflammatory pathogenesis. It is one of the most frequently consumed beverages in North African countries such as Algeria, which is characterized by a high incidence of scorpion envenomation. The aim of this study was to investigate the effects of the green tea against cardiac and hepatic inflammatory response and oxidative stress induced by scorpion venom. **Materials and Methods:** Green tea was administered to mice in drinking water for 4 weeks before the injection of a sublethal dose of venom. The inflammation response and oxidative stress were assessed twenty-four hours after the envenomation by the evaluation of some inflammatory and oxidative stress markers, histological alterations and metabolic enzyme levels. **Results:** The results showed that scorpion venom induced tissue inflammatory response characterized by inflammatory cell infiltration and by an increase in levels of reactive oxygen/nitrogen species and a decreased antioxidant defense. Significant alterations of the cardiac and the hepatic tissues such as edema and hemorrhages associated with increased levels of metabolic enzymes were also observed. The administration of green tea infusions decreased nitric oxide and thiobarbituric acid-reactive substances levels and reduced the incidence of heart and liver tissue alteration and metabolic enzyme levels. These results indicate that green tea exhibits potent protective effects against scorpion venom induced cardiac and hepatic toxicity probably by increasing antioxidant defense. **Conclusion:** The antioxidant properties are due to the synergistic properties of green tea extracts such as catechins, which are increasingly recognized as being potentially important for medicinal benefits.

Key words: Scorpion venom, cardiac and hepatic tissues, inflammation response, oxidative stress, green tea.

RESUME

Introduction: Le thé vert et ses catéchines polyphénoliques ont montré des effets bénéfiques dans le confort ou traitement de plusieurs pathologies inflammatoires. C'est l'une des boissons les plus consommées dans les pays d'Afrique du Nord tels que l'Algérie qui est concernée par une forte incidence d'envenimation scorpionique. Le but de cette étude est d'étudier l'effet du thé vert sur la réponse inflammatoire induite au niveau cardiaque et hépatique et le stress oxydant par le venin de scorpion. **Matériels et Méthodes** : Dans cette étude, le thé vert a été administré, à des souris dans l'eau de boisson pendant 4 semaines avant l'injection d'une dose sublétale de venin. La réponse inflammatoire et le stress oxydant ont été évalués 24 heures après l'envenimation par l'évaluation de certains marqueurs de la réponse inflammatoire et du stress oxydant, par une analyse histopathologique ainsi que par l'estimation du taux de quelques paramètres métaboliques. **Résultats:** Les résultats ont montré que la réponse inflammatoire induite par le venin de scorpion se caractérise par une infiltration de cellules inflammatoires, une augmentation des taux des espèces réactives oxygénées et azotées, une diminution de la défense anti-oxydante ainsi

que par des altérations tissulaires importantes (œdèmes et hémorragies), associées à une augmentation des taux d'enzymes métaboliques au niveau sérique. L'administration du thé vert a diminué la réponse inflammatoire, le stress oxydant, les altérations tissulaires cardiaques et hépatiques ainsi que le taux des enzymes métaboliques. **Conclusion :** L'effet immuno-protecteur du thé vert semble être dû à l'augmentation de la défense anti-oxydante par l'apport de molécules telles que les catéchines connues par leur intérêt thérapeutique et protecteur contre les désordres immuno-inflammatoires.

Mots clés: Venin de scorpion, tissus cardiaque et hépatique, réaction inflammatoire, stress oxydant, thé vert.

* *Auteur correspondant.* Tel.: +213 6 151 6276; fax: +0-000-000-0000.
Adresse E-mail:flaraba@hotmail.com

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1. Introduction

There is currently great interest in the use of phytochemicals from foods and beverages as preventive or curative compounds [1-3]. Green tea provided from the *Camellia sinensis*plant is one of the most popular beverages in several areas of the world such as North Africa and Middle East. The beneficial impact of its regular consumption against various diseases has been reported [4]. Many of these beneficial effects of green tea are related to its significant antioxidant and anti-inflammatory properties reported in numerous human, animal, and *in vitro* studies [5]. These studies reported the contribution of green tea in the prevention of heart and liver diseases and its various physiological and pharmacological properties such as anti-hypertensive effect, antibacterial and antiviral activities, anti-fibrotic properties, and neuroprotective powerful [6-8]. Green tea also prevents some forms of cancer [9], protects against nephrotoxicity and delays memory regression [10, 11]. In this study, we evaluate the role of green tea in protecting against cardiac and hepatic tissue injuries after scorpion envenomation. The cardiac and hepatic inflammatory response and oxidative stress are key processes in scorpion envenoming syndrome and could be a cause of death after scorpion envenomation [12,13]. Inflammatory events were assessed by measuring markers of the influx of inflammatory cells to the cardiac and the hepatic tissues and the oxidative stress status. Furthermore, this study was supported by histological analysis and assessment of enzyme activities reflecting tissue injuries.

2. Materials and methods

2.1. Venom

The venom from the Androctonus australis hector scorpion was provided by the Laboratory of Cellular and Molecular Biology (Biochemistry of Biomolecules: Mode of Action, Immunotherapy and Immunodiagnostic team, code: C0610502) of the *Biological Sciences Faculty at the University of Science and Technology HouariBoumediene (USTHB).*

2.2. Animals

N.M.R.I. mice (20 ± 2 g body weight) were obtained from the animal breeding division of the Biological Sciences Faculty of the USTHB.They were group-housed under controlled temperature and light-dark cycle conditions. Animal care and experimental procedures were performed according to the 2010/63/EU Directive of the European Parliament.

2.3. Green tea

The dried leaves of green tea issued from the plant *Camellia sinensis* (5, 10 or 50 g) were soaked in 500 ml of boiling distilled water for 5 minutes. The obtained extract was filtered and the leaves underwent a further extraction step by the addition of 500 ml of boiling distilled water. The obtained second extractwas filtered and then mixed with the first one. It was subsequently stored at 4 °C.

2.4. Chemicals and reagents

The chemicals and reagents are of a high analytical quality. They are provided from Sigma Aldrich (United States of America) and Merck (Germany).

2.5. In vivo experiments

Mice of group 1 and 2 received green tea (0.5 and 1%, respectively) as the only source of drink for 15 and 30 days, while the third group was given green tea extract (5%) by gavage twice daily during 30 days. On the fifteenth and the thirtieth day, the mice received a subcutaneous injection of physiological saline (NaCl control group) or a sublethal dose (0.5

mg /kg) of the *Androctonus australis hector* venom. Mice were sacrificed within 24 hours of NaCl or venom injection. At necropsy, blood, heart and the liver were collected. The organs were weighed, and then processed for further analysis in the assays outlined below.

2.6. Evaluation of inflammatory cell infiltration

The neutrophil myeloperoxidase as well as eosinophil peroxidase in inflamed mice heart and liver were measured. The estimation of the activity of the myeloperoxidase was carried out according to the protocol of Krawisz et al. (1984) [14], whereas the dosage of eosinophil peroxidase activity was carried out according to the method of Van Oosterhout et al. (1996) [15].

2.7. Evaluation of the heart and the liver redox status

In order to evaluate the redox status in the cardiac and the hepatic tissues, a study of the pro- and antioxidant balance was undertaken. The nitric oxide (nitrites) assay was performed according to the method of Sun et al. (2003) [16], whereas, the levels of the peroxidation marker of membrane lipids (malondialdehyde) were carried out according to the method of Ohkawa et al. (1979)[17].

The study of the antioxidant defense system was undertaken by the estimation of catalase antioxidant activity, according to Aebi (1984) [18]. The reduced glutathione was estimated by the Ellman modified method (Zhang, 2000) [19].

2.8. Tissue alterations analysis and metabolic enzyme measurements

The hearts and livers taken after mice sacrifices were immediately immersed in 10 % formalin for 48h. They were then dehydrated in alcohol baths and impregnated with paraffin. The sections were made and stained with hematoxylin and eosin. They were observed under a photonic (Motic) microscope connected to a camera. Measurements of the serum enzymatic activities of creatine phosphokinase, lactate dehydrogenase, alanine aminotransferase and alkaline phosphatase were performed according to manufacturer's instructions (Spinreact) using an automatic apparatus (HITACHI 902 ISE).

2.9. Statistical analysis of results

The statistical analysis of the results was carried out by the Student's *t*-test. The results are shown as mean and standard error of the mean (mean ± S.E.M).

3. Results

3.1. Effects of green tea on inflammatory cell infiltration induced by scorpion venom

The results showed significant decrease (dose- and time-dependent) of neutrophil and eosinophil infiltration in the cardiac and the hepatic tissues compared to the envenomed mice (**Figure 1: a, b, c and d**).

Indeed, myeloperoxidase activity decreased when green tea at 0.5 and 1 % during 15 and 30 days compared to the estimated values obtained in the cardiac and in the hepatic tissues of envenomed mice (**Figure 1a and 1c**).

It was noted that the doses of 1 and 5 % administered during 30 days are the most effective in preventing eosinophil cell infiltration in the cardiac tissue ($P < 0.01$) (**Figure 1b**).

In the liver tissue, the best preventive effects of green tea were observed at 0.5 and 1% ($P < 0.01$) (**Figure 1d**).

3.2. Beneficial effects of green tea on the imbalance between the pro and the antioxidant system induced by scorpion venom

Nitrite contents produced in the cardiac and the hepatic tissues of the pretreated envenomed mice with green tea showed a decrease in this reactive nitrogen specie level in dose-and time- dependent manner. The pretreatment of the mice during the 30-days appears to be more preventive than those carried out during 15 days (**Figure 2c and 2d**).

Indeed, lower levels of nitrites were noted in the cardiac and the hepatic tissues with green tea at 1 % ($P < 0.05$ and $P < 0.01$, respectively) (**Figure 2a and 2b**). Green tea also reduces lipid peroxidation caused by scorpion venom in the heart and the liver. The most interesting results are those obtained within the 30 days-pretreatment period (**Figure 2c and 2d**).

The status of the anti-oxidant defense system in the cardiac tissue was assessed in the homogenates by the evaluation of catalase activity and the glutathione level (**Figure 3a and 3b**).

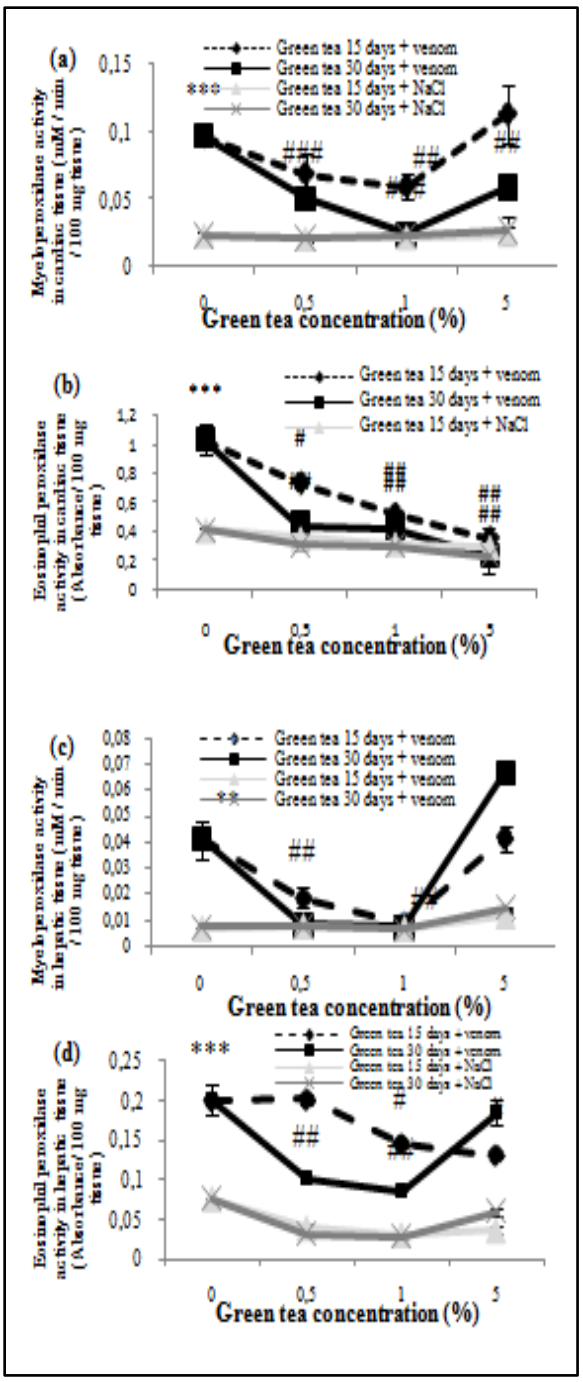

Figure 1: Effects of green tea on myeloperoxidase and eosinophil peroxidase activities reflecting infiltration of neutrophils and eosinophils, respectively, into the cardiac (a, b) and the hepatic (c, d) tissues, induced by a sublethal dose of *Androctonus australis hector* venom (0.5 mg / kg), 24 hours after the injection *(Mean ± S.E.M., n=3 ; Student t test; ***P<0.001; Groups of envenomed mice compared to the control (NaCl alone). #P<0.05; ## P<0.01; ### P<0.001; Groups pretreated with green tea compared to the group of mice receiving the venom alone).*

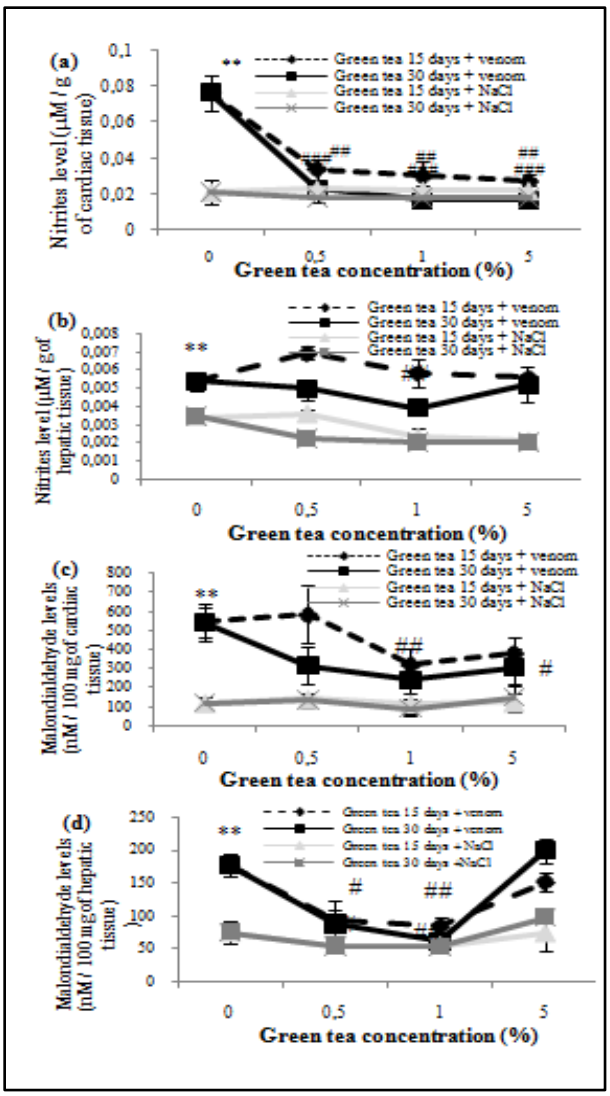

Figure 2: Effects of green tea on the variation of the levels of nitrites (a, b) and malondialdehyde (c, d) in the cardiac and the hepatic tissues after experimental envenomation with a sublethal dose of *Androctonus australis hector* venom (0.5 mg / kg), 24 hours after its injection.*(Mean ± S.E.M., n=3 ; Student t test; ** P < 0.01 ; *** P < 0.001 Groups of envenomed mice compared to the control (NaCl alone). #P<0.05; ## P<0.01; ### P < 0.001; Groups pretreated with green tea compared to the group of mice receiving the venom alone).*

Mice receiving green tea displayed important protective effects against the depletion of the antioxidant system. The study of the non-enzymatic antioxidant system state in envenomed mice showed significant increase in the reduced glutathione levels in the cardiac ($P < 0.001$) and the hepatic ($P < 0.05$) tissues of pretreated animals with 0.5 and 1% of green tea during 15 and 30 days prior to experimental envenomation compared to those of envenomed mice. However, green tea at 5% was less effective in

preventingthe non-enzymatic antioxidant system alteration (**Figure 3a and 3b**). On the other hand, the recorded catalase activity was more important in presence of green tea at 5% than that observed in envenomed mice (**Figure 3c and 3d**).

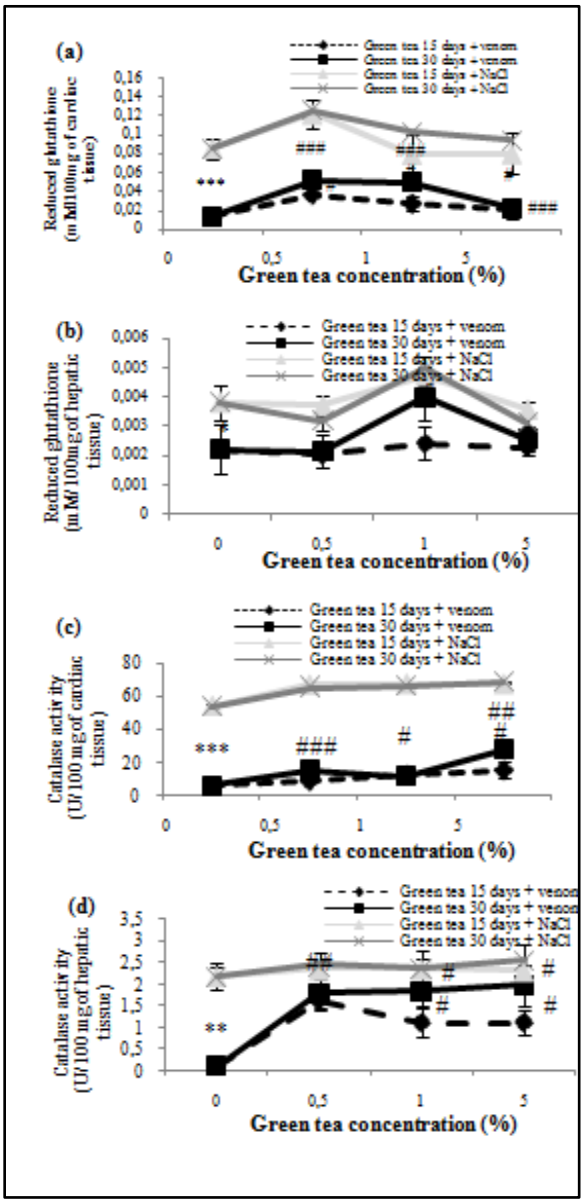

Figure 3: Effects of green tea on the non enzymatic (a, b) and the enzymatic (c, d) antioxidant system in the cardiac and the hepatic tissues after experimental envenomation with a sublethal dose of venom (0.5 mg / kg), 24 hours after its injection. *(Mean ± S.E.M., n=3 ; Student t test; ** P < 0.01 ; *** P < 0.001 Groups of envenomed mice compared to the control (NaCl alone). #P<0.05; ## P<0.01; ### P < 0.001; Groups pretreated with green tea compared to the group of mice receiving the venom alone).*

3.3. Evaluation of the green tea effects on histological damage and organ dysfunction induced by scorpion venom

The anatomopathological analysis of heart and liver tissues of the envenomed mice revealed edema, hemorrhage, congested center-lobe vein and massive leukocyte infiltration into these tissues 24h after venom injection (**Figure 4b and 4e**). Histopathological analysis of the myocardium and the hepatic tissue of mice receiving green tea prior to experimental envenomation revealed fewer alterations in pretreated animal's tissues during the 30-day period than those receiving the same pretreatment during 15 days.

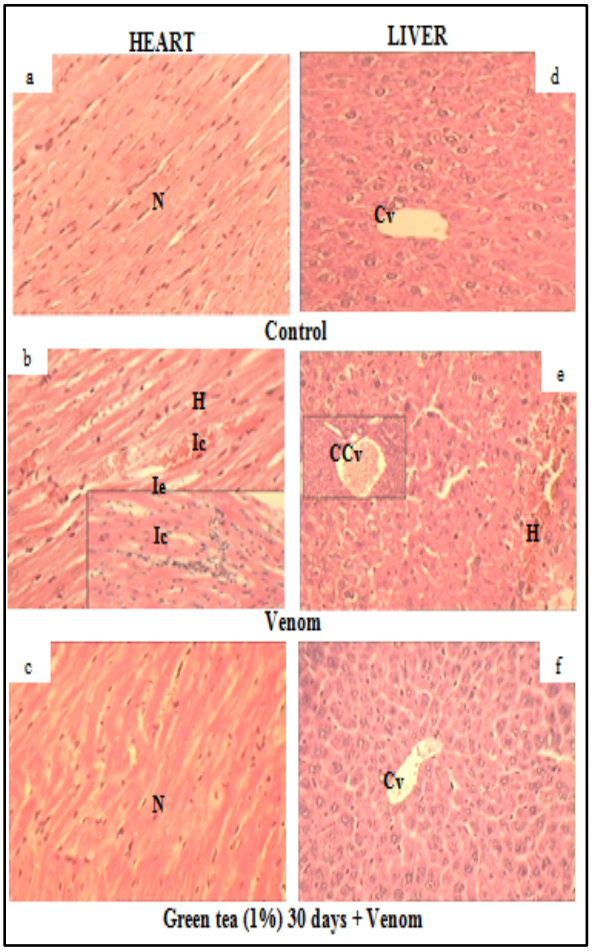

Figure 4: Effects of green tea concentration (1%) and time consumption period (30 days) on changes in myocardium and liver parenchyma structure induced by a sublethal dose of *Androctonus australis hector* venom (0.5 mg / kg). *Ic: Inflammatory cells, H: Hemorrhage: N: Nucleus, Ie: Interstitial edema, Cv: Center-lobe vein, CCv: Congested center-lobe vein (Hematoxylin-eosin staining: magnification: × 400).*

Indeed, hemorrhagic edema and infiltration of inflammatory cells were observed in the myocardium and the liver parenchyma of mice receiving green tea during the 15-day period (data not shown) whereas no alterations were observed in the tissues of pretreated mice during 30-days with green tea at 1% compared to the envenomed animals (**Figure 4c and 4f**). The administration of green tea at 1% during 15 or 30 days appears to be more effective in the reduction of the level of creatine phosphokinase, lactate dehydrogenase, alanine aminotransferase and alkaline phosphatase in the sera of envenomed mice (**Figure 5a, b, c and d**).

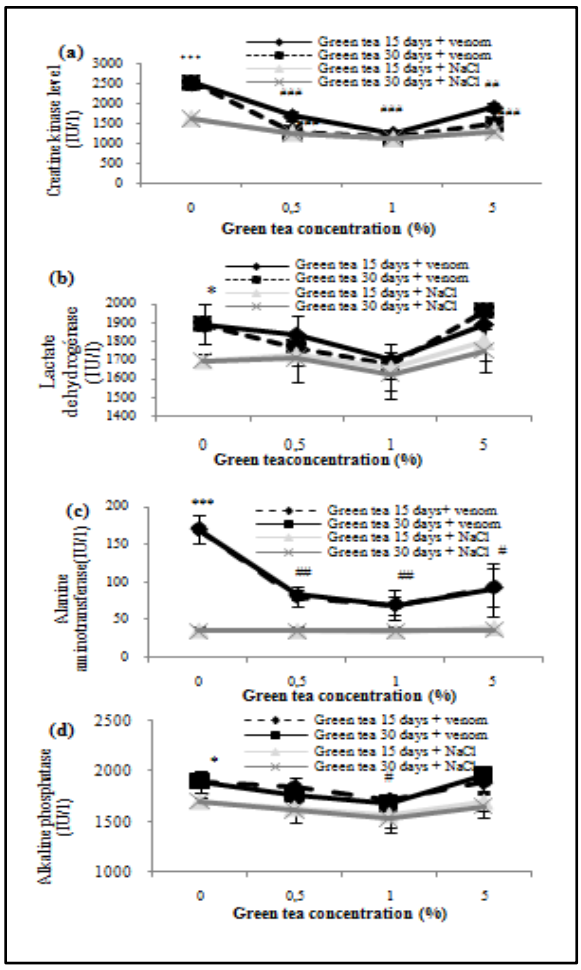

Figure 5: Effects of green tea concentration on the variation in seric levels of creatine kinase (a), lactate dehydrogenase (b), alanine aminotransferase (c) and alkaline phosphatase (d) after experimental envenomation with a sublethal dose of scorpion venom (0.5 mg / kg), 24 hours after its injection.
(Mean ± S.E.M., n=3 ; Student t test; * P < 0.05 *** P < 0,001 ; Groups of envenomed mice compared to the control (NaCl alone). ## P<0.01; ### P < 0.001; Groups pretreated with green tea compared to the group of mice receiving the venom alone).

4. Discussion

Several studies carried out over the past years have shown that the polyphenolic fractions isolated from green tea inhibit oxidative stress and possess anti-inflammatory activity [5, 20]. The administration of green tea to mice for one month prior to the administration of the venom showed that this plant has a preventive effect against the increased infiltration of the inflammatory cells in cardiac and hepatic tissues induced by the scorpion venom. This effect depends on the dose or the consumption time of the *Camellia sinensis* infusions.

The triggering of the immune-inflammatory response during scorpion envenomation is accompanied by the antioxidant system dysfunction [21, 22]. Several studies have demonstrated the beneficial effect of green tea or its polyphenolic extracts commonly known as flavanols or catechins in the reduction of oxidative or nitrosative stress [23]. Donà et al. reported in 2003 [24] that the green tea polyphenols are potent inhibitors of neutrophil activity, and suggest their use as pharmacological agents in preventive therapy for person at risk for inflammatory diseases. Di Paola et al. (2005) [25] also reported that green tea decreased the induced infiltration of neutrophil levels into lung tissue in experimental model of pulmonary inflammation by carrageenan. The infiltration decrease in inflammatory cells can be attributed to the inhibitory effect of the involved enzymes or cytokines in immune-inflammatory responses such as COX-2 and TNF-α by green tea [26,27]. Polyphenols are also able to modulate the activity, expression and/or secretion of various mediators involved in the immune-inflammatory response. For example, they reduce the expression of adhesion molecules, allowing a decrease in the adhesion and recruitment of blood cells to tissues inflammatory foci [25]. The obtained results in this study showed that, in addition to its effect on the reduction of inflammatory cell infiltration, green tea can also prevent the installation of oxidative and nitrosative stress caused by scorpion venom in cardiac and hepatic tissues by decreasing the nitric oxide levels, lipid peroxidation as well as an improvement in antioxidant systems (catalase and reduced glutathione).
The previous study conducted by Tedeschi and collaborators in 2004 [28] showed that green tea and its extracted polyphenols present an inhibitory effect on the activity of the inducible NO synthase. Other studies have shown that the administration of green tea polyphenols induces a decrease in lipid peroxidation due to its antioxidant activity [29].

Green tea catechins can act as free radical scavenger sensors, reducing the tissue damage [30]. In addition to these effects, green tea flavonoids can chelate iron and copper and prevent their participation in the reactions of Fenton and Haber-Weiss reaction types [31].

The anti-inflammatory and the antioxidative effects of green tea resulted in a remarkable prevention of the immune-inflammatory disorders induced by *Androctonus australis hector* scorpion venom in the myocardium and the liver parenchyma. The prevention of these disorders was dose- and time-dependent on green tea consumption. This effect was observed at different concentrations (0.5, 1 and 5 %). However, the protective effects of green tea were observed mainly at 1% administered during 30 days when compared with envenomed mice consuming no green tea.

Conclusion

The present study suggests that *Camellia sinensis* has a beneficial preventive effect against cardiac and hepatic inflammatory disturbances and oxidative stress induced after scorpion envenomation. However, further studies are needed to identify biological active molecules in order to accurately describe the molecular mechanism(s) responsible for this anti-inflammatory and antioxidant effect.

Conflicts of interest

The authors do not declare any conflict of interest.

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