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Protective effect of *Alsidium corallinum* against TNF-α toxicity in H9c2 cells

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Abstract
Sepsis is a principal cause of human mortality. Tumor necrosis factor alpha (TNF-α) is a factor of the myocardial dysfunction induced by sepsis. The present study attempts to determine the role of the macroalga *Alsidium corallinum* (*A. corallinum*) from TNF-α-induced sepsis. The cardiomyocytes H9c2 exposed to *A. corallinum* extract, then these cells have treated with TNF-α. H9c2 viability and antioxidant defense like glutathione peroxidase, superoxide dismutase, catalase and glutathione were evaluated. Main results reported that TNF-α caused a morphological cell modifications and, a reduction in H9c2 viability, and an increase of antioxidant defense. *A. corallinum*-treatment reduced the toxicity induced by TNF-α. These results demonstrated the cytoprotector role of *A. corallinum* against oxidative stress.

Keywords: *Alsidium corallinum*; cardiomyocyte cells; tumor necrosis factor alpha; antioxidant defense

1. Introduction
Sepsis, defined by 'systemic inflammatory response syndrome', is characterized by sever inflammation and coagulation of immune defense [1]. Sepsis may induce cardiac malfunction and serious heart disease [2]. Cardiac degeneration caused by sepsis are principal risk factors of heart toxicity and mortality of effected patients [3]. Various toxic agents, such as cytokines and endotoxins can be induced sepsis [4]. In fact, Cytokines can attack the immune responses, and cause injuries in tissues such as liver and heart [4]. Data demonstrated the association by tumor necrosis factor alpha (TNF-α) and sepsis [5]. TNF-α is an inflammatory cytokine present in the blood circulation, that can be produced essentially by cardiomyocytes [5]. Additionally, TNF-α is responsible for cells apoptosis, and contributes to sepsis-induced cardiac dysfunction. Furthermore, the mechanism of this disease is poorly known. The embryonic cardiomyocyte cells H9c2 is previously used to investigate the sepsis [2].
The control of TNF-α levels has a therapeutic method in sepsis-induced myocardial dysfunction. Medical treatments of sepsis may be efficient, but the mortality levels still elevated. Interestingly, recants studies demonstrated the efficacy effect of natural product against sepsis [2, 5]. Marine algae are good sources of bioactive compounds [2, 6].
Thus, our study aims to evaluate the mechanism of protective effect of *A. corallinum* in TNF-α-induced H9c2 cardiomyocytes damage.

2. Material and Methods
2.1 Maintenance and culture of cells
H9c2 cardiomyocytes cells were put in Dulbecco's Modified Eagle Medium (DMEM). Then fetal bovine serum and antibiotic were supplemented. The melange have incubated at 37 °C under CO_2. Finally, these cells were harvested with trypsin/EDTA [7].

2.2 Treatment of cells
To evaluate the cytoprotective assay of *A. corallinum*, H9c2 cells were exposed to TNF-α (30 ng/ml), dimethylsulfoxide (DMSO), as well as *A. corallinum* (30 µg/ml) at 37 °C for 12 h. All tests were carried out in triplicate.

2.3 Viabilty of cells
Cell viability was evaluated using 3-(4, 5-dimethylthiazol-2-yl)-2, 5 diphenyltetrazolium bromide (MTT) test. The viability of cells was expressed as viable cells (%) to control cells.

The result was analyzed in triplicate.

2.4 Morphology of cells

After 12 h of treatments, morphology of H9c2 cells was observed with an electronic microscope.

2.5 Oxidative stress parameters

2.5.1 Protein quantification

Protein levels were evaluated using the method of Bradford [8]. Then, the cells were stored at 80 °C for biochemical analysis.

2.5.2 Superoxide dismutase activity (SOD)

The activity of SOD was assayed using the Beauchamp and Fridovich method [9]. Result was observed at 580 nm. Results of SOD activity were expressed as units/mg of protein. All assays were analyzed in triplicate.

2.5.3 Glutathione peroxidase activity (GPx)

GPx activity was estimated by the method of Flohe and Gunzler [10]. The results were was observed on 340 nm. Final activity was expressed as nmol of GSH/min/mg protein. The test was measured in triplicate.

2.5.4 Catalase activity (CAT)

The activity of catalase was evaluated using the method of Aebi [11]. The absorbance was measured spectrophotometrically at 240 nm. The GPx activity was expressed as mmol H_2O_2/min/mg protein. The test was assayed in triplicate.

2.5.5 Glutathione levels (GSH)

The GSH contents were evaluated using the method reported by Ellman [12] and modifiated by Jollow *et al.* [13]. The absorbance was measured at 412 nm. The levels of GSH are expressed as µg/mg protein. The assay was performed in triplicate.

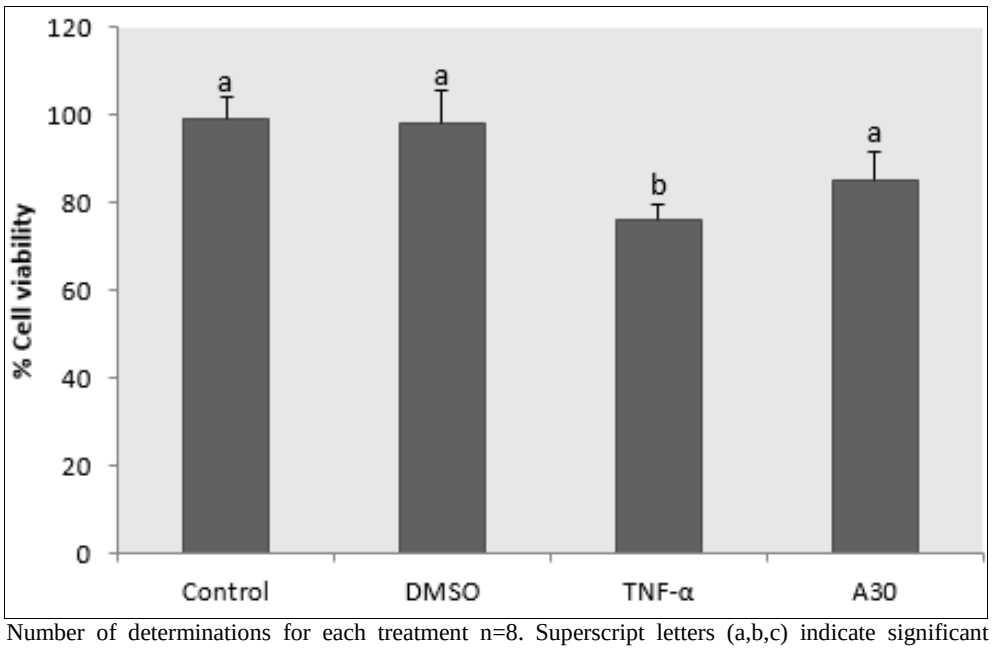
2.6 Statistical data

The results were presented as the mean ± standard deviation (SD). The difference between different groups was determined using one-way ANOVA, according to Duncan's multiple-range test using SPSS 20.0 as a post hoc test for comparison between different groups.

3. Results

3.1 Effect of TNF-α and A. corallinum on H9c2 cell viability

The viability of H9c2 cells after *A. corallinum* treatment (30 µg/ml) has (semblable) to control cells. The treatment of TNF-α (30 ng/ml) have reduced the cell viability. The co-treatment by TNF-α and *A. corallinum* has improved the cell viability in comparison with TNF-α cells (figure 1).

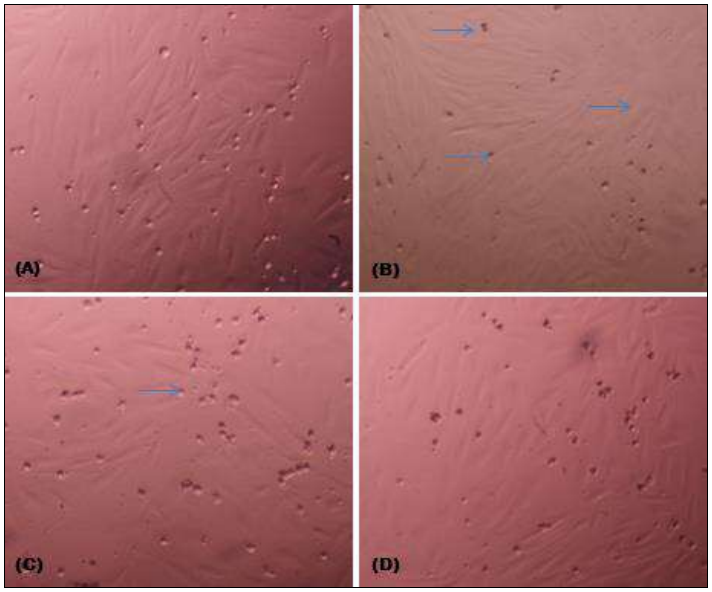


Number of determinations for each treatment n=8. Superscript letters (a,b,c) indicate significant differences at $p < 0.05$

Fig 1: Viabilty of H9c2 Cells treated with DMSO, TNF-α and Alga (%)

3.2 Effect of TNF-α and A. corallinum on cell morphology

The microscopic observation after 12h of treatment demonstrated that the TNF-α induced morphological modifications in H9c2 cells like detachment of cells, and rounding and progressive cell detachment. However, the co-treatment by TNF-α and *A. corallinum* demonstrated a normal structure of cells (Figure 2).



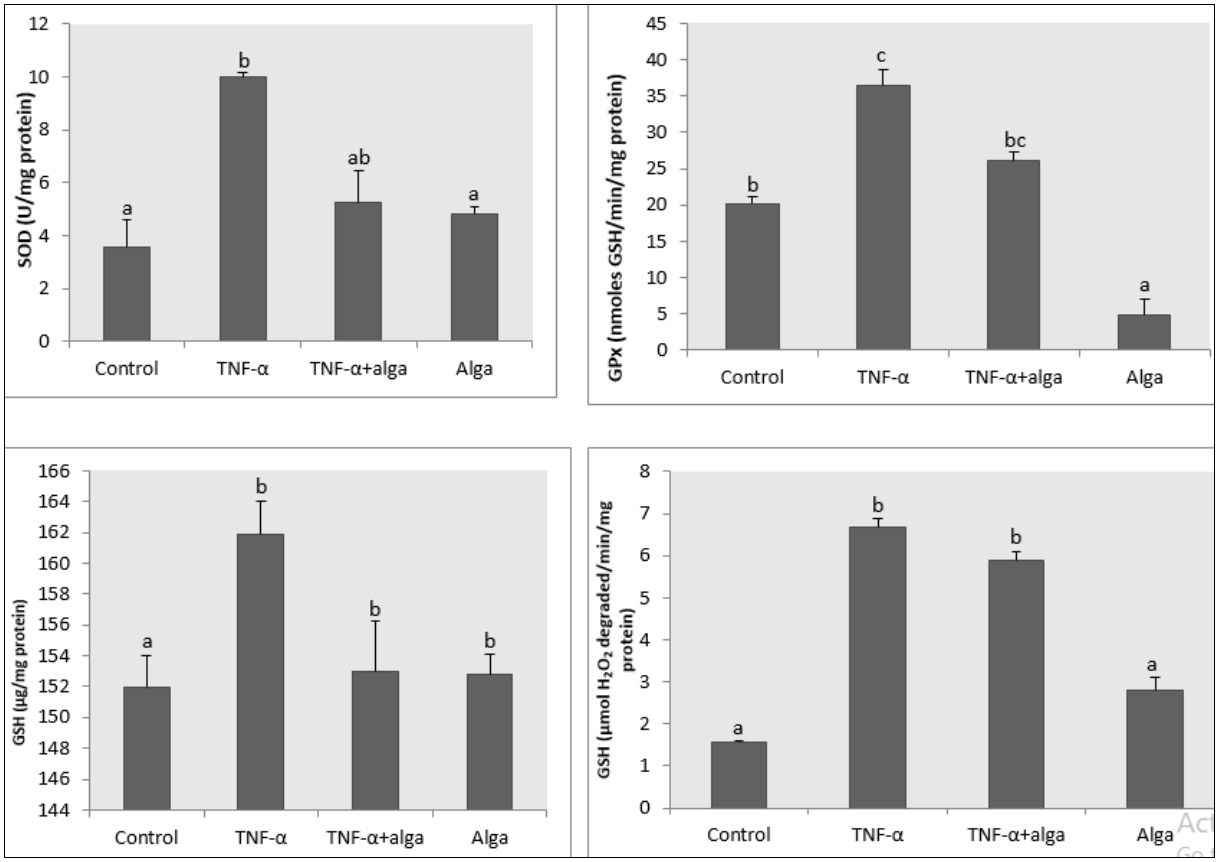
(A) Control cells, (B) TNF-α (30 ng/ml), (C) Alga extract+ TNF-α, Alga extract (30 mg/ml)

Fig 2: H9c2 Morphology by TNF-α with or without *Alsidium corallinum*

3.3 Effect of TNF-α and A. corallinum on antioxidant defense

After 4h of TNF-α exposure, our results demonstrated a significant rise in enzymatic antioxidant defence such as GPx and SOD activities, compared to the untreated cells. The co-treatment by *A. corallinum* and TNF-α showed a significant improve in GPx and SOD activities in comparison by TNF-α treatment (Figure 3). *A. corallinum* alone improved antioxidant enzymatic system (Figure 3).

For the non-enzymatic defence including GSH and catalase levels, we have demonstrated an increase after TNF-α treatment. However, the co-treatment by *A. corallinum* and TNF-α showed a decrease in GSH and catalase levels in comparison with TNF-α-exposure cells. The treatment by *A. corallinum* alone improved the non-enzymatic defence compared to the TNF-α cells.



Number of determinations for each treatment: n=8. Different superscript letters (a,b,c) indicate significant differences at $p < 0.05$

Fig 3: Antioxidant enzymatic and no enzymatic defence of H9c2 cells after treatment with *Alsidium corallinum* (30 µg/ml) associated to TNF-α (30 ng/ml)

4. Discussion

Tumor necrosis factor-alpha is an essential cause of pathophysiological events of sepsis. Recently, inhibition of this factor attracts the attention in medicine sector. The current study was aimed to evaluate the possible therapeutic role of macroalga *A. corallinum* against TNF-α induced sepsis in H9c2 cells.

Our findings indicated that TNF-α induced a modification of antioxidant defense. In fact, an increase of SOD and GPx, GSH and GPx activities in H9c2 cells was noted. Sepsis-induced heart damage and endogenous antioxidant system alteration has been related to mitochondrial dysfunction induced by oxidative stress. Mitochondrial dysfunction has shown as result of sepsis [14]. The mitochondrial toxicity may be activate the cells antioxidant defence system [14]. This system is strictly activated by enzymatic and non-enzymatic responses combination. Administration of *A. corallinum* to TNF-α-treated cells induced an improvement in the antioxidant enzyme activities due to the richness of the red alga in phenolic compounds, including polyphenols, flavonoids, and polysaccharides [15]. These biomolecules are able to trap reactive oxygen species before reaching their cellular targets. The modulation of antioxidant defence by *A. coralinum* in H9c2 treated cells is previously reported by our study [2].

Heart dysfunction induced by sepsis has been related to oxidative stress, which results decline of energy production [16]. In fact, our findings showed that TNF-α exposure caused a reduction in H9c2 cell viability, as shown by modification in cell morphology such as cells detachment. The treatment with *A. corallinum* has improved cell viability confirming that the macroalga have potential antioxidant capacity in cardiomyocytes. Our previous study showed that *A. corallinum* increase cell viability in H9c2 cells exposure to H_2O_2 [2].

5. Conclusion

The present study indicated that *Alsidium corallinum* could be a potential natural treatment for chronic diseases with sepsis.

6. Conflict of interests

All the authors declare any conflict of interests.

7. Acknowledgments

The present study was realized in the Laboratory of Enzyme Engineering and Microbiology, National Engineering School, University of S fax, Tunisia.

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