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Protective and antioxidant potential of the argan oil on induced oxidative stress in *Tetrahymena pyriformis*

Rachida Cadi*, Khadija Mounaji, Fadila Amraoui and Abdelaziz Soukri

Department of Biology, Laboratory of Physiology and Molecular Genetics, associated with CNRST, Ain Chock Faculty of Sciences, Hassan II University, Casablanca, Morocco.

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Since reactive oxygen species (ROS) play an important role in the pathogenesis of many human diseases, attention is given to the development of safe and effective natural antioxidants. In the present study, the protective and antioxidant potential of the argan oil extracted from the fruit of *Argania spinosa,* an endemic tree in Morocco, was evaluated against hydrogen peroxide (H_2O_2) induced oxidative damages in *Tetrahymena pyriformis*. The protozoan cells were cultivated in polypeptone yeast glucose (PPYG) medium supplemented with argan oil (AO) at a dose of 0.5% (v/v) during 4 subcultures and treated with different concentrations of H_2O_2. The effect of this oil on the protozoan growth was followed and the antioxidant enzyme activities of catalase (CAT), superoxyde dismutase (SOD) and glutathione reductase (GR) were evaluated as well as lipid peroxydation (MDA). As compared to the controls, the AO increased both the cell viability and the antioxidant enzyme activities and decreased the MDA level. The co incubation of *T. pyriformis* with AO and H_2O_2 (300 µM) reduced the H_2O_2 cytotoxicity. The cells cultivated in the presence of AO then exposed to H_2O_2 for 3 h, show a significant reduction in MDA level ($p < 0.001$), an important increase in CAT ($p < 0.05$) and SOD ($p < 0.01$) activities. No effect was observed in that GR activity. These results strongly indicate that AO have a protective potential against induced H_2O_2 cell damages. This protective effect may be due to antioxidant compounds present in high concentrations in the argan oil.

Keys words: Antioxidant enzymes, argan oil, hydrogen peroxide, lipid peroxidation, *Tetrahymena pyriformis.*

INTRODUCTION

In stress conditions, many oxidants are produced and can initiate further deleterious effects on biomolecules, and therefore pathogenesis in many diseases. The oxidative stress is involved in various physicochemical processes and diseases such as ageing (Halliwell, 1994), atherosclerosis (Cipollone et al., 2007), diabetes (Bonnefont-Rousselot, 2002), cancer (Valko et al., 2006), cataract (Vinson, 2006), and in cellular death pathways of neurodegenerative diseases such as Alzheimer (Ca and Yan, 2007) and Parkinson (Halda and Lotharius, 2005). Several studies have reported that natural plants have a potent antioxidant and represent an important source of natural antioxidants. They can reduce oxidative damages and thus prevent the apparition of diseases. Also, they could treat diseases caused and/or fostered due to oxygen free radicals. Argan oil is a natural product extracted from the fruit of *Argania spinosa,* an endemic tree in Morocco. Argan tree, of the family Sapotaceae,

*Corresponding author. E-mail: rachidacadi@gmail.com. Tel: +212 5 22 23 06 80/84. Fax: +212 5 22 23 0674.

plays an essential ecological and socio-economic role in south western Morocco. This oil is largely used in cooking, traditional medicine and cosmetic (Charrouf and Guillaume, 1999). During the last decades, scientific work came to support its use. On the one hand, chemical analyses uncovered its wealth of unsaturated fatty-acids as well as antioxidant elements such as tocopherols, polyphenols, sterols, carotenoids, xanthophylls and squalene (Khallouki et al., 2003). On the other hand, several biological studies revealed its antitumoral (Samane et al., 2006; Bennani et al., 2007), hypolipidemic, hypocholesterolemic (Berrougui et al., 2003; Samane et al., 2006) and anti inflammatory effects (Alaoui et al., 1998). In addition, it reduces the cardiovascular risk and may be used as antiatherogenic oil (Cherki et al., 2006; OuldMohamedou et al., 2011). However, the antioxidant potential of argan oil (OA) is not clearly elucidated.

The present study aims to evaluate the antioxidant and protective effect of argan oil against stress induced by H_2O_2. This oil is mainly rich in antioxidant compounds such as tocopherols which are present in a higher proportion (Khallouki et al., 2003). In this study, *Tetrahymena pyriformis,* eukaryotic cell, was used as model which imitates the animal cell, especially mammalian and used widely in toxicant screening studies in environmental and pharmaceutical fields (Sauvant et al., 1999).

MATERIALS AND METHODS

Strains and culture conditions

Tetrahymena pyriformis (Strain E, ATCC 30005) was grown in PPYG medium (1% proteose peptone, 0.25% yeast extract and 0.2% glucose) axenically at 28°C during 72 h. The assay departed from initial densities of about 5,000 individuals' ml^{-1}.

Argan oil

The argan oil was provided from the South of Morocco (Taroudant, Morocco). This oil was extracted traditionally from the fruits of *A. Spinosa* as described by Charrouf and Guillaume (1999). The quality of handmade argan oil is assured by the control oil extraction process.

Analysis of cell viability

The protozoan cells were grown in PPYG medium added with various concentrations of H_2O_2 (200, 250, 300 and 400 µM) in order to determine sub lethal concentration. The growth curves were performed by a daily enumeration cells. For AO, the cells were maintained during 4 subcultures in PPYG medium supplemented with the various concentrations (0.05, 0.1 and 0.5) (v/v%). The AO is preliminary solubilised in dimethyl sulphoxide (DMSO). The final concentration of DMSO in the culture medium was 0.1% (v/v).

Crude extract preparation

Protozoan cells were harvested by centrifugation at 4,000 × g for 10 min and suspended in 20 mM Tris-HCl buffer (pH 7.5) containing 1 mM ethylenediamine tetra-acetic acid (EDTA), 10 mM 2-mercaptoethanol, 1 mM phenylmethylsulfonylfluoride (PMSF) and 1% (v/v) glycerol. The cells were then disrupted in the cold with a Branson model B12 Sonifer (80 W, 60 s). The supernatant obtained after centrifugation at 12,000 × g for 45 min at 4°C was considered as crude extract and used for enzymes assay.

Determination of lipid peroxidation

Lipid peroxidation was quantified in terms of malondialdehyde equivalents (MDA) as described by Samokyszyn and Marnett (1990). Briefly, 100 µl of samples was added to 900 µl solution (0.375% thiobarbituric acid and 15% trichloracetic acid in 0.25 M hydrochloric acid). The reading of supernatant was made to 535 nm. The MDA level was expressed as nanomoles of MDA per milligram of protein.

Determination of antioxidant enzyme activities

Catalase activity

The CAT activity was determined by the method of Aebi (1984) in which the consumption of 7.5 mM H_2O_2 in 50 mM potassium phosphate buffer (pH 7) was measured spetrophotometrically at 240 nm. The enzyme activity was expressed as millimoles of H_2O_2 consumed/min/mg protein.

Superoxide dismutase activity

The SOD activity was assayed as described by Paoletti et al. (1986) with assay conditions: 5 mM EDTA, 2.5 mM $MnCl_2$, 3.9 mM 2-mercaptoethanol in 50 mM potassium phosphate buffer (pH 7). The activity started by the addition of nicotinamide adenine dinucleotide reduced form (NADH) to 0.27 mM as final concentration and monitored at 340 nm. The enzyme activity was calculated as micromoles of NADH consumed/min/mg protein.

Glutathione reductase activity

The GR activity was determined by the method of Di Ilio et al. (1983). Assay mixture contained 0.5 mM oxidized glutathione (GSSG), 1 mM EDTA, 0.1 mM nicotinamide adenine dinucleotide phosphate reduced form (NADPH) and 50 mM potassium phosphate buffer (pH 7.4). The NADPH consumption was monitored at 340 nm. The enzyme activity was calculated as micromoles of NADPH consumed/min/mg protein.

Protein assay

Protein content was measured according to the Bradford procedure (1976) by using bovine serum albumin (BSA) as standard.

Statistical analysis

All the experiments were repeated three times or more, and the

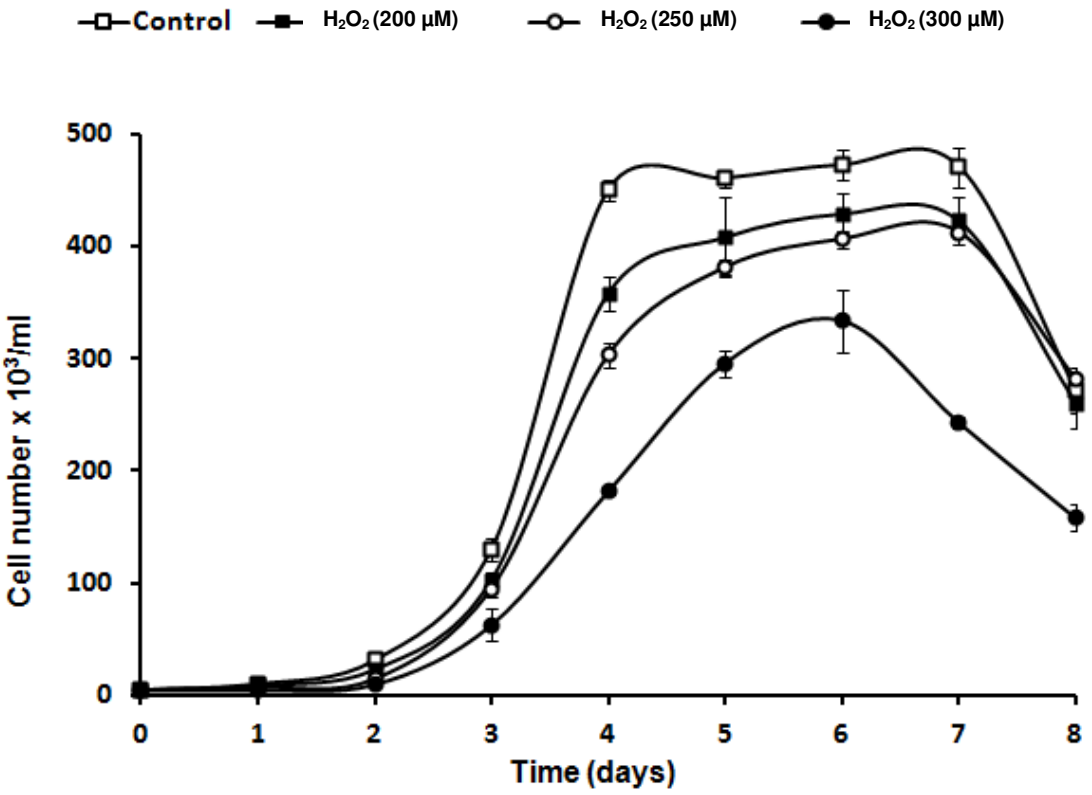

Figure 1. Effect of H_2O_2 on *Tetrahymena pyriformis* growth. The cells were cultivated in PPYG medium in the presence of different concentrations of H_2O_2 (200, 250 and 300 µM). The initial density of the cells is 5 × 10^3 individuals ml^{-1}. Values are given as means ± SEM of three experiments.

results were expressed as mean and standard deviation (SD) values. Student's *t*-test was used to analyze the differences between the groups. A value of $p < 0.05$ was considered as statistically significant.

RESULTS

Cytotoxicity of H_2O_2 on *T. pyriformis*

To evaluate the effect of H_2O_2 on growth of *T. pyriformis*, different concentrations of this oxidant agent (200, 250, 300 and 400 µM) were added to culture. The typical growth curve was remarkably modified by the presence of H_2O_2 (Figure 1). It reduced the growth of the protozoan cells and remarkably decreased the individual's number/ml than the control; particularly for the concentration 300 µM where the number of cells is reduced to 47% on the third day. Concentrations higher than 300 µM are very toxic and lethal (data not show). The effect of H_2O_2 on the lipid peroxidation and the antioxidant enzyme activities was studied. Indeed, at the exponential phase, *T. pyriformis* was exposed to H_2O_2 (300 µM) for 1, 3, 5 and 24 h. Results of this study show a significant increase of MDA level ($p < 0.001$) after 3 h of treatment with the stress compared to control cells (Figure 2A). At the same time, a significant increase in CAT, SOD and GR activities was observed ($p < 0.01$). Where after 5h of treatment with H_2O_2, only the CAT activity was enhanced ($p < 0.05$) (Figures 2B, C and D).

Effect of AO on the growth of *T. pyriformis*

According to the studied concentrations, the AO has no toxic effect on *T. Pyriformis* (Figure 3). The AO treated cultures presented a shorter lag phase and a longer exponential phase that eventually reached higher cell densities than the control. This increase on cell densities is proportional to the concentration of AO.

Effect of AO on H_2O_2 cytotoxicity

The effect of AO on H_2O_2 cytotoxicity is shown in Figure 4. The protozoan cells were beforehand maintained during 4 subcultures in the PPYG medium supplemented with AO (0.5%) then incubated in the presence of AO

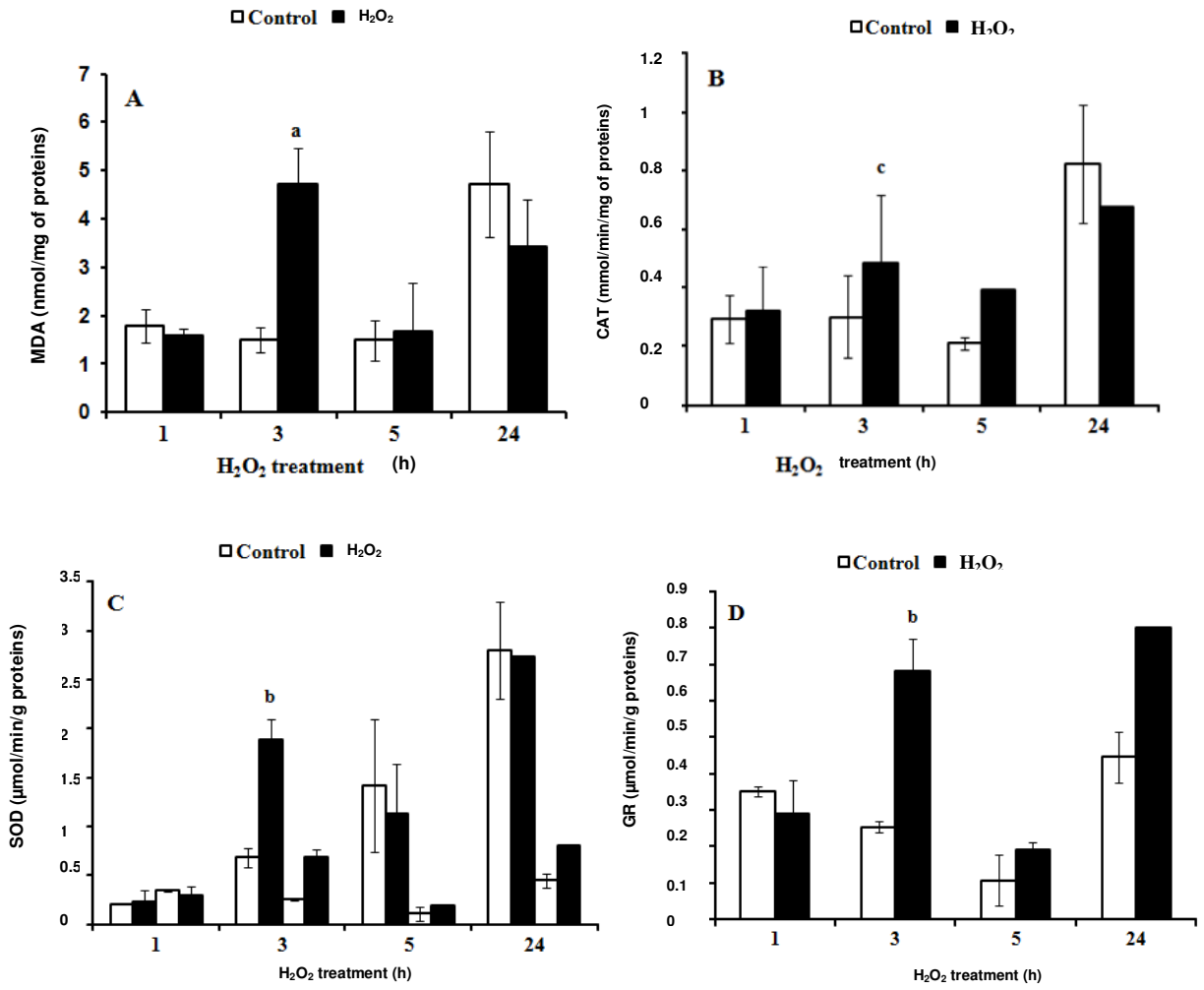

Figure 2. Effect of H_2O_2 on lipidic peroxidation (MDA), catalase (CAT), superoxyde dismutase (SOD) and glutathion reductase (GR) activities in *Tetrahymena pyriformis*. After 3 days of culture in PPYG medium, the cells were treated with H_2O_2 (300 µM) for 1, 3, 5 and 24 h. (A) MDA level; (B) CAT activity; (C) SOD activity; (D) GR activity. Data represents mean ± SEM of triplicate analysis. Significant difference: $^{a}p < 0.001$, $^{b}p < 0.01$, $^{c}p < 0.05$.

(0.5%) and H_2O_2 (300 µM). The growth curves showed that AO remarkably suppressed the H_2O_2 induced decrease in cell viability.

Effect of AO on H_2O_2 induced lipid peroxidation and antioxidant enzyme activities

The cells were grown in the medium supplemented with AO (0.5%) and at the exponential phase they were exposed to H_2O_2 (300 µM) for 3 h. The results are shown in Figure 5. Treatment of *T. pyriformis* with AO alone decreased significantly the MDA level ($p < 0.05$) and increased the activity of CAT, SOD and GR. In the cells treated with AO and exposed to H_2O_2, we noted that AO significantly reduced the lipid peroxidation ($p < 0.001$). Furthermore, the activity of CAT and SOD was higher compared to control cells. However, no significant variations were detected in GR activity between the untreated and AO treated cells.

DISCUSSION

In the present study, the protective and antioxidant activity of argan oil derived from the *A. spinosa* tree was evaluated against cytotoxicity induced by H_2O_2 in *T. Pyriformis*. The balance between antioxidants and oxidants is believed to be a critical concept for maintaining a healthy biological system. Free radicals causes

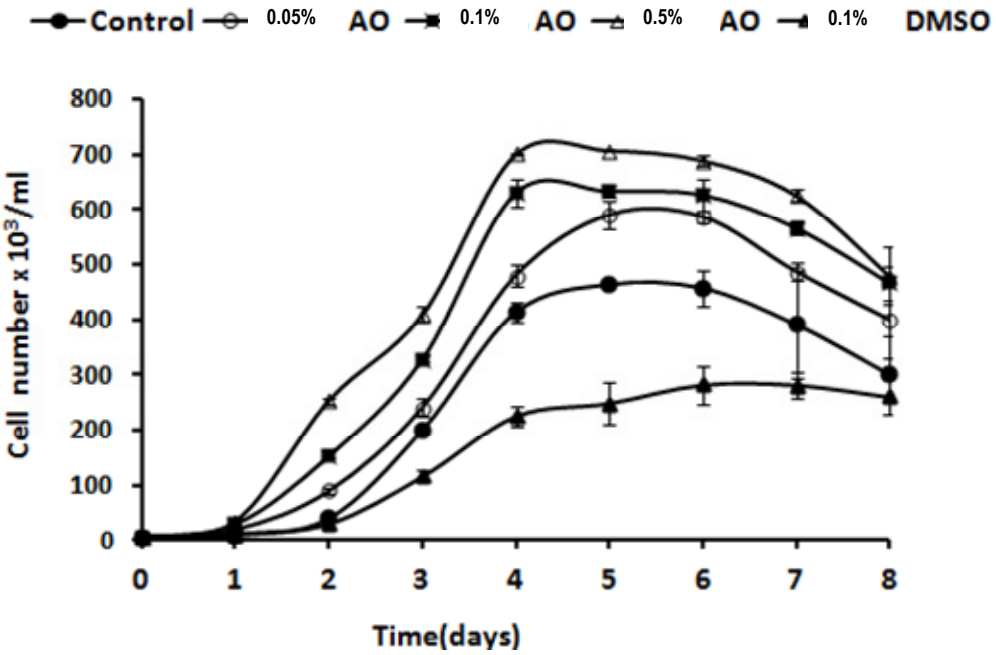

Figure 3. Effect of argan oil (AO) on the growth of *Tetrahymena pyriformis*. The cells were maintained in PPYG medium supplemented with different concentrations of AO (0.05, 0.1 and 0.5%) for four subcultures. The initial density of the cells is 5 × 10^3 individuals' ml^{-1}. Data represents mean ± SEM of triplicate values.

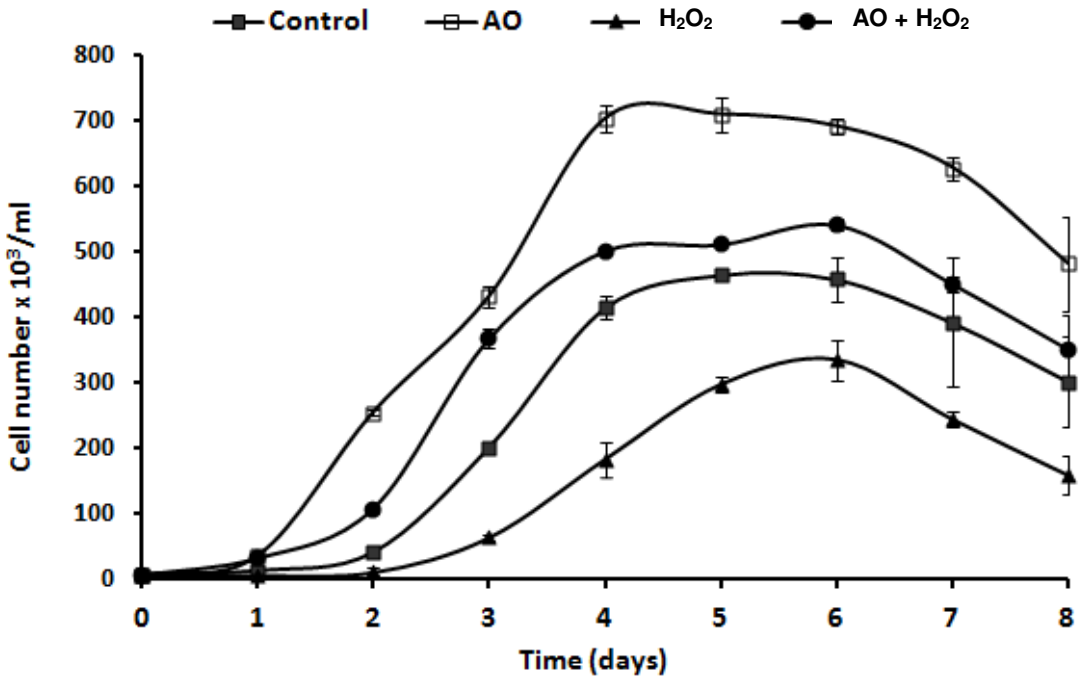

Figure 4. Argan oil (AO) effect on the H_2O_2 cytotoxicity in *Tetrahymena pyriformis*. The cells were maintained in PPYG medium added with 0.5% of AO for four subcultures then co-incubated with AO (0.5%) and H_2O_2 (300 µM). The initial density of the cells is 5 × 10^3 individuals' ml^{-1}. Data represents mean ± SEM of triplicate values.

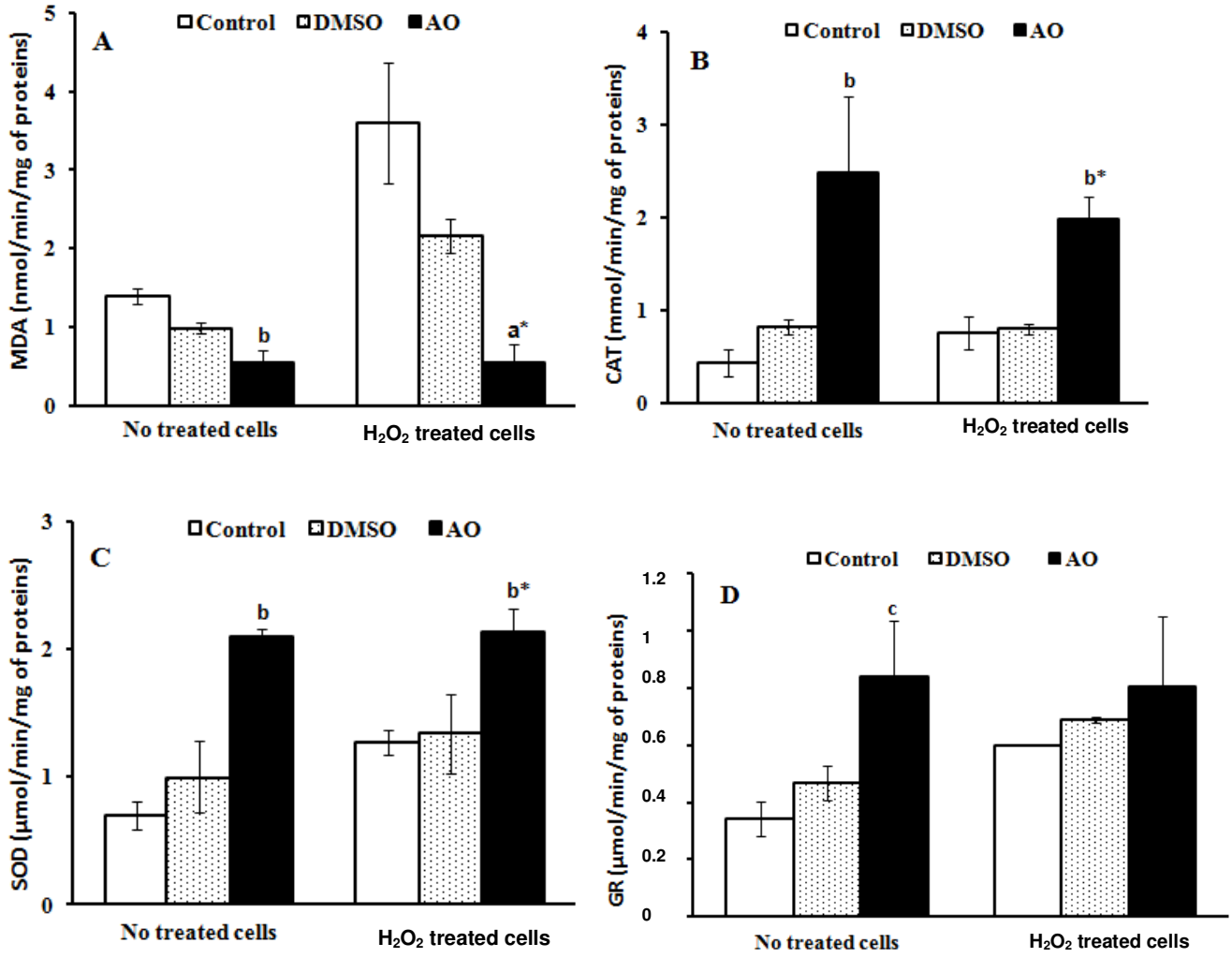

Figure 5. Effect of argan oil (AO) on H_2O_2 induced lipid peroxidation and catalase (CAT), superoxyde dismutase (SOD) and glutathion réductase (GR) activities in *Tetrahymena pyriformis*. The protozoan cells were maintained in PPYG medium added with AO (0.5%) for four subcultures then subjected to H_2O_2 (300 µM) for 3 h. (A) MDA level; (B) CAT activity; (C) SOD activity; (D) GR activity. Data represents mean ± SEM of triplicate values. Significant difference: $^{b}p < 0.01$, $^{c}p < 0.05$ compared with control without AO treatment, $^{a*}p < 0.001$, $^{b*}p < 0.01$ compared with control AO treated.

imbalance between oxidants and antioxidants in the cell. This imbalance leads to oxidative stress involved in ageing and various human diseases (Halliwell, 1994).

In this study, the experimental perturbations in redox status were caused by the H_2O_2, it belongs to the group of reactive oxygen species (ROS). The action of this oxidant agent on *T. pyriformis* showed an increase in MDA level; this result is also observed by Fourat et al. (2007) in *T. pyriformis* after oxidative and nitrosative stress. This increase of lipid peroxidation might be explained by the attack of radicals, such as ROS and reactive nitrogen species (RNS) to polyunsaturated fatty acid residues of phospholipids generating many aldehydes including the MDA (Nair et al., 2007). The cell defence involved the antioxidant systems such as antioxidant enzymes that neutralized the ROS. The protozoan cells react against H_2O_2 by increasing the enzymatic activities of CAT, SOD and GR mainly after 3 h of treatment. These results agree with those obtained by other authors (Cadi et al., 1991, 1992, 1993; Carper et al., 2001) in mice and cell line.

Cell has a non enzymatic defence system constituted of molecules able to inhibit the chain reaction of lipid peroxidation. These molecules are called “substrate suicide” or “scavenger” such as tocopherols, carotenoids, ubiquinones, ascorbic acid and uric acid (Favier, 2003; Genestra, 2007). In fact, the chemical analysis of the argan oil showed the presence of sterols (295 mg/100 g

oil), tocopherols (637 mg/kg oil), polyphenols (3263 µg/kg oil), and carotenes (545 mg/100 g oil), which are strong scavengers (Charrouf and Guillaume, 1999; Khallouki et al., 2003; Yousfi et al., 2009).

In our study, these scavenging properties of AO were revealed by a significant decrease in MDA level in untreated and H_2O_2 treated cells. Simultaneously, significant increase in CAT, SOD and GR activity was noted in the cells treated with AO and not subjected to the action of H_2O_2; this result supports the antioxidant activity of argan oil. In consistency to with findings, Benajiba et al. (2002) showed that AO significantly increased the catalase activity on isolated heart before and after ischemia. Furthermore, Cherki et al. (2005) and OuldMohamedou et al. (2011) found that AO reduced the low density lipoprotein (LDL) susceptibility to oxidation.

The scavenging and antioxidant properties of AO observed in this study could explain its non toxicity at concentrations equivalent to 4.25 mg/ml and the increase in cell viability. Also, Samane et al. (2006) mentioned the non toxicity of this oil on hepatic tumoral cells in vitro culture. The protective effect of argan oil is probably due to its high contents of powerful antioxidants, particularly polyphenols, tocopherols and sterols, which are known as powerful antioxidants (Masella et al., 2001).

Conclusion

Our study shows that argan oil has a protective activity against H_2O_2 toxicity in *T. Pyriformis*. Further studies are needed in order to explore the cellular mechanisms underlying cytoprotective effects of argan oil.

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