# Anti-stress activity of *Asparagus rasemosus* rhizomes against hydrogen peroxide induced oxidative stress in erythrocytes (RBC)

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## ABSTRACT

*Stress is a feedback survival response that strengthens the physical and mental status of an individual. If the level of stress is extreme, the homeostatic mechanisms of the organism become deficit and the survival of the organism is threatened. During stressful situations, the energy requirement of an organism is increased, resulting in enhanced generation of free radicals. The generation of these free radicals induced oxidative stress. Malondialdehyde (MDA) is widely used as a biomarker for assessing oxidative stress in biomedical fields. Lipid peroxidation is a chain phenomenon resulting in the formation of various active compounds that result in cellular damage. Estimation of lipid peroxidation in terms of quantifying malondialdehyde (MDA). Biomonitoring of MDA has been used in both in-vivo and in-vitro studies as a key biomarker for various disease patterns including hypertension, diabetes, atherosclerosis, heart failure and cancer. In the present study to investigate the anti-stress activity of Asparagus rasemosus rhizomes extract against hydrogen peroxide induced oxidative stress in erythrocytes (RBC). Results of the study decreased the MDA content in dose dependent manner in erythrocytes proved as anti-stress activity. The findings suggest that the validity of the MDA assay as a reliable tool in finding out the anti-stress activity against hydrogen peroxide induced oxidative stress.*

***Key words:*** *Asparagus rasemosus rhizomes, Oxidative stress, Free radicals, Malondialdehyde, Lipid peroxidation,*

## INTRODUCTION

Stress is a feedback survival response that strengthens the physical and mental status of an individual (Ravindran *et al*., 2005). It is vital that stress is kept under control and normal functioning is not hampered due to excessive stress. (Verma *et al*., 2009). If the level of stress is extreme, the homeostatic mechanisms of the organism become deficit and the survival of the organism is threatened (Lakshmi *et al*., 2009). Stress has been postulated to be involved in the etiopathogenesis of a variety of disease states, viz; hypertension, peptic ulcer, cancer,

diabetes, immunosuppression, reproductive dysfunctions and behavioral disorders like anxiety due to involvement of the central nervous system (CNS), endocrine system and metabolic syndrome (Rai *et al*., 2003). During stressful situations, the energy requirement of an organism is increased, resulting in enhanced generation of free radicals. The generation of these free radicals induced oxidative stress (Kenjale *et al*., 2007).

Oxidative stress is the state of imbalance between the reactive oxygen species (ROS) and the ability of a biological system to detoxify readily the reactive intermediates. Development of oxidative stress because of free oxygen radical generation has been implicated in the pathogenesis of many diseases including Parkinson's disease, Alzheimer's disease, atherosclerosis, heart failure, myocardial infarction and even cancer (Velavan, 2011; Aruoma, 1998). Lipid peroxidation is a chain reaction occurring during oxidative stress leading to the formation of various active compounds. Lipid peroxides, which are derived from polyunsaturated fatty acids, are unstable. They readily decompose to form a complex series of compounds, which include malondialdehyde (MDA) (Brammer *et al*., 1982). MDA is the major metabolite of arachidonic acid and serves as a reliable biomarker for oxidative stress. MDA is a mutagenic, tumorigenic and highly reactive three-carbondialdehyde produced during polyunsaturated fatty acid peroxidation and arachidonic acid metabolism (Smith *et al*., 1976).

The scope of the present study is to evaluate the anti-stress activity of *Asparagus rasemosus* rhizomes extract against $H_2O_2$-induced oxidative stress in RBCs. In fact, human RBCs, because they are oxygen carriers with high polyunsaturated fatty acid content on their membranes and high cellular concentration of hemoglobin are particularly exposed to oxidative damage. The hemoglobin released from erythrocytes is potentially dangerous because in reacting with $H_2O_2$. It is converted into oxidized forms: methemoglobin (met-Hb) and ferrylhemoglobin, which are powerful promoters of oxidative processes (van der Berg *et al*., 1992). Moreover, the free hemoglobin exposed to $H_2O_2$ causes heme degradation with the release of iron ions catalytically active in initiating free radical reaction and formation of lipid peroxidation product as MDA (Puppo *et al*., 1988).

## MATERIALS AND METHODS

### Preparation of alcoholic extract

*Asparagus rasemosus* rhizomes collected from sengipatti, Thanjavur in 2017. The collected *Asparagus rasemosus* rhizomes were washed several times with distilled water to remove the traces of impurities from the rhizomes. The rhizomes were dried at room temperature and coarsely powdered. The powder was extracted with Ethanol for 24 hours. A semi solid extract was obtained after complete elimination of alcohol under reduced pressure. The *Asparagus rasemosus* rhizomes extract was stored in refrigerator until used (Velavan *et al*., 2007). Doses such as 100, 200 and 400µg/ml were chosen for *in vitro* anti-stress activity.

**Preparation of erythrocytes suspensions** (Edger *et al*., 2001)

Fresh blood sample from healthy volunteers (10–15ml) were collected and centrifuged at 3000 rpm for 15 minutes, plasma and pufy coats were removed. Red cells were washed with PBS (pH 7.00) for three times and erythrocytes were lysed with ice-cold distilled water.

**Experimental design (**Sasikumar *et al*., 2015)

Erythrocyte suspensions obtained from healthy donor were divided into six groups. Group I- Control [Erythrocyte suspension (750µl), PBS (1000µl) and D.$H_2O$ (250µl)]. GroupII- $H_2O_2$[Erythrocyte suspension (750µl), 10mM $H_2O_2$ (50µl), PBS (950µl) and D.$H_2O$ (250µl)]. Group III- [Erythrocyte suspension (750µl), 10mM $H_2O_2$ (50µl), (100µg/ml *Asparagus rasemosus* rhizomes extract in 500µl PBS) and PBS (700µl)]. Group IV- [Erythrocyte suspension (750µl), 10mM $H_2O_2$ (50µl), (200µg/ml *Asparagus rasemosus* rhizomes extract in 500µl PBS) and PBS (700µl)] Group V- [Erythrocyte suspension (750µl), 10mM $H_2O_2$ (50µl), (400µg/ml *Asparagus rasemosus* rhizomes extract in 500µl PBS) and PBS (700µl)] and Group VI- [Erythrocyte suspension (750µl), 10mM $H_2O_2$ (50µl), (100µg/ml Ashwagandha in 500µl PBS) and PBS (700µl)]. These experimental groups were incubated at 37°C for 1 hour. Following the incubation, Malonodialdehyde (MDA) was determined by the Thiobarbituric acid assay (Beuge and Aust, 1978).

## RESULTS

The anti-stress effect of *Asparagus rasemosus* rhizomes extract in MDA level on erythrocyte suspensions was presented in Table 1and Fig 1. The MDA level was higher in group II where $H_2O_2$ induced oxidative damage. The group III, IV and V samples with different concentration (100, 200 and 400µg/ml) of *Asparagus rasemosus* rhizomes extract showed significant anti-stress effect against oxidative stress caused by $H_2O_2$. The group VI Ashwagandha (100µg/ml) treated group showed anti-stress effect against oxidative stress caused by $H_2O_2$. The results of the present study reveal a clear dose dependent decrease in the formation of MDA upon treatment with *Asparagus rasemosus* rhizomes extract. The higher dose (400µg/ml) of *Asparagus rasemosus* rhizomes extract has potential activity and near to silimarin

**Table 1 Anti-stress effect of *Asparagus rasemosus* rhizomes extract in MDA level on erythrocyte suspensions**

| Stress marker parameter | Group I | Group II | Group III | Group IV | Group V | Group VI |
|---|---|---|---|---|---|---|
| **Malondialdehyde (MDA)** (U/g Hb ) . | 6.69±0.32 | 13.20±1.59 | 10.49±0.15 | 8.86±0.33 | 6.65±0.33 | 6.26±0.23 |

Values expressed as Mead ± SD for triplicates;

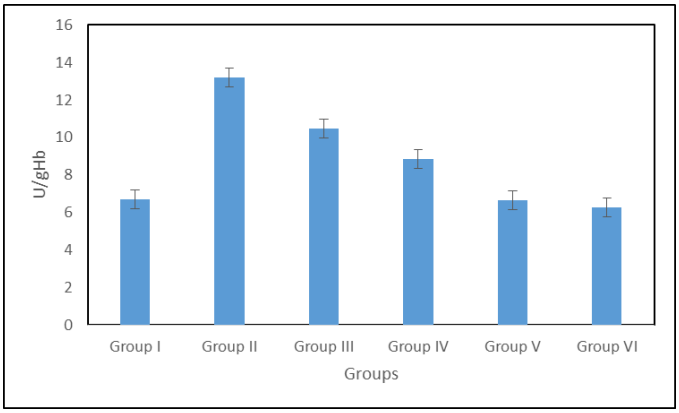


**Fig 1 Anti-stress effect of *Asparagus rasemosus* rhizomes extract in MDA level on erythrocyte suspensions**

## DISCUSSION

Red blood cells are one of the most susceptible biological tissues to oxidative stress due to the presence of both high concentration of polyunsaturated fatty acids (PUFA) in the membrane and the oxygen transport associated with redox active hemoglobin molecules, which are promoters of ROS. Due to their susceptibility to oxidation red blood cells are often used as cellular models to investigate oxidative damage. Moreover, hydrogen peroxide is an established model to induce oxidative stress *in vitro* (Velavan *et al*., 2007; Norsharina Ismail *et al*., 2016). Hydrogen peroxide ($H_2O_2$) is the most effective species for cellular injury (Rao *et al*., 1996). Reactive oxygen species i.e. $H_2O_2$ generated during oxidative stress is known to cause damage to proteins, nucleic acids, and cell membranes and has also been associated with cancer, aging and several chronic neurodegenerative diseases (Kellogg and Fridovich, 1975; Daroui *et al*., 2004).

The monitoring of MDA levels in different biological systems can be used as an important indicator of lipid peroxidation both *in-vitro* and *in vivo* for various health disorders. The endogenous formation of MDA during intracellular oxidative stress and its reaction with DNA forms MDA- DNA adducts which makes it an important biomarker of endogenous DNA damage. Determination of MDA in blood plasma or tissue homogenates is one of the useful methods to predict the oxidative stress levels. MDA falls in the category of Thiobarbituric Acid Reactive Substances (TBARS) and the later are an index of lipid peroxidation. Thiobarbituric acid (TBA) assay is the commonly used method for determination of MDA (Nagulendran *et al*., 2007; Chole *et al*., 2010).

Auto-oxidation induced by $H_2O_2$ in RBCs provides an obvious and convenient model for lipid peroxidation owing to the high concentration of unsaturated lipid in its cell membranes. In the present study, we have estimated the lipid peroxidation in terms of quantifying malondialdehyde (MDA) level. Our results show that MDA release from $H_2O_2$ -treated RBCs.

The observation that MDA formation is an index of lipid peroxidation preceding hemoglobin release from the RBCs is consistent with the precursor–product relationship, i.e. $H_2O_2$ -dependent lipid peroxidation disrupts the RBC membrane, which in turn allows hemoglobin leakage. Similarly, Sato *et al*. (1998) reported that hemolysis was observed only after apparent significant lipid peroxidation induced by oxidizing compounds and suggested a competitive reaction correlation between lipid peroxidation and protein oxidation-induced hemolysis of RBCs. Therefore, the MDA concentration may be higher than the hemoglobin release in peroxidative medium owing to early release, as shown in the present results.

In the present study, a clear dose dependent decrease in the concentration of MDA was obtained following *Asparagus rasemosus* rhizomes extract treatment. A similar results Yasir Sasikumar *et al*. (2015) reported that protective effect of partially purified alkaloids (PPA) from *Amaranthus viridis* against hydrogen peroxide induced oxidative damage in human erythrocytes in vitro conditons. The alkaloids prevent the decline of antioxidant status which in turn decreases LPO levels by preventing MDA formation in erythrocytes. The results confirm the protective effect of alkaloids against free radical induced oxidative stress in human erythrocytes.

## CONCLUSION

Malondialdehyde (MDA) is a useful biomarker for oxidative stress. Increased levels of oxidative stress have been associated with various disease patterns. The anti-stress activity of *Asparagus rasemosus* rhizomes extract was dose dependent manner against $H_2O_2$-induced oxidative stress in RBCs. Conclusively, The findings suggest that the validity of the MDA assay as a reliable tool in finding out the anti-stress activity against hydrogen peroxide induced oxidative stress.

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