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ANTISTRESS ACTIVITY OF *Desmidorchis indica* STEM EXTRACT AGAINST HYDROGEN PEROXIDE INDUCED OXIDATIVE STRESS IN ERYTHROCYTES

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ABSTRACT

Stress can be described as the sum total of all the reactions of the body, which disturb the normal physiological condition and result in a state of threatened homeostasis. Plants have been widely used for medicinal purposes for thousands of years and the reported scientific evidence on their biological effects has increased in recent years. The present study was aimed to examine the antistress effect of *Desmidorchis indica* stem extract against hydrogen peroxide induced oxidative stress in erythrocytes *in vitro* conditons. The experiments are performed in groups (Control,Hydrogen peroxideinduced andHydrogen peroxide+ 100, 200 and 400 mg concentrationsof *Desmidorchis indica* stem extract). The enzymic antioxidants like CAT, SOD, GPxand nonenzymic antioxidants like vitamin C, vitamin E and GSH levels and MDA were evaluated forthe antistress effect of *Desmidorchis indica* stem extract. The *Desmidorchis indica* stem extract enhances the of antioxidant status which in turndecreases lipid peroxidation levels by preventing MDA formation in erythrocytes. The results confirm the antistresseffect of *Desmidorchis indica* stem extract against hydrogen peroxide induced oxidative stress in erythrocytes.

Keywords: Antistress, *Desmidorchis indica* stem extract, Hydrogen peroixide, erythrocytes, enzymatic and non-enzymatic antioxidants

INTRODUCTION

Stress can be described as the sum total of all the reactions of the body, which disturb the normal physiological condition and result in a state of threatened homeostasis. Stress has been postulated to be involved in the etiopathogenesis of a diverse variety of diseases ranging from psychiatric disorders such as anxiety and depression, immunosuppression, endocrine disorders including diabetes mellitus, male sexual dysfunction, cognitive dysfunctions, peptic ulcer, hypertension and ulcerative colitis (Hiroshi Ueno *et al.,* 2019). Benzodiazepines and anxiolytics, despite having significant anti-stress activity, have not proved effective against chronic stress induced adverse effects on immunity, behavior cognition, male sexual function, during pregnancy and lactation. Additionally, the problem of tolerance and physical dependence on their prolonged use, limits the clinical utility of these drugs When we study the history of ancient alternative systems of medicine, Ayurveda and Traditional Chinese Medicine (TCM) are on the forefront (Stuart, 1984). Therefore there is a need for an effective herbal anti-stress agent in the therapy of stress induced disorders (Rai *et al.,* 2003;Bruna de Falco and Riccardo Motti, 2021).Plants have been widely used for medicinal purposes for thousands of years and the reported scientific evidence on their biological effects has increased in recent years (Masek *et al.,* 2017).Antioxidant is a molecule capable of slowing or preventing the oxidation of other molecules. In a biological system, they protect cells from the

damage caused by unstable molecules known as free radicals (Velavan Sivanandham, 2011). Antioxidants terminate the chain reactions by removing free radical intermediates, and inhibit other oxidation reactions by being oxidized themselves. They are believed to play a role in preventing the development of chronic diseases (Chakraborty *et al*., 2009).

During the last 20 years, the research in the field of natural products has re-emergeddue to the discovery and development of new molecules with pharmaceutical interestbased on the ethno-medical knowledge. The use of medicinal plants has been a constant since ancient times, to such a degree that the World Health Organization (WHO) recognizes its important value. Nowadays, it is calculated that 80% of the world population makes use of these types of plants to treat illnesses and diseases (Muhammad*et al.*, 2018; Velavan, 2015). Of the nearly 250,000–500,000 species of plants on earth, 20,000 have medicinal properties and function as a drug source (Fabricant and Farnsworth, 2011). Many plant extracts and plant products are known tohave promising anti-stress and antioxidant activities (Koppula and Choi, 2012; Ravishankar and Parvathi. 2012) Hence, the present research aimed to explore the antistress effect of *Desmidorchis indica* stem extract against hydrogen peroxide induced oxidative stress in erythrocytes.

MATERIALS AND METHODS

Collection of plant materials

The *Desmidorchis indica*stem was collected from Kathattipatti (Palaiyapatti North), Sengipatti Village at Thanjavur District in the month of March-2020. The plant was identified and authenticated by Dr. S. John Britto. The Director, the Rabiant Herbarium and Centre for molecular Systematic. St. Joshphe's college, Trichy - Tamil Nadu, India. A voucher specimen (RSV01) has been deposited at the Rapinat Herbarium St. Joshph's college, Trichy, Tamil Nadu, India.

Preparation of extract

The *Desmidorchis indica*stem was first washed well and dust was removed from the stem. Then the stemwas dried at room temperature and coarsely powdered. The powder was extracted with hydro-alcoholic (ethanoland water(70:30))for 24 hours. A semi solid extract was obtained after complete elimination of alcohol under reduced pressure. The extract was stored in desiccator until used.

Preparation of erythrocytes suspensions

Fresh blood sample from myself (10–15ml) werecollected and centrifuged at 3000 rpm for 15 minutes, plasma andpufy coats were removed. Red cells were washed with PBS (pH 7.00)for three times and erythrocytes were lysed with ice-cold distilledwater.

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

Erythrocyte suspensions obtained from myself weredivided into five groups.

Group I- Control [Erythrocytesuspension (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), (100mg/ml - *Desmidorchis indica* stemextract in500µl PBS) and PBS (700µl)].

Group IV- [Erythrocyte suspension(750µl), 10mM H_2O_2 (50µl), (200mg/ml - *Desmidorchis indica* stemextract in500µl PBS) and PBS (700µl)]

Group V- [Erythrocyte suspension(750µl), 10mM H_2O_2 (50µl), (400mg/ml -*Desmidorchis indica* stem extract in500µl PBS) and PBS (700µl)]

These experimental groupswere incubated at 37°C for 1 hour. The antioxidant activities in erythrocytes were evaluated.

Analysis of stress markers

Malondialdehyde was estimated by the thiobarbituric acid assay method of Beuge and Aust (1978). Superoxide dismutase activity was determined by the procedure of Kakkar *et al*. (1984). The activity of catalase was assayed by the method of Beers and Sizer, (1952). The activity of glutathione peroxidase was assayed by the method of Rotruck *et al* (1973). Reduced glutathione was estimated by method of Moron *et al.*, (1979).The level of ascorbic acid was estimated by the method of Omaye *et al* (1979). Vitamin E was estimated by the method of Baker *et al* (1980).

RESULTS AND DISCUSSION

Under normal conditions, the continuous production of freeradicals is compensated by the powerful action of protective enzymeslike superoxide dismutase, catalase and gluthathione peroxidase thatare believed as major antioxidant enzymes present in the humanbody that protect against the oxygen toxicity (Abraham *et al.*,2015)As a result, a lipidperoxidation (LPO) process occurs. Therefore, antioxidant enzymeactivities and lipid peroxidation levels are accepted as importantparameters in the evaluation of oxidative stress in aerobic organisms.The current study was carried out with the main aim of evaluating theantioxidant status of *Desmidorchis indica*stem extract. The *Desmidorchis indica*stem proved to protect the erythrocytes from the oxidative stress causedbyH_2O_2.The results of the present study, i.e., the effect of variousantioxidants onH_2O_2induced oxidative damage on erythrocytes wasdepicted in Figures: 1-3.

MDAis formed in high amounts during the lipid peroxidation process andits quantity reveals the extent of cell damage by peroxidation. MDA isa highly reactive, bifunctional molecule that can effectively cross linkwith the membrane phospholipids and proteins of the erythrocytes,thus impairing the membrane related functions ultimately leading todiminished survival of the erythrocytes (Ault and Lawrence, 2003).The production of MDAwas high in group II where the erythrocyte cells are treated withH_2O_2alone (Figure 1).Treatment with *Desmidorchis indica* stem preventedH_2O_2inducedMDA production and this inhibition was strongly dose dependent.The values in group IV are reversed and nearer to the control whichindicates that *Desmidorchis indica*stem inhibits the membrane lipid peroxidationtriggered by the injurious oxygen radicals generated from hydrogenperoxide.

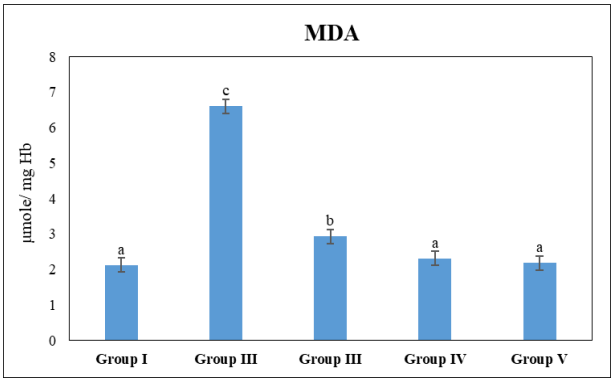

Figure 1: Effect of *Desmidorchis indica* stemon MDA activity in experimental group in RBC

Enzymicantioxidants, such as superoxide dismutase, catalase and glutathione peroxidaseare play important roles in the scavenging of reactiveoxygen species, such as superoxide radical, hydrogen

peroxide, lipidhydroperoxides and so forth. An aerobic organism can cope withthe metabolic production of ROS under normal conditions via itsantioxidant defense system; however, overproduction of ROS causes adangerous process called “oxidative stress” (Rajasekaran,2005). The antioxidant effectof SOD, CAT and GPX on erythrocyte suspensions were presentedin Table 3. The effect of all these three antioxidants, viz., SOD, CAT and GPX was lowered in group II where H_2O_2induced oxidativedamage was high. The group IV samples with high concentration of*Desmidorchis indica*stemshowed more protective effect against oxidativedamage caused byH_2O_2.The activity of the enzymic antioxidants inpresence of the *Desmidorchis indica*stem was high and it is dose dependent, higher the concentration is higher free radical scavenging activity (Figure2).

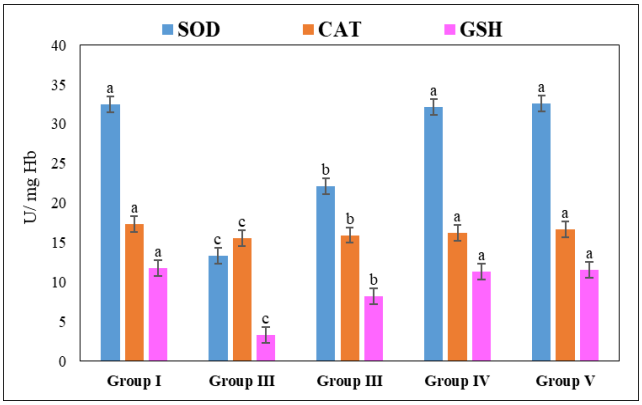

Figure 2: Effect of *Desmidorchis indica* stemon SODand CAT, GSH activity in experimental group in RBC

VitaminC, E and Glutathione are some of the non-enzymic antioxidants ofanimals which help those organisms to combat cellular damage dueto oxidative stress. Vitamin C may accumulate at high concentrationin photosynthetic tissues, in which it is intimately in the regulation ofphotosynthesis and protect the chloroplasts against damage causedby ROS such as
O_2, H_2O_2,hydroxyl radicals (OH) and singlet oxygen(Sivanandham Velavan, 2012; Müller *et al.*, 2002).The effect of non-enzymic antioxidants on the erythrocytesuspension was presented in Figure3. Vitamin C is proved to havehigher free radical scavenging activity in presence of *Desmidorchis indica*stem. The protective effect of those nonenzymicantioxidants was preserved even in presence ofH_2O_2andtheir activity was nearly normal to that of the control sample . Glutathione is also a non-enzymic antioxidant which also possesses higher free radical scavenging activity and its antioxidant effect wasfound to be preserved at higher rates in presence of increased amount of *Desmidorchis indica*stem extract.

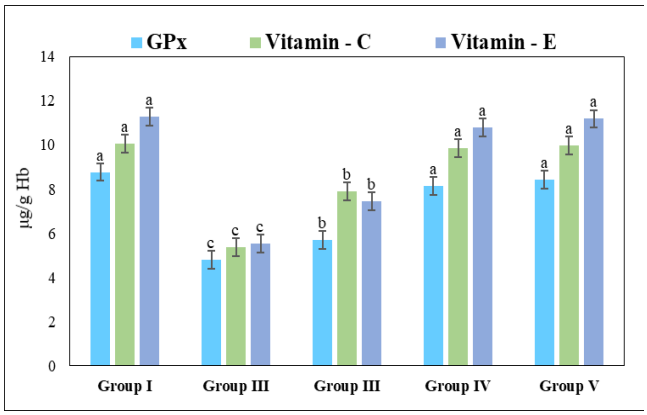

Figure 3: Effect of*Desmidorchis indica* stemon GPx Vitamin Cand Vitamin Eactivity in experimental group in RBC

CONCLUSION

Over all, it can be concluded that the anti-stress activity of *Desmidorchis indica* stem 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 and enzymatic and non-enzymatic antioxidants as a reliable tool in finding out the anti-stress activity against hydrogen peroxide induced oxidative stress. The antistress activity of *Desmidorchis indica* stem extract due to the presence of phytochemicals.

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