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ANTIOXIDANT EFFECT OF *ADIANTUM CAPILLUS VENERIS* LINN. ON HUMAN LYMPHOCYTE : AN *IN VITRO* STUDY

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Abstract: Free radicals induce damage due to lipid peroxidation in biomembranes and DNA leading to a number of diseases. Antioxidants neutralize the effect of free radicals through different ways and may prevents the body from various disease. The present study evaluates the antioxidant potential of leaf extract of Adiantum capillus veneris Linn against hydrogen peroxide induced oxidative damage in peripheral blood lymphocytes. Incubation of peripheral blood lymphocytes with 100 µM H_2O_2 for 2 hours significantly increased lipid peroxidation and decreased the levels of glutathione and the antioxidant enzymes (SOD, CAT, GPx). Pretreatment with plant leaves extract for 18 hours could effectively inhibit lipid peroxidation and enhance the activities of antioxidant enzymes and glutathione content significantly. The results indicate that this may be due to its direct action in scavenging free radicals and thereby modulating the antioxidant defence system.

Key words: *Adiantum capillus veneris* Linn., Human lymphocyte,

INTRODUCTION

During organic evolution aerobic organisms have evolved certain defense mechanisms to face the challenges of products of cellular oxidation. Though the aerobic cells have great advantage but free radicals produced by aerobic respiration are a serious confront for the cell. An imbalance between free radicals and defense mechanism in the cell leads to oxidative stress [1]. There are several enzymatic and non enzymatic systems that contribute to the inactivation of free radical reactions.

About 5% or more of inhaled Oxygen (O_2) is converted to reactive oxygen species (ROS) such as O_2-, H_2O_2 and OH by univalent reduction of O_2 [2]. Antioxidants can act by scavenging reactive oxygen species (SOD removing O_2^-), by inhibiting their formation (e.g. by blocking activation of phagocytes), by binding transition metal ions and preventing formation of OH and/or decomposition of lipid hydroperoxidase, by repairing damage (e.g. α-tocopherol repairing peroxyl radicals and so terminating the chain reaction of lipid peroxidation) or by any combination of the above [3].

In Ayurveda some rasayanas with well defined antioxidant properties have been prepared [4]. Rasayanas are a group of non-toxic poly-herbal drug preparations, many of them are immunostimulatory and prevent the causation of disease and promote health and longevity [5].

The leaf extract of the plant *Adiantum capillus – veneries* Linn. (Adiantaceae) is known for the treatment of fever, cough and bronchial disorders. It is also used as a stimulant, emollient, purgative, demulcent, general tonic and hair tonic [6]. It has anticancerous, hypoglycemic, aphrodisiac, antifungal, antibacterial and antiviral properties [7]. In the present paper the antioxidant property of leaf extract of this plant has been investigated against hydrogen peroxide induced oxidative damage in peripheral blood lymphocytes (PBLs).

MATERIALS AND METHODS

Plant Extract: The plant was collected from sandy alluvial soil deposits in rock – crervices from Chitrakut waterfall of Jagdalpur (Bastar), Chhattisgarh, India. Its leaf extract was prepared in 59% alcohol by Soxhlet Extraction apparatus and used for the present study.

***In vitro* study:** Blood samples were collected from human volunteers (with their kind permission through medical doctors) following density gradient method in heparinized sterlized tube. It was washed in phosphate buffer saline and then placed in Dulbercco's Modified Eagles Media (DMEM) along with 10 % fetal calf serum. Cells were cultured in a humidified CO_2 incubator at 37 ^{0}C and 5% CO_2 for 18 h with known concentration for *Adiontum cappilus veneris* leaf extract. After incubation, cells were resuspended in fresh media and exposed to oxidative stress with 100µM H_2O_2 for 2 hours [8]. For the experiment cultured lymphocytes were divided into five groups and in each group five samples were processed.

Group I: Only lymphocyte

Group II: Lymphocyte + 100µM H_2O_2 for 2 hours.

Group III: H_2O_2 treated lymphocytes pretreated with 5 µ/10000 cells of *Adiantum cappilus veneris* leaf extract.

Group IV: H_2O_2 treated lymphocyte pretreated with 10 µ/10000 cells of *Adiantum capillus veneris* leaves extract.

Group V: H_2O_2 treated lymphocyte pretreated with 20µL/10000 cells of *Adiantum capillus veneris* leaves extract.

The cells were collected, washed twice in ice cold phosphate buffer and used for biochemical assays.

Biochemical studies: To evaluate the effect of leaf extract of *Adiantum capillus veneris* on oxidative stress caused by H_2O_2 to cultured lymphocytes following biochemical studies were made: Lipid peroxidation in term of malondialdehyde (MDA) was determined by thioborbituric acid reaction following Ohkama et al. [9] method, the reduced glutathione (GSH) by Moron et al. [10] method, superoxide dismutase (SOD) by Misra et al. [11] method, catalase (CAT) by Bergmeyer et al. [12] method and glutathione peroxide (GPx) by Rotruck et al. [13] method.

Statistical analysis: The data was statistically analyzed using ANOVA followed by Duncans Multiple Range Test of 5% P-level [14].

RESULT

The malondialdehyde was found to be significantly higher in H_2O_2 treated lymphocytes, as compared to control. But its level was recovered significantly ($P < 0.05$) in all three sets of experiments with increasing conc. (5 µl/10 µ/20 µl) of leaf extract of *Adiantum capillus veneris* (Table-1) as compared to H_2O_2 treated cells (Table 1).

The concentration of reduced glutathione decreased significantly in H_2O_2 treated cells as compared to control. However, after treatment with leaf extract it recovered consideravely. The recovery was progressive with increasing concentrations of leaf extract (significant ($P < 0.05$)) as compared to H_2O_2 treated cells (Table 1).

The superoxide dimutase, catalase and glutathione peroxidase activities also decreased significantly ($P < 0.05$) during treatment with H_2O_2 in comparison to control cells. Nevertheless, levels of all the three enzymes were restored significantly ($P < 0.05$) after

Table 1: Effect of leaves extract of *Adiantum capillus veneris Linn.* on the level of lipid peroxides and glutathione in human lymphocyte in a *in vitro* study.

Parametets	Group I (Control)	Group III (H_2O_2) treated	Group III (5µ of PLE & H_2O_2)	Group IV (10µl of PLE & H_2O_2)	Group V (20µl of PLE & H_2O_2)
Lipid peroxides (n mole MDA/mg protein)	0.84 ± 0.05*	4.24 ± 0.06#	3.80 ± 0.05 #	2.20 ± 0.03 #	1.24 ± 0.04 #
Reduced Glutathione (µ moles/mg proteins)	5.60 ± 0.02*	2.60 ± 0.40 #	3.0 ± 0.03 #	4.40 ± 0.02 #	4.90 ± 0.03 #

PLE = Plant leaves extract, * Compared with control # Compared with H_2O_2

Table 2: Effect of leaves extract of *Adiantum capillus veneris* Linn. on the activities of superoxide dismutase, catalase and glutathione peroxidase in human lymphocytes in a *in vitro* study.

Parameters	Group I (Control)	Group II (H_2O_2 treated)	Group III (5µl of PLE &H_2O_2)	Group IV (10 µl of PLE & H_2O_2)	Group V (20 µl of PLE & H_2O_2)
Superoxide dismutase (Units/mg protein)	3.68 ± 0.06	1.80 ± 0.04*	2.00 ± 0.02 #	2.90 ± 0.02 #	3.20 ± 0.05 #
Catalase (µ moles of H_2O_2 consumed / min / mg proteins)	5.20 ± 0.03	3.02 ± 0.02 *	3.60 ± 0.03 #	4.12 ± 0.04 #	4.80 ± 0.02#
Glutathione peroxidase (µg of glutathione utilised / min / mg protein)	12.24 ± 0.01	6.40 ± 0.04 *	7.60 ± 0.05 #	8.62 ± 0.04#	10.20 ± 0.05#

PLE = Plant leaves extract.. Value are expressed as Mean ± SD of ANOVA followed by DMRT. $P < 0.05$. * compared with control. # Compared with H_2O_2

treatment with leaf extract and the results were better with higher concentrations of leaf extract (Table 2).

DISCUSSION

The imbalance between reactive oxygen species and antioxidant defense system may increase the oxidative burden and lead to the damage to macromolecules such as DNA, corbohydrates or proteins. Such processes are thought to play a role in pathological processes of various diseases. Cellular antioxidant significantly delays or prevents oxidation of macro-molecules.

Antioxidants can be classified in to three main types : first line defence antioxidants (SOD, GAT, GTx, glutathione reductase and some minerals like Se, Mn, Cu, Zn), second line defence antioxidants (GSH, Vit. C, uric acid, albumin, bilirubin, vitamin. E, carbotenoids flavonoids etc.) and third line defence antioxidants (complex group of enzymes for repair of damaged DNA, damaged protein, oxidised lipid and peroxides and also to stop chain propagation of peroxyl lipid radicals [15].

Lymphocytes contain a diversified redox and free radical scavenging system [16,17], and are extensively screened in human population exposed to a variety of toxicants [18]. In the present study hydrogen peroxide was used to induce oxidative stress in lymphocyte because hydrogen peroxide can cross the cell membrane easily and stimulate hydroxyl radical formation [19,20]. Leaf extract of *Adiantum capillus veneris* can also cross the membrane *in vitro* and neutralize the free radicals and check the lipid peroxidation leading to decrease in malondialdehyde in H_2O_2 stressed cells.

In the present study we found increased malondialdehyde which indicates activation of lipid peroxidation in H_2O_2 treated lymphocytes. During this process there is excessive free radical formation that attacks the fatty acid components of membrane lipids resulting in membrane rigidity and receptor realignment [21,22]. Increased free radical formation and lipid peroxidation was also indicated by decrease in reduced glutathione in H_2O_2 treated lymphocyte [23-25]. This impairs the cellular defense and renders the cell more vulnerable to oxidative stress [26].

The activities of antioxidant enzymes like superoxide dimutase, catalase and glutathione peroxidase in H_2O_2 treated lymphocytes also decreased which indicates marked disturbances in GSH metabolism. Same condition was reported by Koke et al. [27] and Hashim et al. [28]. Further, the lymphocytes pre-treatment with different doses of *Adiantum, capillus veneris* leaf extract prior to H_2O_2 intoxiction showed amelio-rative effect with increased dose. Thus the lympho-cytes pre-treatment with higher concentration of leaf extract showed the least increase in malondialdehyde as well as disturbance in GSH metabolism.

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