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PHYTOREMEDIATION OF OXIDATIVE STRESS BY ROOT EXTRACT OF *ACHYRANTHES ASPERA* IN LYMPHOCYTES OF *ORYCTOLAGUS CUNICULUS* L.

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ABSTRACT

Oxidative stress plays an important role in the various pathological processes leading to many dreadful diseases. It arises due to the excessive damages of bio membranes and macromolecules caused by the harmful effects of free radicals. Free radicals (ROS & RNS) are generated during cellular oxidation and metabolic processes. Their decomposition exhibits deleterious effects on various physiological systems of living organisms and human health. The free radicals are scavenged by the cellular antioxidants that constitute the strong antioxidant defense system of the body. The antioxidants from natural sources such as the medicinal plants have been of prime importance in the biological world. The aim of the present study is to evaluate the antioxidant activity of the root extract of *Achyranthes aspera,* a well-known medicinal herb, for the phyto- remediation of hydrogen peroxide induced oxidative stress in lymphocytes of *Oryctolagus cuniculus* L. (rabbit). Incubation with H_2O_2 (hydrogen peroxide) for 2 hours increased lipid peroxidation, affecting the concentrations of antioxidant enzymes and glutathione, in terms of malondialdehyde (MDA – 3.90 ± 0.04), superoxide dismutase (SOD – 1.36 ± 0.04), reduced glutathione (GSH – 2.36 ± 0.06), catalase (CAT – 3.73 ± 0.02) and glutathione peroxidase (GPx – 4.83 ± 0.09). Pretreatment with the methanolic root extract of *Achyranthes aspera* for 18 hours was found to control lipid peroxidation and positively enhances the activities of antioxidant enzymes, with gradual increase in dosages, ultimate being 20µl / 10,000 cells, viz., MDA (1.00 ± 0.03), GSH (5.12 ± 0.12), SOD (1.75 ± 0.02), CAT (5.03 ± 0.05) and GPx (8.45 ± 0.36). The high antioxidant activity and the efficient free radical scavenging ability of the methanolic root extract of *Achyranthes aspera* contributed to the minimization of oxidative stress in lymphocytes. The rich phytochemical content present in the roots is usually considered to be responsible for the antioxidant efficiency of root extract of *Achyranthes aspera,* and hence ideal for phytoremediation of oxidative stress.

KEYWORDS: Lipid peroxidation, Oxidative stress, Antioxidants, Free radicals

The cellular oxidation and free radical generation leads to impaired antioxidant defense system. The oxygen free radicals induce damage to biomembranes and biomolecules such as proteins, lipids, carbohydrates and DNA (Young *et al.,* 2001 ; Mc Cord *et al.,* 2000), which are responsible for various kinds of cellular dysfunctions. The imbalance and the deleterious effects developed due to free radicals, in the living system, give rise to "Oxidative Stress" (Kovacic *et al.,* 2001 ; Ridnour *et al.,* 2005). The continuous and rapid production of free radicals leads to deficient antioxidants. Once the natural antioxidant defense system becomes weak, there is biochemical lesion in many metabolic pathways (Sood *et al.,* 2004). Thus, the conditions of oxidative damage and oxidative stress are responsible for pathological processes of various dreadful diseases and fast ageing as well (Tiwari *et al.,* 2001 ; Mohammad *et al.,* 2004).

The strong and efficient antioxidant system is significantly required for the control of the toxic and harmful state caused by oxidative stress. Antioxidants are the substances that scavenge the free radicals, and thus reduce oxidative stress by inhibiting cellular damages and lipid peroxidation (Irshad *et al.,* 2002), thereby preventing the condition of pathogenecity (Rock *et al.,* 1996). The harm caused to significant antioxidant enzymes of the antioxidant system, *viz.,* MDA (Malondialdehyde), SOD (Superoxide dismutase), CAT (Catalase), GSH (Reduced Glutathione) and GPx (Glutathione peroxidase) is compensated by the routine intake of antioxidants through food, tonics, supplements and medicines.

There are many plants with medicinal values that have been the major part of the traditional medicines since ages, as well as form the basis of many of the modern pharmaceuticals. The medicinal value of such plants has been attributed to their rich phytochemical constituents and antioxidant properties. Thus in recent era, there is an inclination to use antioxidants from natural sources such as medicinal and dietary plants (Brown *et al.,* 1998; Herrera *et al.,* 2001) and their characterization for successful phytoremediation of oxidative stress.

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In the string of such antioxidant sources, one of the very commonly available plants in India is *Achyranthes aspera.* It has been traditionally used in the treatment of many cutaneous, respiratory and digestive disorders (Nadkarni, 2009; Pankaj, 2003). The rich antioxidant activity and medicinal property of the root extract of *Achyranthes aspera* is reported due to the presence of phytochemical compounds such as saponin, sterols, alkaloids and polysaccharides (Priya *et al.,* 2010).

In human population, the lymphocytes are considered to be important for encountering the various toxicants and free radicals. The lymphocytes are used in the present study for inducing H_2O_2 into them as the hydroxyl radical formation is easily stimulated in them, leading to oxidative stress. The present paper focuses on the evaluation of antioxidant efficacy of the methanolic root extract of *Achyranthes aspera* to assess phytoremediation of H_2O_2 induced oxidative stress in lymphocytes of *Oryctolagus cuniculus.*

MATERIALS AND METHODS

The healthy plants of *Achyranthes aspera* were collected from the open fields and grounds of Durg (Chhattisgarh), and the roots were separated. After proper washing and sterilization in 70% alcohol, the roots were shade dried. The extract was prepared in 59% methanol by Soxhlet extraction apparatus.

For *in vitro* study, blood sample was collected from *Oryctolagus cuniculus,* and stored in heparinized sterilized tube. This was followed by centrifugation for isolation of lymphocytes and thereafter washing in phosphate buffer saline.

Table 'A' showing effect of Methanolic Root Extract of *Achyranthes aspera* on the activity of the antioxidant enzymes and in recovery of Oxidative Stress

	Group I (Control)	**Group II (H_2O_2 treated)**	**Group III (5µl ARE + H_2O_2)**	**Group IV (10µl ARE + H_2O_2)**	**Group V (20µl ARE + H_2O_2)**
Lipid peroxides in mole MDA/mg protein **(MDA)**	0.80 ± 0.02	3.90 ± 0.04*	3.02 ± 0.06*	2.19 ± 0.015#	1.00 ± 0.03#
Reduced Glutathione (µ moles / mg protein) **(GSH)**	5.96 ± 0.04	2.36± 0.06*	2.72 ± 0.05#	3.37 ± 0.13#	5.12 ± 0.12#
Superoxide dismutase (units / mg protein) **(SOD)**	3.88 ± 0.06	1.36 ± 0.04*	2.68 ± 0.11#	2.70 ± 0.14#	2.75 ± 0.12#
Catalase (µ moles of H_2O_2 consumed / min./ mg protein) **(CAT)**	5.56 ± 0.10	3.73 ± 0.02*	3.88 ± 0.03#	4.09 ± 0.04#	5.03 ± 0.05#
Glutathione peroxidase (µg of Glutathione utilized / min. / mg protein) **(GPx)**	9.56 ± 0.11	4.83 ± 0.09*	4.86 ± 0.03#	5.61 ± 0.18#	8.45 ± 0.36#

DMEM medium alongwith 10% fetal serum was used for the culture of lymphocytes. The culture was maintained in a humidified CO_2 incubator at 37˚C temperature and 5% CO_2 for 18

hours. After incubation, the cells/lymphocytes were exposed to oxidative stress by 100µM H_2O_2 induced lipid peroxidation for 2 hours (Sohi *et al.,* 2003). The experiment was designed with five groups of cultured lymphocytes for the analysis of each of the considered antioxidant enzymes. The samples taken were in replicates of five. Pretreatment with methanolic root extract of *Achyranthes aspera* in gradual increasing dosages as 5µl, 10µl, and 20µl/ 10,000 cells successively in the third, fourth and fifth groups of the experiment aided in evaluation of its antioxidant property and ability of phytoremediation of oxidative stress. The enzymatic parameters taken into consideration for antioxidant assay are:

MDA (Malondialdehyde)	determined by following Okhawa *et al.* method.
GSH (Reduced Glutathione)	following Misra *et al.*method
SOD(Superoxide dismutase)	following Moron *et al.* method
CAT (Catalase)	following Bergmeyer *et al.* method, and
GPx (Glutathione)	following Rotruck *et al.* method

The collected data for all the antioxidant enzymatic parameters were statistically validated by ANOVA. ARE – Achyranthes Root Extract ; * Compared with Control ; # Compared with H_2O_2 treated Group

RESULTS AND DISCUSSION

The H_2O_2 induced lymphocytes give rise to oxidative stress and the hydroxyl radicals increased the lipid peroxidation by their constant attacks on lipid components of the membrane. This increased the levels of MDA, the final product of peroxide process, The state of oxidative stress so developed decreased the antioxidant activities of GSH, GPx, SOD and CAT. The pretreatment with the methanolic root extract of *Achyranthes aspera* in gradual and successive increasing dosages (5µl, 10 µl and 20 µl/10,000 cells), regulated the activity of MDA, and the antioxidant activity of glutathione as well as other antioxidant enzymes, increased and was restored accordingly. This indicated the state of recovery of lymphocytes from oxidative stress ($P<0.05$) **[Table A].**

The fact that *Achyranthes aspera* is known for its rich medicinal values makes it significant for its use in traditional medicinal system. The plant has been traditionally used in the treatment of asthama, cough, digestive and skin diseases. The paste of roots in water is reported to be effective in curing diarrhoea and eye disorders. The roots of *Achyranthes aspera* are used by the natives of Chhattisgarh in the form of Herbal Mala and Tabiz in order to treat fever and hasten the process of delivery. This is attributed to its wound healing property. Freshly harvested roots are used in preparation of herbal oil (Pankaj, 2003; Neeru *et al.,* 2006). Infusion of root is known as mild astringent in curing bowel complaints, and juice of root in the treatment of diabetes and mild type of leprosy, bleeding in delivery and menstrual disorders (Chopra *et al.,* 1958 ; 1986; Ojha, 1966).

The whole plant of *Achyranthes aspera* exhibit good antioxidant effect by presenting the formation of free radicals. Rich source of phytochemicals present in the plant is responsible for its antioxidant properties (Edwin *et al.,* 2008). Ecdysterone and ecdysone isolated from the Methanolic extract of roots and whole plant attributes to its antioxidant potentials (Banerji *et al.,* 1970 ; Ikan *et al.,* 1971 ; Rastogi *et al.,* 2004). A new aliphatic acid responsible for antioxidant properties was isolated from the ethanolic extract of roots (Sharma *et al.,* 2009). Ethanolic extract of root and aerial parts of *Achyranthes aspera* are also well known for its antibacterial activity (Kumar *et al.,* 2003). Also the alcoholic root extract has been reported to exhibit anti-inflammatory activity (Kumar *et al.,* 2009; Mehta *et al.,* 2009).

Antioxidant activity of leaves and roots have been reported by Gayathri *et al.* (2009). Malarvili *et al.* (2009) reported antioxidant efficiency in the seeds of *Achyranthes aspera* for phytoremediation. Free radical scavenging activity of the ethanolic and aqueous extract of roots were assessed using two methods, *viz.,* DPPH radical scavenging activity and superoxide scavenging activity (Paul *et al.,* 2010). Antioxidant ability of *Achyranthes aspera* was established by using different extracts of root, stem, leaf and inflorescence, evaluated by DPPH assay. With the increase of time and concentration, evaluation of antioxidant ability was reported to be in order of root > stem > inflorescence > leaf (Beaulah *et al.,* 2011).

Root did not show antioxidant activity in chloroform extract, whereas stem extract showed high radical scavenging potential. In ethyl acetate extract, the antioxidant activity of root was higher than stem. Antioxidant potential of stem and root extracts for phytoremediation was evaluated in methanol and water by measuring DPPH radical scavenging activity. Methanol extract showed high antioxidant activity than that of aqueous extract (Priya *et al.,* 2010).

DPPH and superoxide assay methods explain the antioxidant properties of the plant extracts, but significant direct free radical scavenging enzymes are MDA, GSH, SOD, CAT and GPx. The present work holds its novelty in getting the antioxidant property evaluated comprehensively, considering all directly acting enzymes for free radicals in methanolic root extract of *Achyranthes aspera.* Thus, it was observed that the amleorative effect of antioxidant enzymes was very effective on exposure of methanolic extract of root of *Achyranthes aspera.* Hence, the root extract was found suitable source of phytoremediation of oxidative stress in lymphocytes.

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