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Antioxidant Activity of Root of *Achyranthes Aspera* and its Comparison with Melatonin in Recovery of Oxidative Stress

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Abstract: ***The free radicals produced during the cellular oxidation and metabolic processes cause excessive damage to biomembranes and macromolecules like DNA, carbohydrates, lipids and proteins. Such a condition of oxidative stress and the harmful effects of free radicals bring about imbalance in the antioxidant system of the human body. The pathological conditions so developed are implicated into many dreadful diseases and fast ageing as well. In the present study, the antioxidant activity of the Chloroformic root extract of the well known medicinal herb, Achyranthes aspera is evaluated in recovery from H_2O_2 induced oxidative stress in lymphocytes of Oryctolagus cuniculus. The H_2O_2 incubated lymphocytes showed increased lipid peroxidation in terms of Malondialdehyde (MDA - 3.83 ± 0.09), and also altered the activities of other antioxidant enzymes and glutathione systems, viz., Superoxide dismutase (SOD – 1.47 ± 0.08), Reduced Glutathione (GSH – 2.42 ± 0.03), Catalase (CAT – 3.75 ±0.04) and Glutathione peroxidase (GPx – 4.66 ± 0.62). Pretreatment with Chloroformic root extract of Achyranthes aspera in increasing concentrations (5 µl, 10 µl, 20 µl / 10,000 cells) for 18 hours was found to decrease lipid peroxidation and increase the antioxidant activities of the significant enzymes, viz., MDA (1.07 ± 0.04), GSH (5.03 ± 0.09), SOD (3.15 ± 0.06), CAT (5.16 ± 0.06) and GPx (8.16 ± 0.17). The decline in the state of oxidative stress attributed to the high antioxidant activity and efficient free radical scavenging ability of the Chloroformic root extract of Achyranthes aspera. Further, the antioxidant activity of root of Achyranthes aspera was compared with the powerful antioxidant, Melatonin. The melatonin exposure (5 µl, 10 µl, 20 µl / 10,000 cells) also enhanced the antioxidant activity of the enzymes, viz., GSH (3.78 ± 0.17), SOD (2.94 ± 0.04), CAT (4.60 ± 0.05) and GPx (7.66 ± 0.19), and decreased lipid peroxidation in terms of MDA (1.60 ± 0.10). Both the Chloroformic root extract and melatonin as well, were found effective in recovery from oxidative stress, but the root extract of Achyranthes aspera was reported to exhibit high antioxidant activity as compared to melatonin.***

Keywords: Free radicals, Oxidative stress, Antioxidant enzymes, Peroxidation, Melatonin.

1. Introduction

The enzymatic and non-enzymatic reactions during the natural cellular processes by the endogenous system of the body produce oxygen free radicals continuously [1]. The harmful effects of free radicals give rise to the state of oxidative stress by causing potential biological damage [2]. The rapid and continuous production of free radicals reduce the antioxidants and their activity, as well as give rise to many physiological imbalances and metabolic disruptions [3]. Thus, the state of oxidative damage and oxidative stress lead to pathogenesis and rapid ageing [4].

The antioxidants with strong free radical scavenging ability constitute the characteristic components of the efficient antioxidant defense system. It helps to reduce the cellular damages, oxidative stress and lipid peroxidation [5] thereby preventing the condition of pathogenecity. The significant antioxidant enzymes of the antioxidant defense system and their activity is usually restored by the intake of antioxidants through food, tonics, supplements, and also by intake of many herbs and spices of medicinal importance. Some of the such important antioxidant enzymes are MDA (Malondialdehyde) SOD (Superoxide dismutase), CAT (Catalase), GSH (Reduced Glutathione) and GPx (Glutathione peroxidase).

There are many medicinal plants known since ages, for their therapeutical potentials, because of which they are used traditionally to cure many diseases, and also in the modern pharmaceuticals. The rich phytochemical constituents and efficient antioxidant properties attribute to the remarkable medicinal values of such medicinal and dietary plants [6]. So, there have been continuous attempts towards the use of antioxidants from the natural sources of medicinal plants, that increase the antioxidant activity of the enzymes and other components of the defense system. This helps in rapid scavenging of free radicals, and thereby in recovery of oxidative stress. One of the very well known antioxidant sources is the commonly available medicinal herb of India, i.e., *Achyranthes aspera*. It has been traditionally used in the treatment of respiratory, digestive and cutaneous problems. *Achyranthes aspera* is also well known for its anti-bacterial, spermicidal, anti-allergic and anti-inflammatoy activity [7,8].

At the same time, Melatonin, the neurohormone, is the powerful antioxidant, known for its free radical scavenging abilities [9]. It is primarily produced by the pineal gland, and also synthesized in bone marrow, retina and lymphocytes. Melatonin is easily available to all tissues and cells because of its remarkable ability to cross the blood-brain-barriers with ease [10]. Melatonin is known to stimulate many antioxidant enzymes, and also promote the glutathione activity [11]. It acts as the most efficient detoxific agent that can easily suppress the oxidative effects of free radicals by increasing the production of endogenous antioxidants [12].

The present paper focuses on the evaluation of antioxidant efficacy of the root extract of *Achyranthes aspera* in the

solvent Chloroform, against H_2O_2 induced oxidative stress in lymphocytes of *Oryctolagus cuniculus.* The antioxidant activity of root of *Achyranthes aspera* is, further, compared to the antioxidant efficacy of Melatonin for the better and more intricate evaluation.

2. Materials and Methods

A few plants of *Achyranthes aspera* with healthy growth were collected from the open fields of Durg (Chhattisgarh, India), and the roots were separated. The roots were shade dried after proper washing and sterilization in 70% alcohol. Chloroform was the solvent selected for the preparation of root extract by using Soxhlet Extraction Apparatus. For *in vitro* study, blood sample was collected from *Oryctolagus cuniculus,* and stored in heparinized sterilized tube. This was followed by isolation of lymphocytes by centrifugation, and their washing in phosphate buffer saline. The culture of lymphocytes was done by using DMEM medium alongwith 10% fetal serum. Thereafter 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 100 µM H_2O_2 induced lipid peroxidation for 2 hours [13].

The experiment was designed with eight groups of cultured lymphocytes for the analysis of each of the considered enzymes. The samples taken were in replicates of five. Group I was considered as Control set, with only lymphocytes. Group II was with lymphocytes induced with 100 µM H_2O_2 for 2 hours, to develop oxidative stress. Group III, IV and V comprised of H_2O_2 treated lymphocytes pretreated with 5 µl, 10 µl and 20 µl / 10,000 cells of the Chloroformic root extract of *Achyranthes aspera,* respectively. Group VI, VII and VIII consisted of H_2O_2 treated lymphocytes pretreated with Melatonin exposure in the concentrations of 5 µl, 10 µl and 20 µl / 10,000 cells respectively for experimentation. The cells were collected, washed twice in ice cold phosphate buffer and used for antioxidant assay.

The following biochemical enzymatic parameters were analyzed for the antioxidant assay :
MDA (Malondialdehyde) – 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
GPx (Glutathione peroxidase) – following Rotruck *et al.* method

The collected data for all the antioxidant enzymatic parameters were statistically validated by ANOVA.

Table 1: Effect of Chloroformic Root Extract of *Achyranthes aspera* on the antioxidant activity of different enzymes and their comparison with Melatonin

Enzymatic parameters Expt. Groups	Lipid peroxides in mole MDA/mg protein **(MDA)**	Reduced Glutathione (µ moles/mg protein) **(GSH)**	Superoxide dismutase (units/mg protein) **(SOD)**	Catalase (µ moles of H_2O_2 consumed/ min./mg protein) **(CAT)**	Glutathione peroxidase (µg utilized/min./mg protein) **(GPx)**
Group I (Control)	0.78 ± 0.03	5.58 ± 0.05	3.63 ± 0.06	5.54 ± 0.07	8.90 ± 0.41
Group II (H_2O_2 treated)	3.83 ± 0.09*	2.42 ± 0.03*	1.47 ± 0.08*	3.75 ± 0.04*	4.66 ± 0.62*
Group III (5µl ARE + H_2O_2)	3.01 ± 0.03#	2.79 ± 0.06#	1.82 ± 0.04#	3.95 ± 0.05#	4.48 ± 0.30#
Group IV (10µl ARE + H_2O_2)	1.98 ± 0.05#	3.45 ± 0.12#	2.51 ± 0.05#	4.64 ± 0.04#	5.94 ± 0.18#
Group V (20µl ARE + H_2O_2)	1.07 ± 0.04#	5.03 ± 0.09#	3.15 ± 0.06#	5.16 ± 0.06#	8.16 ± 0.17#
Group VI (5µl MEL + H_2O_2)	3.60 ± 0.44#	2.70 ± 0.09#	1.58 ± 0.13#	3.76 ± 0.05#	4.75 ± 0.11#
Group VII (10µl MEL + H_2O_2)	2.39 ± 0.12#	3.15 ± 0.04#	2.40 ± 0.10#	4.00 ± 0.03#	5.53 ± 0.17#
Group VIII (20µl MEL + H_2O_2)	1.60 ± 0.10#	3.78 ± 0.17#	2.94 ± 0.04#	4.60 ± 0.05#	7.66 ± 0.19#

ARE – *Achyranthes* Root Extract ; MEL – Melatonin ; * - Compared with control ; # - Compared with H_2O_2

3. Result and Discussion

The lymphocytes of *Oryctolagus cuniculus,* when treated with H_2O_2 gave rise to the condition of oxidative stress. The hydroxyl radicals initiated peroxidation of lipids, and the final product of the peroxide process, Malondialdehyde (MDA), showed increased levels (Group II). The state of increased lipid peroxidation and oxidative stress, led to the reduced levels of Glutathione (GSH) and Glutathione peroxidase (GPx), i.e., the co-factor of several detoxifying enzymes against oxidative stress, and the scavenger of H_2O_2 and lipid peroxides respectively (Group II). The free radical scavenging activity of antioxidant enzymes, Superoxide dismutase (SOD) and Catalase (CAT) also decreased due to the weak antioxidant defenses (Group II). The pretreatment with the Chloroformic root extract of *Achyranthes aspera* in the increasing concentrations (5 µl, 10 µl and 20 µl / 10,000 cells), regulated lipid peroxidation. This enabled in reducing the levels of MDA, and in increasing gradually the antioxidant activity of the enzymes, GSH, SOD, CAT and GPx (Group III, IV & V). This indicated the state of lymphocytes with restored antioxidant activity of enzymes and recovery from oxidative stress ($P < 0.05$) [Table 1]. Table 1 also showed the results of Melatonin exposure to

H_2O_2 treated lymphocytes in increasing dosages (5 µl, 10 µl and 20 µl / 10,000 cells). It too helped in reducing lipid peroxidation in terms of MDA, and also in restoring the antioxidant activity of GSH, SOD, CAT and GPx ($P < 0.05$) (Group VI, VII & VIII). However, better efficacy of antioxidant activity and recovery from oxidative stress was observed under the influence of Chloroformic root extract of *Achranthes aspera* in comparison to the Melatonin treatment (Table 1).

The popularly known medicinal herb, *Achyranthes aspera* possess rich medicinal properties as it is the rich source of antioxidants and phytochemicals [14]. So the natural constituents can be derived from any part of the plant, the root being the most significant [15]. Aqueous paste of roots is reported to be effective in treatment of cornea and ophthalmic problems [16]. The bowel complaints are cured by the infusion of the root, and the juice of root is known for the treatment of diabetes, mild type of leprosy and menstrual disorders [17]. The natives of Chhattisgarh use the roots of *Achyranthes aspera* in the form of Herbal Mala and Tabiz in order to treat fever and hasten the process of delivery [7]. Dried and powdered roots are used as astringent to cure leprosy, antifertility and bleeding in delivery [18].

The seeds, leaves and significantly the roots of *Achyranthes aspera* have been reported for antioxidant efficiency in reducing oxidative stress [19]. The root extracts prepared in different solvents are well known for good antioxidant activity. Free radical scavenging ability of Ethanolic and Aqueous extracts of roots was determined by using two methods, *viz.,* DPPH radical scavenging and Superoxide scavenging activity methods [20]. Antioxidant efficacy of root extract in Hexane was found to indicate the best as compared to stem, inflorescence and leaf. Ethyl acetate extract of root also exhibited good antioxidant ability, but less as compared to inflorescence and leaf [21]. Beaulah evaluated the antioxidant property of the different parts of *Achyranthes aspera* using DPPH assay, and was reported to be in the order of root > stem > inflorescence > leaf. Methanol extracts of root showed high antioxidant activity than that in Aqueous extracts, when evaluated by DPPH radical scavenging activity method [22].

The root extract of *Achyranthes aspera* prepared in Chloroform as solvent showed varied activities. Highest anti-bacterial and anti-inflammatory activity have been reported for root extract in Chloroform [21]. The presence of flavonoids polyphenolic compounds and steroids are hopefully considered to be responsible for such activity [23]. Evaluation by DPPH radical-scavenging activity method did not show any antioxidant activity for Chloroformic root extract of *Achyranthes aspera* [21]. However, in the present work, high antioxidant activity of root extract of *Achyranthes aspera* in Chloroform have been reported in terms of antioxidant enzymes such as MDA, GSH, SOD, CAT and GPx.

Melatonin has been reported as the powerful antioxidant, efficient in scavenging free radicals, and thus able to protect nuclear DNA, membrane lipids and cytosolic proteins from oxidative damage [24,25]. It efficiently stimulates the activities and m-RNA levels of antioxidant enzymes, such as SOD, CAT, GPx and GSH [11,26]. The ability of melatonin to detoxify hydroxyl radical and neutralize its precursor, i.e., hydrogen peroxide (H_2O_2) has also been quite remarkable [27]. Melatonin efficacy in terms of protecting cellular damages against free radical attacks and decline of oxidative stress has been found better at a lower dose than other classical antioxidants [28].

Thus, in the present study, antioxidant activity of Chloroformic root extract of *Achyranthes aspera* is compared with Melatonin that has been already known as the most established antioxidant with protective effects against lipid peroxidation and oxidative stress [29, 30]. The antioxidant properties of the plant extracts of *Achyranthes aspera* mostly reported, have been evaluated usually by DPPH and Superoxide assay methods. But it is also known that antioxidant enzymes, *viz.,* MDA, GSH, SOD, CAT and GPx are the power sources of radical scavenging and antioxidant activities. So, the present work holds the unique feature in getting the antioxidant property evaluated comprehensively, taking into consideration all directly acting enzymes against free radicals in Chloroformic root extract of *Achyranthes aspera.* It was also further compared with the antioxidant activity of Melatonin in recovery of oxidative stress. Thus, it was observed that the antioxidant enzymes showed strong amleorative effects under exposure of Chloroformic extract of root of *Achyranthes aspera.*

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