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RESEARCH ARTICLE

In vitro effects of Indian ayurvedic medicinal preparation against UV B induced cytotoxicity

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ABSTRACT

The object of the present study was to investigate the effect(s) of UV-B irradiation on chicken splenocyte culture and the ameliorating effect of Indian Ayurvedic medicinal preparation. The medicinal preparation *i.e.* aqueous extracts from *Andrographis pnaiculata* (Acanthaceae) and hydromethanolic extract of *Acacia catechu* (Fabaceae) namely ANDRO and ACQ, were used against UV B rays induced cytotoxicity. Isolated lymphocytes were subjected to UV-B irradiation and in combination of medicinal preparations *in vitro* for 0, 250, 500, 1250, 2500 and 3750 Joules/m^2 which correspond to the irradiation time of 0, 1, 2, 5, 10 and 15 minutes. 3-[4,5-dimethylthiozol-2yl]-2,5-diphenyltetrazolium bromide [MTT] assay performed for viability of lymphocytes in both treatments. Result show that the significant reduction in cell viability with increase in exposure of UV B rays. While the treatment of medicinal plants *i.e.* the dose of 0.3 mg/ml of both ANDRO and ACQ significantly reduces the per cent reduction in cell viability due to exposure of UV B rays.

Key words : Ultraviolet B irradiation, Viability

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INTRODUCTION

Oxidative stress also results from exposure of radiation due to generation of ROS (Cunningham *et al.*, 1985; Tyrrell, 1991). There is an increased concern over the potential destruction of stratospheric ozone layer (National Academy of Sciences, 1982) due to human-directed activities. Decrease in stratospheric ozone are expected to result in enhanced levels of UVB (290-320 nm) reaching the earth's surface and concomitant increase in skin cancer is predicted (Scotto *et al.*, 1981). UV radiation localized to a limited skin area has been shown to inhibit the induction of the immune response in a distant skin area not exposed to UV radiation (Noonan *et al*., 1981). This phenomenon is called systemic immunosuppression. Also, this systemic immunosuppressive effect is transferred in T lymphocytes from one animal to another (Fisher and Kripke, 1982).

The health risks associated with ozone depletion are

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caused by the enhanced UVA radiation in the environment and increased penetration of UV radiation of shorter wavelength (between 280 nm and 320 nm, UVB rays). UV-B irradiation has shown to cause apoptosis by its oxidative property and UV-C has shown to cause apoptosis by damaging the DNA (Pamphilon *et al.*, 1991). Hence, compounds capable of protecting cellular membranes against ionizing radiation in particular and free radicals in general will have potential benefits as radioprotectors, antioxidants and anti-mutagens (Stavric, 1994 and Odin, 1997). There is sufficient evidence to suggest that adequate antioxidant defense by vitamin E and the other antioxidants can provide protection from the high levels of free radicals generated. Regardless of the sources of antioxidants, all the antioxidants have a similar function, which is to prevent damage done by free radicals. Currently, great interest centres on the possible protective value of a wide variety of plant-derived antioxidant compounds, particularly those from fruits and vegetables and medicinal plants against radiation damage (Stavric, 1994; Maulik *et al*., 1997).

Medicinal plant extracts *viz.,* aqueous extracts from *Andrographis pnaiculata* (Acanthaceae) and hydromethanolic extract of *Acacia catechu* (Fabaceae), namely ANDRO and ACQ have been widely evaluated for their biological properties as these are reported to possess therapeutic potentials due to presence of different phyto-constituents. These plants are the potential source of antioxidants (Kruawan and Kangsadalampai, 2006) which can help in counteracting UV B rays induced cytotoxicity. Due to their significant therapeutic values (like antioxidant, anticancer and immuno-stimulant), there is a renewed interest to explore their efficacies under different conditions.

Acacia catechu Wild.(Family) : Fabaceae and Subfamily : *Mimosoideae*) known as Black cutch, a deciduous thorn like tree mainly found in India and also found in deciduous forest around the world. It is an astringent, having cooling and digestive properties, beneficial in cough and diarrhea, applied externally to ulcer, boils and skin eruption and shows hypotensive effect (Syed and Mohammed, 2009).

Andrographis paniculata (Kalmegh) is a traditional herbal medicine which is widely used for the treatment of many diseases in Asia and it has immunostimulatory activity, anti-inflammatory effect, cytokine induction or deduction, a potential cancer therapeutic agent and T cell suppressant (Youhong, 2009).

Assessing cell membrane integrity is one of the most common ways to measure cell viability and cytotoxic effects. Compounds that have cytotoxic effects often compromise cell membrane integrity. Vital dyes, such as trypan blue or propidium iodide are normally excluded from the inside of healthy cells; however, if the cell membrane has been compromised, they freely cross the membrane and stain intracellular component. Cytotoxicity can also be monitored using the MTT assay. This assay measures the reducing potential of the cell using a colorimetric reaction. Viable cells will reduce the MTT reagent to a coloured formazan product. The MTT test has been widely used as a rapid and sensitive method for screening anticancer drugs as well as for the assessment of cytotoxicity of materials. The reproducibility of the MTT test is statistically significant (Fotakis *et al.*, 2006).

MATERIALS AND METHODS

DMSO, potassium chlorite, potassium di-hydrogen phosphate, sodium bicarbonate, sodium carbonate, rodium chlorite, sodium hydroxide and MTT dye (Hi-Media) were used during present study.The authentic plant materials were collected from MRDC and AFRC and CIMAP Pantnagar. The aerials part of plant *viz.,* stem and leaves of *Andrographis paniculata* (Kalmegh); leaves of *Acacia catechu* (Kath) were freshly collected. The chicken spleens were collected from healthy birds aseptically in sterilized vial containing phosphate buffer saline from slaughter house at Pantnagar and brought to the laboratory for the separation of lymphocytes.

Preparation of extracts of medicinal plants :

Plant extract were prepared as by methodology given by Aboaba *et al.* (2006) per cent yield of extract was calculated.

Biochemical analysis :

A small portion of the extracts were subjected to different phyto-chemical tests as per the methods described by Harborne (1973); Sofowara (1993); Trease and Evans (1989) to test the presence of proteins, carbohydrate, alkaloids, tannins, flavonoids, steroids and saponins.

Preparation of chicken splenocytes culture :

Chicken splenocyte culture was performed as per

the method described by Mellon *et al.* (1999). RPMI-1640 medium was used for culturing the splenocytes.

Cell viability assay :

Percentage cell viability was determined by 0.1 per cent trypan blue dye exclusion test using haemocytometer (Kaltenbach *et al.*, 1958) and final cell count was adjusted to $1x10^7$ cells /ml in RPMI-1640 medium.

Determination of UV B rays induced *in vitro* cytotoxicity in avian splenocyte cell culture system:

In vitro UV B rays induced cytotoxic dose was determined by MTT cytotoxicity assay in ckicken splenocyte culture system as per the method described by Fotakis *et al.* (2006). Splenocyte culture was prepared as described above. UV B ray was exposed for 0, 250, 500, 1250, 2500 and 3750 Joules/m^2 which correspond to the irradiation time of 0, 1, 2, 5, 10 and 15 minutes. The lymphocytes were treated in quadruplicate for each dilution of UV B rays in 96 well cell culture plates and incubated for 48 hours in CO_2 incubator at 37^0 C. The cytotoxicity caused by UV B rays was evaluated by MTT assay. The rate of growth inhibition was calculated as per the following formula:

$$\text{Per cent growth rate } Z \frac{\text{OD of extract treated cells}}{\text{OD of control}} \times 100\%$$

The values of per cent cytotoxicity are expressed with respect to the control cells (untreated cells) *i.e.*

$$\text{Per cent cytotoxicity} = (100 - \text{Per cent growth rate}) \%$$

Determination of non-cytotoxic dose of plant extracts :

Various dilutions of the plant extracts were prepared in RPMI-1640 (L-Glutamine 25 millimolar (mM) HEPES buffer, Hi-Media, Mumbai) medium under aseptic conditions, which were then used for *in vitro* exposure in 96 well cell culture plate for treating the isolated chicken lymphocytes. *In vitro* noncytotoxic dose for each plant extract in chicken splenocytes was determined by MTT cytotoxicity assay as per the method described by Scoger (1988).

Determination of ameliorating potentials of the plant extracts against i*n vitro* exposure of UV B rays :

Amelioratory effects of each plant extract against UV B rays induced immunotoxicity was evaluated by giving simultaneous exposure of UV B rays and different plant extracts for different time periods in the similar manner as described above.

Statistical analysis :

Statistical analysis was based on comparing the values of control group with that of the exposed groups. The results were expressed as means OD ± SE. The statistical significance of the data has been determined using one way analysis of variance (ANOVA-LSD) using SPSS statistical software package version 15. Pearson correlation test was used to determine the significant correlations between variables. The level of significance was set at $p < 0.05$.

RESULTS AND DISCUSSION

The results obtained from the present investigation as well as relevant discussion have been summarized under following heads :

Extract preparation and per cent yield of medicinal plant extracts :

Recently great attention is paid towards the natural antioxidants and immunomodulators. The use of medicinal plants as traditional medicines is well known in rural areas of many developing countries (Sandhu and Heinrich, 2005 and Gupta *et al.*, 2005). Traditional healers claim that their medicine is cheaper and more effective than modern medicine. Therefore, the real challenge lies not in proving whether herbs have health benefits, but in defining what these benefits are and developing the methods to expose them by scientific means (Tapsell *et al.*, 2006).

Therefore, medicinal plants *viz.*, *Acacia catechu, Andrographis paniculata* are examined for their antioxidative properties against UV B rays induced oxidative stress.

While preparation of medicinal plant extract 5.23 g of the hydromethanolic extract was recovered from 50 g dry weight of stem and leaves of *Acacia catechu*. The per cent yield was found to be 10.46 per cent showing minimum yield. 8.87 g of the aqueous extract was recovered from 50 g of *Andrographis paniculata* aerial part. Therefore, the per cent yield was found to be 17.75 per cent as depicted in Table 1.

Biochemical analysis of medicinal plant extract :

Biochemical analysis of ACQ and ANDRO revealed

presence of various phytochemicals like Resins, Saponin, Flavonoid, Alkaloids, Steroids, glycoside, protein, carbohydrate and phenol as shown in Table 2.

All these phytochemicals helped in preventing many diseases and showed that these medicinal plants have anti-oxidative potential. The presence of protein, glycosides and carbohydrate are indicative of palatability of the material.

Non-cytotoxic dose of medicinal plant extracts in chicken splenocytes :

The avian lymphocytes were exposed to various dilutions of hydromethanolic/ aqueous extract of medicinal plant extracts to determine its maximum non-cytotoxic dose for further *in vitro* studies.

The data indicated dose-dependent cytotoxicity induced by *Acacia catechu* in splenocyte cell culture. Since maximum concentration of *Acacia catechu* extract that showed 100 per cent cell viability was 0.3mg/ml, this was selected for further *in vitro* analysis. The similar experiment was conducted for ANDRO and ACQ showing cent per cent cell viability at 0.3 mg/ml doses.

Effect of Indian Ayurvedic medicinal preparation on chicken splenocyte culture in UV B rays induced cytotxicity :

One of the hallmark events of exposure to ultraviolet B radiation (UVB, 290–320 nm) is the induction of apoptotic cell death. UV-B irradiation has shown to cause apoptosis by its oxidative property (Odin, 1997). As the medicinal plant preparation have potential antioxidants like resins, saponin, flavonoid, alkaloids, Steroids etc., (Table 2) they are evaluated for their protecting effect against UV B rays induced cytotoxicity. Exposure of the splenocyte cell culture to UVB resulted in apoptosis, which was significantly reduced when cells were preincubated with medicinal plant extracts with their non-cytotoxic before irradiation (Fig. 1a).

Acacia catechu :

UV radiation causes systemic immunosuppression also, this systemic immunosuppressive effect is transferred in T lymphocytes from one animal to another (Fisher and Kripke, 1982). The antioxidant present in *Acacia catechu* evaluated for counteracts the effect of oxidative stress due to UV B radiation on lymphocyte culture system.

Medicinal plant extract increase the viability of cell up to 115 per cent at time interval of 1 minute exposure of UV B rays. Similarly medicinal plant extract increase the viability of cell up to 105 per cent at time interval of 15 minutes exposure of UV B rays. The data indicates that medicinal plants show potential antioxidative proeprties and counteract the oxidative potential of UV B induced oxidative stress.

Table 1 : Per cent yield of different plant extracts

Sr. No.	Medicinal plants	Dry weight	Final weight of extract	Per cent yield
1.	Hydromethanolic extract *Acacia catechu* (ACQ)	50	5.23	10.46
2.	Aqueous extract *Andrographis paniculata* (ANDRO)	50	8.87	17.75

Table 2 : Phytochemicals of various medicinal plant extracts

Plants / *Biochemical tests*	*Acacia catechu* (ACQ)	*Andrographis paniculata* (ANDRO)
Test for protein	+	+
Test for carbohydrates	+	+
Test for resins	+	+
Test for tannins	+	+
Test for Sapomims	+	+
Test for flavonoids	+	+
Test for alkaloids	+	+
Test for steroids	+	+
Test for phenols	+	+
Test for glycosides	+	+

Andrographis paniculata :

The present data showed that the rate of reduction of viability was up to 99 per cent at 1 min and it was significantly reduced to 75 per cent at 15 minutes exposure of UV B rays in control. Whereas, after treatment of extract, it was up to 97 per cent and significantly reduced to 80 per cent at 15 min exposure of UV B rays. In extract treated culture reduction rate was minimum as compare to control as shown in Fig. 1b.

The present data indicate that medicinal plant extract minimized the rate of reduction of viability after exposure of UV B rays. Since ACQ extract of ANDRO had potential antioxidative properties and counteracted the oxidative stress induced by UV B rays. Induction of apoptosis is an additional protective mechanism as it eliminates cells that were unable to repair the DNA damage or membrane damage completely. There are several ways of inhibiting UV-induced apoptosis - by overexpressing heat-shock proteins or anti-apoptotic proteins, by disrupting p53 function or by blocking death-receptor activation, for example. Here we provide the evidence that medicinal plant preparation protect the cell from UVB rays induced apoptosis may be by reducing the DNA and cell membrane damages. Biochemical analysis of medicicnal plant preparation shows the presence of potential source of antioxidants, they may scavenge out the ROS generated by the UV B rays and protect the cell from oxidative damage.

The low dose of *Acacia catechu* was more effective compared to the high dose. The reason for exact mechanism of this cannot be explained. There are many constituents present in the *Acacia catechu,* the main constituents are catechins that include catechin and epicatechin. Both these catechins are present in many other plants and these plants are reported for different activities. The tea catechins are known to possess antibacterial, antiviral, anticancer, antiinflammatory and antioxidant activity to name few (Fisher and Kripke, 1982). The present results indicate that ACQ ameliorate the effect of UV B rays induced cytotoxicity may be due to antioxidative properties. As ACQ is potential source of antioxidants, it may counteract the oxidative stress induced by the UV B rays in chicken splenocyte culture.

Andrographis paniculata contained flavonoid and their derivatives and reported to possess anti-oxidative properties (Gorter, 1911). *Andrographis paniculata* also contained diterpenes, lactones and flavonoids. Flavonoids mainly exist in the root, but have also been isolated from the leaves. Aerial parts contain alkanes, ketones and aldehydes and the bitter principles in the leaves were due to presence of the lactone andrographolide, which have therapeutic value and antioxidative potential (Kumar *et al.*, 2012). *Andrographis paniculata* had been reported for their therapeutic value. It had multiple pharmacological activities such as protozoacidal, antihepatotoxic, anti HIV, immunostimulative, anticancer, hypoglycemic and hypotensive activities (Handa and Sharma, 1990; Siripong *et al.*, 1992), anti-viral (Calabrese *et al.*, 2000) and anti-bacterial (Singha *et al.*, 2003). Das *et al.* (2009) also reported that *A. paniculata* or andrographide had antioxidative potential.

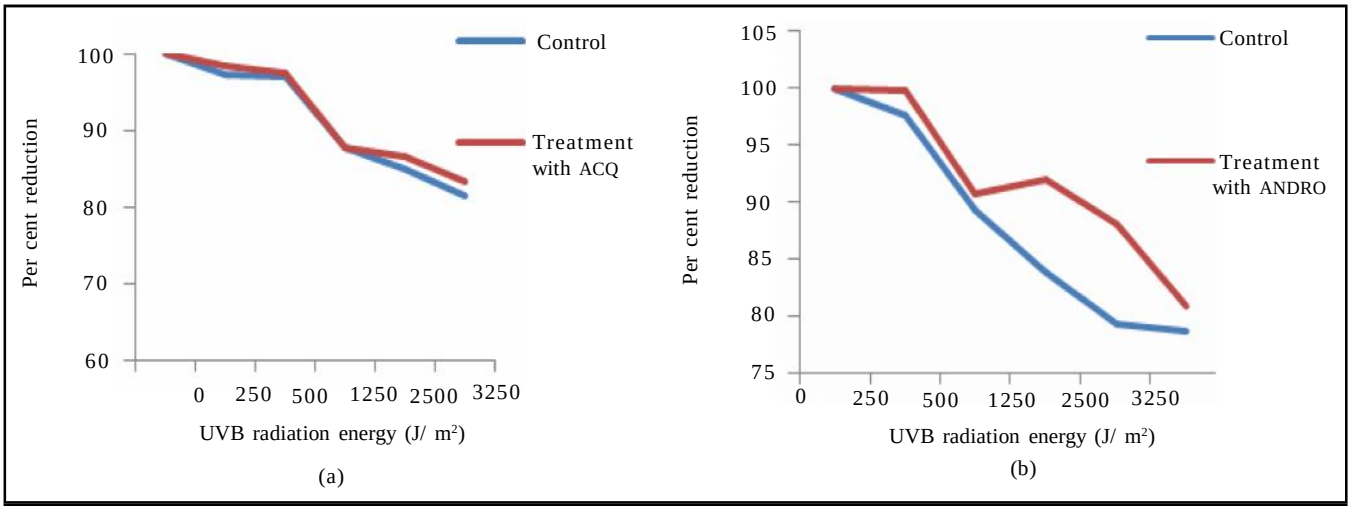

Fig. 1 : Antioxidative potential of ACQ (a) and ANDRO (b) against *in vitro* exposure of UVB rays

Conclusion :

The results of the present work indicate that the extracts have various phytochemicals like resins, saponin, flavonoid, alkaloids, steroids, glycoside, protein, carbohydrate and phenol which have potential therapeutic value and antioxidative activity. These assays are useful for establishing the ability of medicinal plants extract to chelate and reduce the oxidative stress induced by the UV B rays and have important applications for the pharmaceutical and human health.

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