Applied Biological Research 14 (2):220-222, 2012 **Short Note**

IMMUNORESTORATIVE EFFECT OF NATURAL AND *in vitro*-RAISED *Eupatorium cannabinum* L., A HIGH ALTITUDE MEDICINAL PLANT

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Eupatorium cannabinum L. (Asteraceae), a common wild plant growing in high altitude areas of Asian and Europian continents, is a well known traditional medicinal plant used as a source of alternative medicine and febrifuge. Herbalists have recognize its wound healing, cathartic and diuretic properties. The herb when used either alone or in combination with other herbs is a good remedy for purifying blood. Its leaves contain a volatile oil compound which acts on kidneys, and likewise some tannin and a bitter chemical principle are present which cut short the chill of intermittent fever. The chemical constituent and pharmacological studies of the herb in rats have revealed its choleretic and hepatoprotective properties (Zdero and Bohlmann, 1986; Lexa *et al*., 1989). Investigations on anti-cancer action of Eupatoriopicrin against Lewis lung tumour has previously been conducted (Woerdenbag *et al*., 1987). With increase in the incidence of immunological disorders like AIDS, malaria, tuberculosis, cancer, inflammatory problems, presently the focus is back on alternative medicines (Atal *et al.*, 1986). Many plant products, indispensable as pharmaceuticals and materials for diagnostic purposes, are in increasing demand. Plant cell culture has been considered as alternative source to agricultural production. Plant cell cultures serves a useful alternative route for production of such medicinal substances (Koul *et al.*, 1983, Wagner *et al.*, 1988). In present study the aqueous extracts of natural and *in vitro*-raised *Eupatorium cannabinum* plants were evaluated for their immuno-restorative potential after suppressing the immune system by hydrocortisone *in vivo* in swiss albino mice so as to explore the possibility of exploiting tissue culture raised plants for medicinal value.

Eupatorium cannabinum was collected from Darjeeling (altitude: 2200 m masl), West Bengal, India. The leaves and stem were air-dried, powdered and preserved at 4°C till use. The sterilized shoot tips and internodes (1.5-2.5 cm) were cultured under laminar flow for the initiation of shoot organ cultures in MS medium supplemented with auxins and cytokinins (Murashige and Skoog, 1962). The cultures were maintained at 25±2ºC under a 16/8 h light/darkness cycle. The optimum medium for shoot organ culture (MS medium supplemented with BAP and IAA each @ 1.0 mg L^{-1} was used for further studies. The aqueous extract was prepared by boiling dried powdered plant material in distilled water on a water bath for 24 h. The filterate was concentrated on a flash-rota evaporator under vaccum at 40ºC and dry concentrated extracts stored at 4ºC. Six to eight week old disease-free Swiss albino mice, weighing 20-25 g obtained from Animal House, Haryana Agriculture University, Hissar, were used. The animals were maintained on standard diet. For induction of immunosuppression**,** hydrocortisone (HC) [Wyeth Lederie Limited] was used as immunosuppressive agent. The mice were given HC @ 200 mg kg^{-1} body weight in single dose of 0.2 ml HC^{-1} $dose^{-1}$ $mouse^{-1}$ i.p. On 5th day of HC dose the mice were given plant extracts of natural and *in vitro*-raised *E. cannabinum.* The bioactivity was checked in 3 animal groups having 10 animals each, *i.e.* group I, without any extract inoculation, group II, *E. cannabinum* natural

extract treated animals, and group III, *E. cannabinum in vitro*-shoot organ culture extract treated animals. The 6-8 week old swiss old Swiss albino mice were inoculated with 10 non-lethal equal doses of extracts over a span of 30 days. Each dose was 0.2 ml so each mouse received a total of 2 ml dose and the dose inoculated was 1800 mg kg^{-1} body weight. The animals were immunized with single dose of 1 x 10^8 sheep red blood cells after 6-7 doses of extract. The animals were bled on 5^{th} day post-SRBC inoculation to check humoral response. The mice were sacrificed and dissected from ventral side to collect their spleens to study cell mediated immunological response. Spleens were perfused with MEM and teased to get cells. The suspension was made to get 2 x 10^6 number of cells ml^{-1}. For development of anti-SRBC antibody, direct haemagglutination (HA) test was followed (Hudson and Hay, 1989). For complement receptor bearing B (CRLB) lymphocytes, numeration method of Gravely *et al*. (1977) was followed. For determination of nitroblue terazolium (NBT) reduction, inducible nitric oxide (iNO's) test and phagocytosis, the methods of Hudson and Hay (1989), Bhatia and Jha (2000) and Raghuramulu *et al*. (1983) were followed, respectively.

The results of evaluation and comparison of immunorestorative potential of the group - without any extract inoculation (control animals), group II - *Eupatorium cannabinum* natural extract treated animals and group III - *Eupatorium cannabinum in vitro* shoot organ culture extract treated animals are given in the table below. The tests used to evaluate the macrophage functions revealed that NBT reduction activity in group II test animals with natural EC extract was 47.62% and with group III it was 43.7%. The INO's activity was 14.77% in control animals and 12.15% in group II and 13.67% in group III treated test animals. Phagocytosis was 24% in group II treated animals and -13% phagocytic activity in group III extract treated animals as compared to control animals. The immune system of the test animals was suppressed by hydrocortisone and the inoculation of the natural plant extract of *Eupatorium cannabinum* positively modulated the immune system of the test animals. Similarly, Benencia *et al.* (1994) and Akbay *et al.* (2002) have used the respiratory burst test to study the cell mediated immune response and Phagocytosis test to assess the bactericidal activity of leucocytes, Wagner and Jurcic (1991), Shen *et al.* (1991) and Olafsdottir *et al.* (1999) had similarly studied the effect of plant extracts on Phagocytosis to evaluate the cell mediated immune response.

Table 1: Comparison of immunorestorative potential of the natural extract of *Eupatorium cannabinum* plants and the plant extracts from *in vitro* raised shoot organ cultures of *Eupatorium cannabinum*

Tests used	Group I (control animals)	Group II Natural *Ec* plant extract	Group III *In vitro Ec* plant extract
Anti SRBC antibody titre	1-3, +ve	1-3, +ve	1-3, +ve
CRLB enumeration (%)	22.00	23.00	24.00
Wt. of spleen (g)	0.13	0.13	0.14
NBT reduction (%)	40.89	47.62	43.70
INO'S (%)	14.77	12.15	13.67
Phagocytosis (No. of colonies)	34.00	25.60 (24%)	38.40 (-13%)

Ec: Eupatorium cannabinum

Results revealed that though there is no significant difference in anti- SRBC antibody titre, CRLB enumeration, INO'S activity and the weight of spleen between control and the extract treated animals. But it has been observed that there is a significant increase in NBT reduction and the phagocytic potential in the animals treated with natural plant extract of *Eupatorium cannabinum*. The NBT reduction test is employed to measure phagocytosis and metabolic pathway responsible for microbial killing. The increase in respiratory burst of neutrophils i.e. increase in neutrophil intracellular killing activity (NBT reduction) suggests the positive modulation of functions of macrophages, T as well as B cells, Sharma *et al.* (1994). In phagocytosis the bactericidal activity of leucocytes is proved. Hence our

results reveal that native aqueous *Eupatorium cannabinum* could be a potent immunorestorator of functions of immunocytes especially macrophages.

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