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Effect of *Solanum nigrum* leaf extract on the non-enzymic antioxidant profile of experimental mice induced with tumour

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SUMMARY

Free radicals are the main contributing factor in most of the diseases. Antioxidants help in eradicating the effects of free radicals. In the present study, the hypothesis that *Solanum nigrum* leaf extracts are equipped to scavenge Reactive Oxygen Species (ROS) in tumour bearing mice was tested. The oxidative status of liver of female Swiss Albino mice (6-8 weeks old) subjected to DLA injection intraperitoneally and subsequent oral administration of *Solanum nigrum* leaf extract (500 mg/kg) for 21 days was studied. The antioxidant activity of leaves of plants bearing red berries and black berries was assessed by analyzing the non-enzymic antioxidants and by 2, 2'-diphenyl picryl hydrazyl (DPPH) assays. Vitamins A, E, C, reduced glutathione and protein thiols level and DPPH scavenging activity decreased significantly in DLA injected mice. Administration of *Solanum nigrum* leaf extracts was found to elevate the antioxidants level. Black berry leaf extract showed higher antioxidant potential than red berry leaf extract except in reduced glutathione assay where red berry leaf extract showed a higher activity than black berry leaf extract. The results suggest that *Solanum nigrum* leaves are a potential source of antioxidant and help in quenching ROS levels.

Key words : DLA, *Solanum nigrum*, Non-enzymic antioxidants

Oxygen Free Radicals (OFR) produced at metabolic sites are neutralized by non-enzymic scavengers such as certain vitamins or converted into non-harmful products such as water by the action of detoxifying enzymes such as superoxide dismutase, catalase, oxidases, etc. (Das, 2002). When the equilibrium between the radicals (oxidants) and antioxidants shifts towards the oxidants, the condition, which arises, is known as oxidative stress (Schaller, 2005). Accumulation of ROS is very dangerous to health. Antioxidants have the capacity to prevent, delay or ameliorate many disorders (Delanty and Dichter, 2000). Antioxidants can be derived either directly or indirectly from the diet. Plant and plant products have been used as a source of medicine for a long time. Human beings rely on traditional medicine for their primary health care need for a long time (Ranga *et al*., 2005).

Solanum nigrum also called black night shade and petty morel belongs to the family *Solanaceae*. It grows to a foot or so in height and is highly branched. The leaves are egg shaped and stalked and the stem is green and hollow. *Solanum nigrum* has been found to exhibit hydroxyl radical scavenging capacity (Kumar *et al*., 2001). It has been used as a medicinal plant for a long time. The fruits of *Solanum nigrum* have been found to possess protection against gastric ulcers (Jainu and Devi, 2006). It has been found to be protective against ROS induced disorders such as cirrhosis (Huseini *et al*., 2005). This study was designed to specifically investigate the antioxidant efficacy of *Solanum nigrum* leaf extract by investigating non-enzymic antioxidants in Swiss Albino mice liver tissue when it is administered *in vivo*.

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MATERIALS AND METHODS

Experiments were carried out on female Swiss Albino mice (6 – 8 weeks old) of 25 – 30g body weight, which were procured from Small Animal Breeding House, Kerala Agricultural University, Thrissur. The mice were maintained under standard laboratory conditions. They were placed in polypropylene cages bottomed with husk, maintained at room temperature and were given standard rat feed pellets supplied by M/s. Hindustan Lever Ltd., Bangalore, India and water *ad libitum*. The mice were first randomized into various groups and then acclimatized for a period of 1 week in the new environment before the experiments were carried out.

Preparation of plant extract:

Fresh leaves of *Solanum nigrum* were collected from plants grown in pots. The leaves were cleaned under running tap water and dried with filter paper. 1 g of the leaves was chopped finely, ground to a smooth paste with

mortar and pestle and then 2.5ml water was added to it. Centrifuged the ground substance and collected the aqueous layer. This was then fed to the mice depending on their body weight at a dose of 500 mg / kg body weight.

Treatment groups:

The experimental animals were divided into six groups, each containing six mice.

Group I: Control rats

Group II: DLA treated

Group III: DLA + BBLE (Black berry leaf extract) treated

Group IV: DLA + RBLE (Red berry leaf extract) treated

Group V: BBLE treated

Group VI: RBLE treated.

The experiment was carried out for a period of 21 days. DLA (Daltons Lymphoma Ascites) cells administration was carried out as a single injection at a dose of 1 x 10^6 DLA cells / mouse intraperitoneally. The Black berry leaf extract (BBLE) and Red berry leaf extract (RBLE) was administered orally to the animal with the help of an intragastric catheter for 21 days. The mice were sacrificed at the end of 25 days.

Processing of tissues for analysis:

At the end of 25 days, the mice were sacrificed after an overnight fast. The animals were dissected and tissues were removed. The liver was taken out carefully and washed in sterile ice-cold saline to remove the blood. The liver slices were blotted dry and were transferred to cryovials containing 0.1M Tris–HCl buffer (pH 7.4) and then stored in deep freezer (-80^{0}C) till analysis. All the analysis was carried out within a week period to avoid deterioration of specimen.

Parameters analysed:

The parameters analyzed were vitamin C (Roe and Keuther, 1953), vitamin E (Varley *et al*., 1981), vitamin A (Bayfield and Cole, 1980), reduced glutathione (Moron *et al*., 1979), Protein thiols (Sedlack and Lindsay, 1968) and DPPH activity (Mensor *et al*., 2001). The results obtained were statistically analyzed using students 't' test.

RESULTS AND DISCUSSION

The data of Table 1 clearly shows that the administration of *Solanum nigrum* leaves caused a significant increase in ascorbic acid content in DLA injected mice. The vitamin E content increased considerably in DLA injected mice fed with black berry leaf extract as compared to those that were treated with red berry leaf extract. DLA administration caused a significant decrease in the vitamin A content in the liver tissues. BBLE was able to bring up the vitamin A level almost to the control level in DLA injected mice when compared to RBLE. Both treated and untreated mice, fed with leaves of *Solanum nigrum* showed considerable increase in GSH content. However, unlike other estimated

Table 1 : Effect of *Solanum nigrum* leaves on the non-enzymic antioxidants and free radical scavenging activity in liver of DLA treated and untreated mice

Parameters	Groups					
	Control		BBLE		RBLE	
	Without DLA	With DLA	Without DLA	With DLA	Without DLA	With DLA
Ascorbic acid (mg / g liver)	0.085 ± 0.021	0.038 ± 0.001[a]	0.152 ± 0.029 [a]	0.085 ± 0.17 [b, c]	0.131 ± 0.046	0.070 ± 0.001 [b, c]
Vitamin E (µg/g liver)	7.29 ± 0.554	3.8 ± 0.95[a]	9.82 ± 0.542 [a]	6.33 ± 1.46 [b, c]	8.55 ± 1.65	4.43 ± 0.548 [a, c, d]
Vitamin A (µg / g liver)	46.38 ± 0.84	16.23 ± 1.15[a]	82.63 ± 1.91 [a]	44.08 ± 3.21[b, c]	64.98 ± 1.17[a,d]	38.23 ± 1.08 [a, b, c, d]
GSH (nanomoles / g liver)	155.29 ± 15.8	77.13 ± 15.5[a]	217.6 ± 15.4 [a]	139.38 ± 15.8[b, c]	279.85 ± 15.58[a,d]	186.18 ± 15.8 [b, c, d]
Protein thiols (mg / g liver)	0.512 ± 0.002	0.45 ± 0.005 [a]	0.723 ± 0.024 [a]	0.539 ± 0.005 [a, b, c]	0.712 ± 0.009 [a]	0.504 ± 0.003 [a, b, c, d]
DPPH (per cent scavenging activity)	4.73 ± 0.312	1.92 ± 0.295[a]	7.26 ± 0.46 [a]	5.75 ± 0.297[a, b, c]	6.39 ± 0.346[a,d]	4.48 ± 0.235 [b, c, d]

BBLE – Black berry leaf extract RBLE – Red berry leaf extract.

a – Statistically significant ($P < 0.05$) compared to untreated control.

b – Statistically significant ($P < 0.05$) compared to DLA treated group.

c – Statistically significant ($P < 0.05$) compared to respective non – DLA group.

d – Statistically significant ($P < 0.05$) compared to respective BBLE group.

non–enzymic antioxidants, the increase was more in the case of red berry leaf extract than black berry leaf extract. Both red berry leaf extract and black berry leaf extract were able to significantly bring up the levels of protein thiol. A significant increase in the extent of scavenging activity in both DLA injected and DLA untreated mice fed with *Solanum nigrum* leaves was observed. However BBLE has a higher scavenging activity than RBLE.

The concentration of vitamin C in the serum was significantly lowered on alcohol supplementation, but on treatment with *Cassia auriculata* leaf extract, vitamin C content was elevated to that of experimental control animals (Rajagopal *et al*., 2003). *Aloe vera* administration increased the levels of vitamins C and E in human blood serum (Vinson *et al*., 2005). Extracts prepared from the fruits of *Cycium* species (Kosar *et al*., 2003) and *Anno squamosa* leaves (Shirwaikar *et al*., 2004) showed DPPH scavenging activity in a dose-dependent manner. *Saussarea costus*, roots are of high medicinal value, showing high DPPH scavenging activity (Pandey *et al*., 2005).

Conclusion:

Vitamins A, E, C, reduced the glutathione and protein thiols level and DPPH scavenging activity decreased significantly in DLA injected mice. Administration of *Solanum nigrum* leaf extracts was found to elevate the antioxidants level. Black berry leaf extract showed higher antioxidant potential than red berry leaf extract except in reduced glutathione assay where red berry leaf extract showed a higher activity than black berry leaf extract. The results suggest that *Solanum nigrum* leaves are a potential source of antioxidant and helps in quenching ROS levels.

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