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Antioxidant potential of Lactuca sativa

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Abstract

The present study is based on the evaluation of antioxidant potential of a well known plant *Lactuca sativa.* Methanolic leaf extract was investigated for in vitro inhibition of oxidative damage induced by UV-radiations to the *salmonella typhi* bacteria and in vivo effect on the production of body enzymes i.e. catalase and superoxide dismutase. The lipid peroxidation masurement was also done in terms of thiobarbituric acid reactive substances (TBARS) in blood and brain of male albino wistar rats. The plant extract has shown significant antioxidant potential both *in vitro* and *in vivo.*

INTRODUCTION

Lactuca sativa Linn. (Lettuce) is a well known plant worldwide due to its use in the preparation of salad, soup and vegetable curries[-1]. Also, this plant exhibit excellent medicinal properties. The latex sap of *Lactuca sativa possess* antifungal properties[-2.] Stem of *Lactuca sativa* has shown depressant effects[-3.] The seed oil is reported to have sedative, hypnotic, analgesic and anticonvulsant properties[4]. *Latuca sativa* is also proved to have hypoglycaemic effect[5].

Lattuce is a rich source of carotene and vitamin C. It is also a fair source of vitamin E [1,6,7]. In the light of presence of these valuable phytoconstituents, it was thought that the plant may possess antioxidant activity.

In the present study, vacuum dried methanolic extract of *Lactuca sativa* was investigated for its antioxidant potential. In vitro study was carried out on UV-induced oxidative damage on *salmonella typhi* and in vivo study was carried out to estimate enzyme levels catalase and superoxide dismutase (SOD) and formation of malondialdehyde (MDA) was measured in terms of thiobarbituric acid reactive substances (TBARS) to estimate lipid peroxidation in blood and brain of male albino *wistar* rats.

MATERIALS AND METHODS

Animals

Male albino *wistar* rats (250-300mg) were obtained from animal house, J.S.S. College of Pharmacy, Ooty maintained under standardized environmental conditions (22-28^{o}C, 60-70% relative humidity) at 12hr dark/light cycle and fed with standard rat feed and water ad *libitum.*

Preparation of the plant extract

Lactuca sativa leaves were collected from surrounding areas of Ooty and identified by

Mr. Rajan at Govt. medicinal plant collection centre, Ooty. The dried leaves were subjected to soxhelation by using methanol as solvent. The extract obtained was concentrated and vacuum dried extract with dimethyl sulphoxide (DMSO) for in vitro study and suspended in 0.3% carboxymenthyl cellulose (CMC) for *in vivo* studies.

In vitro evaluation on UV-induced damage

Salmonella typhi bacteria was standardized by serial dilution method (105 cells in 0.1ml) and inhibition of UV-induced free radicals by methanolic plant extract was determined. As per procedure[-8], the different concentrations of plant extract i.e. 1mg/ml, 2mg/ml and 4mg/ml in DMSO were incorporated into the nutrient agar plates during their preparation. When the plates were at room temperature, bacteria (10^5 cells in 0.1ml) were spread on all the petridishes using a sterile loop and the petridishes were irradiated under a 15W UV germicidal tube for 15 seconds at a distance of 33 cm. The irradiated petridishes were incubated at 37^oC for 24 hrs. At the end of 24 hrs, the number of colonies in each plate were counted and increase in number of colonies in the extract incorporated petridishes were compared with control and standard. Ascorbic acid (1mg/ml in DMSO) was used as standard free radical scavenger. (Table 1)

***In vivo* antioxidant evaluation**

a) Experimental design

Animals were divided into three groups of six animals each. One group was kept as control and given only vehicle. Group 2 and 3 animals received two concentrations i.e. 200mg/kg body weight and 400mg/kg body weight. (bd. Wt.) of plant extract respectively. The animals were given drug for 5 days continuously and on 6^{th} day 1hr after administration of the drug by oral routs; the rats were sacrificed for biochemical estimation.

b) Isolation of blood serum

The animals were anaesthetized with diethyl ether and blood was collected through sino-ocular vein. The blood was centrifuged at 2500 rpm for 20 min to get clear supernatant solution which was used for analysis.

c) Preparation of the brain homogenate

Rats were sacrificed with euthanasia and brains were removed after decapitation, weighted and homogenized immediately with Teflon plunger in ice chilled 10% KCI solution (3ml/gm tissue). After centrifugation at 2000 rpm for 10 minutes, clear supernatant liquid was used for determination of enzyme levels.

d) Catalase assay[-9]

2.25ml of potassium phosphate buffer (65mM, pH 7.8) and 0.1ml of brain homogenate/serum as test sample or sucrose (for blank) were incubated at 25^0C for 30 minutes. H_2O_2 (7.5mM) 0.65ml was then added to initiate the reaction and change in absorption at 240 nm was measured for 3 minutes. Results are expressed as CAT unitis/0.1ml of the sample.

e) Superoxide dismutase assay[-10]

Pipetted out 2.8ml carbonate buffer, 0.1ml homogenate/serum as sample solution or sucrose (for blank) incubated at 30^oC for 45 minutes. Absorbance was set at zero and then added 0.1 ml adrenaline solution to the sample cells and readings were taken at 480 nm at intervals of one minutes for 12 minute. Standard calibration curve was prepared for comparison. Results are expressed as units of SOD activity/0.1 ml of sample.

f) MDA estimation as TBARS-11

0.1 ml of brain homogenate or serum was added to 2ml of cold 15% w/v trichloroacetic acid solution to precipitate the proteins. The resultant solution was then centrifuged and to the supernatant solution, 0.375% w/v thiobarbituric acid solution was added and boiled at 100^{o}C for 15 minute. After cooling, absorbance was measured at 532nm. Standard calibration curve was prepared by using stock MDA solution and values for sample solution were calculated. Results are expressed as malondialdehyde formation (micromoles/0.1 ml of sample).

RESULTS

Data from table I reveals that *Lactuca sativa* has significantly raised the number of colonies by protecting the bacteria form oxidative damage by UV radiations. From table II it is clear that the plant extract has significant results at the dose of 400 mg/kg bd. Wt. by raising the Catalase an SOD levels both in blood and brain of male albino wistar rats and reduced the MDA formation in both blood and brain.

DISCUSSION

Lactuca sativa-a herb of common food possesses sedative, hyphotic, analgesic, anticonvulsant, hypoglycemic and antifungal properties. Presence of high amount of carotene, Vitamin C and Vitamin E inspired us to carry out these investigations, which reveal that *Lactuca sativa* possesses noticeable antioxidant property. Thus it can be well concluded that in the light of the nutritive values and presently investigated antioxidant/free radical scavenging properties, *Lactuca sativa* can be graded as a potential nutraceutical of tomorrow.

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Table I: Effect of *Lactuca sativa* leaf extract on UV-induced damage on Salmonella Typhi

Test Sample	Average no. of colonies / plate mean ± S.D.	% Protection
Control	8.50 ± 2.07	----------
1mg/ml Standard Ascorbic acid	61.17 ± 5.70	82.66
1mg/ml test extract	22.00 ± 3.16	29.7b
2 mg/ml test extract	36.00 ± 6.84	48.6b
4mg/ml test extract	42.83± 9.60	57.87b

Data are expressed as Mean SD. (n=6) Statistical significance in comparison to control b=p<001

Table II: Effect of *Lactuca sativa* leaf extract on catalase, superoxide dismutase and MDA formation

Group	Catalase		MDA		SOD	
	Blood	Brain	Blood	Brain	Blood	Brain
Control	3.80 ±	0.511±	0.143±	0.001	0.911±	0.201
	2.133	0.439±	0.2605±	0.008	0.727±	0.059
Lactuca sativa 200 mg/kg	3.859±	0.487±	0.143±	0.001	0.963±	0.077
bd.wt.	2.281±	0.413±	0.229±	0.007	0.799±	0.205
Lactuca sativa 400	5.347±	0.277c±	0.130±	0.001d	1.479±	0.085a
Mg/kg bd.wt	3.825±	0.445c±	0.214±	0.009c	1.280±	0.147c

Data are expressed as Mean± S.D. (n=6) statistical significance in comparison to control a=p<0.5, c=p<0.01, d=P<0.001.