

RESEARCH ARTICLE

ANTIOXIDANT ACTIVITY OF *IPOMOEA LEARI*

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ABSTRACT

The antioxidant properties of four successive extracts of *Ipomoea leari* Paxton and the successive chloroform extract fraction, ILCF-28, were tested using standard *in vitro* and *in vivo* models. The amount of the total phenolic and flavonoid content was also determined. The successive chloroform extract, ILC and its fraction ILCF-28 exhibited strong scavenging effect on 2,2-diphenyl-2-picryl hydrazyl (DPPH), Nitric oxide, 2,2 -azino-bis (3ethylbenzo-thiazoline-6-sulphonic acid) diammonium salt (ABTS) radical cation, Reducing power, p-NDA and hydrogen peroxide methods. The free radical scavenging effect of ILC and ILCF-28 was comparable with that of reference antioxidants. The ILCF-28 having the highest content of phenolic compounds and strong free radical scavenging effect when administered orally to male albino rats at 100, 200 and 400mg/kg body weight for 7 days, prior to carbontetrachloride (CCl₄) treatment, caused a significant increase in the levels of catalase (CAT) and superoxide dismutase (SOD) and a significant decrease in the levels of lipidperoxidation (LPO) in serum, liver and kidney, in a dose dependent manner, when compared to CCl₄ treated control. These results clearly indicate the strong antioxidant property of the plant *Ipomoea leari*. The study provides a proof for the ethnomedical claims and reported biological activities. The plant has, therefore, very good therapeutic potential.

Key words: *Ipomoea leari*; free radicals; *in vivo*; Ccl4

INTRODUCTION

The role of free radicals and active oxygen is becoming increasingly recognized in the pathogenesis of many human diseases, including cancer, aging and atherosclerosis¹. Human body can be protected from these harmful compounds by enzymatic system, catalase, scavengers and antioxidants. Antioxidants are capable of preventing oxidative processes by inhibiting the initiation and propagation of an oxidative chain reaction. They are important in the prevention of many oxidative-stress related diseases². Several studies have shown that the therapeutic effects of some medicinal plants, fruits and even vegetables which are commonly used in folklore remedies against many diseases can be attributed to the antioxidant properties of their phytoconstituents^{3,4}.

An extract of vine *Ipomoea squamosa*, a member of the Morning Glory genus, has been found to exhibit strong cytotoxicity. After initial investigations by the then pharmaceutical partner Bristol-Myers Squibb, the extract was reinvestigated at Virginia Polytechnic Institute and State University and was found to contain a series of six resin glycosides named Ipomoeassins A-F with potent antiproliferative activities⁵. The compounds were biologically interesting because in spite of their very similar chemical structures, they had antiproliferative activities differing by about 2 orders of magnitude. This finding indicated that their antiproliferative activities were not due to some general detergent effect. A follow up experiment in the NCI 60-cell-line panel followed by a COMPARE analysis indicated a previously unknown mechanism of action. Ipomoeassin F and ipomoeassins B and E have been synthesized^{6, 7, 8}. *Ipomoea muricata*, *Ipomoea bahiensis* and *Ipomoea squamosa* are from same genus show biological properties such as cardiac depressant, spasmolytic antimicrobial, anticancer, cytotoxic activity^{9,10}.

We have selected from the same genus the plant *Ipomoea leari*. The Plant belongs to, Family Convolvulaceae, is twining perennial vine. This plant is famous as Blue Dawn and morning glory vine with a striking purple-blue color flowers. The flowers open in the morning and fade to magenta in the evening. The Origin of the plant is uncertain, but it is widespread throughout the tropical world and commonly found at hill stations of India like Ooty, Nainital and Mussoorie, scrambling over fences and over woody plants. Ethnopharmacological information available on the plant indicates that the root is used for dysentery, blood pressure and Waker carcinoma 256 in rat¹¹.

In view of this ethnopharmacological information on the plant, it was proposed screen *Ipomoea leari* successive extracts and its fraction(s) for the *in vitro* and *in vivo* antioxidant activity using standard procedures.

MATERIALS AND METHODS

Plant material

The plant *Ipomoea leari* was purchased from Abirami Botanicals, Tuticorin, Tamilnadu, India, and authenticated by Dr. D. Suresh Baburaj, Survey of Medicinal Plants and Collection Unit, Ootacamund, India. A voucher specimen (TIFAC 21) has been deposited for further reference at J.S.S College of Pharmacy herbarium, Ootacamund, India.

Chemicals

2,2-Diphenyl-2-picryl hydrazyl (DPPH) and 2,2-azinobis(3-ethylbenzo-thiazoline-6-sulphonic acid) diammonium salt (ABTS) were obtained from Sigma-Aldrich Co., St. Louis, USA. Rutin and *p*-nitroso dimethyl aniline (*p*-NDA) were obtained from Acros Organics, NJ, USA. Naphthyl ethylene diamine dihydrochloride (NEDD) was from Roch-Light Ltd.,

Suffolk, UK, ascorbic acid, nitro blue tetrazolium (NBT) and butylatedhydroxyanisole (BHA) were from SD Fine Chemicals Ltd., Mumbai, India and 2-deoxy-d -ribose was from Hi-Media Laboratories Pvt. Ltd., Mumbai, India. Sodium nitroprusside and Silymarin were from Ranbaxy Laboratories Ltd., Mohali, India. Sulphanilic acid used was from E-Merck (India) Ltd., Mumbai, India. All chemicals used were of analytical grade.

Animals

Healthy male albino rats of wistar strain (180–220 g) were obtained from the animal house, J.S.S. College of Pharmacy, Ootacamund, India, and were maintained under standard environmental conditions (22–28°C, 60–70% relative humidity, 12-h dark:12-h light cycle) and were fed with standard rat feed (M/S Hindustan Lever Ltd., Bangalore, India) and water *ad libitum*. The experiments were conducted as per the guidelines of CPCSEA, Chennai, India (approval no. JSSCP/IAEC/Ph.D/Phyto pharmacy/02/2010–2011).

Extraction procedure

The plant was chopped to small pieces and dried in shade. The dried root was powdered and passed through sieve no. 20 and extracted (600g) successively with 4.8 L each of n-hexane (60–80°C), chloroform, ethyl acetate and hydromethanolic (1:1) solvents in a Soxhlet extractor for 18–20 h. The extracts were concentrated to dryness under reduced pressure and controlled temperature (40–50°C). The n-hexane extract yielded a yellowish green sticky semisolid (2.37g). The chloroform extract yielded green semisolid (6.07g). The ethyl acetate extract yielded dark blackish green solid (6.73g) and hydromethanolic extract yielded (21.54g), respectively. All the extracts were preserved in a refrigerator till further use.

Isolation and fractionation

The chloroform extract was concentrated and loaded in a column after adsorbing silica gel 60-120 mesh for isolation. About 35 different fractions were collected with different solvent system ranging from low to high polarity by gradient technique. Among all the fractions, the fraction showing good *in vitro* antioxidant (ILCF-28) was chosen for further studies. Repeated re-column chromatography of ILCF-28 yielded a colourless crystalline compound. This was characterized by melting point, IR, NMR and mass spectrum.

Preparation of test and standard solutions

All the four extracts of *Ipomoea leari*, fraction ILCF-28 and the standard antioxidants (ascorbic acid, rutin and butylated hydroxy anisole) were dissolved in distilled dimethyl sulphoxide (DMSO) separately and used for the *in vitro* antioxidant assays using five different methods except the hydrogen peroxide method. For the hydrogen peroxide method (where DMSO interferes with the method), the extracts and the standards were dissolved in distilled methanol and used. The stock solutions were serially diluted with the respective solvents to obtain lower dilutions. A suspension of ILC, ILCF-28 and standard drug silymarin were prepared in sodium CMC (0.5%, w/v) using distilled water and used for *in vivo* experiments.

Estimation of total phenolic content

The amount of phenolic compounds in all the four extracts was estimated by using Folin–Ciocalteu reagent¹². In a test tube, 0.4mL of the extract in methanol was taken, mixed with 2 mL of Folin–Ciocalteu reagent and 1.6mL of sodium carbonate. After shaking, it was kept for 2h and the absorbance was measured at 750 nm using a Shimadzu-UV-160 spectrophotometer. Using gallic acid monohydrate, a standard curve was prepared. The linearity obtained was in the range of 1–10 g/mL. Using the standard curve, the total phenolic compounds content was calculated and expressed as gallic acid equivalent in mg/g of the extracts.

Estimation of Total flavonoid content

Aluminum chloride colorimetric method was used for the estimation of flavonoids¹³. The sample (1mL) was mixed with 3 mL of methanol, 0.2 mL of 10% aluminum chloride, 0.2 mL of 1 M potassium acetate and 5.6 mL of distilled water. The mixture was kept at room temperature for 30 minutes and the absorbance of the reaction mixture was measured at 415 nm with PerkinElmer UV/Visible spectrophotometer. A calibration curve was prepared by preparing quercetin solutions at 20, 40, 60, 80 and 100 µg/mL concentrations in methanol. The concentration of flavonoid was found using the standard curve and the results were expressed as mg of quercetin equivalents (QE) per g dry weight (mg QE/g DW) of the sample.

In vitro antioxidant activity

The four extracts and the fraction ILCF-28 were tested for their *in vitro* antioxidant activity using standard methods. In all these methods, a particular concentration of the extract or standard solution was used which gave a final concentration of 1000–0.45 g/mL after all the reagents were added. Absorbance was measured against a blank solution containing the extract or standard, but without the reagents. A control test was performed without the extracts or standards. Percentage scavenging and IC₅₀ values ± S.E.M. (IC₅₀ value is the concentration of the sample required to inhibit 50% of radical) were calculated.

DPPH radical scavenging method

A 10µL aliquot of the extracts, or the standards was added to 200 µL of DPPH in methanol solution (100 µM) in a 96-well microtitre plate (Tarson Products (P) Ltd., Kolkata, India). After incubation at 37°C for 20 min, the absorbance of each solution was determined at 490 nm using ELISA reader (Bio-Rad Laboratories Inc., CA, USA, Model 550)¹⁴.

Nitric oxide radical inhibition assay

The reaction mixture (6 mL) containing sodium nitroprusside (10 mM, 4 mL), phosphate buffer saline (1 mL) and the extract or the standard (1 mL) was incubated at 25°C for 150 min. After incubation, 0.5mL of the reaction mixture was removed and 1mL of sulphanilic acid reagent (0.33% in 20% glacial acetic acid) was mixed and allowed to stand for 5 min for completion of diazotization reaction and then 1mL of NEDD was added, mixed and allowed to stand for another 30 min in diffused light. The absorbance was measured at 540 nm against the corresponding blank solution in a 96-well microtitre plate (Tarsons Product (P) Ltd., Kolkata, India) using ELISA reader (Bio-Rad Laboratories Inc., Model 550)^{15, 16}.

Scavenging of ABTS radical cation

To 0.2 mL of the extract or standard, 1.0 mL of distilled DMSO and 0.16 mL of ABTS solution were added and incubated for 20 min. Absorbance of these solutions were measured spectrophotometrically at 734 nm¹⁷.

Reducing power assay

The extract (1mL) or the standard was added to 2.5 mL of 1% potassium ferricyanide and the mixture incubated for 20 minutes at 50°C. Aliquots of trichloroacetic acid (2.5 mL, 10%) was added to the mixture and centrifuged for 10 minutes. The upper layer (2.5 mL) was mixed with 2.5 mL of water and 0.5 mL of 0.1% ferric chloride solution. Absorbance was measured at 700 nm against the corresponding blank solutions¹⁸.

Scavenging of hydroxyl radical by p-NDA method

The extracts or the standard in distilled DMSO (0.5 mL) was added to a solution mixture containing ferric chloride (0.1 mM, 0.5 mL), EDTA (0.1 mM, 0.5 mL), ascorbic acid (0.1 mM, 0.5 mL), hydrogen peroxide (2 mM, 0.5 mL) and p-NDA (0.01 mM, 0.5 mL) in phosphate buffer (pH 7.4, 20 mM), to produce a final volume of 3 mL. Absorbance was measured at 440 nm¹⁹.

Scavenging of hydrogen peroxide

A solution of hydrogen peroxide (20 mM) was prepared in phosphate-buffered saline (PBS at pH 7.4). The extract / standard in methanol (1 mL) was added to 2mL of hydrogen peroxide solution in PBS. After 10 min the absorbance was measured at 230 nm²⁰.

In vivo antioxidant activity

Animals were divided into six groups comprising of six animals in each group. Group I served as normal and received 1mL of 0.5% sodium carboxy methyl cellulose (CMC). Group II served as CCl₄ treated control and received 1mL of 0.5% sodium CMC. Groups III received standard silymarin at 100 mg/kg body weight. Group IV, Groups V and VI received the successive chloroform extracts at 100, 200 and 400 mg/kg body weight, respectively. All these treatments were given orally for 7 days. On day 8, except for group I, all the other groups received 1 mL/kg body weight of CCl₄, intraperitoneally. On the day 9, the rats were anesthetized using diethyl ether and blood was collected from abdominal artery and kept at 37°C in the incubator for 30 min. Later, it was cold centrifuged at 2000 rpm for 15 min to get clear supernatant serum, which was used for biochemical estimations. Liver and kidneys were removed, weighed and homogenized immediately with Elvenjan homogenizer fitted with Teflon plunger, in ice-chilled 10% KCl solution (10 mg/g of tissue). The suspension was centrifuged at 2000 rpm at 4 °C for 10 min and the clear supernatant was used for biochemical estimations. Catalase was estimated by following the breakdown of hydrogen peroxide according to the method of beers et al.,²¹. Superoxide dismutase (SOD) was assayed based on the inhibition of epinephrine autooxidation by the enzyme²². Lipid peroxidation was measured in terms of malondialdehyde (MDA) content following the thiobarbituric acid method²³.

Statistical analysis

Results were expressed as mean \pm S.E.M. Comparisons among the groups were tested by one-way ANOVA using Graph Pad Prism, Version 4.0 (Graph Pad Software, San Diego, CA, USA). When the *p*-value obtained from ANOVA was significant (*p* < 0.05), the Tukey test was applied to test for differences among groups.

RESULTS

Isolation of stigmasterol

The melting point of isolated compound is 162°C. IR bands (KBr): 3336, 2936, 2891, 1591, 1459, and 1051 cm⁻¹; FAB-MS:*m/z* 412 (M + 1). In the ¹H-NMR spectrum of the compound, the upfield region exhibited the presence of two tertiary methyls (δ 0.68 and 1.01), three secondary methyls (δ 0.98, 0.85 and 0.83) and a primary methyl (δ 0.85, overlapped with the secondary methyl group). The olefinic protons were observed at δ 5.34 (1H, br.s), 5.16 (1H, dd) & 5.01 (1H,dd), suggesting the presence of two double bonds, while the carbonylic proton centered at δ 3.55 as a multiplet. ¹³C-NMR spectra showed recognizable signals at δ 14.60 (C-5), 138.26(C-23), 121.65(C-22) and 121.63 (C-6) confirming the presence of two double bonds. Among them the signal at δ 14.60 (C-5) is of weak intensity, indicating that it is a quaternary atom. The signal at δ 71.71 indicates the presence of the carbon atom under the hydroxyl function (C-3). These data confirm the structure of the compound as stigmasterol (Figure 1).

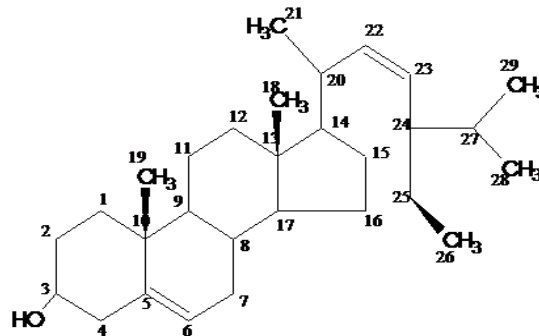


Figure 1: Structure of Stigmasterol

Total phenolic content estimation

The total phenolic content of the four successive extracts were expressed as gallic acid equivalent in mg/g of extracts. Chloroform extract had the highest phenolic content, namely 29.2 \pm 0.8 mg/g, followed by ethyl acetate extract 16.5 \pm 0.2 mg/g. Ethyl acetate extract had the highest flavonoid content 4.7 \pm 0.1 mg/g followed by chloroform extract 19.2 \pm 0.15 mg/g.

In vitro antioxidant assay

The four successive extracts and the fraction ILCF-28 were tested for *in vitro* antioxidant activity. The results are given in Table I. The data reveal that in the DPPH method potent antioxidant activity was observed for ILCF-28, ILC and ILE with IC₅₀ value of 19.3 \pm 0.91, 25.02 \pm 0.05 and 90.69 \pm 0.72 μ g/ml, respectively. ILE and ILHM showed good to moderate antioxidant activity with IC₅₀ value of 120.20 \pm 0.17 and 176.34 \pm 0.61 μ g/ml, respectively. The successive n-hexane extract of *Ipomoea leari* ILH showed poor antioxidant activity.

Table 1: *In vitro* antioxidant activity of different extracts and fractions

Sample	IC ₅₀ values ± SEM* (µg/mL)					
	DPPH	Nitric oxide	ABTS	Reducing power	p-NDA	Hydrogen peroxide
ILH	225.27±1.20	>1000	278.36±0.31	>1000	>1000	>1000
ILC	25.02±0.05	37.97±2.11	65.19±0.21	195.34±2.01	402.01±0.22	516.21±3.11
ILCF-28	19.31±0.91	26.72±0.72	54.22±1.12	125.15±1.04	341.01±0.32	480.21±2.01
ILE	90.69±0.72	117.12±0.31	287.03±1.22	362.56±0.02	589.21±0.55	>100
ILHM	176.34±0.61	187.43±1.32	353.87±2.10	416.77±1.22	>1000	790.31±2.11
Ascorbic Acids	10.41±0.41	7.32±0.36	7.22±1.11	>1000	550	-
Rutin	0.72±0.31	19.49±0.22	1.86±0.15	-	196.32±0.32	-
BHA	-	-	-	-	-	22.43±1.02

*Average of three independent determinations, three replicates and values are mean ± SEM

In the Nitric oxide method, potent antioxidant activity was observed for ILCF-28 with an IC₅₀ value of 26.72 ± 0.72 µg/ml. ILC also showed potent antioxidant activity (but lower than both the standards) with an IC₅₀ value of 37.97 ± 2.11 µg/ml. ILE showed good to moderate antioxidant activity with IC₅₀ value of 117.12 ± 0.31 and 165.21 ± 0.41 µg/ml, respectively. ILH and ILHM show no or weak antioxidant activity.

In ABTS method, ILCF-28 exhibited potent antioxidant activity with, IC₅₀ value of 54.22 ± 1.12 µg/ml. ILC also showed potent antioxidant activity with IC₅₀ value of 65.19 ± 0.21 µg/ml. ILC, ILE showed moderate antioxidant activity with IC₅₀ value of 287.03 ± 1.22 and 310.57 ± 2.01 µg/ml, respectively. ILP and ILHM showed weak activity even at the highest concentrations tested.

In the reducing power method ILCF-28 exhibited good antioxidant activity with IC₅₀ value of 125.15 ± 1.04 µg/ml. ILC and ILE, showed moderate antioxidant activity with IC₅₀ value of 195.31 ± 2.01 and 362.56 ± 0.02 µg/ml, respectively. ILH showed no antioxidant activity. ILHM showed weak activity even at high concentrations tested.

In the p-NDA method ILCF-28 exhibited moderate activity with IC₅₀ value of 341.01 ± 0.32 µg/ml. ILC and ILE showed weak activity with IC₅₀ value of 402.01 ± 0.22 and 589.21 ± 0.55 µg/ml, respectively. ILHM showed weak activity even at high concentrations tested.

In H₂O₂ method ILC-28 exhibited moderate activity with IC₅₀ value of 480.21 ± 2.01 µg/ml. The remaining extracts and fractions are inactive.

***In vivo* antioxidant assay**

ILC and ILCF-28 has showed potent *in vitro* antioxidant activity compared to other extracts. ILC showed total phenolic content. Hence, ILC was selected for the *in vivo* antioxidant screening. The administration of ILC at 100, 200 and 400 mg/kg bodyweight for 7 days prior to CCl₄ treatment caused a significant increase in the levels of catalase and SOD and a significant decrease in the levels of LPO in serum, liver and kidney.

***In vivo* lipid peroxidation**

The localization of radical formation resulting in lipid peroxidation is measured as MDA in serum, liver and kidney. MDA content in the serum, liver and kidney significantly increased in CCl₄ control group compared to

the normal group (p<0.001). Pretreatment with ILC at all the three doses (100, 200 and 400 mg/kg), however, significantly inhibited the MDA level by 42.70, 50.88 and 84.16%, respectively, for serum, 44.77, 61.29 and 83.04 %, respectively for liver and 44.99, 59.24 and 74.25 %, respectively for kidney when compared to CCl₄ control. The percentage inhibition for silymarin (100 mg/kg) on MDA levels was, however, 91.64, 89.52 and 85.71 %, for serum, liver and kidney, respectively.

***In vivo* antioxidant enzymes**

The SOD levels of serum, liver and kidney in CCl₄ control group were significantly lower than in normal group (p<0.001). Pretreatment with ILC at the entire three dose levels (100, 200 and 400 mg/kg), however, significantly increased the SOD levels by 31.46, 55.94 and 87.41 %, respectively for serum, 32.12, 55.15 and 72.72 %, respectively for liver and 52.94, 66.01 and 79.73 %, respectively for kidney when compared to CCl₄ control. The percentage increase for silymarin (100 mg/kg) on SOD levels was, however, 93.70, 84.24 and 86.92 %, for serum, liver and kidney, respectively.

Catalase activity of CCl₄ control group was seen to be strikingly lower than the normal group (p<0.001). Pretreatment with ILC at all the three dose levels (100, 200 and 400 mg/kg), however, significantly increased the catalase levels by 21.92, 69.79 and 88.19%, respectively for serum, 36.15, 59.50, 86.85 %, respectively for liver and 27.19, 56.61 and 72.09 %, respectively for kidney when compared to CCl₄ control. The percentage increase for silymarin (100 mg/kg) on catalase levels was, however, 85.14, 87.78 and 74.32 %, for serum, liver and kidney, respectively.

The analysis of the results reveal, alterations in the activities of antioxidant enzymes (SOD and catalase), lipid peroxidation, serum, liver and kidney of CCl₄ intoxicated rats. Administration of ILC at 100, 200 and 400 mg/kg for 7 days prior to CCl₄ treatment showed significant protection.

DISCUSSION

CCl₄ hepatotoxicity depends on the reductive dehalogenation of CCl₄ catalysed by Cyt 450 in the liver cell endoplasmic reticulum leading to the generation of an unstable complex CCl₃• radical. This trichloromethyl radical has been shown to be a highly reactive species, capable of attacking microsomal lipids leading to its peroxidation. This also covalently binds to microsomal lipids and proteins initiating secondary biochemical

processes which is the ultimate cause for the unfolding of the panorama of pathological consequences of CCl₄ metabolism²⁴.

Further, oxidative stress, the consequence of an imbalance of pro oxidants and antioxidants in the organism, is also gaining recognition as a key phenomenon in chronic illnesses like inflammation, heart diseases, hypertension and some forms of cancer²⁵. ROS produced through mechanism of signaling leads to deleterious effects. Hydrogen peroxide (ROS) has been reported as an important mediator of signaling oriented to the activation of transcription factors which are sensible to redox cycle and activators of responsible genes of cancerigenic cells growth and of some inflammatory processes. Oxidative stress results in toxicity when the rate at which the ROS are generated exceeds the cell capacity for their removal. Lipid peroxidation is an autocatalytic process, which is a common consequence of cell death. This process may cause peroxidative tissue damage in inflammation, cancer and toxicity of xenobiotics and aging. MDA is one of the end products in the lipid peroxidation process²⁶.

The data obtained in our present study (Table 2) clearly show an increase in the MDA level in serum, liver and kidney of rats treated with CCl₄ suggesting enhanced lipid peroxidation leading to tissue damage and failure of antioxidant defense mechanisms to prevent formation of excessive free radicals. Treatment with ILC, however, is seen to significantly reverse these changes in a dose dependent manner. ILC at 400 mg/kg significantly ($p < 0.001$) inhibited the formation of MDA levels in CCl₄ treated group and this is seen to be comparable with the standard drug silymarin.

Biological systems protect themselves against the damaging effects of activated species by several means. These include free radical scavengers and chain reaction terminators; enzymes such as SOD and CAT system²⁷. The SOD converts superoxide radicals (O₂⁻) into H₂O₂ plus O₂, thus participating with other antioxidant enzymes, in the enzymatic defense against oxygen toxicity. The data obtained in the present study (Table 2) reveals that there is an increase of SOD activity in a dose dependent manner suggesting that the ILC has an efficient protective effect in response to ROS. ILC at 400 mg/kg and silymarin at 100 mg/kg significantly ($p < 0.001$) restores the SOD activity in CCl₄ treated groups.

CAT is a key component of the antioxidant defense system. Inhibition of this protective mechanism results in enhanced sensitivity to free radical induced cellular damage. The reduction in the activity of CAT may, therefore, result in a number of deleterious effects due to the accumulation of superoxide radicals and hydrogen peroxide²⁸. The data obtained in the present study reveals that administration of ILC increases the CAT level in CCl₄ induced liver damage to rats thus preventing the accumulation of excessive free radicals and protects the liver from CCl₄ intoxication. ILC at 400 mg/kg and silymarin at 100 mg/kg almost significantly ($p < 0.001$) restore the enzyme activity to the near normal levels.

During hepatic injury, superoxide radicals generate at the site of damage and modulate SOD and CAT, resulting in the loss of activity and accumulation of superoxide radical, which damages liver. Decreased CAT activity is linked to unexhaustion of the enzyme as a result of oxidative stress caused by CCl₄. The reduced levels of SOD and CAT, in CCl₄ treated rats, significantly increases when treated with plant extracts thus showing that antioxidant property of the extract^{29,30}.

The phytochemical studies carried out on ILC reveal the presence of carbohydrates, flavonoids, glycosides, steroids and phenols etc. An analysis of the data given in Table 1 reveals that the observed *in vitro* antioxidant activity of four successive extracts of *Ipomoea leari* correlates with its phenolic content. A number of scientific reports indicate certain terpenoids, steroids and phenolic compounds such as tannins, coumarins and flavonoids have protective effects due to its antioxidant properties^{31, 32, 33, 34}.

In the present study successive chloroform extract, ILC shows the presence of stigmasterol as a major compound which has been shown well to moderate antioxidant activity in different *in vitro* models by earlier workers³⁵. Stigmasterol thus contributes to antioxidant nature of ILC. In conclusion, the present study clearly reveals that ILC has potent *in vitro* free radicals scavenging effect in different *in vitro* models and exhibits a dose dependent antioxidant activity by inhibiting lipid peroxidation and enhancing antioxidant enzymes such as SOD and CAT level, in CCl₄ intoxicated rat model. ILC is, therefore, a potential therapeutic, thus making it an excellent candidate for more detailed investigations.

Table 2: Effect of ILC and silymarin on antioxidant enzymes and lipid peroxidation in rat with carbon tetrachloride–induced hepatotoxicity

Treatment	Dose (mg /kg body wt)	Catalase (IU/min/mg of tissue)			SOD (Unit/min/mg of tissue)			LPO (n mole of MDA/mg of tissue)		
		Serum	Liver	Kidney	Serum	Liver	Kidney	Serum	Liver	Kidney
Normal	0.5 mL sod.CMC	1.513 ± 0.034	3.698 ± 0.252	1.865 ± 0.435	0.298 ± 0.079	0.301 ± 0.108	0.311 ± 0.029	3.672 ± 0.135	3.942 ± 0.139	3.385 ± 0.183
Control (CCl ₄)	1 mL	0.692 ± 0.031 ⁺⁺⁺	1.431 ± 0.062 ⁺⁺⁺	0.790 ± 0.075 ⁺⁺⁺	0.155 ± 0.018 ⁺⁺⁺	0.136 ± 0.013 ⁺⁺⁺	0.158 ± 0.056 ⁺⁺⁺	5.983 ± 0.620 ⁺⁺⁺	8.306 ± 0.450 ⁺⁺⁺	6.143 ± 0.238 ⁺⁺⁺
Silymarin+CCl ₄	100	1.392 ± 0.052 ^{***} (85.14)	3.421 ± 0.172 ^{***} (87.78)	1.589 ± 0.089 ^{***} (74.32)	0.289 ± 0.028 ^{***} (93.70)	0.275 ± 0.013 ^{***} (84.24)	0.291 ± 0.011 ^{***} (86.92)	3.865 ± 0.111 ^{***} (91.64)	4.399 ± 0.345 ^{***} (89.52)	3.779 ± 0.154 ^{***} (85.71) ¹
ILC + CCl ₄	100	0.872 ± 0.039 (21.92)	2.533 ± 0.164 [*] (49.49)	1.058 ± 0.069 (24.93)	0.298 ± 0.009 [*] (31.46)	0.189 ± 0.013 (32.12)	0.239 ± 0.041 ^{**} (52.94)	4.996 ± 0.270 (42.70)	6.354 ± 0.166 ^{**} (44.77)	4.902 ± 0.154 ^{**} (44.99)
ILC + CCl ₄	200	1.265 ± 0.039 ^{***} (69.79)	2.891 ± 0.132 ^{***} (64.40)	1.389 ± 0.094 ^{***} (55.72)	0.235 ± 0.006 ^{***} (55.94)	0.227 ± 0.025 ^{***} (55.15)	0.259 ± 0.039 ^{***} (66.01)	4.807 ± 0.136 ^{**} (50.88)	5.631 ± 0.149 ^{***} (61.29)	4.509 ± 0.152 ^{***} (59.24)
ILC + CCl ₄	400	1.375 ± 0.068 ^{***} (83.19)	3.202 ± 0.153 ^{***} (78.12)	1.565 ± 0.147 ^{***} (72.09)	0.275 ± 0.014 ^{***} (87.41)	0.256 ± 0.008 ^{***} (72.72)	0.280 ± 0.006 ^{***} (79.73)	4.038 ± 0.188 ^{***} (84.16)	4.682 ± 0.191 ^{***} (83.04)	4.101 ± 0.143 ^{***} (74.03)

*The data in the parenthesis indicate percent protection in individual biochemical parameters from their elevated values caused by CCl₄.
The % of protection is calculated as 100 X (values of CCl₄ control - values of sample) / (values of CCl₄ control - values of vehicle control)
Results are Mean ± SEM (n = 6) * p<0.05, ** p<0.01, *** p<0.001, When compared with ccl₄ treated control +++ p<0.001, When compared to normal group*

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