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In Vitro Hepatoprotective Activity of Methanolic Leaf Extract of *Acalypha indica* Against CCl_4 Induced Hepatotoxicity in Goat Liver Slice Culture

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Abstract

Plants used in traditional medicine may constitute an important source of new biologically active compounds. *Acalypha indica* is one of the weed plants that contain important medicinal values for human health applications. The present study was undertaken to assess the hepatoprotective activity of methanolic leaf extract of *Acalypha indica* in goat liver slice culture against carbon tetrachloride (CCl_4) to prove its efficacy against liver disorders CCl_4 was used to induce hepatotoxicity in liver slice of goat. The cytotoxicity induced by CCl_4 was estimated by quantifying the release of marker enzymes such as alanine transaminase (ALT), aspartate transaminase (AST), alkaline phosphatase (ALP), acid phosphatase (ACP) and lactate dehydrogenase (LDH). Also, the degree of hepatic damage was measured by estimating the levels of lipid peroxidation (LPO) of hepatocytes membrane lipids. The treatment of liver cells with CCl_4 caused twice increase in LPO of cells besides release of LDH, ALT, AST, ACP and ALP by 6.55, 3.89, 3.31, 2.69 and 2.50 times, respectively as compared to untreated liver cells. Thus, a toxic effect of CCl_4 was significantly reduced by the treatment of methanolic leaf extract of *Acalypha indica*, silymarin and quercetin. The free radical scavenging activity of plant extract was estimated by 2, 2-diphenylpicrylhydrazyl (DPPH) method and IC_{50} is 65.12 ± 2.27 µg/mL. Qualitative analysis of flavonoid indicates the presence of flavonoids in plant extract. These results indicate that all concentration of plant extract of *Acalypha indica*, silymarin and quercetin protect the liver cells from CCl_4 induced oxidative/free radical mediated damage *in vitro*. The hepatoprotective activity of methanolic extract of *Acalypha indica* extract is due to the presence of flavonoids which have remarkable antioxidant and anti-inflammatory activity.

Keywords: *Acalypha indica,* Quercetin, Silymarin, Hepatoprotective activity, *In vitro* goat liver slice culture assay, Carbon tetrachloride, Hepatotoxicity

Introduction

The liver is considered to be one of the most vital organs that functions as the centre of metabolism of nutrients such as carbohydrates; proteins and lipids; and excretion of waste metabolites [1]. The liver tolerates maximum insult in detoxifying the various toxins present in the food, drinks, drugs and environment. Many risk factors predispose an individual to hepatic drug injury such as pre-existing liver disease, aging, female sex, and genetics [2]. Liver disorders are the most common health hazard found in developing countries due to dietary habits, alcohol ingestion, poor hygiene, unsupervised drug use and smoking. Liver diseases can be non-inflammatory, inflammatory and degenerative. High levels of plasma total cholesterol (LDL-C) and triacylglycerols (TGs) are associated with high risk of atherosclerosis and cardiovascular disease owing to the hepatic insufficiency [3]. Hepatotoxicity caused by many toxins like carbon tetrachloride, thioacetamide, acute or chronic alcohol consumption, results in various infections like hepatitis A, B, C. Free radicals generated through alcohol use result in the development of hepatitis leading to cirrhosis [1].

Plants used in traditional medicine may constitute an important source of new biologically active compounds. Plants are widely used by all sections of the population either directly as folk medicines or indirectly in the pharmaceutical preparation of modern medicines [4]. Herbal medicines play a vital role in the treatment and cure for various diseases and physiological conditions in traditional methods practiced such as Ayurveda, Unani and Siddha. This type of treatment, also known as conventional treatment, was the main source of medical treatment. *Acalypha indica* is one of the weed plants that

contain important medicinal values for human health applications. It can be found commonly in India, Sri Lanka, Thailand and Pakistan. The extracts of various parts of the plant, leaves, roots and stem are used for medicinal purposes to treat various diseases such as eye infections, respiratory problems, rheumatism, skin problems and to decrease blood sugar level [5]. The biochemical constituents of *Acalypha indica* are tannins, flavonoids, cyanogenic glucoside acalyphin, acalyphamide, aurantiamide, succinimide and the pyranoquinolinone alkaloid flindersin. Further, 4 known kaempferol glycosides such as biorobin, nicotiflorin, clitorin and mauritianin have been isolated from the dried methanol extract of freeze-dried flowers and leaves of *Acalypha indica* [6].

Acalypha indica commonly known as Indian Acalypha belongs to the family Euphorbiaceae. It is a common annual herb, found mostly in the backyards of houses and waste places throughout the plains of India. *Acalypha indica* leaves have significant antibacterial activity against both gram-negative and gram-positive bacteria. The plant is reported to have a post-coital antifertility effect, anti-venom properties, wound healing effects, antioxidant activities, anti-inflammatory effects acaricidal effects, diuretic effects and anti-bacterial activities [7].

Many *in vivo* studies have been reported regarding the assessment of hepatoprotective activity of *Acalypha indica*. But there is no evidence regarding the evaluation of *in vitro* hepatoprotective activity of the methanolic leaf extract of *Acalypha indica* against CCl_4 induced hepatotoxicity in goat liver slice culture. The liver slice is a microcosm of the intact liver consisting of highly organized cellular communities in which the different cell types are subject to mutual contact. Therefore, liver slice culture is an *in vitro* technique that offers the advantages of *in vivo* situation over maintained cell line cultures. It is a simple and suitable model for the experimental analysis of hepatotoxic conditions [8]. Therefore, the present study was undertaken to assess the hepatoprotective activity of methanolic leaf extract of *Acalypha indica* in goat liver slice culture against CCl_4 to prove its efficacy against liver disorders.

Materials and methods

Chemicals and reagents

All chemicals used were of analytical grade having high purity and purchased from either Sigma chemicals (Bangalore, India) or Hi Media Ltd. (Mumbai, India).

Preparation of methanolic leaf extract of *Acalypha indica*

The methanolic extract of *Acalypha indica* was prepared by Soxhlet extraction method [9]. 50 g of finely grounded *Acalypha indica* powder was subjected to Soxhlet extraction by placing in extraction thimble and then transferred to a Soxhlet extractor. The bottom of extraction flask was filled with 250 mL of solvent. The extraction was carried out with methanol as extraction solvent in 2:10 powder to solvent ratio at temperature 65 °C for 8 h. This methanolic extract was filtered through Whatman filter paper No.1, then the filtrate was concentrated using a rotary vacuum evaporator at 45 °C. The concentrated methanolic extract was stored in desiccator until further use.

Qualitative test for flavonoids

1) Aluminium chloride test: Three millilitre of 1 % Aluminium chloride solution were added to 5 mL of extract. A yellow coloration was observed indicating the presence of flavonoids. 5 mL of dilute ammonia solution were added to the above mixture followed by addition of concentrated H_2SO_4. A yellow coloration disappeared on standing. The yellow coloration which disappeared on standing indicates a positive test for flavonoids [10].

2) Shibat's test: One millilitre of the extract is dissolved in 5 mL of 50 % methanol by heating. Then add magnesium metal and 6 drops concentrated H_2SO_4. Red colour or orange colour indicates the presence of flavonoids [11].

3) Shinoda's test: One millilitre of plant extract dissolved in 5 mL methanol. Add fragments of magnesium ribbon and few drops of concentrated H_2SO_4. Presence of pink to crimson coloured solution indicates the presence of flavonoids [12].

4) Pew's test: Few mL of extract is added with 0.1 g metallic zinc and 8 mL of concentrated H_2SO_4. A red colour indicates the presence of flavonoids [11].

Free Radical Scavenging Activity by 2, 2-diphenylpicrylhydrazyl (DPPH) Method

The free radical scavenging capacity of the extracts was determined using DPPH method [13]. The DPPH solution (1 mM) was prepared in 95 % methanol. The methanol extract of the *Acalypha indica* leaves was mixed with methanol to prepare the stock solution (1 mg/mL). 500 µl of freshly prepared

DPPH solution (1 mM) added to each of these test tubes, which contained 1 mL of extract (5 - 100 µg/mL) and 500 µl phosphate buffered saline (10 mM, pH 7.4). The reaction mixture was incubated in the dark for 30 min at room temperature and thereafter the optical density was recorded at 520 nm against the blank. For the control, 500 µl of DPPH solution in ethanol was mixed with 500 µl buffered saline and the optical density of the solution was recorded after 30 min. The assay was carried out in triplicate. Ascorbic acid used as standard. The decrease in optical density of DPPH on addition of test samples in relation to the control was used to calculate the antioxidant activity, as percentage inhibition (% IP) of DPPH radical. The capability of scavenging DPPH radical was calculated using the following equation;

Percentage (%) inhibition = $[1-(A_T / A_C)] \times 100$

Where, A_T is the absorbance of the test sample and A_C is the absorbance of the control sample.

***In vitro* hepatoprotective activity**

The goat liver was selected as the mammalian tissue to determine the *in vitro* effect of different concentration of methanolic leaf extract of *Acalypha indica* using cytotoxicant CCl_4.

Liver slice culture

According to the method described by Chaudhari and Mahajan [8], liver slice culture was performed. The fresh liver was collected from local slaughter house soon after the animal was sacrificed. The liver was transferred to sterilized Krebs Ringer Hepes medium (KRH 2.5 mM Hepes, pH 7.4, 118 mM NaCl, 2.85 mM KCl, 2.5 mM $CaCl_2$, 1.15 mM KH_2PO_4, 1.18 mM $MgSO_4$ and 4.0 mM glucose). The liver was cut into thin slices using sharp blade, and slices ranging from 8 to 10 mg were used for this study. Each set of experiments contained 15 tissue slices total weight of 100 mg. These tissue pieces were washed with 10 mL KRH medium every 10 min. over a period of 1 h and incubated for 60 min in small beakers containing 10 mL KRH on a shaking water bath at 37 °C.

Experiment design

The experimental designs were made according to the method of Chaudhari and Mahajan, [8] with slight modification. The washed liver pieces were further divided into 10 individual culture groups for respective treatment. 1 mL of 15 mM cytotoxic CCl_4 and 1 mL of different concentrations of plant extract *Acalypha indica*, quercetin and standard drug Silymarin were used for the experiment. Group 1 was toxicant free control group. Group 2 was administrated by 1 mL of 15 mM CCl_4. Group 3 - 5 was administrated by 1 mL of CCl_4 and 1 mL of different concentration of plant extract (10, 15 and 25 µg/mL). Group 6 and 7 was administrated by 1 mL of CCl_4 and 1 mL of quercetin (10 µg/mL) and silymarin (10 µg/mL) respectively. Group 8, 9 and 10 was received by 1 mL of plant extract (25 µg/mL), quercetin (10 µg/mL) and silymarin (10 µg/mL) respectively. All the groups except group 1, 8, 9 and 10 incubated with CCl_4 for 1 h at 4 °C and after the incubation period treated groups received different concentration of plant extract, quercetin and silymarin. At the end of the treatment, all the cultures were incubated on a water bath at 37 °C for 2 h. At the end of incubation, the culture medium was homogenized with ice cold normal saline using a glass teflon homogenizer at 4 °C. After homogenization each set was centrifuged at 10,000 rpm for 20 min at 4 °C to remove cell debris and other suspended particles and clear supernatants were collected and assayed for leakage of biochemical markers such as alanine transaminase (ALT), aspartate transaminase (AST), alkaline phosphatase (ALP), acid phosphatase (ACP), lactate dehydrogenase (LDH) and lipid peroxidation.

Determination of lipid peroxidation

The degree of lipid peroxidation was assayed by estimating the Thiobarbituric acid reactive substances (TBARS) by using the standard method Fraga *et al.* [14]. According to this method 1.0 mL of the tissue homogenate was treated with Thiobarbituric acid: Trichloro acetic acid: Hydrochloric acid (TBA-TCA-HCl) (1:1:1) reagent and mixed thoroughly. The mixture was kept in boiling water bath for 15 min. After cooling, the tubes were centrifuged at 3,000 rpm for 10 min and the supernatant was taken for measurement. A series of standard malondialdehyde solution in the range 2 - 10 µmoles concentrations were treated in the similar manner. The absorbance of pink complex formed was read at 535 nm against the reagent blank. The result was expressed as µmoles of malondialdehyde (MDA) produced/min/mg protein.

Estimation of acid phosphatase activity

The activity of Acid Phosphatase was determined by following the methods of Anon [15]. Reaction mixture for ACP analysis contained 0.1 mL of tissue homogenate and 1 mL of ρ-NitroPhenyl Phosphate (1 %) substrate in 0.1 M citrate buffer at pH 4, which was incubated at 37 °C for 30 min. Then 1.5 mL of 0.1 N sodium hydroxide was added to stop the reaction. The hydrolytic product, yellow ρ-nitrophenol, was measured at 405 nm by using spectrophotometer (Shimadzu UV-1,900i UV-VIS spectrophotometer, Japan). The enzyme activity was determined by using a standard graph of ρ-nitrophenol. The ACP activity was expressed as dephosphorylation of ρ-Nitrophenyl Phosphate (PNPP) to ρ-nitrophenol (PNP) in µMoles/min/mg protein.

Activities of aspartate transaminase (AST) and alanine transaminase (ALT)

Activities of AST and ALT were assayed by the method of Reitmann and Frankel [16]. One mL of substrate, aspartate transaminase for AST and alanine transaminase for ALT was added with 0.2 mL of homogenate and incubated for 1 h in case of aspartate transaminase and 30 min for alanine transaminase, then added 2 drops of aniline-citrate reagent to test. The reaction was arrested using 1.0 mL of 1 mM 2, 4 Dinitrophenyl Hydrazine (2, 4 DNPH) solution and the tubes were kept at room temperature for 20 min. 1.0 mL of 0.4 N sodium hydroxide was added to all the tubes. Sets of sodium pyruvate standards were also treated in the similar manner. The colour developed was read at 540 nm. Activities of aspartate and alanine transaminase were expressed as µmoles/min/mg protein.

Activity of alkaline phosphatase (ALP)

Alkaline phosphatase was assayed by the method of King and Armstrong [17] using disodium phenyl phosphate as the substrate. An incubation mixture containing bicarbonate buffer (0.1 M pH 10) and substrate (0.01 M) in 2.9 mL distilled water was pre incubated at 37 °C for 10 min. 0.2 mL of tissue homogenate was added and incubated at 37 °C for 15 min. The reaction was arrested by the addition of 1.0 mL of Folin-phenol reagent. The suspension was centrifuged at 3,000 rpm for 5 min and 10 % sodium carbonate was added to the supernatant. The solution was incubated at 37 °C for 10 min. Standard phenol solutions (2.5 - 10.0 µg) were also treated with Folin-phenol reagent and sodium carbonate. The blue colour developed was read at 680 nm. The enzyme activity was expressed as µmoles/min/mg protein.

Estimation of lactate dehydrogenase (LDH)

Lactate dehydrogenase activity was estimated by the method of King [18]. To 1 mL of buffered lithium lactate substrate (0.1 M pH 7.4) add 0.1 mL of homogenate and 0.25 % NAD^+ were added and incubated at 37 °C for 5 min. Then DNPH (0.02 %) was added and incubated for 15 min at 37 °C. Finally, 0.4 N NaOH was added and the colour developed was measured at 420 nm against reagent blank. Sets of standard sodium pyruvate were also treated in a similar manner. The activity of LDH was expressed as lU/L or µmols/min/mg protein.

Statistical analysis

Statistical analysis were performed using one-way analysis of variance (ANOVA) followed by Duncan's Multiple Range test using statistical package SPSS 25.0 (IBM, USA). Differences were considered to be significant at $p < 0.05$ against CCl_4 treated group. Data are presented as mean ± SD for n = 3. All biochemical estimations were carried out in triplicates.

Results and discussion

Herbal drugs are safe and have the potential to cure liver diseases, so they have gained popularity in recent years. Moreover, the plant derived drugs are cost-effective too. So many medicinal plants, present in different parts of India have been mentioned as hepatoprotective drugs and these are extensively used to treat liver disorders. Various plants and polyherbal formulations have hepatoprotective activity. Approximately 160 phytoconstituents and other phytochemicals have been claimed to possess hepatoprotective activity. In India more than 87 plants are used, out of which 33 are patented and have proprietary multi-ingredient plant formulations [2].

In the present study, *in vitro* effect of different concentration of methanolic leaf extract of *Acalypha indica,* quercetin and silymarin on CCl_4 induced hepatotoxicity in goat liver slice culture was carried out. The fresh liver slices were washed with 10 mL of Krebs Ringer Hepes medium (KRH) for different time intervals and washed liver slices were used for the evaluation of *in vitro* hepatoprotective activity of different concentration of methanolic leaf extract of *Acalypha indica.* The hepatotoxicity was induced by

administrating CCl_4 and all the treated groups received different concentration of methanolic leaf extract of *Acalypha indica,* quercetin and silymarin. Moreover, all of the previously mentioned liver biomarkers together with lipid peroxidation were measured (**Table 2**). After 1 h of CCl_4 administration, AST, ALT, LDH, ACP, ALP and lipid peroxidation levels significantly increased, compared to control group (**Table** 1; $p < 0.001$), which indicated CCl_4-induced liver injury in goat liver slice culture. Interestingly, CCl_4-induced increase in liver enzymes was improved by *Acalypha indica* extract supplementation. AST, ALT, LDH, ACP and ALP are sensitive indicators of CCl_4- induced liver injury and are the most important parameters in routine clinical liver function tests. The results indicated the increase of these liver enzymes among CCl_4-induced goat liver slice, and that the extract, quercetin and silymarin significantly ameliorated enzymatic activities at the doses of 25, 10 and 10 µg/mL ($p < 0.05$). These mentioned doses of the plant extract, quercetin and silymarin brought back these liver enzymes to normal levels.

Preliminary flavonoid screening

Flavonoids are polyphenol compounds that are considered essential nutrients. Their basic chemical structure consists of 2 benzene rings bound by a 3-atom heterocyclic carbon chain. The oxidation of the structure produces several families of flavonoids (flavones, flavonoles, flavanones, anthocyanins, flavanoles, and isoflavones). Flavonoids have the capability to scavenge free radicals and prevent lipid peroxidation. The biological activity of medicinal plants is often related with the presence of flavonoids. There are many different types of flavonoids that are known as various effects on health. Therefore, flavonoid content can be used as a parameter or index in evaluating the quality of medicinal plant used [19]. Preliminary flavonoid screening of the extract revealed the presence of flavonoids in the extract, and the result of the phytochemical test has been summarized in **Table 1**.

Table 1 Preliminary flavonoid screening from methanolic leaf extract of *Acalypha indica*.

Test	Procedure	Observation	Inference
Aluminium chloride test	3 mL of 1 % Aluminium chloride + 5 mL of extract + 5 mL of dilute ammonia solution + concentrated H_2SO_4	A yellow coloration was observed and on standing yellow coloration disappeared	Presence of flavonoid
Shibat's test	1 mL extract + 5 mL 50 % methanol + metal magnesium + 6 drops concentrated H_2SO_4	A red or orange colour	Presence of flavonoid
Shinoda's test	1 mL extract + 5 mL methanol + magnesium ribbon + few drops concentrated H_2SO_4	A pink to crimson coloured solution	Presence of flavonoid
Pew's test	Few mL extract + 0.1 g metal Zinc + 8 mL concentrated H_2SO_4	A red colour	Presence of flavonoid

The phytochemical analysis is very much important to evaluate the possible medicinal utilities of a plant and also to determine the active principles responsible for the known biological activities exhibited by the plants. Further, it provides the base for targeted isolation of compounds and to perform more precise investigations.

Free radical scavenging activity by DPPH method

Free radical scavenging activity (expressed as IC_{50}) of *Acalypha indica* methanol extract evaluated and the free radical scavenging activity of methanolic leaf extract of *Acalypha indica* have IC_{50} concentration is 65.12 ± 2.27 µg/mL and standard ascorbic acid have IC_{50} concentration is 45.76 ± 3.79 µg/mL. Proton radical scavenging action is an important attribute of antioxidants, which is measured by DPPH radical scavenging assay. Hydrogen donating ability of the antioxidants molecules contributes to its free radical scavenging nature. In order to characterize antioxidant activity of a plant extract, it is desirable to subject it for the tests that evaluate the range of activities such as scavenging of the reactive

oxygen species, inhibition of membrane lipid peroxidation and metal ion chelation. Antioxidant-rich plant extracts serves as sources of nutraceuticals that alleviate the oxidative stress and therefore prevent or slow down the degenerative diseases [20]. The result indicates the potential of the *Acalypha indica* methanol leaf extract as a source of natural antioxidants or nutraceuticals with potential application to reduce oxidative stress with consequent health benefits.

***In vitro* hepatoprotective activity**

CCl_4 is a prototypical lipid peroxidative agent Induced early lipid peroxidation in the liver. The level of lipid peroxidation in the liver slice was evaluated by Thiobarbutyric acid reactive substances (TBARS) assay. Lipid peroxidation was calculated in terms of TBARS and was expressed as μmoles of malondialdehyde formed/min/mg protein [21]. **Table 2** showed the result of lipid peroxidation in different treatments. The level of lipid peroxidation was significantly increased by 2.98 fold ($p < 0.05$) in the liver tissue homogenates treated with CCl_4 as compared to control. Such increased level of lipid peroxidation has been documented earlier in the liver of rat after oral administration of CCl_4 [22] and in liver cells treated with CCl_4 *in vitro* [21,8]. After treatment with different concentration of methanolic leaf extract (10, 15 and 25 μg/mL) level of lipid peroxidation decreased significantly ($p < 0.05$) compared to CCl_4 treated group. The level of lipid peroxidation reduced significantly ($p < 0.05$) in liver slice homogenate treated with CCl_4 with the presence of methanolic leaf extract of *Acalypha indica,* Quercetin, and Silymarin. The lipid peroxidation in liver tissue homogenate due to CCl_4 decreased significantly ($p < 0.05$) by 25, 36 and 48 % with treatment of 25 μg/mL concentration of plant extract and 10 μg/mL concentration of Quercetin, and Silymarin.

Biological materials, particularly membranes, contain high concentrations of unsaturated lipids. In the presence of a free radical initiator and oxygen they may be oxidized. This process, known as lipid peroxidation, has been implicated as a general biological degenerative reaction, and may be an important *in vivo* process [22]. Hepatic damage caused by carbon tetracloride is a common experimental method to evaluate the hepatoprotective activity of medicinal plants and drugs [23]. Cytochrome P-450 dependent monoxygenase enzyme system metabolized CCl_4 to trichloromethyl radical (CCl_3). This CCl_3 reacted with molecular oxygen and converted to highly reactive trichloromethyl peroxy radical. Functional and morphological changes occur in the cell membrane when fatty acids present in the cytoplasmic membrane phospholipids oxidized by the free radicals formed after the metabolism of CCl_4 in liver [24]. Moreover, these radical forms covalent bonds with sulfhydryl groups of various membrane molecules like GSH leading to depletion and increased the level of malondialdehyde and causes lipid peroxidation [25].

Reactive free radicals initiate cell damage through the following 2 major mechanisms: Covalent binding to cell membrane lipids and lipid peroxidation. In addition, oxidative stress may cause reversible or irreversible alterations of sensitive proteins that may lead to increased susceptibility to proteolytic attacks [26]. Lipid peroxidation and free radicals are involved in the main mechanisms by which hepatotoxins injure hepatocytes. The hepatoprotective activity of *Acalypha indica* could be attributed to its antioxidant capacity. The antioxidant activity of *Acalypha indica* leaf extracts was assessed by measuring their DPPH scavenging activity. The extracts were found to have potent DPPH radical scavenging activity comparable to ascorbic acid, the positive control. This effect could be due to their hydrogen donating capacity. This capacity of the extracts to scavenge DPPH radical can mediate inhibition of lipid peroxidation [27].

Table 2 Effect of methanolic leaf extract of *Acalypha indica*, quercetin and silymarin treatment on release of biochemical markers of hepatotoxicity induced by CCl_4 in the goat liver tissue *in vitro*.

Set no	Lipid peroxidation	Lactate dehydrogenase	Alkaline phosphatase	Alanine transaminase	Aspartate transaminase	Acid phosphatase
Control	1.13 ± 0.05	10.19 ± 1.36	15.15 ± 1.51	72.96 ± 2.79	55.99 ± 1.90	23.84 ± 2.50
CCl_4	3.37 ± 0.34[a]	66.78 ± 1.55[a]	37.87 ± 2.61[a]	247.27 ± 2.10[a]	185.18 ± 4.81[a]	64.24 ± 4.41[a]
CCl_4+ PE 10 μg/mL	3.24 ± 0.11*	61.19 ± 0.93*	34.72 ± 1.8*	224.10 ± 3.55*	173.16 ± 2.85*	61.24 ± 0.94*
CCl_4+ PE 15 μg/mL	2.86 ± 0.08*	52.55 ± 2.31*	30.45 ± 0.7*	218.47 ± 2.6*	152.63 ± 2.5*	52.85 ± 1.4*
CCl_4+ PE 25 μg/mL	2.51 ± 0.08*	19.15 ± 1.49*	23.87 ± 1.7*	187.83±2.2*	107.07 ± 2.3*	45.35 ± 1.0*

Set no	Lipid peroxidation	Lactate dehydrogenase	Alkaline phosphatase	Alanine transaminase	Aspartate transaminase	Acid phosphatase
CCl_4+ Q 10 µg/mL	2.14 ± 0.05*	45.87 ± 2.69*	21.36 ± 1.2*	142.71 ± 2.3*	82.33 ± 1.22*	37.15 ± 0.5*
CCl_4+ S 10 µg/mL	1.72 ± 0.03*	31.78 ± 2.11*	18.55 ± 0.8*	116.66 ± 1.8*	62.43 ± 1.63*	31.64 ± 1.3*
PE 25 µg/mL	1.35 ± 0.03*	16.81 ± 1.29*	32.36 ± 2.2*	86.30 ± 1.97*	58.21 ± 0.95*	27.30 ± 1.2*
Q 10 µg/mL	1.07 ± 0.05*	12.66 ± 2.46*	16.99 ± 1.2*	82.29 ± 2.14*	51.15 ± 1.07*	21.48 ± 0.5*
S 10 µg/mL	0.93 ± 0.04*	9.26 ± 1.20*	11.66 ± 1.3*	46.97 ± 1.68*	42.75 ± 2.67*	17.86 ± 0.7*

PE- Plant Extract, Q- Quercetin, S- Silymarin.
(All values are expressed as Mean ± S.D. (n = 3); Superscript (a) in columns indicates value differ significantly ($p < 0.001$) from control mean; Superscript asterisk in columns indicate value differ significantly (*$p < 0.05$) from CCl_4 mean).

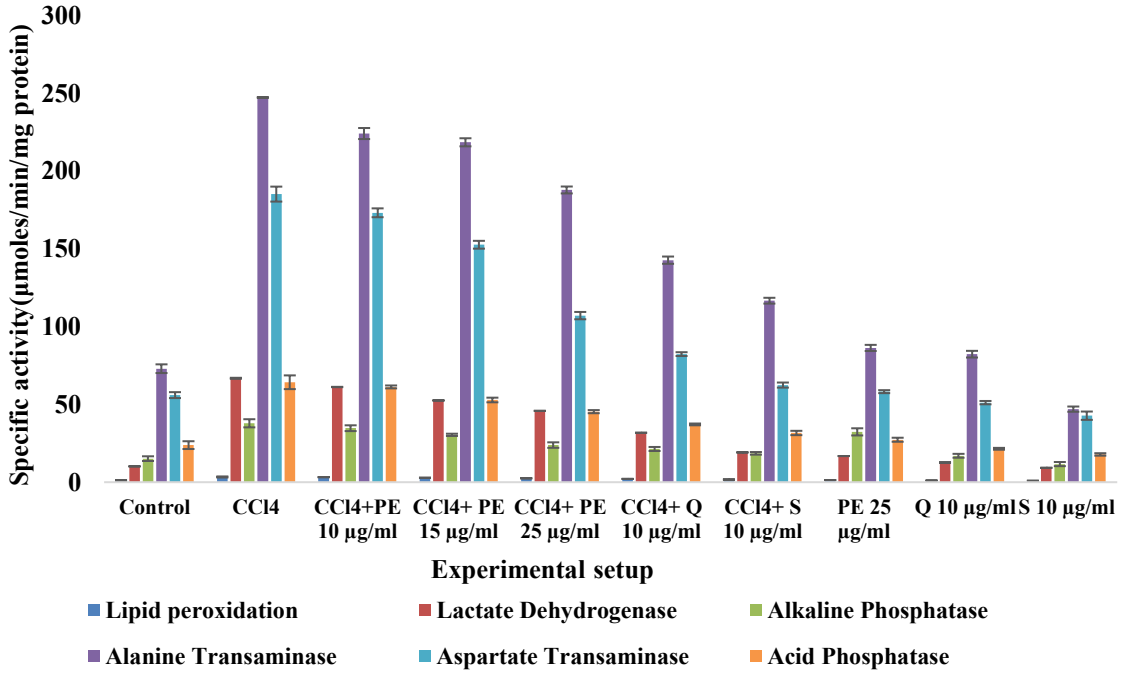

Figure 1 Effect of methanolic leaf extract of *Acalypha indica*, quercetin and silymarin treatment on release of biochemical markers of hepatotoxicity induced by CCl_4 in the goat liver tissue *in vitro*.

In the absence of reliable hepatoprotective drugs in modern medicine, a large number of medicinal plants with well-established traditional use have been recommended for the treatment of liver disorders. The present study demonstrated the hepatoprotective efficacy of *A. indica* leaf extracts in *in vitro* model of hepatotoxicity. Our results are correlated with the result obtained by Chaudhari and Mahajan [8]. They showed that methanolic stem bark extract of *Terminalia arjuna* inhibits lipid peroxidation due to CCl_4 by 55 % at a concentration of 100 µg/mL and at 10 µg/mL concentration of quercetin inhibit lipid peroxidation by 51 %.

Reactive oxygen species (ROS) or reactive nitrogen species (RNS) can initiate lipid peroxidation in liver cells and lipid peroxidation starts a series of reactions leading to liver injury [28]. A number of enzymes are produced in the liver and are normally distributed with in the cells of liver. The change in the activities of these enzymes is taken as the sensitive biomarkers of liver injury. The estimation of various liver enzymes in serum, as AST, ALT, ALP, ACP and LDH are used to determine the functional status of

the liver and to detect liver damage [29]. Administration of CCl_4 markedly increased ($p < 0.05$) the activity of liver serum marker enzymes such as AST, ALT, ALP, ACP and LDH as compared with the control group. The liver tissue treated with CCl_4 in presence of methanolic leaf extract of *A. indica* (10, 15 and 25 µg/mL), Quercetin, and Silymarin (10 µg/mL) significantly reduced ($p < 0.05$) the ACP, LDH, AST, ALT, ALP levels compared to the CCl_4 induced set. Overall depend on the concentration of test samples, methanolic leaf extract of *A. indica,* Quercetin, and Silymarin reduces the elevated ACP, LDH, AST, ALT and ALP levels (**Figure 1**).

Previous studies on the mechanism of hepatic injury indicated that CCl_4 is first metabolized by cytochrome P450 in the liver endoplasmic reticulum to the highly reactive trichloromethyl (CCl_3^-) radical, which reacts readily with O_2 to form the peroxytrichloromethyl (CCl_3OO^-) radical. These free radicals are known to interact with cellular macromolecules, especially with unsaturated fatty acids, and initiate lipid peroxidation, thereby disrupting the structure and function of cell membrane. The present study showed that CCl_4 challenge caused hepatocellular damage, which was clearly indicated by the marked elevation of enzyme (AST, ALT, ACP, LDH and ALP) activities. ALT, AST, ACP, LDH and ALP are considered the most sensitive markers for the diagnosis of hepatic injury, since they are located in cytoplasm, and deficiency of these enzymes occurs rapidly after cellular damage [30].

Results of this study are in agreement with those from previous studies of Chaudhari and Mahajan [8] and Abdel-Ghany *et al*. [26] that showed severe liver damage (assessed by significant elevation in ALT, AST, ACP, LDH and ALP levels) following administration of CCl_4. ALT is considered more specific for hepatic disorders because it is mainly present in the cytosol of hepatocytes but in low concentration elsewhere. AST has cytosolic and mitochondrial forms and is present in the liver, heart, skeletal muscles, brain, and pancreas. Elevation of ALT and AST levels induced by CCl_4 administration may be attributed to the hepatocellular damage. In addition, the oxidative stress caused by CCl_4 has been shown to induce mitochondrial dysfunction and depletion of adenosine triphosphate. Abdel-Ghany *et al*. [26] reported that CCl_4 reduces glutathione content by increasing lipid peroxide generation. Furthermore, the enzymes that scavenge free radicals, such as glutathione reductase, peroxidase, and catalase are decreased during the induction of cirrhosis.

Metabolism of various metabolites and exogenous toxic chemicals (pesticides, drugs, metals), are takes place inside the hepatic tissue causes the formation of free radicals which may be extensively toxic than the parent compound. CCl_4, an extensively studied hepatotoxin is converted into its metabolites such as CCl_3 radicals which are involved in the liver pathogenesis including cirrhosis, genotoxicity of hepatic tissue and hepatic carcinoma [31]. Our present results showed that exposure of liver slice culture to CCl_4 caused significant increase in the secretion of hepatic biomarkers profile due to hepatic injuries caused by their free radicals. The hepatotoxicity induced by CCl_4 increases level of biochemical markers, LDH, ALT, AST, ACP and ALP by 6.55, 3.89, 3.31, 2.69 and 2.50 times in culture medium as compared to control. The hepatoxicity of CCl_4 in terms of biochemical markers ALT, AST, ACP and ALP is remarkably reduced by silymarin followed by quercetin and different concentration of methanolic leaf extract of *A. indica.* However, the level of LDH elevated by CCl_4 toxicity in culture medium is highly reduced by plant extract (25 µg/mL) as compared to silymarin and quercetin and presenting its role in cardio protection. The result of treatment of liver slice culture with CCl_4 along with methanolic leaf extract of *A. indica,* Quercetin, and Silymarin showed reduction in the elevated level of LDH and other hepatic biomarkers like AST, ALT, ACP and ALP level in the medium indicating the hepatoprotective action. Quercetin, the most abundant of the flavonoids consists of 3 rings and 5 hydroxyl groups. Quercetin is a member of the class of flavonoids called flavonoles and forms the backbone for many other flavonoids including the citrus flavonoids like rutin, hesperidins, Naringenin and tangeritin. It is widely distributed in the plant kingdom in rinds, leaves and barks. Quercetin acting as free radical scavengers was shown to exert a protective effect in reperfusion ischemic tissue damage. Quercetin prevents free radical induced tissue injury by various ways. One way is the direct scavenging of free radicals. By scavenging free radicals, Flavonoid; particularly Quercetin can inhibit LDL oxidation in vitro [32]. Quercetin has been described to have hepatoprotective property against ethanol-induced liver injury through antioxidant, anti-inflammation, down-regulation of CYP2E1 and CYP3A, and increase glutathione levels [33]. One of the mechanisms of liver damage caused by alcohol is the generation of free radicals formed by the metabolism of this xenobiotic. Silymarin is an antioxidant that protects the liver from the free radical damage produced by alcohol metabolism. Silymarin is the most used natural compound for the treatment of hepatic diseases worldwide due to its antioxidant, anti-inflammatory, and anti-fibrotic activities. Silymarin functions by stabilizing biological membranes and increasing protein synthesis [34]. Quercetin and silymarin are natural hepatoprotective agents and apart from these drugs methanolic leaf extract of *Acalypha indica* with concentration of 25 µg/mL showed potential *in vitro*

hepatoprotective effects against CCl_4 induced liver injury in goat liver slice, which is likely due to the presence of flavonoids and also its antioxidant and anti-inflammatory properties.

Conclusions

The present investigation indicates that, the methanolic leaf extract of *Acalypha indica* exhibit hepatoprotective effect against CCl_4 induced hepatic damage. The hepatoprotective role of *Acalypha indica* leaf may be due to its flavonoids and antioxidant properties. Evidently, flavonoids produce antioxidant activity, so this mechanism signifies that the plant extract may help prevent oxidative stress-induced liver damage. Our observations of *in vitro* hepatoprotection support and enrich the findings of earlier researchers. Thus this plant should be recorded for its global acceptance as an herbal drug.

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