Chemopreventive effect of *Fumaria indica* that modulates the oxidant−antioxidant imbalance during N−nitrosodiethylamine and CCl_4−induced hepatocarcinogenesis in Wistar rats

Talib Hussain[1,2], Hefazat H Siddiqui[1], Sheeba Fareed[1], K Sweety[2], M Vijayakumar[2], Chandana V Rao[2*]

[1] Department of Pharmacology and Toxicology, Faculty of Pharmacy, Integral University, Kursi road, Lucknow−226026, Uttar Pradesh, India
[2] Pharmacognosy and Ethnopharmacology Division, National Botanical Research Institute (CSIR), Rana Pratap Marg, P. B. No. 436, Lucknow 226 001, Uttar Pradesh, India

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ABSTRACT

Objective: To investigation the chemopreventive efficiency of *Fumaria indica* extract (FIE) on the antioxidant status of N−nitrosodiethylamine (NDEA) and CCl_4−induced hepatocarcinogenesis in Wistar rats. **Methods:** The experimental animals were divided into six groups ($n = 6$). HCC was induced by single intraperitoneal injection of NDEA in normal saline at a dose of 200 mg/kg body weight followed by weekly subcutaneous injections of CCl_4 (3 mL/kg/week) for 6 weeks, as the promoter of carcinogenic effect. After administration of the carcinogen, 200 and 400 mg/kg of FIE were administered orally once a day throughout the study. At the end of 20 weeks, the body weight, liver weight and relative liver weight were measured. The level of hepatic malondialdehyde (MDA) formation, reduced glutathione (GSH) and the activities of antioxidant enzymes viz. CAT, SOD, GPx, and GST in the liver of NDEA and CCl_4−treated rats were assessed. **Results:** Obtained results demonstrated that the cotreatment with FIE (200 and 400 mg/kg) significantly prevented the decrease of the body weight and also increased in relative liver weight caused by NDEA and CCl4 administration. FIE also significantly prevented hepatic malondialdehyde (MDA) formation and reduced glutathione (GSH) in NDEA−treated rats which were dose dependent. Additionally, FIE also markedly increased the activities of antioxidant enzymes such as CAT, SOD, GPx, and GST in the liver of NDEA and CCl_4−treated rats. **Conclusions:** These finding powerfully supports that *Fumaria indica* exert a chemopreventive effect by reversing the oxidant−antioxidant imbalance during hepatocarcinogenesis induced by NDEA and CCl_4.

1. Introduction

Hepatocellular carcinoma (HCC) or liver cancer is the sixth most common cancer and the third leading cause of cancer mortality in the world [1]. The burden of cancer is increasing in economically developing countries as a result of population aging and growth as well as, increasingly, an adoption of cancer−associated lifestyle choices including smoking, physical inactivity, and ''westernized'' diets. Liver cancer in men/ women is the fifth/seventh most frequently diagnosed cancer worldwide but the second/ sixth most frequent cause of cancer death. An estimated 748 300 new liver cancer cases and 695 900 cancer deaths occurred worldwide in 2008[2]. Hepatitis viral infection, food additives, alcohol, fungal toxins (aflatoxins), toxic industrial chemicals, air and water pollutants are the major risk factors of liver cancer[3]. Human liver is the major site in the body that metabolizes ingested material. It has pore to carcinogenic insult. Moreover, due to the high tolerance of liver, HCC is seldom detected at the early stage and once detected treatment faces a poor prognosis in most cases [4]. N−Nitrosodiethylamine (NDEA) is a potent hepatocarcinogenic nitrosamine present in tobacco smoke, water, cheddar cheese, cured and fried meals, occupational settings, cosmetics, agricultural chemicals and pharmaceutical agents [5].

N−Nitrosodiethylamine is widely accepted for induction of preneoplastic lesions and hepatic tumors in rats and it is initiated by perturbations of nuclear enzymes involved in DNA repair or replication [6]. It is also reported that the generation of reactive oxygen species (ROS) is apparent during the metabolic biotransformation of NDEA resulting in oxidative stress. Oxidative stress leads to carcinogenesis

*Corresponding author:
Chandana V. Rao, Pharmacognosy and Ethnopharmacology Division, National Botanical Research Institute (CSIR), Rana Pratap Marg, Lucknow, India.
  Tel.: +91 522 2297976
  Fax: +91 522 2205836
  E−mail: mdth_ah@yahoo.com

by several mechanisms including DNA, lipid and protein damage, change in intracellular signaling pathways and even changes in gene expression. Together, these oxidative modifications promote abnormal cell growth and carcinogenesis [7]. DNA damage includes the formation of 8−hydroxydeoxyguanosine (8−OHdG), which is the most studied DNA oxygen adduct implicated in carcinogenesis. Lipid peroxidation (LPO) result in several sequelae, including structural and functional membrane modifications, protein oxidation and generation of oxidation products such as acrolein, crotonaldehyde, malondialdehyde (MDA) and 4−hydroxy−2−nonenal (HNE), which are considered strong carcinogens[8].

Many pharmaceutical agents have been discovered by screening natural products from plants, animals, marine organisms and microorganisms. Vincristine, vinblastine, irinotecan, etoposide and paclitaxel are examples of plant−derived compounds that are being employed in cancer treatment as well as several chemicals are also known to possess chemopreventive properties against a broad spectrum of cancer [9]. Recently, identification of bioactive ingredients from medicinal plants to inhibit tumorigenesis in a variety of animal models of carcinogenesis, involving organ sites, such as the skin, lungs, oral cavity, esophagus, stomach, liver, pancreas, small intestine, colon, and prostate is gaining considerable attention[10]. Moreover, in recent years, naturally occurring plant products have getting increased attention for the intervention of malignant invasive progression in the late stage of neoplastic diseases [11].

Fumaria indica pugsely (Syn: Fumaria parviflora Fumariaceae) (*F. indica*) is a small, scandent, branched annual herb growing wild in plains and lower hills. It has long been used as a household remedy and forms a constituent of many common Ayurvedic, Unani medicinal preparations and polyherbal liver formulation. The plant considered to be diuretic, diaphoretic, anthelmintic, laxative and used to purify blood and in obstruction of liver [12]. Pharmacological studies show that *F. indica* is a smooth muscles relaxant and possess antipyretic [13], analgesic, anti−inflammatory[14], antidiarrhoeal[15], and hepatoprotective properties[16-19]. Phytochemical investigation revealed the presence of alkaloids viz. protopine, parfumine, fumariline, fumaranine, fumaritine, cryptopine, paprafumicin, paprarine, papraline, reddeanine[14], narlumicine, fumarophycine, steroids *viz.* β−sitosterol, stigmasterol, campesterol, organic acid viz. caffeic acid and fumaric acid[20]. Traditionally and scientifically proved reports suggested that *F. indica* is a successful and safe hepatoprotective agent and acts in a manner similar to that of silymarin and it is a more effective inducer of Phase II enzymes indicating its anticarcinogenic properties. Therefore, present study was designed to evaluate the chemopreventive potential of *F. indica* extract (FIE) against NDEA and CCl4−induced hepatocarcinogenesis using lipid peroxidation product and antioxidants: GSH, SOD. CAT, GPx and GST as biochemical end point of chemoprevention.

2. Materials and methods

2.1. Chemicals and their sources

All the chemicals used were of analytical grade and procured from Sigma chemicals Co., USA and Qualigens fine chemicals, Mumbai, India.

2.2. Preparation of plant extract

The fresh plant of *F. indica* was collected in the botanical garden of National Botanical Research Institute, India in January 2010. The plant material was identified and authenticated and the voucher specimen number (NAB 180023) was deposited in the departmental herbarium. The air dried powdered material (1 000 g) was extracted with petroleum ether thrice to remove fatty material and further marc was exhaustively extracted thrice with 50% aqueous ethanol by cold percolation method at room temperature. The extract was separated by filteration, concentrated at (40 ± 1) ℃ on rotavapour (Buchi, USA) to yield 9.85% (w/w) of FIE. Preliminary qualitative phytochemical screening of FIE has given the positive testes for alkaloids, glycosides, flavonoids, tannins, saponins, steroids and triterpenoids.

2.3. Animals

Wistar albino rats (150−170 g) and Swiss albino mice (25−30 g) were procured from the National Laboratory Animal Centre (NLAC), Central Drug Research Institute, Lucknow, India. The animals were housed separately in polypropylene cage at temperature of (22 ± 2) ℃ and 50%−60% relative humidity, with a 12 h light/dark cycle respectively, for one week before and during the experiment. Animals were allowed to access standard rodent pellet diet (Dayal animal feed, India) and drinking water. Food was withdrawn 18−24 h before the experiment though water was allowed ad libitum and allocated to different experimental groups. All studies were performed according to the guidelines for the care and use of laboratory animals, as adopted and promulgated by the Institutional Animal Care Committee, CPCSEA, India (Reg. No. 1213/ac/2008/CPCSEA/IU).

2.4. Toxicity studies

Acute toxicity study was performed for FIE according to the OECD guideline for acute toxic classic method [21]. Three female albino mice were used for each step in this study. The animals were kept fasting for overnight only on water, after which the extracts were administered orally at the single dose of 300 mg/kg and closely observed for the initial 4h after the administrations, and then once daily for 14 days. If mortality occurred in two out of three animals, then this dose was assigned as toxic dose. If the mortality occurred in one animal, then this same dose was repeated to confirm the toxic dose. If mortality did not occur, the procedure was repeated for further higher dose, i.e., 2 000 mg/kg. One−tenth and one−fifth of the maximum tolerated dose of the extract tested for acute toxicity were selected for evaluation of chemopreventive effect of FIE, i.e., 200 and 400 mg/kg.

2.5. Experimental design and induction of hepatocellular carcinoma (HCC)

The animals were divided into six experimental groups, each group comprising of six animals (n = 6) for a study period of 20 weeks. Group I served as normal control and treated with 0.9% normal saline throughout the study. HCC was induced in groups II, III, IV and V with single intraperitoneal injection of NDEA in normal saline at a dose of 200 mg/kg body weight followed by weekly subcutaneous injections of CCl_4 (3 mL/kg/week) for 6 weeks, as the promoter of carcinogenic effect[22]. After administration of NDEA, test groups III and IV were administered orally 200 and 400 mg/kg FIE, respectively, in the form of aqueous suspension daily once a day throughout the study. Group V received silymarin, the known hepatoprotective and anti−hepatocellular carcinoma compound at a dose of 200 mg/kg [4,23]. The dose of FIE (400 mg/kg alone) was administered orally to rats of group VI. The experiment was terminated at the end of 20 weeks of experimental period, the body weight of each rat was taken before sacrifice. The overnight fasted animals were anaesthetized and sacrificed 48 h after the last dose of the drug. The liver tissue was washed twice with ice cold saline, blotted, dried, and then weighed. The relative liver weight was calculated as the percentage ratio of liver weight to the body weight.

2.6. Preparation of PMS of rat liver

A part of the liver of rats was homogenized (10%) in phosphate buffer (pH 7.4) with a Potter−Elvenhjem glass homogenizer. The homogenate was centrifuged at 12 000 × g for 20 minutes at 4 ℃ to obtain post mitochondrial supernatant (PMS) and it was used to measure malondialdehyde (MDA), reduced glutathione (GSH) level, catalase (CAT), superoxide dismutase (SOD), glutathione peroxidase (GPx) and glutathione−S−transferase (GST).

2.7. Assessment of antioxidant parameters

2.7.1. Assessment of lipid peroxidation (LPO)

A volume of the homogenate (0.2 mL) was transferred to a vial and was mixed with 0.2 mL of a 8.1% (w/v) sodium dodecyl sulphate solution, 1.5 mL of a 20% acetic acid solution (adjusted to pH 3.5 with NaOH) and 1.5 mL of a 0.8% (w/v) solution of thiobarbituric acid (TBA) and the final volume was adjusted to 4.0 mL with distilled water[24]. Each vial was tightly capped and heated in a boiling water bath for 60 min. The vials were then cooled under running water. Equal volumes of tissue blank or test samples and 10% trichloroacetic acid were transferred into a centrifuge tube and centrifuged at 1 000 × g for 10 min. The absorbance of the supernatant fraction was measured at 532 nm (Beckman DU 650 spectrometer). Control experiment was processed using the same experimental procedure except the TBA solution was replaced with distilled water. Malonyldialdehyde (MDA) is an end product of lipid peroxidation, which reacts with thiobarbituric acid to form pink chromogen thiobarbituric acid reactive substance. 1,1,3,3−tetra ethoxypropan was used as standard for calibration of the curve and is expressed as nmole/mg protein.

2.7.2. Assessment of reduced glutathione (GSH)

Reduced glutathione (GSH) level was measured by the method of Ellman[25]. The PMS of rat liver (720 μL) and 5% TCA were mixed to precipitate the protein content of the supernatant. After centrifugation at 10 000 × g for 5 min, the supernatant was taken. DTNB (5,5′−dithio−bis (2−nitrobenzoic acid) Ellman′s reagent was added to it and the absorbance was measured at 412 nm. A standard graph was drawn using different concentrations of a standard GSH solution. GSH contents were calculated in the PMS of rat liver and expressed as nmol/mg of protein.

2.7.3. Assessment of catalase (CAT)

Catalase activity was measured according to the method of Aebi[26]. One unit of catalase was defined as the amount of enzyme required to decompose 1.0 μM of hydrogen peroxide (H_2O_2) in 1 min. The reaction was initiated by the addition of freshly prepared 20 mM H_2O_2 (1.0 mL). The rate of decomposition of H_2O_2 was measured at 240 nm for 1 min, at 25 ℃. The enzyme activity was expressed as U/mg of protein.

2.7.4. Assessment of superoxide dismutase (SOD)

The activity of superoxide dismutase in the PMS of liver was assayed[27] based on the oxidation of epinephrine adrenochrome transition by enzyme. The post−mitochondrial suspension of rat liver (0.5 mL) was diluted with distilled water (0.5 mL). To this, chilled ethanol (0.25 mL) and chloroform (0.15 mL) were added. The mixture was shaken for 1 min and centrifuged at 2 000×g for 10min. The PMS (0.5 mL) was added with PBS buffer (pH 7.2; 1.5 mL). The reaction initiated by the addition of epinephrine (0.4 mL) and change in optical density (O.D., min^{-1}) was measured at 470 nm. SOD activity expressed as U/mg of protein. Change in O.D. (min^{-1}) at 50% inhibition to adrenochrome transition by the enzyme was taken as one enzyme unit.

2.7.5. Assessment of Glutathione peroxidase (GPx)

Glutathione peroxidase activity was measured by the method described by Rotruck[28]. To 0.2 mL of buffer, 0.2 mL of EDTA, 0.1 mL of sodium azide and 0.5 mL of PMS were added. To that mixture, 0.2 mL of glutathione solution and 0.1 mL of H_2O_2 were added. The contents were mixed well and incubated at 37 ℃ for 10 minutes along with the control tubes containing the entire reagent but no enzyme. After 10 minutes, the reaction was arrested by the addition of 0.4 mL of 10% TCA. Then 0.2 mL of PMS was added to the control tubes and the tubes were centrifuged. To 0.5 mL of supernatant, 3.0 mL of sodium hydrogen phosphate and 1.0 mL of DTNB were added and the color developed was read at 412 nm immediately in spectrophotometer. Graded concentrations of the standard were also treated similarly. Glutathione peroxidase activity in liver homogenate was expressed as U/mg of protein.

2.7.6. Assessment of glutathione–S–transferase (GST)

The activity of glutathione–S–transferase was measured according to the method of Habig[29]. The PMS of liver (0.1 mL), PBS buffer (pH 7.4; 1.0 mL), double distilled water (1.7 mL), and 1–chloro–2,4–dinitrobenzene (CDNB; 0.1 mL) reagent were taken in the screw cap tubes and incubated at 37 ℃ for 15 min. the reaction was started by the addition of GSH (1.0 mL) was added and increase in optical density was recorded at 340 nm from 0 to 3.0 minutes. The reaction mixture without the enzyme was used as blank. The activity of GST was expressed as U/mg of protein.

2.7. Statistical analysis

The data were represented as mean ± S.E.M. for six rats. Analysis of variance (ANOVA) test was followed by individual comparison by Newman–Keuls test using Prism Pad software (Version 3.0) for the determination of level of significance. The value of $P < 0.05$ was considered statistically significant.
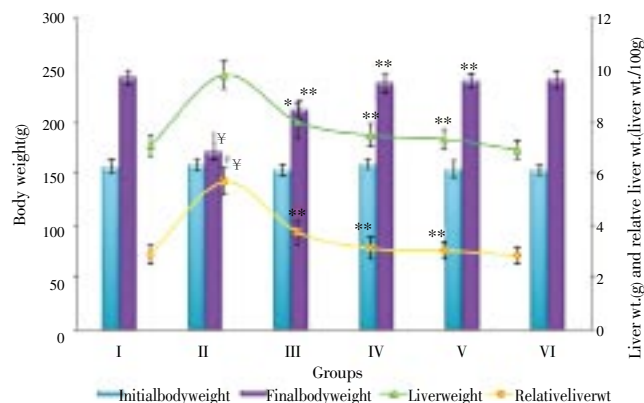
3. Results

3.1. Effect of FIE on body weight, liver weight and relative liver weight

Figure 1 shows the body weight (initial and final) and liver weights of control and experimental groups of animals. The final body weight of normal group I rats showed (243.0 ± 7.4) g which was significantly decreased to ($P < 0.001$) (172.0 ± 8.6) g comparatively in group II rats following NDEA treatment. In FIE–treated group III (200 mg/kg) and IV (400 mg/kg) rats, the final body weights became significantly increased ($P < 0.01$) (212 ± 9.8) g and ($P < 0.001$) (238 ± 9.5) g when compared to the NDEA group II, respectively. Moreover, NDEA treatment significantly increased the relative liver weight to ($P < 0.001$) (5.71 ± 0.52)/100 g body

Figure 1. Effect of FIE on body weight, liver weight and relative liver weight of control and NDEA induced HCC in rats.
Values are expressed as mean ± SEM of 6 rats in each group, ¥$P<0.01$ compared with respective control group I, *$P<0.05$, **$P<0.01$, compared with group II (NDEA +CCl₄).
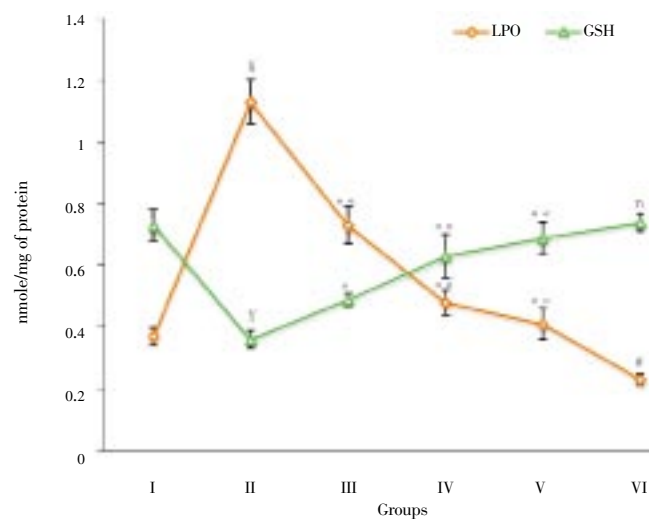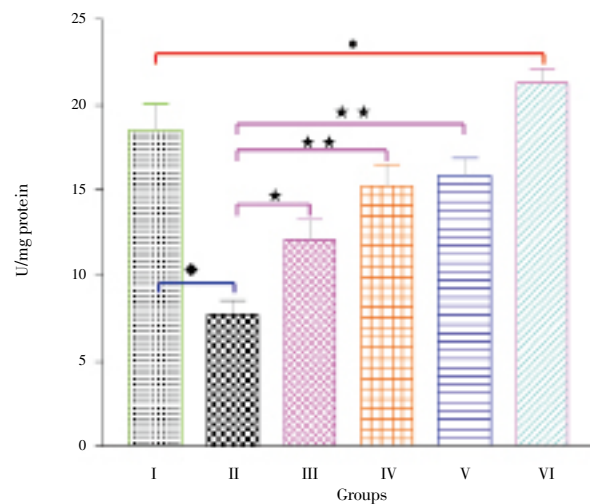

Figure 2. Effect of FIE on the levels of lipid peroxide and glutathione in the PMS of control and NDEA induced HCC in rats.
Values are expressed as mean ± SEM of 6 rats in each group.
¥$P<0.001$ compared with respective control group I, *$P<0.05$ and **$P<0.001$ compared with group II (NDEA + CCl4), #$P<0.05$ compared with respective control group I, n: non significant when compared to respective control group I.

Figure 3. Effect of FIE on the antioxidant activities of SOD (50% inhibition of chromogen/min/mg protein) in the PMS of control and NDEA induced HCC in rats.
Values are expressed as mean ± SEM of 6 rats in each group, ♦ P <0.001 compared with respective control group I, ★P <0.01 and ★★ $P<0.001$ compared with group II (NDEA +CCl₄), • P <0.05 compared with respective control group I.
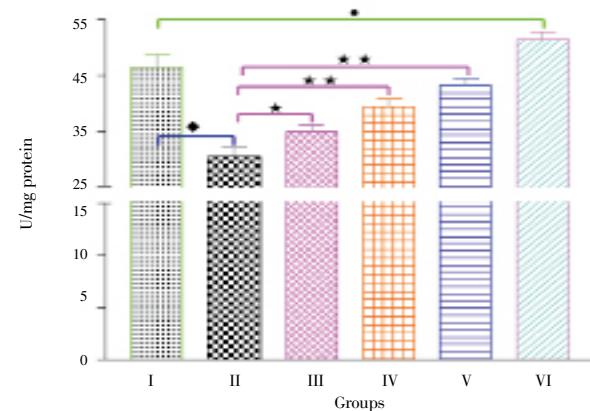

Figure 4. Effect of FIE on the antioxidant activities of CAT (μ mol H₂O₂ consumed/min/mg protein) in the PMS of control and NDEA induced HCC in rats.
♦ P <0.001 compared with respective control group I, ★ P <0.05 and ★★ P <0.001 compared with group II (NDEA +CCl₄), • P <0.05 compared with respective control group I.

weight when compared to the control (group I, (2.93 ± 0.38)/100 g body weight). However, administration of 200 and 400 mg/kg FIE significantly reduced (P< 0.01 and P< 0.001) the relative liver weight to (3.78 ± 0.47) and (3.15 ± 0.43/100) g body weight, respectively, compared to (5.71 ± 0.52/100) g in NDEA treatment. FIE treated group IV activity was less to standard silymarin−treated group V rats at the concentration used. The animals treated with FIE alone (400 mg/kg) showed no significant change in the body weight and relative liver weight when compared to control group I.
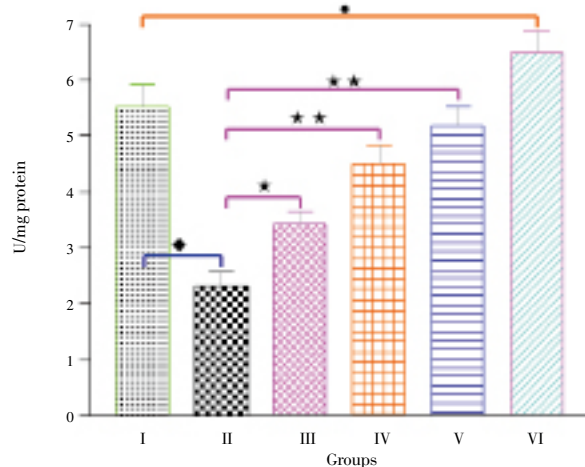

Figure 5. Effect of FIE on the antioxidant activities of GPx (μ mol GSH utilized/min/mg protein) in the PMS of control and NDEA induced HCC in rats.
♦ P <0.001 compared with respective control group I, ★ P <0.05 and ★★ P <0.001 compared with group II (NDEA +CCl4), • P <0.05 compared with respective control group I.
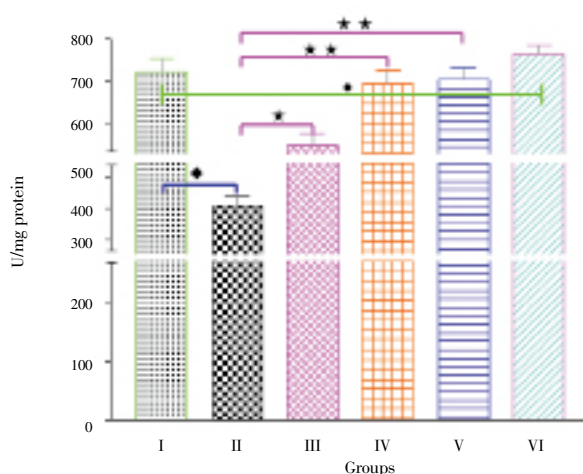

Figure 6. Effect of FIE on the antioxidant activities of GST (1−chloro−2,4−dinitrobenzene nmol/min/mg protein) in the PMS of control and NDEA induced HCC in rats.
Values are expressed as mean ± SEM of 6 rats in each group.
♦ P <0.001 compared with respective control group I, ★ P <0.05 and ★★ P <0.001 compared with group II (NDEA +CCl4), • P <0.05 compared with respective control group I.

3.2. Effect of FIE on LPO and levels of antioxidant enzymes in liver

The LPO levels in liver homogenate were found to be significantly increased in the NDEA−treated group II rats. The LPO value of control group I was (0.37 ± 0.03) U/mg

protein which increased to 1.13 ± 0.07 U/mg protein (P < 0.001) (Figure 2). Administration of FIE showed significant reduction in LPO as (0.73 ± 0.06) and (0.48 ± 0.04) U/mg protein, P < 0.001 and P < 0.001 at doses 200 and 400 mg/kg, respectively. NDEA treatment (group II) decreased the levels of hepatic GSH, CAT and SOD from (0.73 ± 0.05) to (0.36 ± 0.03) (P < 0.001), (46.56 ± 2.27) to (30.41 ± 1.53) (P < 0.001), and (18.58 ± 1.54) to (7.71 ± 0.83) U/mg protein (P < 0.001), respectively when compared to group I animals (Figures 2−4). However, FIE at 200 and 400 mg/kg significantly increased the levels of GSH, CAT and SOD from (0.49 ± 0.02) to (0.63 ± 0.07) (P < 0.05 to P < 0.001), (34.85 ± 1.13) to (39.43 ± 1.34) (P < 0.05 to P < 0.001) and (12.14 ± 1.13) to (15.31 ± 1.21) U/mg protein (P < 0.01 to P < 0.001), respectively when compared to group II rats. Levels of GPx and GST were significantly decreased in NDEA−induced HCC bearing rats [(2.33 ± 0.26) and (410.83 ± 24.13) U/mg protein, P < 0.001, respectively] which is presented in Figures 5 and 6. On the contrary, the FIE (200 and 400 mg/kg) also increased the level of GPx from (3.42 ± 0.23) to (4.49 ± 0.36) U/mg protein (P < 0.05 to P < 0.001) and GST from (547.76 ± 29.11) to (693.81 ± 32.12) U/mg protein in liver (P < 0.01 and P < 0.001) as compared to NDEA−treated group II rats, respectively. Silymarin at 200 mg/kg significantly reduced the elevated LPO level [(0.41 ± 0.05) U/mg protein, P < 0.001), but increased the levels of GSH, CAT, SOD, GPx and GST [(0.69 ± 0.05] (P < 0.001), (43.21 ± 1.23) (P < 0.001), (15.87 ± 1.06) (P < 0.001), (5.17 ± 0.35) (P < 0.001) and (703.62 ± 31.29) (P < 0.001) U/mg protein], respectively compared to NDEA−treated group II rats. Surprisingly, when FIE alone at a dose of 400 mg/kg was administered to the non−NDEA−treated rats (Group VI), gentle significant changes (P < 0.05) were observed in LPO, SOD, CAT and GPx when compared to normal control group I rats (Figures 2−5). This indicates that *F. indica* extract contributes to exert antioxidant defense mechanism by metabolizing lipid peroxides and scavenging endogenous peroxides as well as its depicting the non−toxic nature of the plant extract.

4. Discussion

The present investigation showed that oral administration of FIE counteracts lipid peroxidation (LPO) and prevents the development of hepatocellular carcinoma (HCC) that is usually induced by N−nitrosodiethylamine (NDEA) and CCl4 in experimental rats. NDEA on metabolic biotransformation produces promutagenic products, O6−ethyldeoxyguanosine and O4 and O6−ethyldeoxythymidine in liver which are responsible for their carcinogenic effects[30]. In addition, NDEA purportedly induces sustained production of oxidative stress in liver of rats, involving changes in lipid peroxidation and antioxidant enzymes[6]. It is well established that CCl4 induces hepatotoxicity by metabolic activation and therefore selectively causes toxicity in liver cells maintaining a semi−normal metabolic function. CCl4 is bio−transformed by cytochrome P450 (CYP) enzyme system in the endoplasmic reticulum to produce trichloromethyl free radicals. Trichloromethyl free radicals (CCl3•) then combine with

cellular lipids and proteins in the presence of oxygen to form trichloromethyl peroxyl radical, which further attack lipids on the membrane of endoplasmic reticulum faster than trichloromethyl free radical. Thus, trichloromethyl peroxyl free radical leads to elicitation of lipid peroxidation (LPO) and destruction of Ca^{2+} homeostasis, resulting in cell death[31].

The results of the present study seem to provide support for the chemopreventive effects of FIE against NDEA and CCl4–induced hepatocarcinogenesis in rats. There is an appreciable reduction in body weight and increase in liver weight observed in HCC bearing group II rats as compared to control group I rats. Decreased appetite and food intake contribute to the weight loss which could be an indirect indication of the declining hepatic function, an increase in the liver weight of the animals, which could be attributed to the formation of nodules and tumors in the liver following carcinogen exposure. Sreepriya and Bali[9] have also reported marked loss of body weight and increase in liver weights. The steadily increase in body weight and decrease in liver weight after FIE treatment (groups III and IV) indicate that, increased appetite and reduced tumor incidence shows its anticancer effectiveness.

Lipid peroxidation (LPO) is a useful marker of oxidative stress because it is linked to increased production of ROS when CYP metabolizes NDEA[32]. The level of LPO increases with the administration of NDEA during hepatocarcinogenesis has been reported by Sivaramakrishnan[5]. Interestingly, we also found that the level of LPO increases in group II rats when compared to the control group I rats, the rats cotreated with FIE exhibited significantly reduced LPO levels induced by NDEA and CCl$_4$, suggesting that counteracting this oxidative step is decisive in avoiding the development of HCC. The products of LPO are considered mutagenic and carcinogenic as they cause damage to cellular macromolecules by generating ROS[31,32]. The present results are in conformity with previous studies on *Fumaria indica* extracts which showed the inhibition of lipid peroxidation[19]. In this study, we observed that FIE treatment affected all the components of the antioxidant defense system analyzed in the same 'direction', namely towards counteracting oxidative stress. GSH is an important factor in this system, which is required to maintain the normal reduced state of cells and to counteract the deleterious effects of oxidative stress[23]. GSH is said to be involved in many cellular processes including detoxification of endogenous and exogenous compounds[32]. NDEA, an electrophilic carcinogen may interact with the large nucleophilic pool of GSH thereby reducing the macromolecules and carcinogen interaction[4]. In FIE–cotreated rats, a significantly high level of hepatic GSH was observed when compared to NDEA–induced rats consistent with the idea of reduced DNA–carcinogen interaction and thereby averting a favorable environment for carcinogenesis. SOD acts as the first line of defense against superoxide radicals, which dismutates two superoxide radicals to H_2O_2 and O_2. In addition, CAT and GPx acts as supporting antioxidant enzymes by converting H_2O_2 to H_2O, thereby providing protection against ROS[32]. The reduction

in activity of these enzymes may be caused by the increase in radical production during NDEA metabolism. In the present investigation, an increase in MDA formation was presumably associated with increased ROS, consistent with the observation that these free radicals reduce the activity of hepatic SOD[33]. Biochemical results of hepatic SOD, showed a decrease in activity of SOD in NDEA–induced group II rats compared to control group I and FIE–cotreated group III and IV rats. GPx is another endogenous antioxidant selenoprotein present in the cytosol and mitochondrial matrix that participates in the defense mechanism. It is generally activated before the initiation of chronic oxidative stress and catalyzes the reduction of lipid and non–lipid hydroperoxides using two molecules of GSH and thereby curtails the quantity of biomolecules having destructive properties[34].

Similarly, GST is a soluble protein located in cytosol and plays an important role in detoxification and excretion of xenobiotics[35]. GST catalyzes the conjugation of the thiol functional groups of GSH to electrophilic xenobiotics and results in increasing solubility. The xenobiotic–GSH conjugate is then either eliminated or converted to mercapturic acid[31]. Since GST increases solubility of hydrophobic substances, it plays an important role in storage and excretion of xenobiotics. Induction of xenobiotic detoxifying enzymes is an additional mechanism by which antioxidant rich extracts may act as anticarcinogens as they compete with steps in xenobiotic activation and metabolize toxic compounds to non–toxic ones[4]. As the activity of GST significantly increased in FIE treated group III and IV rats, it appears that the drug induces greater coupling of electrophilic intermediates with GSH.

From our study, it is concluded that the administration of *Fumaria indica* possesses antihepatocellular carcinoma activity as evidenced by the significant and dose dependent decrease in lipid peroxidation (LPO) and increase in the level of antioxidant enzymes (GSH, CAT, SOD, GPx and GST) through scavenging of free radicals, or by enhancing the activity of antioxidant, which then detoxify free radicals. These factors protect cells from ROS damage in NDEA and CCl4–induced hepatocarcinogenesis. Thus, present investigation suggested that the *Fumaria indica* would exert a chemoprotective effect by reversing the oxidant–antioxidant imbalance during hepatocarcinogenesis induced by NDEA and CCl4.

Conflict of interest statement

The authors declare that there are no conflicts of interest.

Acknowledgements

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