RESEARCH

Exploration of anti-breast cancer effects of *Terminalia chebula* extract on DMBA-induced mammary carcinoma in Sprague Dawley rats

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Abstract

Background: Plant extracts are effectively acting as the natural medicinal cocktail, non-side effective, efficacious, and freely available. The present study aimed to unveil the pharmacological and medicinal effects of *Terminalia chebula* plant extract in 7,12-dimethylbenzanthracene (DMBA)-induced mammary carcinoma in Sprague Dawley rats. The plant extract obtained was subjected to in vivo antioxidant and anticancer studies in various concentrations after an analytical technique such as FTIR, GCMS, and HPLC-based chemo-profiling in Sprague Dawley rats.

Results: Apart from the antiproliferative effect on breast cancer cell line (MCF-7) and normal breast epithelial cells (MCF-10a), we have measured the changes in body weight, along with other tumor parameters such as tumor volume, tumor incidence, tumor weight, tumor burden, serum biochemical parameters, and histopathological findings of breast tissue. As the oxidative stress further enhances the development of cancer, the antioxidant property of the plant extract demonstrates its use against cancer treatment. One hundred fifty milligrams per milliliter (IC$_{50}$ 250 μg/mL) concentration of the ethanolic extract was vital for the proliferation of MCF-7 cell lines (Fig. 7a). Meanwhile, 300 μg/mL (IC$_{50}$ 150 μg/mL) was an effective dose to attain a maximum HDAC inhibition of 78%. Also, the normal liver and kidney functioning revealed the non-toxicity nature of the plant.

Conclusion: *Terminalia chebula* could be one of the effective naturally obtained anti-breast cancer medications. Isolation and characterization of individual bioactive compounds of *T. chebula* would be the future perspective.

Keywords: Anti-proliferation, Breast cancer, Dimethylbenzanthracene (DMBA), MCF-7, MCF-10, Phytomedicine, Sprague Dawley rats, *Terminalia chebula*

Background

Cancer is one of the major public health burdens in both developed and developing countries that arise due to molecular changes within the cell. The search for a new pharmacotherapy from medicinal plants to treat cancers has considerably progressive. A wide range of researchers who keenly discover synthetic medicines to treat breast cancer

[1–6]. But still, the safety is questioned. Plants synthesize numerous medicinally important chemical compounds that are functioning as defensive agents against pathogens and other disease precursors [7–9]. The crude extracts of many plants have been reported to harbor a multi-beneficial effect on diseases and disorders. Most of the recent researches are focusing on obtaining effective anticancer therapeutics [10–13]. Breast cancer is the second leading cause of death among women. High rates of breast cancer burden have created a deep impact on society [14–17]. Most breast cancers are estrogen-dependent and there has been a relapse in

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30-40% of patients receiving adjuvant tamoxifen therapy. So, resistance to endocrine therapy seems to be a clinical problem [18].

The phytochemical screening of medicinal plants is important in identifying new sources of therapeutically important compounds [19]. Presently, various therapies are available for the treatment of cancer, such as chemotherapy, radiotherapy, besides these expensive therapies phytotherapy plays a significant role in the treatment of cancer [20]. The main aim of our present research work is to determine the anticancer potential of the ethanolic extract of the fruits of *Terminalia chebula*. It is generally known as black or chebulic myrobalan, native to South Asia [21]. Anticancer and antiulcer effects of *T. chebula* fruits were previously evaluated in a rat model [22–24]. There exists a strong relationship between cancer and oxidative stress and many reports dealing with the antioxidant properties of *T. chebula* in a recent decade [25–28]. So, the effect of antioxidant enzymes investigated with liver and kidney toxicity profile analysis.

Methods
Plant habitat, plant material, and extract preparation
The focus of the study area situates between the two districts of Salem and Tiruchirappalli of Tamil Nadu, India. The hills lie between 78.31 longitudes and 11.28 latitude, the plant found in the selective area of Pachamalai hills, Tamil Nadu, India. The fruits of *Terminalia chebula* Retz were obtained from the Pachamalai hills of the Tiruchirappalli district. The fruits identified by the help of Dr. John Britto, Director, Rapinet Herbarium, Department of Botany, St. Joseph College, Trichy but a voucher specimen of this material has not been deposited. The experiment was designed by choosing low, moderate, high, and below the lethal or toxic dose to evaluate the comparative effect of the extract. The oral LD_{50} value of plant extract in the case of SD rats was found to be 800 mg/kg. Based on the MTT assay, the IC_{50} value of ethanolic extract was found to be 334.54 μg/ml.

Analytical techniques-based chemo-profiling—chromatography and mass spectrometry
All organic chemicals and solvents required for all assays and biological studies procured from Sigma-Aldrich, Merck, and Himedia. The crude biomass obtained was analyzed with thin layer chromatography (TLC) followed by GCMS (gas chromatography and mass spectrometry). TLC analysis was executed with Merck pre-coated plates (silica gel 60 F254). FTIR (Fourier-transform infra-red spectroscopy) and HPLC (high-performed liquid chromatography) based chemo-profiling was also established. The experiment was executed on an Agilent 1260 series HPLC (Agilent extend-C18 column). Column chromatography is used to extract individual fractions. The

individual fractions (single spot in TLC) were subjected to NMR (nuclear magnetic resonance) studies. GC-MS library (NIST) search result-based chemo profiling was taken an account to confirm the single product by comparing the molecular formula and molecular weight in comparison with *rf* values obtained from TLC experiments. Direct methods and drawn using the ChemDraw 15.0 software, solved the structures.

Animal study
For the experiments, 21 days old female Sprague Dawley rats of 65-70 g were used. All rats were kept at room temperature of 22 °C under 12 h light/12 h dark cycle in the animal house. Rats were fed with standard food. "Principles of laboratory animal care" (NIH publication No. 85-23, revised 1985) were followed or comply with standards equivalent to the UKCCCR guidelines for the welfare of animals in experimental neoplasia [29]. All animals were euthanized using a chemical method (chloroform).

Preparation of DMBA and experimental design for treatment-oriented study
The DMBA was purchased from Sigma Chemicals, Mumbai, India; DMBA was stored at −20 °C to prevent its decomposition. The tamoxifen citrate (standard drug) dissolved in a 0.2-ml peanut oil vehicle. Experimental rats were divided into 5 groups of six animals each. Table 1 describes the divided animal group along with other experimental design parameters. A tumor formed animals selected to given treatment for 30 days [30]. To all the 24 animals, except control group was induced mammary carcinogen (25 mg/kg) by single gastric intubation in 1 ml olive oil, during which the Latency period (the number of days between the DMBA injection and the appearance of the first tumor in each rat) was noted. The tumor was identified by palpation. A tumor formed animals were selected for treatment for 30 days.

Induction of cancer and experimental design for the treatment-oriented study
On the 31st day, the animals were anesthetized, blood was collected through the retro-orbital sinus and used for the estimation of hematological parameters and then the collected blood was centrifuged and the serum was collected [31]. The collected serum was used to study the biochemical parameters. After the blood collection, animals were sacrificed through cervical decapitation, and the entire liver and kidney were perfused immediately with ice-cold 0.9% sodium chloride, thereafter, carefully removed, trimmed free of extraneous tissue finally the mammary tumors were excised out and the parameters such as tumor incidence (% of animals that develop at least one tumor), tumor burden (average

Table 1 Animal study design details

Animal groups	Treatment
Group I	Control rats were given only saline
Group II	Rats given DMBA (25 mg/kg) was induced by single gastric intubation in 1 ml olive oil
Group III	Rats treated with DMBA (25 mg/kg) was induced by single gastric intubation in 1 ml olive oil + tamoxifen citrate 10 mg/kg (PO)
Group IV	Rats treated with DMBA (25 mg/kg) was induced by single gastric intubation in 1 ml olive oil + extract 250 mg/kg
Group V	Rats treated with DMBA (25 mg/kg) was induced by single gastric intubation in 1 ml olive oil + extract 500 mg/kg

number of tumor/animal), tumor volume, tumor weight, and histopathological effects were studied.

Histopathology
Thin pieces of 3 to 5 mm thickness were collected from tissues showing gross morbid changes along with normal tissue. Kept the tissue in fixative (10% formalin) for 24-48 h at room temperature. Deparaffinized the section by xylol 5 to 10 min and removed xylol by absolute alcohol [32, 33]. The cleaned section was stained with hematoxylin for 3-4 min and then counterstained with 0.5% eosin until the section appears light pink 15 to 30 s. Blotted and dehydrated in alcohol and cleared with xylol 15 to 30 s. Mounted on a Canada balsam or DPX mutant and kept the slide dry and remove air bubbles.

In vivo antioxidant activity
The animals were sacrificed after treatment. Liver samples were isolated and washed with normal saline and stored for 12 h for in vivo antioxidant studies [34]. The separated liver was homogenized with motor-driven Teflon coated homogenizer in ice-cold 10% (w/v) 0.1 M Tris-HCl buffer pH 7.4 to get 10% homogenate. The homogenate was centrifuged at 10,000 rpm for 10 min at 5 °C. The supernatant was collected and used for following in vivo studies.

Enzymatic anti-oxidant activity
Estimation of superoxide dismutase (SOD) activity was carried out by using 0.5 ml of supernatant tissue homogenate added to 1.5 ml of carbonate buffer pH 10.2, 0.5 ml of 0.1 Mm EDTA, and 0.4 ml of epinephrine then OD read at 480 nm. The activity of SOD was expressed as units/min/mg protein. One unit of the enzyme is defined as the amount of enzyme, which inhibits the rate of adrenaline autooxidation by 50% [35]. Estimation of catalase activity was analyzed using an assay mixture contained 4 ml of hydrogen peroxide, 5 ml of phosphate buffer, and 1 ml of homogenate. One milliliter portions of the reaction mixture were withdrawn and blown into 2 ml of dichromate/acetic acid reagent at 1 min intervals. Then the mixture was incubated for 30 min later, the OD was measured at 570 nm. The activity of catalase was expressed as μmole of the H_2O_2 consume/mg protein [36]. To estimate glutathione peroxidase (GPx) activity, a reaction mixture consisted of

0.2 ml each of EDTA, sodium azide, H_2O_2, 0.4 ml of phosphate buffer, 0.1 ml homogenate/mitochondria and was incubated at 37 °C at different time intervals. The reaction was arrested by the addition of 0.5 ml of TCA and the tubes were centrifuged at 2000 rpm. To 0.5 ml of supernatant, 4 ml of disodium hydrogen phosphate, and 0.5 ml DTNB were added and the color developed was read at 420 nm immediately. The activity of GPx was expressed as μmoles of glutathione oxidized/mg protein [37].

Non-enzymatic anti-oxidant activity
Estimation of reduced glutathione (GSH) activity carried out using 1 ml of the homogenated precipitate. With 1 ml of TCA and the precipitate was removed by centrifugation. To 0.5 ml of supernatant, 2 ml of DTNB was added and the total volume was made up to 3 ml with phosphate buffer. The absorbance was read at 412 nm. The level of glutathione was expressed as μg/mg protein [38]. In brief, 0.1 ml of tissue homogenate (Tris-HCl buffer, pH 7.5) was treated with 2 ml of 1:1:1 ratio TBA-TCA-HCl reagent (thiobarbituric acid 0.37%, 0.25 N HCl, and 15% TCA) and placed in the water bath for 15 min, cooled. The absorbance of the clear supernatant was measured against reference blank at 535 nm. The level of lipid peroxides was expressed as nmoles of MDA formed/mg protein [39]. Estimation of ascorbic acid (vitamin C) was conducted by adding 0.5 ml of water and 1 ml of TCA was added to 0.5 ml of homogenate, mixed thoroughly, and centrifuged. To 1 ml of the supernatant, 0.2 ml of DTC reagent (2,4 DNPH, thiourea, $CuSO_4$) was added and incubated at 37 °C for 3 h. Then 1.5 ml of sulfuric acid was added, mixed well, and the solutions were allowed to stand at room temperature for another 30 min. The color developed was read at 520 nm. The level of ascorbic acid was expressed as μg/mg protein [40].

Determination of serum biochemical parameters for the liver function and aspartate aminotransferase (AST)/SGOT
The serum collected after centrifugation of collected blood was analyzed for various biochemical parameters like SGOT, SGPT, and ALP. Serum glutamate oxaloacetate transaminase (SGOT) catalyzes the transamination of L-aspartate and α-ketoglutarate to form oxaloacetate and L-glutamate. Oxaloacetate formed is coupled with 2, 4-dinitrophenylhydrazine (DNPH) to form a hydrazone,

a brown-colored complex in an alkaline medium that can be measured calorimetrically [41].

AST (GOT) activity in IU/L = [(Absorbance of the test −Absorbance of control)/(Absorbance of standard−Absorbance of blank)] × concentration of the standard

Determination of alanine aminotransferase (ALT)/SGPT and alkaline phosphatase (ALP)

Serum glutathione peroxidase (SGPT) catalyzes the transamination of L-alanine and α ketoglutarate to form pyruvate and L-glutamate. Pyruvate so formed is coupled with 2,4–dinitrophenylhydrazine to form a corresponding hydrazone, a brown-colored complex in an alkaline medium that can be measured calorimetrically [41].

ALT (GPT) activity in IU/L = [(Absorbance of a test −Absorbance of control)/(Absorbance of standard−Absorbance of blank)] × concentration of the standard

Alkaline phosphatase from serum converts phenyl phosphate to inorganic phosphate and phenol at pH 10.0. Phenol so formed reacts in an alkaline medium with 4-aminoantipyrine in presence of the oxidizing agent potassium ferricyanide and forms an orange-red colored complex, which can be measured spectrometrically. The color intensity is proportional to the enzyme activity [42].

ALP = [(O.D. Test−O.D. Control)/(O.D. Standard−O.D. Blank)] × 10

Colorimetric histone deacetylase activity (HDAC) assay

A commercial HDAC assay kit was used to determine the effect of HDAC inhibition potential of *T. chebula* extracts. The experimental protocol was followed as per the manufacturer's instructions (BioVision, Inc., USA). The different amounts of extracts (0.25-5.0 mg/mL) taken in each reaction well, ddH$_2$O was added to have a final volume of 150 μL. For the negative control, 150 μL of ddH$_2$O was used. In the assay well, 25 μL of the HDAC assay buffer and 5 μL of the HDAC substrate was added and mixed thoroughly by pipetting. The mixture was incubated at 37 °C for 90 min. Further, the reaction was stopped by adding 5 μl of developer solution to each reaction well and carefully mixed by pipetting and incubated at 37 °C for another 30 min. Absorbance was read at 405 nm on a plate reader (xMARK ELISA reader, Bio-Rad). Calculate sample HDAC activity.

Sample HDAC activity (OD$_{405nm}$/μg) = ΔOD$_{405nm}$, sample/sample mass (μg)

Cell culture conditions and antiproliferative activity assay

MCF-cell lines procured from NCCS, Pune, India. All experiments were performed with mycoplasma-free cells. MCF-7 cell line has been authenticated using STR (or SNP) profiling within the last 3 years has been included. The name MCF-7 cell lines are the official name and its Research Resource Identifier (RRID) is Cellosaurus MCF-7 (CVCL_0031). MCF-7 cells obtained were preserved and maintained in DMEM medium supplemented with 10% fetal bovine serum, streptomycin (100 μg/ml), and penicillin (100 U/ml) [43–45]. The cells were further incubated at 37 °C in a humidified (5% CO$_2$) atmosphere. Cells were seeded in a 96-well microtiter plate at a cell density of about 5000 cells/well and incubated for 24 h. Sample groups were treated with different concentrations of *T. chebula* extracts (25-125 μg/mL). The extracts were dissolved in 100 μL of dimethyl sulfoxide (DMSO) in sterile distilled water containing tubes. Vehicle control groups were added with DMSO (final concentration of 0.05%) or ddH$_2$O. Each concentration of the extract was added to the cultured well in triplicate. DMEM was served as the negative control and doxorubicin (100 μg/mL) was served as a positive control. The cells harvested after confluent (± 3 days) were stained using Trypan Blue Dye and assessed further.

Statistical analysis

All results were compared by performing one-way ANOVA with Dunnett's post test. All the results were stated as a proportion/ratio increase or decrease concerning the control result values. The GraphPad Prism software, version 8.1 was used for all statistical analyses. A variance was deliberated statistically significant if $p \leq 0.05$.

Results

Effect of ethanolic extract of fruits of *T. chebula* Retz on tumor parameters

Based on the assessment parameters and the results obtained from the tumors excised from all the tested rats, it was found that the tumor volume, tumor weight, tumor burden, and tumor incidence was found to be increased in the DMBA-treated groups when compared with the control group. There was a significant decrease in the above-tabulated tumor parameters in animals administered with tamoxifen and ethanolic extract of *T. chebula*. The decrease was also dose-dependent compared with the two doses used for the study, 500 mg/kg demonstrated a rapid and significant decrease in tumor volume, tumor weight, and tumor burden, and tumor incidence when compared with DMBA-induced tumor rats (Table 2).

Chemo-profiling through analytical techniques

Crude mass was analyzed for chemo-profiling by the subject it to various analytical techniques such as FT-IR, GC-MS (Ref. Figure S3 for method), and HPLC (Ref. Figure S4 for sample analysis). Table S1 depicts the results of GCMS chemo-profiling (Ref. Figure S1 for obtained GC). The given extract was combusted up to a retention time (RT) of 35. Within this RT range, the compound peaks started from 2.94, 16.26 to 31.02. The corresponding peaks were used for the NIST library search, and the obtained

Table 2 Effect of ethanolic extract of *T. chebula* on tumor parameters

Group	Tumor volume (mm³)	Tumor weight (mg)	Tumor burden (n)*	Tumor incidence (%)
Group I	0.00 ± 0.00[b]	0.00 ± 0.00[b]	0.00 ± 0.00[b]	0.00 ± 0.00[b]
Group II	0.83 ± 0.07[a]	122.7 ± 3.01[a]	7.83 ± 0.75[a]	6.00 ± 0.89[a]
Group III	0.27 ± 0.07[ab]	34.00 ± 2.36[ab]	3.83 ± 0.75[ab]	2.66 ± 0.51[ab]
Group IV	0.47 ± 0.12[ab]	66.00 ± 2.36[ab]	5.00 ± 0.89[ab]	5.00 ± 0.89[ab]
Group V	0.44 ± 0.01[ab]	54.67 ± 2.58[ab]	4.66 ± 1.36[ab]	4.83 ± 0.75[ab]

*n denotes an average number of tumors per animal; values are expressed as mean ± S.D
Statistical significance (p) was calculated by one-way ANOVA followed by Tukey's multiple comparison tests
The control group was compared with normal; standard and test groups were compared with control; $P < 0.05$ considered significant

compounds were tabulated. The individual compound structures were drawn by the Chemdraw 15.0 software. Starting from phenolic compounds, esters, fatty acids (saturated and unsaturated organic) based compounds were found in the extract (Figure S2a-c). Exactly twelve elevated peaks were remarkably found both in GCMS and HPLC (Fig. 1) analysis.

This shows the resemblance of both chromatography technique results. According to FTIR results (Fig. 2), 3344 and 3201 peaks are indicating C-H stretch. The remarkable 2922 and 2852 peaks are representing C=N groups availability in the *T. chebula* extract. C=O presence is also recognized by the availability of peak range from 1722 to 1178.

Histopathology of breast tissue

In the histopathology reports, the section from breast obtained from normal rats displayed no evidence of malignancy. Tumor-induced rats containing only DMBA treatment showed an infiltrating neoplasm composed of cells arranged in glands as seen in the picture (Fig. 3). Individual cells were round to oval with moderate eosinophilic cytoplasm and round oval vesicular nuclei with some showing nucleoli. Areas of comedo necrosis were also seen (Fig. 3a). The sections of breast tissue of tamoxifen-treated DMBA-induced SD rats revealed a circumscribed lesion with areas of adenosis and fibrosis. No evidence of malignancy was observed (Fig. 3b). Two hundred fifty milligrams per kilogram of a crude ethanolic extract of fruits of *T. chebula*-treated DMBA-induced SD rats tissue sections from breast developed circumscribed lesion with diffusing hyperplasia of ducts (adenosis) separated by fibro collagenous stroma. No sign of malignancy was reported (Fig. 3c). A high dose of the crude extract (500 mg/kg) treated DMBA rat sections revealed a cystic lesion composed of diffused
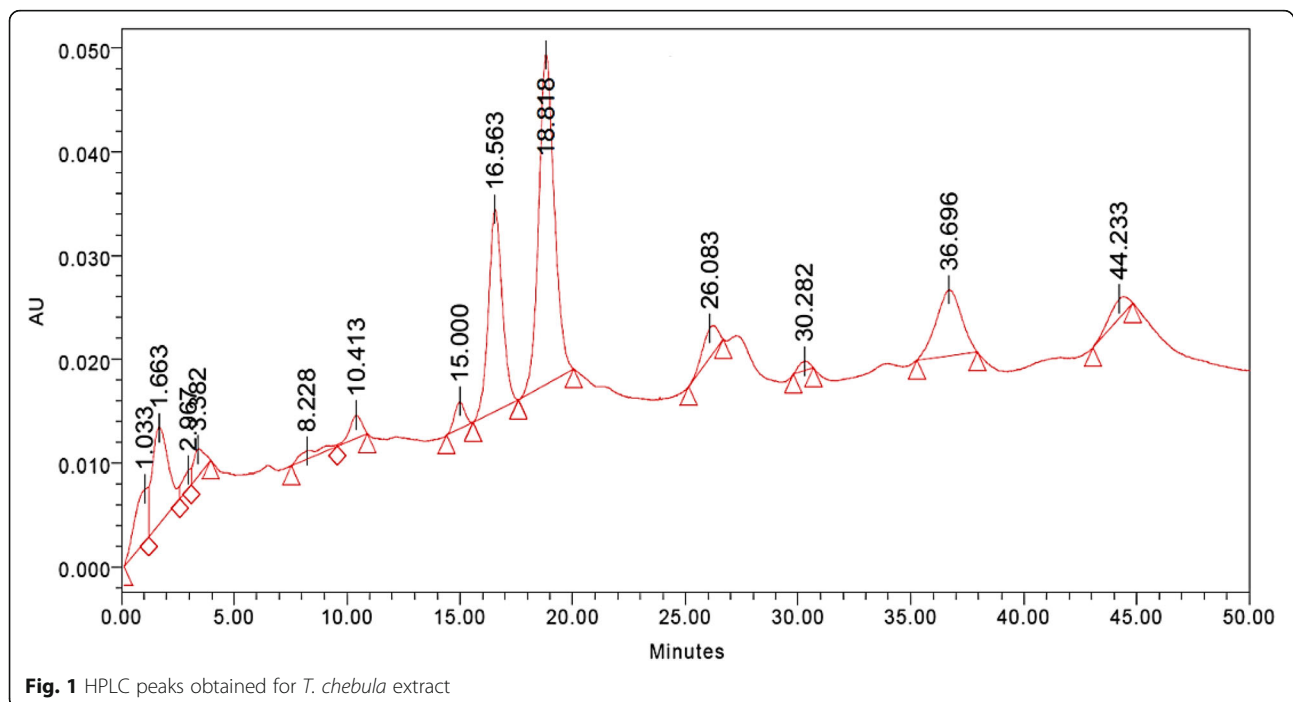
Fig. 1 HPLC peaks obtained for *T. chebula* extract

Fig. 2 FTIR results of *T. chebula* extract
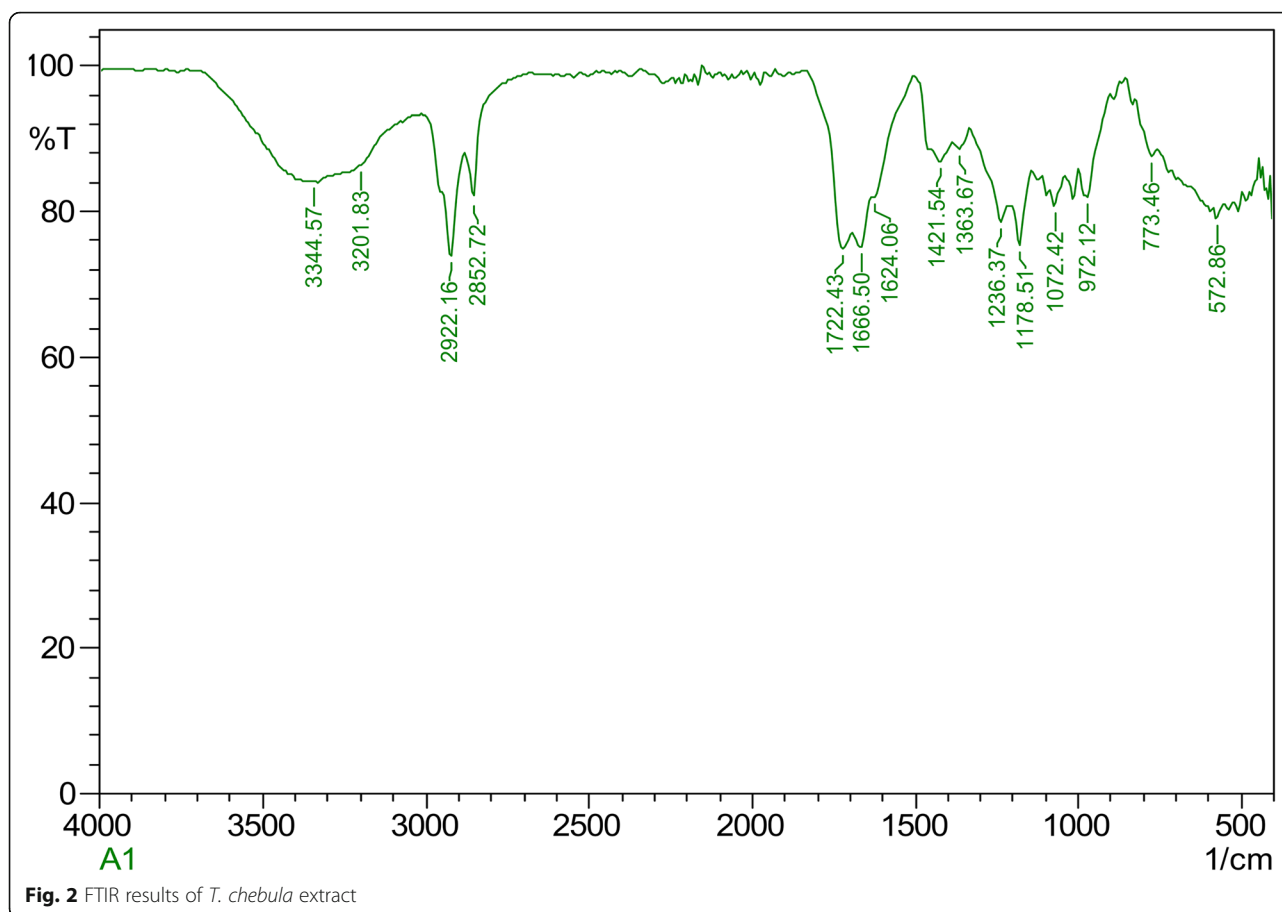
infiltration of neutrophils. Stroma showed congested vessels and fibro-collagenous tissue. No sign of malignancy was seen (Fig. 3d).

In vivo antioxidant activity
In vivo, antioxidant studies were carried out on DMBA-induced mammary carcinoma in female Sprague Dawley rats because oxidative stress is associated with cancer. The following antioxidants enzymes were determined in the liver tissues of all tested rats. Figure 4 represents all the obtained results.

Effect of ethanolic extract of *T. chebula* on superoxide dismutase (SOD)
A deep decrease in the SOD level was observed in DMBA-induced rats 3.05 ± 0.16 unit/mg compared to normal rats. The decrease seems to be statistically significant. When DMBA-induced rats were treated with *T. chebula* extract (250 mg/kg and 500 mg/kg), the SOD levels increased. When 200 mg/kg of the extract was administered, the SOD level was 3.78 ± 0.14unit/mg and when the extract concentration was increased to 500 mg/kg, there was a change in the SOD level as seen in Fig. 4a (Table S1). A significant

increase in SOD was observed in the case of tamoxifen-treated DMBA rats that coincide with the data obtained from the ethanolic extract-treated group.

Effect of ethanolic extract of *T. chebula* on catalase (CAT)
A deep decrease of CAT level was observed in DMBA-induced rats 60.67 ± 1.03 unit/mg when compared to normal rats. The decrease seems to be statistically significant. When DMBA-induced rats were treated with *T. chebula* extract (250 mg/kg and 500 mg/kg), the CAT levels increased. When 200 mg/kg of the extract was administered, the CAT level was 73.67 ± 1.63 unit/mg and when the extract concentration was increased to 500 mg/kg, there was a change in CAT level as seen in Fig. 4b (Table S2). A significant increase in CAT was observed in the case of tamoxifen-treated DMBA rats that coincide with the data obtained from the ethanolic extract-treated group.

Effect of ethanolic extract of *T. chebula* on lipidperoxidase (LPO)
A deep increase of LPO level was observed in DMBA-induced rats 35.83 ± 1.47 nMMDA/g of protein when compared to normal rats. The decrease seems to be
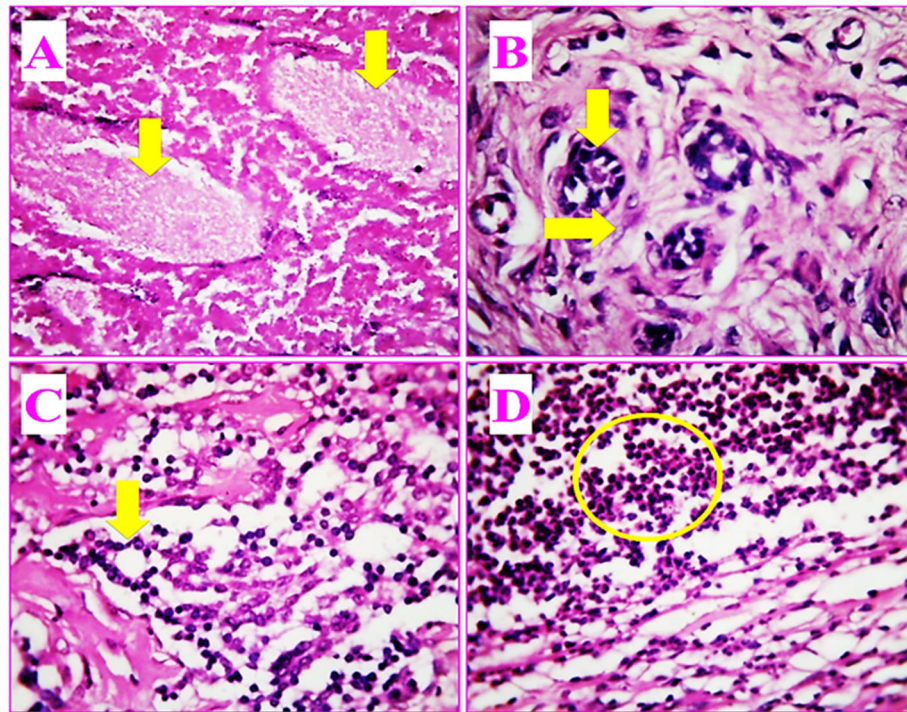
Fig. 3 Histopathology of breast tissues after *T. chebula* treatment. **a** A section from breast shows infiltrating ductal carcinoma (down arrows) (group II). **b** A section from breast shows areas of adenosis (down arrow) and fibrosis (straight arrow) (group III). **c** A section from the breast shows micro glandular adenosis (down arrow) (group IV). **d** A section from the breast shows infiltration of neutrophils (group V)

statistically significant. When DMBA-induced rats were treated with *T. chebula* extract (250 mg/kg and 500 mg/kg), the LPO levels decreased. When 200 mg/kg of the extract was administered, the LPO level was 25.67 ± 1.21 nMMDA/g of protein and when the extract concentration was increased to 500 mg/kg, there was a change in LPO level as seen in Fig. 4c (Table S2). A significant decrease in LPO was observed in the case of tamoxifen-treated DMBA rats that coincide with the data obtained from the ethanolic extract-treated group.

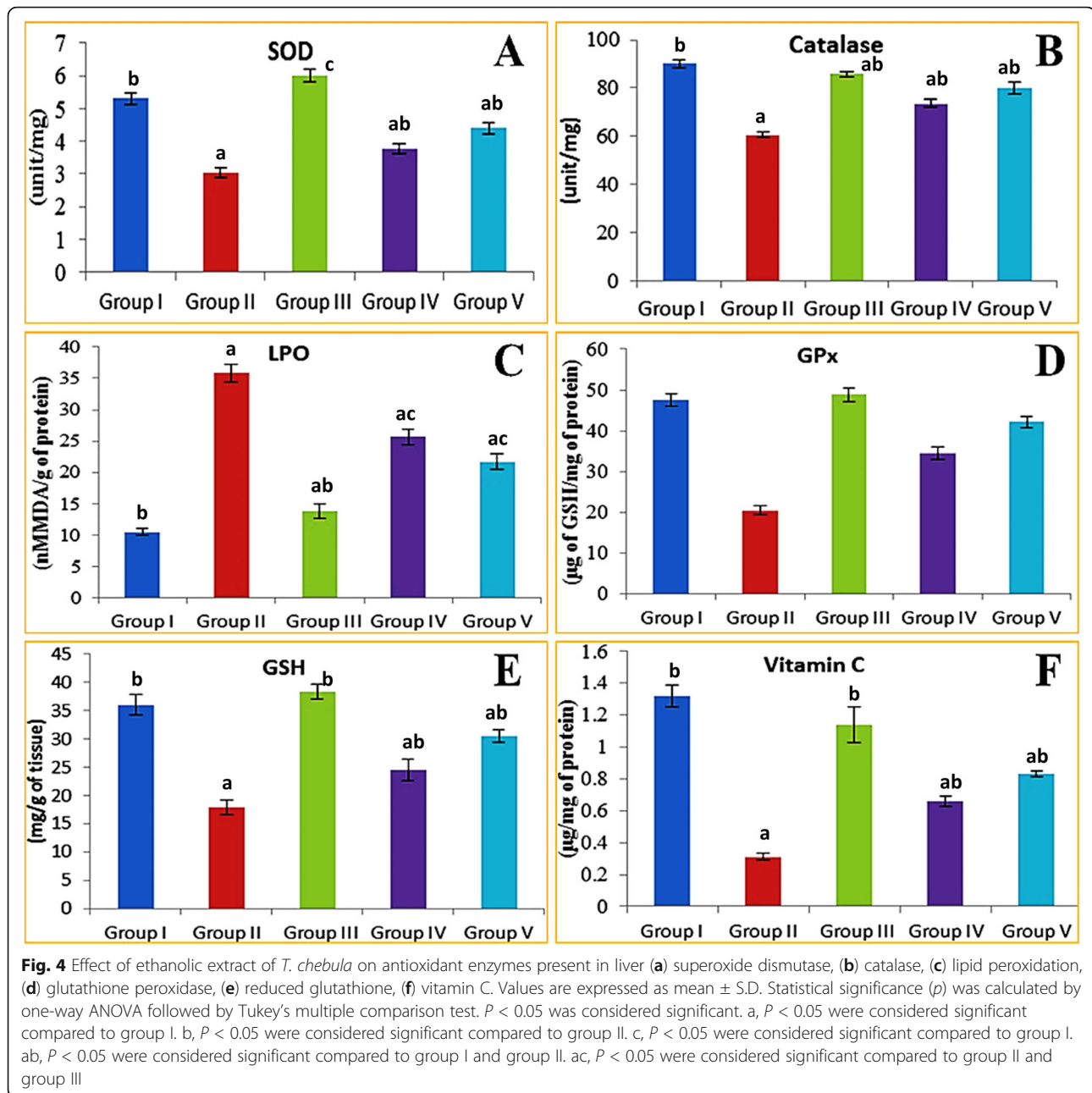### Effect of ethanolic extract of *T. chebula* on glutathione peroxidase (GPx)

A deep decrease of GPx level was observed in DMBA-induced female SD rats 20.50 ± 1.04 µg of GSH/mg of protein when compared to normal rats. The decrease seems to be statistically significant. When DMBA-induced rats were treated with *T. chebula* extract (250 mg/kg and 500 mg/kg), the GPx levels increased. When 200 mg/kg of the extract was administered, the GPx level was 34.50 ± 1.64 µg of GSH/mg of protein and when the extract concentration was increased to 500 mg/kg, there was a change in GPx level as seen in Fig. 4d (Table S1). A significant increase in GPx was observed in the case of tamoxifen-treated DMBA rats that coincides with the data obtained from the ethanolic extract-treated group.

Effect of ethanolic extract of *T. chebula* on reduced glutathione (GSH)

A deep decrease of GSH level was observed in DMBA-induced rats 17.83 ± 1.32 mg/g of tissue when compared to normal rats. The decrease was found statistically significant. When DMBA-induced rats were treated with *T. chebula* extract (250 mg/kg and 500 mg/kg), the GSH levels increased. When 200 mg/kg of the extract was administered, the GSH level was 24.50 ± 1.87 mg/g of tissue and when the extract concentration was increased to 500 mg/kg, there was a change in GSH level as seen in Fig. 4e (Table S2). A significant increase in GSH was observed in the case of tamoxifen-treated DMBA rats that coincides with the data obtained from the ethanolic extract-treated group.

Effect of ethanolic extract of *T. chebula* on vitamin C

A deep decrease of vitamin C level was observed in DMBA-induced female SD rats 35.83 ± 1.47 µg/mg of protein when compared to normal rats. The decrease seems to be statistically significant. When DMBA-induced rats were treated with *T. chebula* extract (250 mg/kg and 500 mg/kg), the vitamin C levels decreased. When 200 mg/kg of the extract was administered, the vitamin C level was 0.66 ± 0.03 µg/mg of protein and when the extract concentration was increased to 500 mg/kg, there was a change in LPO level as seen in Fig.

Fig. 4 Effect of ethanolic extract of *T. chebula* on antioxidant enzymes present in liver (**a**) superoxide dismutase, (**b**) catalase, (**c**) lipid peroxidation, (**d**) glutathione peroxidase, (**e**) reduced glutathione, (**f**) vitamin C. Values are expressed as mean ± S.D. Statistical significance (*p*) was calculated by one-way ANOVA followed by Tukey's multiple comparison test. $P < 0.05$ was considered significant. a, $P < 0.05$ were considered significant compared to group I. b, $P < 0.05$ were considered significant compared to group II. c, $P < 0.05$ were considered significant compared to group I. ab, $P < 0.05$ were considered significant compared to group I and group II. ac, $P < 0.05$ were considered significant compared to group II and group III

4f (Table S2). A significant increase in vitamin C was observed in the case of tamoxifen-treated DMBA rats that coincide with the data obtained from the ethanolic extract-treated group.
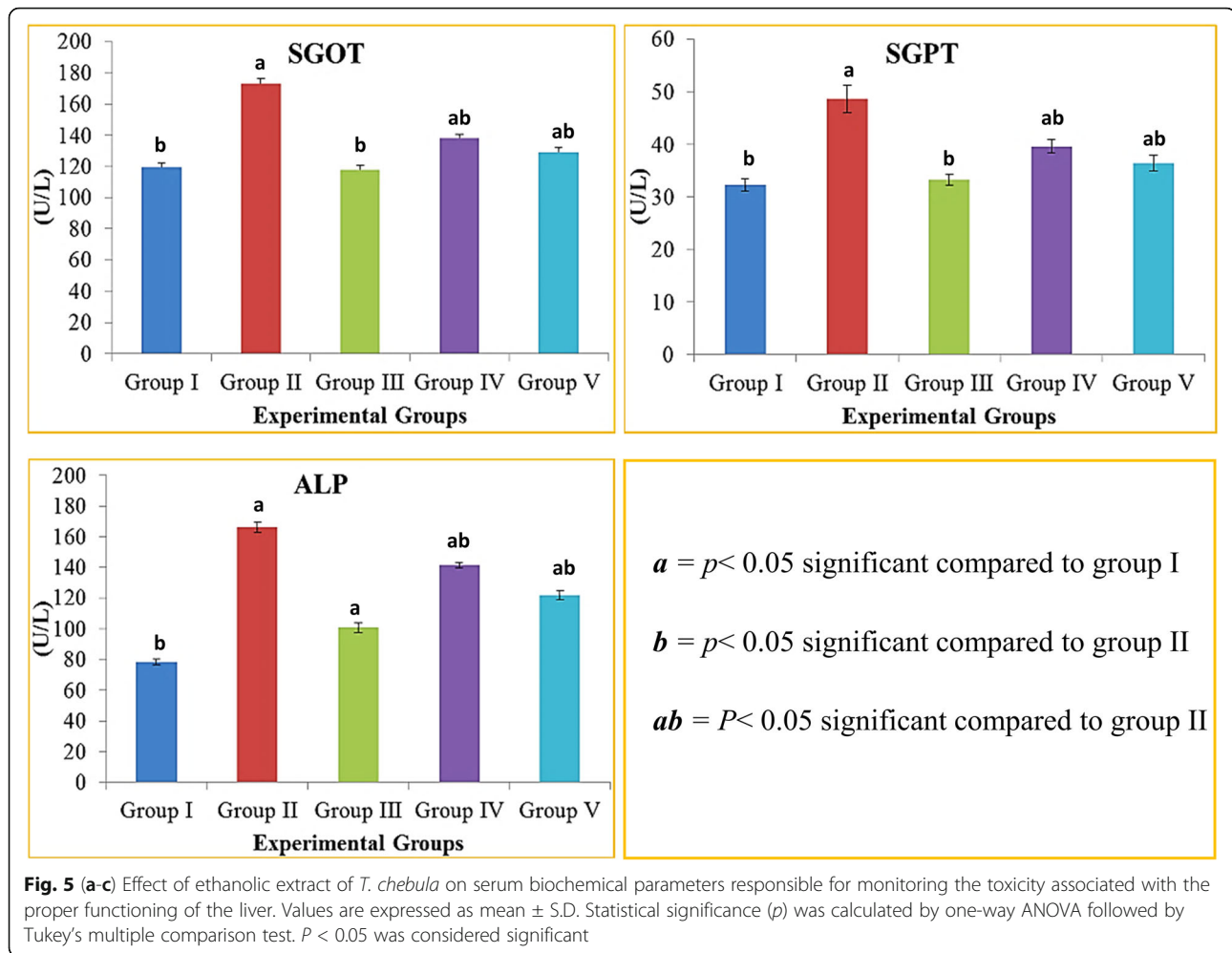
In our study, group II (DMBA-induced rats) showed a significant decrease in SOD, CAT, GSH, and vitamin C levels compared to group I (normal). Conversely, group III (tamoxifen-treated DMBA-induced rats), group IV (ethanolic extract 200 mg/kg treated rats), and group V (ethanolic extract 500 mg/kg treated rats) showed a significant increase in the level of antioxidant enzymes such as SOD, CAT, GSH, GPX, and ascorbate levels

compared to group II. Also, group II showed a significant increase in LPO enzyme compared to group I. Group III, IV, and V showed a significant decrease in the level of liver LPO when compared to group II.

Effect of *T. chebula* on liver functioning (SGOT, SGPT, and ALP)
The present study in DMBA-induced female Sprague Dawley rats represents elevated levels of SGOT, SGPT, and ALP in DMBA induced rats. Figure 5 displays all obtained results of levels of SGOT, SGPT, and ALP in DMBA-induced rats

Fig. 5 (**a**–**c**) Effect of ethanolic extract of *T. chebula* on serum biochemical parameters responsible for monitoring the toxicity associated with the proper functioning of the liver. Values are expressed as mean ± S.D. Statistical significance (*p*) was calculated by one-way ANOVA followed by Tukey's multiple comparison test. *P* < 0.05 was considered significant

Effect of T. chebula extract on serum glutamic oxaloacetic transaminases (SGOT)

The SGOT level of 119.5 ± 3.01 U/L in the normal rats was elevated to 172.9 ± 3.15 U/L in DMBA-induced rats. This increase was found to be statistically significant. Tamoxifen-treated rats demonstrated a decrease in SGOT 118.0 ± 2.75 U/L when compared to the DMBA-induced group. The SGOT levels were found to be 138.3 ± 2.16 U/L and 129.2 ± 2.99 U/L (Fig. 5a; Table S3) in 250 mg/kg and 500 mg/kg of extract-treated rats respectively.

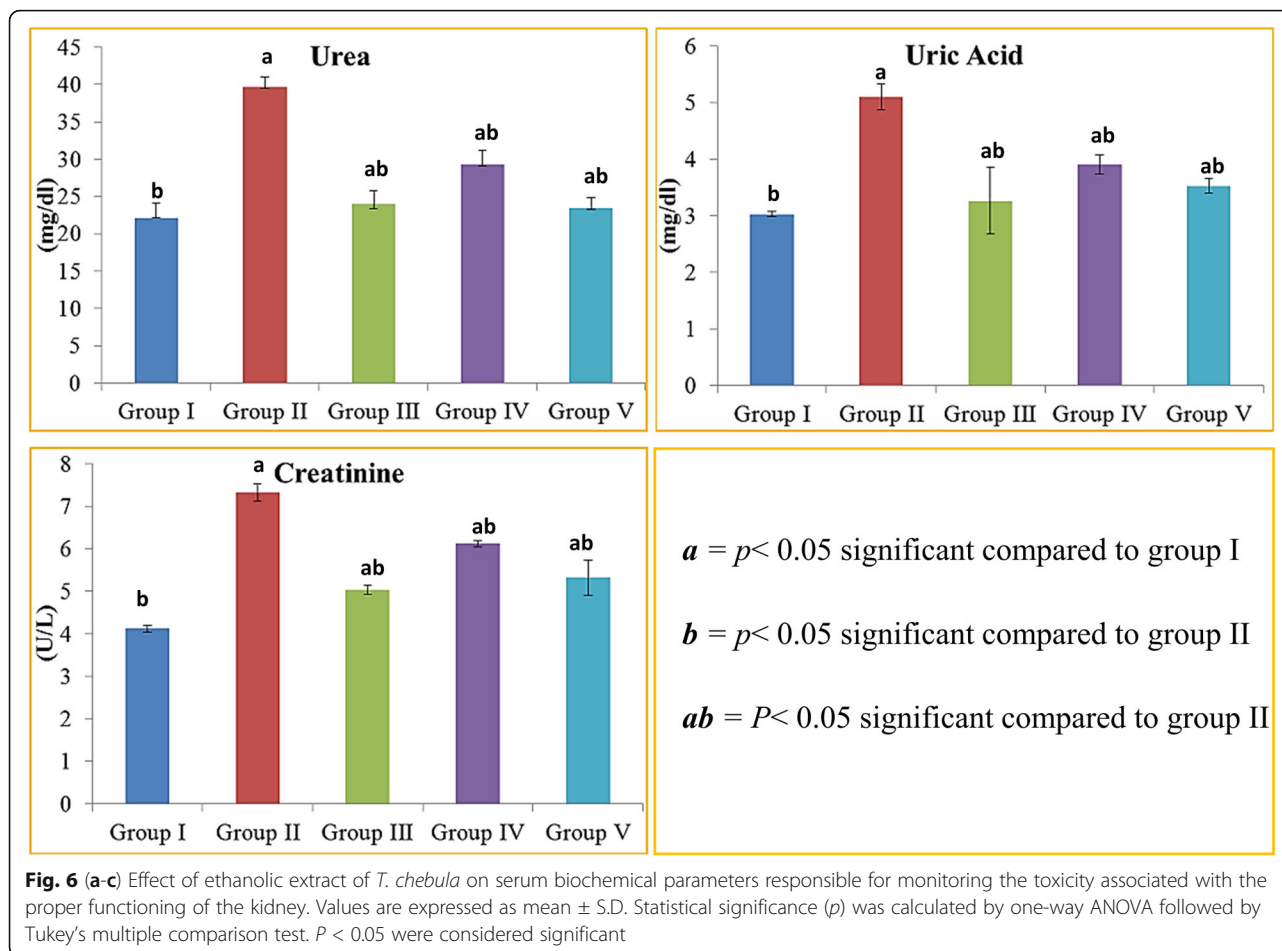### Effect of T. chebula extract on serum glutamic pyruvic transaminases (SGPT)

The SGPT level of 32.33 ± 1.21 U/L in the normal rat was elevated to 48.67 ± 2.58 U/L in DMBA-induced rats. This increase was found to be statistically significant. Tamoxifen-treated rats demonstrated a decrease in SGPT 33.33 ± 1.03 U/L when compared to the DMBA-induced group. The SGPT levels were found to be 39.67 ± 1.21 U/L and 36.50 ± 1.51 U/L (Fig. 5b; Table S3) in 250 mg/kg and 500 mg/kg of extract-treated rats respectively.

Effect of ethanolic extract of T. chebula on alkaline phosphatase (ALP)

The ALP level of 78.57 ± 1.66 U/L in the normal rat was elevated to 166.2 ± 3.54 U/Lin DMBA-induced rats. This increase was found to be statistically significant. Tamoxifen-treated rats demonstrated a decrease in ALP 100.7 ± 3.38 U/L when compared to the DMBA-induced group. The ALP levels were found to be 141.3 ± 1.36 U/L and 121.8 ± 2.92 U/L (Fig. 5c; Table S3) in 250 mg/kg and 500 mg/kg of extract-treated rats respectively.

Effect of ethanolic extract of T. chebula on kidney functioning
Effect of ethanolic extract of T. chebula on urea

Urea level of 22.17 ± 2.04 mg/dL in the normal rat was elevated to 39.70 ± 1.37 mg/dL in DMBA-induced rats. This increase was found to be statistically significant. Tamoxifen-treated rats demonstrated a decrease in urea 24.03 ± 1.79 mg/dL compared to the DMBA-induced group. Urea levels were found to be 29.33 ± 1.86 mg/dL and 23.43 ± 1.34 mg/dL in 250 mg/kg and 500 mg/kg of ethanolic extract-treated rats respectively (Fig. 6a; Table S4).

Fig. 6 (**a**-**c**) Effect of ethanolic extract of *T. chebula* on serum biochemical parameters responsible for monitoring the toxicity associated with the proper functioning of the kidney. Values are expressed as mean ± S.D. Statistical significance (*p*) was calculated by one-way ANOVA followed by Tukey's multiple comparison test. *P* < 0.05 were considered significant

Effect of ethanolic extract of T. chebula on uric acid

The uric acid level of 3.03 ± 0.05 mg/dL in the normal rat was elevated to 5.10 ± 0.23 mg/dL in DMBA-induced rats. This increase was found to be statistically significant. Tamoxifen-treated rats demonstrated a decrease in uric acid 3.26 ± 0.59 mg/dl when compared to DMBA-induced group. The uric acid levels were found to be 3.90 ± 0.17 mg/dL and 3.53 ± 0.13 mg/dL in 250 mg/kg and 500 mg/kg of ethanolic extract-treated rats respectively (Fig. 6b; Table S4).

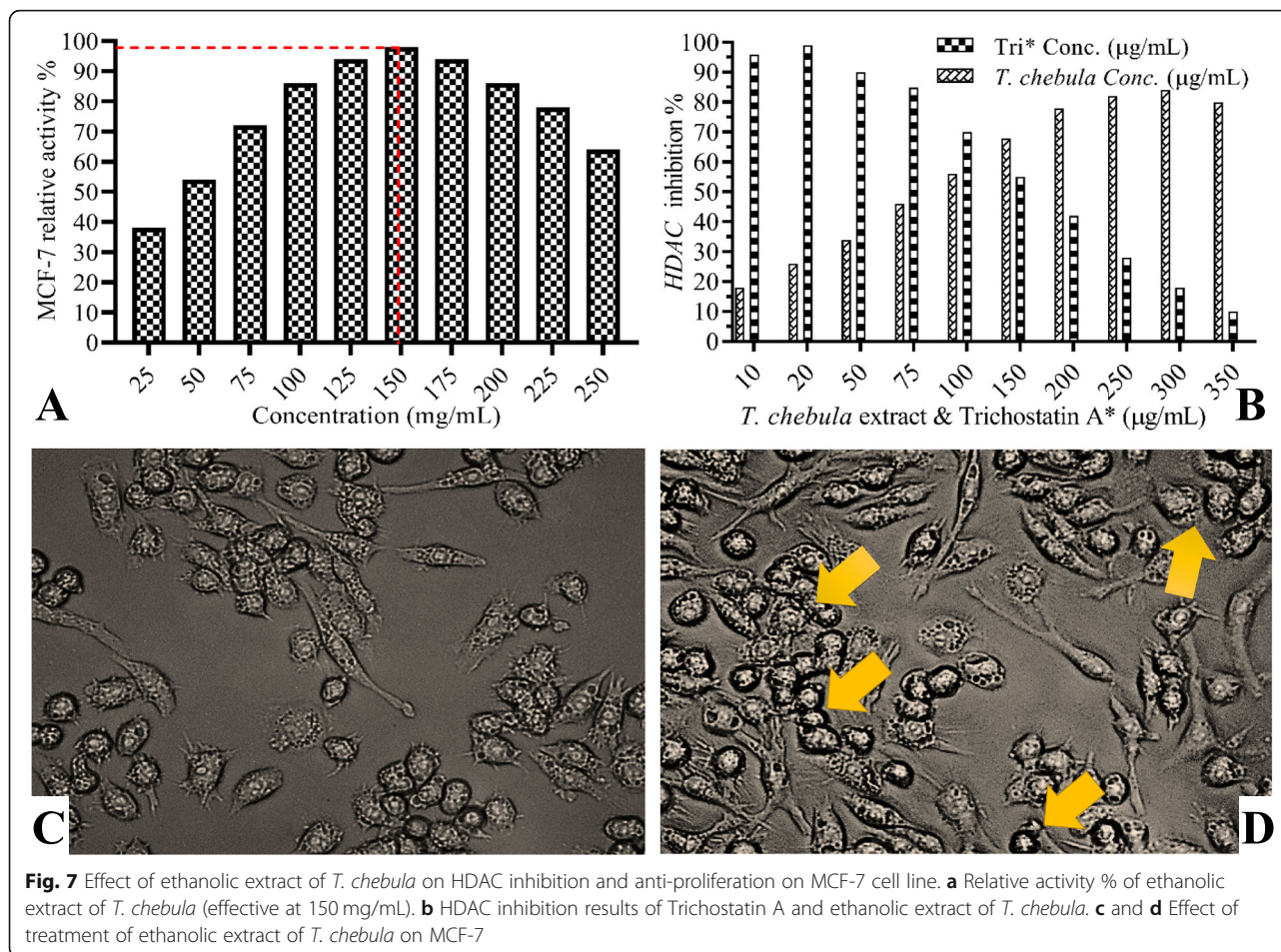### Effect of ethanolic extract of T. chebula on creatinine

The creatinine level of 4.12 ± 0.07 mg/dL in the normal rat was elevated to 7.32 ± 0.21 mg/dL in DMBA-induced rats. This increase was found to be statistically significant. Tamoxifen-treated rats demonstrated a decrease in urea 5.03 ± 0.11 mg/dL when compared to DMBA-induced group. The urea levels were found to be 6.12 ± 0.08 mg/dL and 5.32 ± 0.41 mg/dL in 250 mg/kg and 500 mg/kg of ethanolic extract-treated rats respectively (Fig. 6c; Table S4). Thus, the different doses of ethanolic extract of *T. chebula* proved to be much effective when compared with tamoxifen.

HDAC inhibition and anti-proliferative effects of fruit ethanolic extract of T. chebula

The results depicted in Fig. 7 illustrates the HDAC inhibition and anti-proliferative effect of ethanolic extract of *T. chebula*. One hundred fifty milligrams per milliliter (IC$_{50}$ 250 μg/mL) concentration of ethanolic extract was vital for the proliferation of MCF-7 cell lines (Fig. 7a). Meanwhile, 300 μg/mL (IC$_{50}$ 150 μg/mL) was an effective dose to attain a maximum HDAC inhibition of 78%. Figure 7c (untreated) and Fig. 7d (treated up to 350 μg/mL of extract) illustrates the effect of the treatment of the ethanolic extract of *T. chebula* on MCF-7 cells. These remarkable results denote the effective medicinal values in the treatment of breast cancer.

Discussion

In the case of cancer, the transport function carried out by cell organelles including hepatocyte undergo alteration leading to the release of enzymes owning to altered permeability of the plasma membrane [46]. Recent researches have reported an increase in tumor volume along with elevated levels of SGOT, SGPT, and ALP in tumor-induced rats [42]. ALP's activity affects membrane permeability

Fig. 7 Effect of ethanolic extract of *T. chebula* on HDAC inhibition and anti-proliferation on MCF-7 cell line. **a** Relative activity % of ethanolic extract of *T. chebula* (effective at 150 mg/mL). **b** HDAC inhibition results of Trichostatin A and ethanolic extract of *T. chebula*. **c** and **d** Effect of treatment of ethanolic extract of *T. chebula* on MCF-7

and transport of metabolites and acts as the specific tumor marker during diagnosis and early detection. It plays a key role not only in the transfer of metabolites across the cell membrane but also in the synthesis of proteins and glycogen metabolism. In our study, the ethanolic extract of the fruits of *T. chebula*-mediated suppression of elevated levels of ALP in DMBA-induced rats suggests the possibility of this plant extract to stabilize the plasma membrane [47]. In DMBA-induced rats, treatment with the *T. chebula* showed a 40% tumor volume inhibition and the difference seems to be statistically significant ($p < 0.05$). This demonstrates the tumor growth inhibition potential of the crude extract as reported earlier [48]. The present study of ethanolic extract also demonstrated a dose-dependent activity, which was incomparable to other studies [49, 50].

In a recent investigation, the ethanolic extract of *Argemone Muxicana* L (Papaveraceae), *Polyathia longifolia* (Annonaceae), *Terminalia bellerica*, and *Terminalia chebula* (Combretaceae) were tested for anticancer and antimicrobial activity in vitro [51]. Usage of 80% aqueous acetone extract of cranberry increased apoptosis of MCF-7 cells by 25% at a concentration of 50 g/L with significant arrest in the G1 phase. Evidence suggests that cranberry phytochemicals have a deleterious effect on certain types of tumors by inducing apoptosis, inhibiting proliferation, and colony formation [52]. Another work demonstrated that extracts of the plant *T. occidentalis* decrease tumor weight and volume compared to cancerous control with enhanced body weight and longevity compared to cancerous control and doxorubicin-treated groups [53].

Though some differences are there between rat mammary adenocarcinoma, the degrees of morphological aggressiveness, and other parallel correlations observed between these two parameters suggest DMBA-induced rat model as a current tool for understanding breast cancer [54]. Previously, the anti-tumorigenic potential of *T. chebula* has been assessed, the extract in chemically induced Swiss albino skin tumor mice model [55]. Recently, a triherbal formulation of seed coats of *T. chebula*, dry seeds of *E. ganitrus*, and leaves of *P. cineraria* has reported treating DMBA-induced mammary carcinoma in female Sprague Dawley rats [56]. In the present study, deviated from the abovementioned studies, we have used the ethanolic extract from *T. chebula* fruit to treat DMBA-induced mammary carcinoma in female

Sprague Dawley rats. All these scientific data support the idea of the use of plant-based compounds in the treatment of cancer specifically mammary carcinoma.

Conclusion

In conclusion, we have been able to arrive at a better understanding of the use of *T. chebula* as a carrier of the potential anticancer agent. Chemo-profiling through analytical techniques demonstrated the presence of efficacious bioactive compounds in *T. chebula* extract. The evidence of our in vivo study serves to support our ideology that if further explored this may lead to the development of nouveau drugs of plant origin that could claim medically economic significance beneficial to save the lives of distressed and oppressed mankind.

Supplementary information

The online version contains supplementary material available at https://doi.org/10.1186/s43094-020-00124-z.

Additional file 1: Table S1. GCMS based chemo-profiling of *T. chebula* Retz extract. **Table S2.** Effect of ethanolic extract of T. chebula Retz on antioxidant enzymes. **Table S3.** Effect of ethanolic extract of T.chebula Retz on biochemical parameters. **Table S4.** Effect of ethanolic extract on biochemical parameters. **Figure S1.** GCMS results of Terminalia chebula. **Figure S2a.** Chemoprofiling of T. chebula extract through GCMS-NIST Library search. **Figure S2b.** Chemoprofiling of T. chebula extract through GCMS-NIST Library search. **Figure S2c.** Chemoprofiling of T. chebula extract through GCMS-NIST Library search. **FigureS3.** GCMS method and parameters adopted. **FigureS4.** HPLC sample information.

Plant authentication
The fruits of *Terminalia chebula* Retz were obtained from the Pachamalai hills of the Tiruchirappalli district. The fruits identified by the help of Dr. John Britto, Director, Rapinet Herbarium, Department of Botany, St. Joseph College, Tiruchirappalli, India.

Authors' contributions
MA has designed and completed data acquisition, analysis, interpretation, and drafted the work; RK processed the analytical chemistry work; JR substantively revised the draft; AS carried out in vitro antioxidant assay of plant extract; DPH involved in plant extract preparation, bioactivity, and animal study evaluations. All authors have read and approved the manuscript.

Availability of data and materials
Data and material are available upon request

Ethics approval and consent to participate
All animal procedures performed after approval from the IAEC (Institution of the animal ethical committee) and following the recommendations for the proper care and use of laboratory animals (IAEC NO: KMCRET/Ph.D/11/2014-2015).

Consent for publication
Not applicable

Competing interests
All authors declare that they have no competing interests

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