RESEARCH ARTICLE

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Antioxidant and Anticancer Activity of *Tamarix Indica* Aerial Extracts against DMBA/croton Oil-Induced Skin Carcinogenesis in Mice

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Abstract

Background: The largest organ, skin, acts as the body's first line of protection against various environmental threats and harmful substances, which may be carcinogenic. The widespread acceptance of conventional chemotherapy has been hindered by its high cost and severe side effects, which have encouraged the need for alternatives. The phytoconstituents produced by plants promise to prevent skin carcinogenesis by targeting multiple steps. They are widely accepted, safe, and have few side effects. **Objective:** The current study aimed to determine the antitumor potential of the aerial extracts of *Tamarix indica* in Swiss albino mice by DMBA/croton oil-induced carcinogenesis. **Method:** The antioxidant activity of the extracts was determined by DPPH radical scavenging assay. The animals were randomly divided into 7 groups such as Group I, Normal Control, applied acetone; Group II, Disease Control, applied DMBA and croton oil; Group III, IV, V, and VI given methanol and aqueous extract in 250 and 500 mg/kg doses and Group VII, methotrexate in addition to Group II treatment. At the end of the study, tumor morphological and biochemical parameters were determined. **Results:** The DPPH free radical assay showed antioxidant activity of methanol and aqueous extract of *T. indica* having IC_{50} values of 7.98±0.87 and 14.49±1.01 µg/mL respectively. The results showed a decline in % tumor incidences, burden, and yield, while an increase in average latency period and % inhibition of tumor multiplicity in treatment groups compared to disease control group. Serum biochemical parameters i.e. total protein, LPO, SOD, catalase, GSH, SGOT, SGPT, creatinine, urea, and bilirubin were found to improve towards normal range in the treatment groups compared to disease control group mice. **Conclusion:** The study concluded that *T. indica* extracts have significant antioxidant and chemopreventive activity against DMBA/croton oil-induced carcinogenesis. However specific phytoconstituents and mechanisms of chemoprevention are further to be identified.

Keywords: *Tamarix indica*- Chemoprevention- Carcinogenesis- Antioxidant- Skin cancer

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Introduction

Skin cancer is the leading and fatal public health problem rising continuously throughout the world with special concern for the white skin population [1]. Generally, skin cancer is categorized as nonmelanoma skin cancer (NMSC) and melanoma skin cancer (MSC). Over the past few decades, epidemiologic studies have demonstrated an increase in the incidences of melanoma and NMSC although the reporting and diagnosis rates are low [2]. Skin, being the outermost layer, is exposed continuously to various environmental carcinogens, sun radiations, and microorganisms, increasing the risk of skin cancer [3, 4]. Based on the increasing incidences of skin cancer and the lack of efficient treatments, the urge for new and multiple treatment options developed.

The word "chemoprevention" was first used by Michael Sporn in 1976 and now refers to the use of natural products or synthetic chemicals to prevent tumor initiation or inhibit tumor promotion and progression [5]. Since then, chemoprevention continued to be the subject of ongoing research especially for cancer prevention. Throughout history, people have utilized plants and herbal products for therapeutic purposes. Plants contain a variety of phytoconstituents in addition to their nutritional value. These phytoconstituents are biologically active and may have a potential role in anticipation of various disease conditions via different mechanisms such as modulation of cell signaling or inhibition of cell proliferation and angiogenesis [3]. According to WHO reports, 80% of the world's population still relies on herbal medicine or vegetable extracts for treatment and several drugs used

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nowadays are of plant origin [6]. In 2023, U.S. Food and Drug Administration (FDA) approved 55 new drugs out of which 10 were from natural origin [7].

Tamarix indica Willd. (Family- Tamaricaceae), commonly known as Jhau or Jhav, is a shrub or small tree with reddish-brown bark, glabrous. It is about 1.5 to 6 m tall and is distributed in Afghanistan, India, Pakistan, Bangladesh, Sri Lanka, and Myanmar. In India, it is distributed in Delhi, Punjab, Rajasthan, Uttar Pradesh, Andhra Pradesh, Gujrat, Odisha, Tamil Nadu, and West Bengal [8, 9]. Traditionally different species of Tamarix have been used in various medical conditions like cough, diarrhoea, dysentery, and as an astringent and laxative. The main phytoconstituents in bark, aerial parts, and flowers are flavonoids, phenolic acids, alkaloids, essential oils, saponins, terpenoids, and tannins. [8, 10]. Preliminary studies have proven the pharmacological efficacy of different parts of Tamarix species as antinociceptive, cytotoxicity, diuretic, wound healing, hepatoprotective, antirheumatic, antihypertensive, anti-fisher and skin diseases [11, 12]. The present study aims to evaluate the antioxidant and anticancer potential of aerial extracts of *T. indica* against DMBA and croton oil-induced skin carcinogenesis in mice.

Materials and Methods

Collection and Extraction of the plant material

After authentication of *Tamarix indica* Willd. by the expert botanist (Specimen No. 2021/0921) from Sri Venkateswara University, Tirupati, Andra Pradesh (India), the aerial parts of the plants were collected locally. The aerial parts were shed dried and crushed into coarse powder via a mechanical grinder. 500 g of the powdered material underwent successive hot extraction using solvents i.e. petroleum ether, methanol, and water as per their increasing polarity. The obtained extracts were cooled and condensed using a rotary vacuum evaporator. The obtained extract was stored at 2-8°C till further usage.

Preliminary Phytochemical Screening

The collected extracts of *T. indica* were screened qualitatively for the presence of different classes of phytoconstituents using methods specified in Trease and Evans [13].

Antioxidant Activity via DPPH Free Radical Scavenging Assay

The antioxidant activity of *T. indica* extracts was determined by DPPH Free radical scavenging assay [14, 15]. The ability of the plant extractives to donate the hydrogen atoms was determined by measuring the decoloration of the 2,2-diphenyl-1-picrylhydrazyl (DPPH) methanol solution. DPPH gives a violet/purple colour in methanol which converts to yellow hues when antioxidants are present. 2.4 mL of the 0.1 mM DPPH solution in methanol was combined with 1.6 mL of petroleum ether, methanol, and aqueous extract in methanol at varying doses, ranging from 10-50 µg/mL. The absorbance of the mixtures was observed after 30 minutes of incubation at 517 nm. Butylated hydroxytoluene (BHT) serves as a reference. The percentage of DPPH free radical scavenging activity was determined by the following formula:

$$\% \, inhibition = \left[\frac{A_{Control} - A_{Sample}}{A_{Control}}\right] X \, 100$$

Where Acontrol and Asample are the absorbances of the control and extracts or standard.

The concentration versus percentage of inhibition graph was plotted and IC_{50} was determined by analyzing the curve.

Animal Procurement and Acclimatization

A total of 42 female Swiss albino mice (5-7 weeks old), weighing 20-25 g, were used for the study. The mice were kept in the Animal House of Adarsh Vijendra Institute of Pharmaceutical Sciences, Shobhit University, Gangoh (Saharanpur)-UP under the controlled conditions of temperature (25±2°C) and light (12/12 h light and dark cycle). The animals were randomly divided into 7 groups having 6 mice in each and housed in transparent polypropylene cages of standard size (530 cm^2). The adaptation period of one week was provided with free access to food and water ad libitum. The ethical clearance was granted by the Institutional Animal Ethical Committee (IAEC), Committee for Control and Supervision of Experiments on Animals (CCSEA) with protocol reference number IAEC-AVIPS/2022/V/0001 (PLC-D). Before the start of the study, the dorsal hairs of all the mice (2 cm X 2 cm area) were shaved with the electric trimmer. The animals having cut on shaved surfaces were excluded from the study.

Acute Oral Toxicity Study

The acute oral toxicity study was performed as per the Organization for Economic Cooperation and Development (OECD) 425: up- and down-procedure [16]. A 2000 mg/Kg BW oral dose of extract was chosen for the limit test according to the OECD guidelines for the testing of chemicals. The extract was administered only once and the animals were supervised for the next 14 days for any sign of toxicity.

Experimental Protocol

The study was executed as per the method specified by Berenblum and regularized by Agrawal et al. [17, 18]. As per CCSEA guidelines, minimal use of animals is recommended to avoid unnecessary pain and suffering during experiments and ≤5 animals are sufficient to check the significance of results [19]. Thus, mice were randomly divided into 7 groups, consisting of 6 mice each. The tumor was initiated by a single application of DMBA (100 µg/100 µl) on the dorsal, shaved surface and promotion by applying 1% croton oil in acetone twice weekly starting after 2nd week of DMBA application to the end of the study. The treatment was given as shown in Figure 1.

Morphological Parameters

Each mouse was weighed and shaved weekly during the promotion stage to make the easier application of the cancer promoter and to monitor skin lesions during the induction and therapy stage. Morphological skin cancer

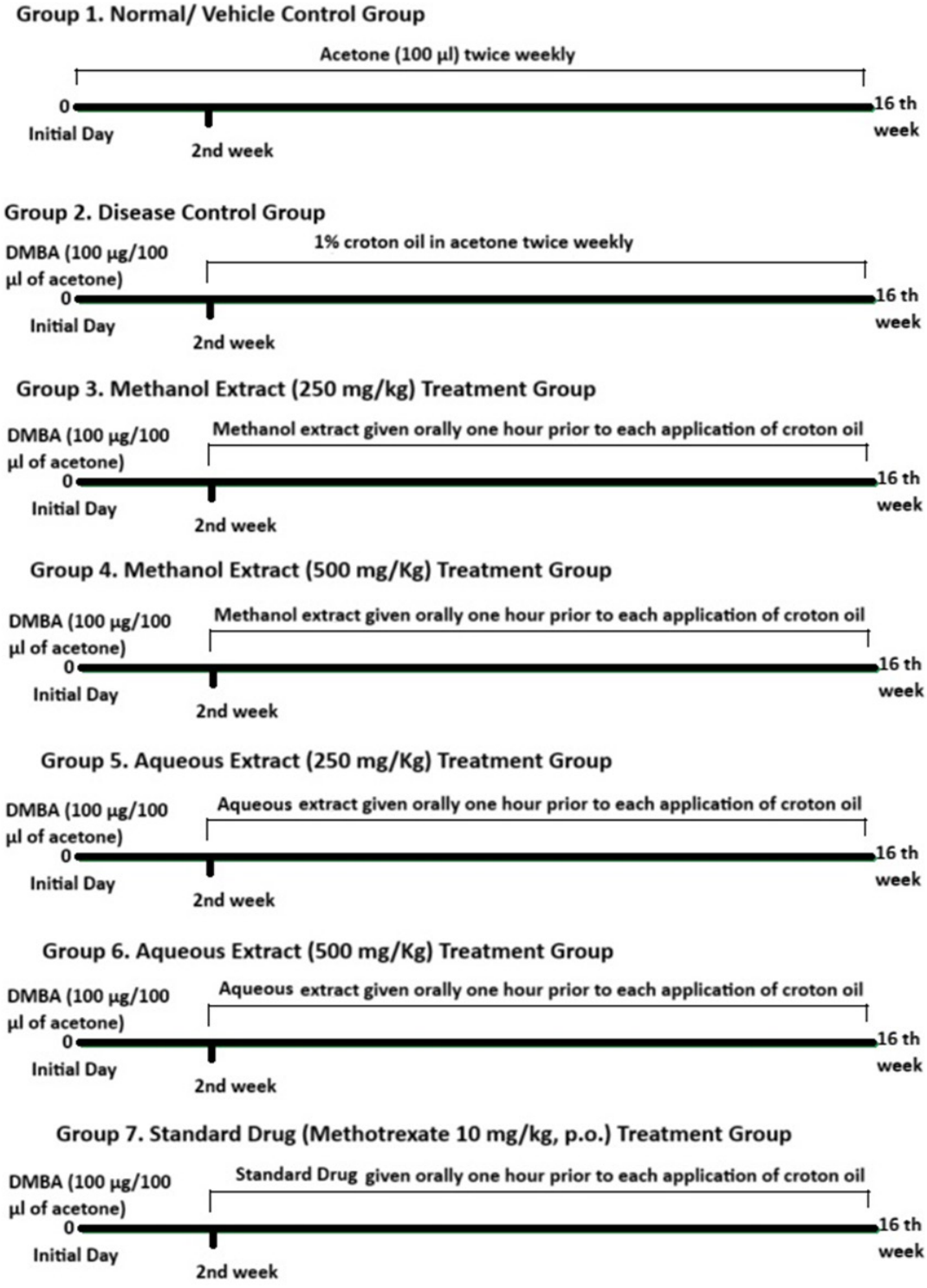

Figure 1. Experimental Protocol for Evaluating Anti-Cancer Activity Using DMBA/croton Oil-Induced Skin Carcinogenesis in Mice

parameters such as tumor burden, yield, cumulative number of tumors, and tumor incidences, were monitored, quantified, and documented weekly. The cumulative counts covered tumors that persisted for at least two successive observations with a diameter larger than 1 mm. From recorded data, the tumor latency period and inhibition of tumor multiplicity were calculated at the end of the study. The data designated as:

* Tumor burden- number of tumors per tumor-bearing mice

* Tumor yield- average number of tumors per mice

* % tumor incidences calculated by the formula,

$$\%\ tumor\ incidences = \frac{No.\ of\ mice\ with\ tumor}{Total\ Number\ of\ mice}\ X\ 100$$

*Tumor latency period- determined by multiplying

the number of tumors that become visible weekly by the time in weeks after promoter application and divided by the total tumor count.

$$Average\ tumor\ latency\ period = \frac{\Sigma NX}{n}$$

Where N is the number of tumors that become visible weekly, X is the number of weeks, and n is the total tumor count

* % inhibition of tumor multiplicity=

$$\frac{(Total\ no.of\ papillomas\ in\ disease\ control - Total\ no.of\ papillomas\ in\ treated)}{Total\ no.of\ papilomas\ in\ disease\ control} X\ 100$$

Blood biochemical parameters

At the end of the study, blood samples were collected from each group of animals using BD Ultra-Fine TM syringes (needle 25 X 5 mm) and collected into heparinized tubes. The serum was separated by centrifugation for 5 minutes at 2500 rpm. Then the serum sample was analyzed for total protein, serum glutamic-oxaloacetic transaminase (SGOT), serum glutamic pyruvic transaminase (SGPT), creatinine, urea, and bilirubin levels using Erba Chem 5X semi-automated analyzer and their kits via protocol described by the manufacturer. While lipid peroxidase (LPO), superoxide dismutase (SOD), catalase (CAT), and glutathione reductase (GSH) were determined by the method specified by Okhawa et al. [20], McCord and Fridovich, [21], Aebi, [22], and Moron et al. [23] respectively.

Statistical analysis

The results are accompanied by the number of observations and are expressed as the mean ± standard error of the mean (SEM). The data was analyzed using one-way analysis of variance (ANOVA) followed by Dunnet's t-test to check the statistical significance. The results were considered significant when the p-value was less than 0.05.

Results

Preliminary Phytochemical Screening

Both methanol and aqueous extracts of *T. indica* showed the presence of carbohydrates alkaloids, flavonoids, tannins, steroids, phenols, terpenoids, flavanones, and isoflavones.

DPPH Free Radical Scavenging Assay

The free radical scavenging activity of aerial extracts i.e. petroleum ether extract (TIPEE), methanol extract (TIME), and aqueous extract (TIAE), is shown in Figure 1a using BHT as standard. Among all the three extracts, TIME showed the highest free radical scavenging activity. At the highest concentration i.e. 50 µg/mL, the percentage free radical scavenging activity of TIPEE, TIME, TIAQ, and BHT was observed to be 66.97±1.04, 96.03±1.05, 91.56±0.89, and 98.96±0.32% respectively (Figure 2a). The IC_{50} value of TIPEE, TIME, TIAQ, and BHT was found to be 32.76±0.96, 7.98±0.87, 14.49±1.01, and 7.03±0.36 µg/mL respectively (Figure 2b). Thus, the free radical scavenging activity was observed in the order of BHT>TIME>TIAE>TIPEE.

Acute Oral Toxicity Study

The extract was tested for acute oral toxicity in mice (n=6) using a single dose of 2500 mg/Kg b.w. and observed for ant toxicity for two weeks. The animals didn't show any sign of toxicity. Thus, two doses i.e. a lower median dose, 250 mg/Kg, and a higher median dose, 500 mg/Kg b.w. were selected for further study.

Anti-skin cancer study

Effect of T. indica Aerial Extracts on Body Weight

The results showed a gradual increase in the body weight of all animal groups, but the carcinogen control group showed a sluggish gradual increase in body weight (Figure 3). The results showed that the gain in the body weight of mice had no significant change irrespective of the treatment given but the methanol extract (500 mg/Kg

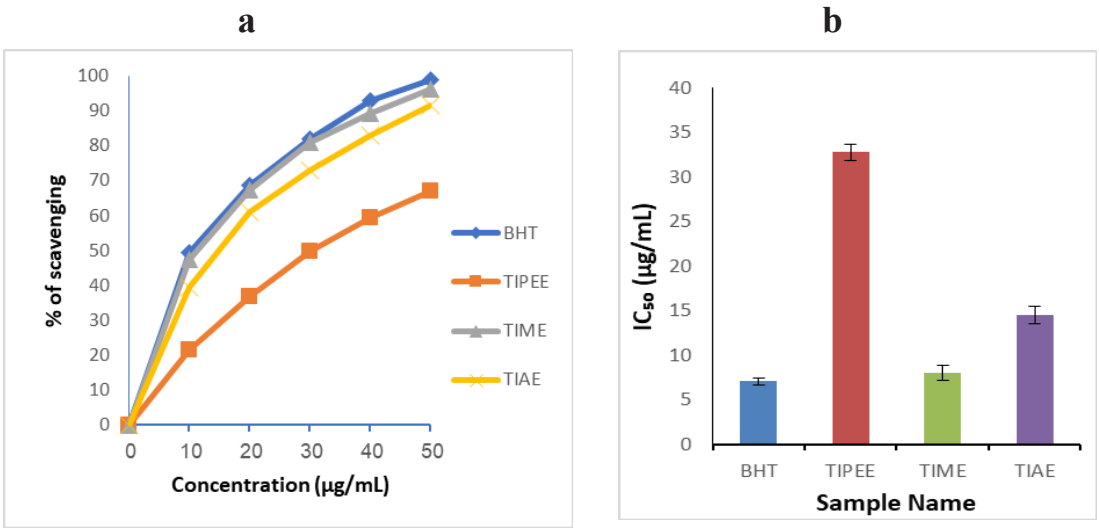

Figure 2. Calculation of (a) DPPH Radical Scavenging Activity and (b) IC_{50} values of petroleum ether (TIPEE), methanol (TIME), and aqueous (TIAE) extracts of *T. indica* using butylated hydroxytoluene (BHT) as standard. All studies were done in triplicate. Data are expressed as mean ± SD for all tested concentrations.

Table 1. Effect of Different Extracts of the Areal Parts of *Tamarix indica* on Body Weight, Tumor Multiplicity, and Average Latency Period of Skin Papilloma-Induced Mice

Groups	Body Weight (g)			% Inhibition of Tumor Multiplicity	Average Latency period (week)
	Initial	Final	% change		
Group I (Normal control)	23.17±0.6	31.67±0.71	36.68%	-	0
Group II (Disease control)	24.83±0.83	30.5±0.76	22.84%	-	10.53
Group III (TIME 250 mg/kg treatment)	23±0.58	31±0.73	34.78%	55.26	12.06
Group IV (TIME 500 mg/kg treatment)	22.67±0.71	31.5±0.92	38.95%	76.32	12.78
Group V (TIAE 250 mg/kg treatment)	23.17±0.48	30.83±0.83	33.06%	50	11.32
Group VI (TIAE 500 mg/kg treatment)	23.33±0.49	31±0.86	32.88%	63.16	12.5
Group VII (Standard treatment, Methotrexate 10 mg/kg)	23.5±0.62	32.17±0.6	36.89%	78.95	13.38

Values expressed as mean ± SEM (n=6).

b.w.) showed persistent gain in body weight of the mice. The percentage change in body weight was observed in all groups of mice in comparison to the initial day body weight of mice (Table 1). The highest percentage gain in body weight was observed in *T. indica* methanol extract (TIME) 500 mg/Kg dose, treatment group i.e. 40.76%.

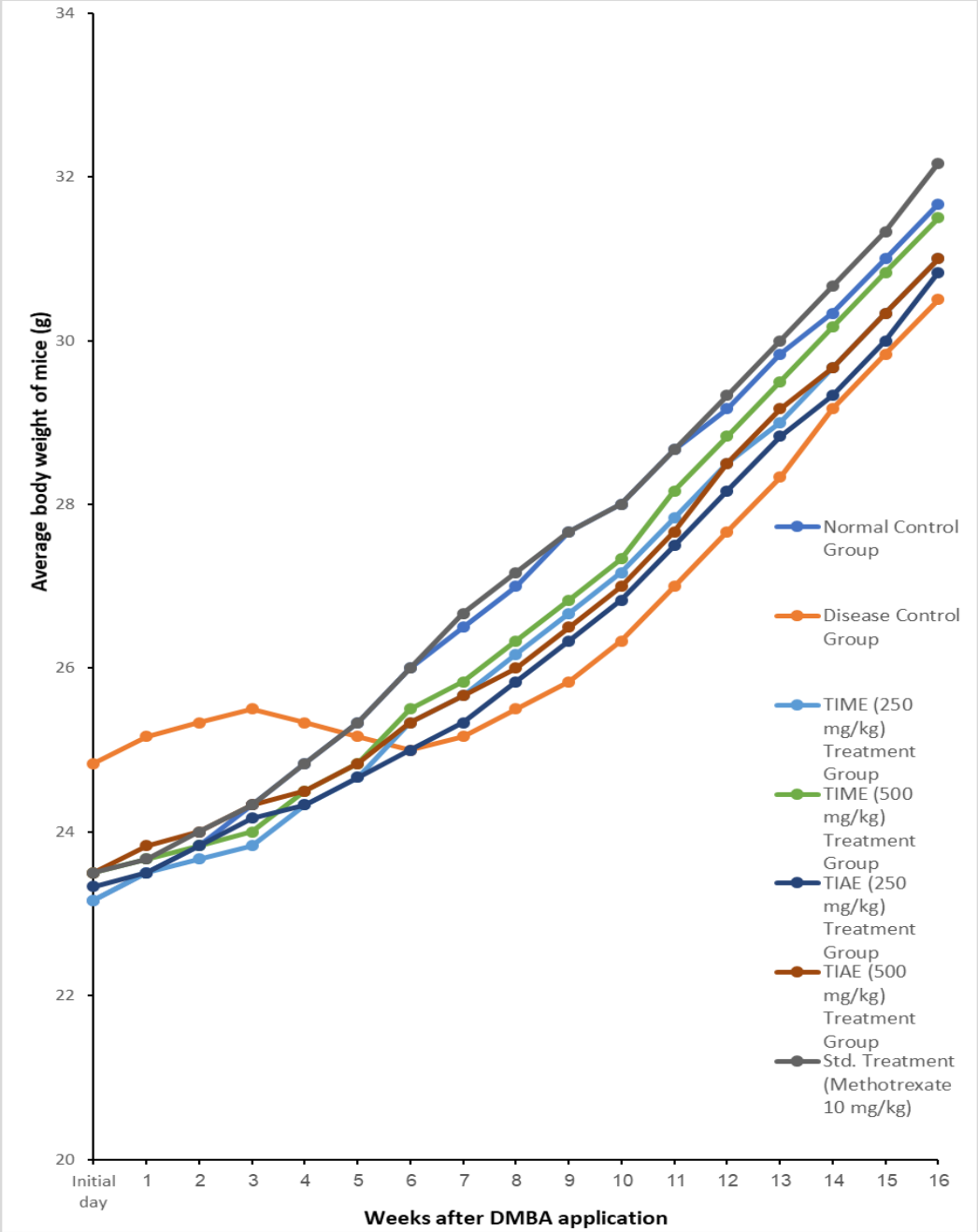

Figure 3. Effect of Treatment on Mice Body Weight (g) in Different Groups (n=6). Data expressed as mean ± SEM.

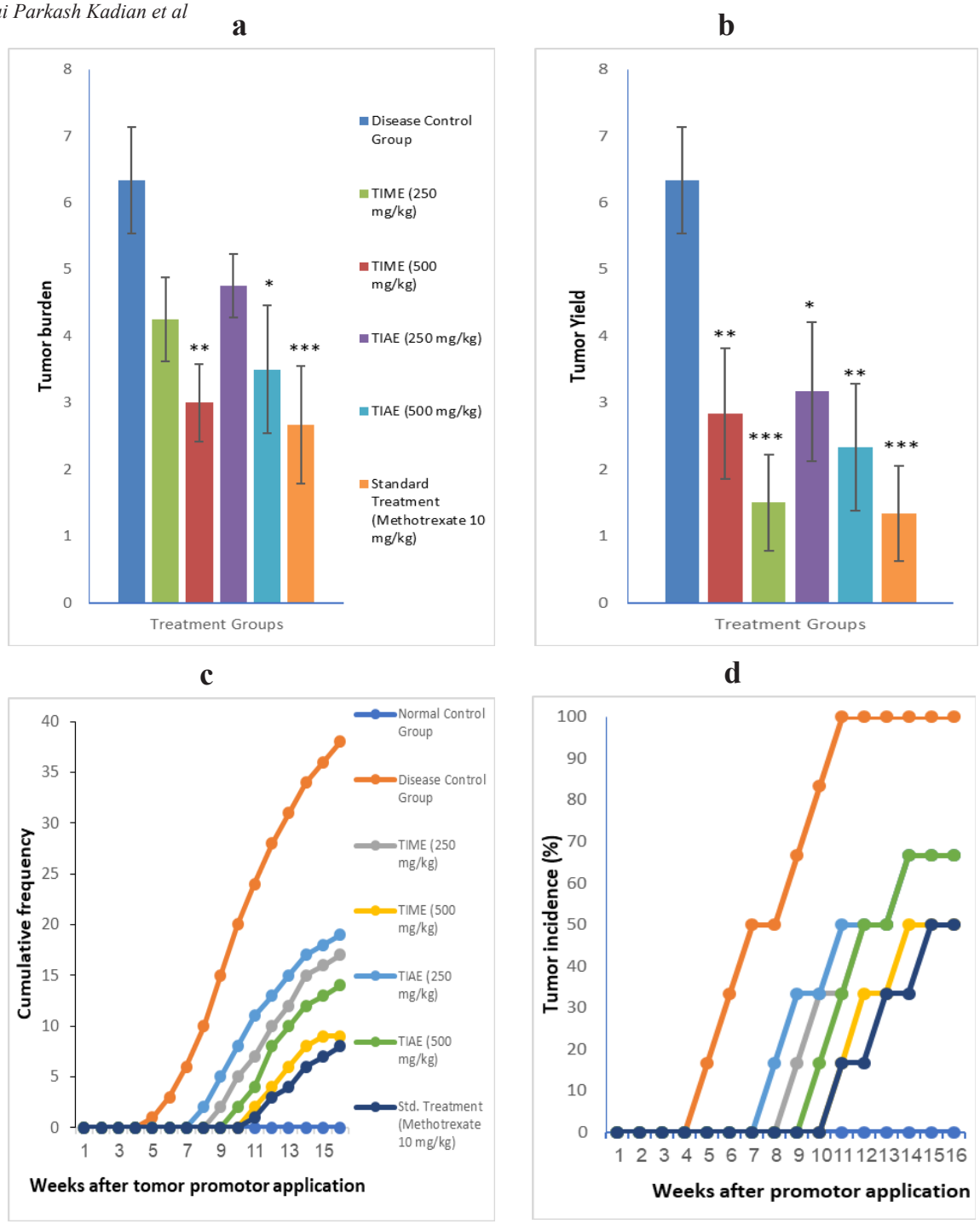

Figure 4. Effects of Different Treatment Groups on (a) tumor burden, (b) tumor yield, (c) cumulative frequency, and (d) tumor incidences in mice (n = 6). The graph represents values as mean ± SEM. The level of significance is determined by ANOVA followed by Dunnet's t-test. *p <0.05, **p <0.01, and ***p <0.001 represented the level of significance between each group in comparison to the disease control group

Effect of T. indica Aerial Extract on Skin Tumor Morphological Parameters

Tumor Burden and Yield

The results showed a maximum tumor burden and yield of 6.33±0.8 observed in the disease control group (Figure 4a & 4b). Both extracts and standard treatment groups showed a dose-dependent decrease in tumor burden and yield compared to the disease control group. At the end of the study, the most significant reduction in tumor burden and yield was observed with the TIME 500 mg/kg dose, showing values of 3.0 ± 0.58 and 1.5 ± 0.72, respectively. The standard treatment resulted in a tumor burden and yield of 2.67 ± 0.88 and 1.33 ± 0.71, respectively.

Cumulative Number of Tumors and Tumor Incidences

The effects of different treatments on the cumulative number of tumors and their incidences induced by DMBA/croton oil application from the initial day to the end of the study are shown in Figures 4c and 4d. The disease control group showed a maximum cumulative number of tumors and incidences, but extract and standard treatment groups represented a dose-dependent reduction in both. The maximum decrease in the cumulative number of tumors

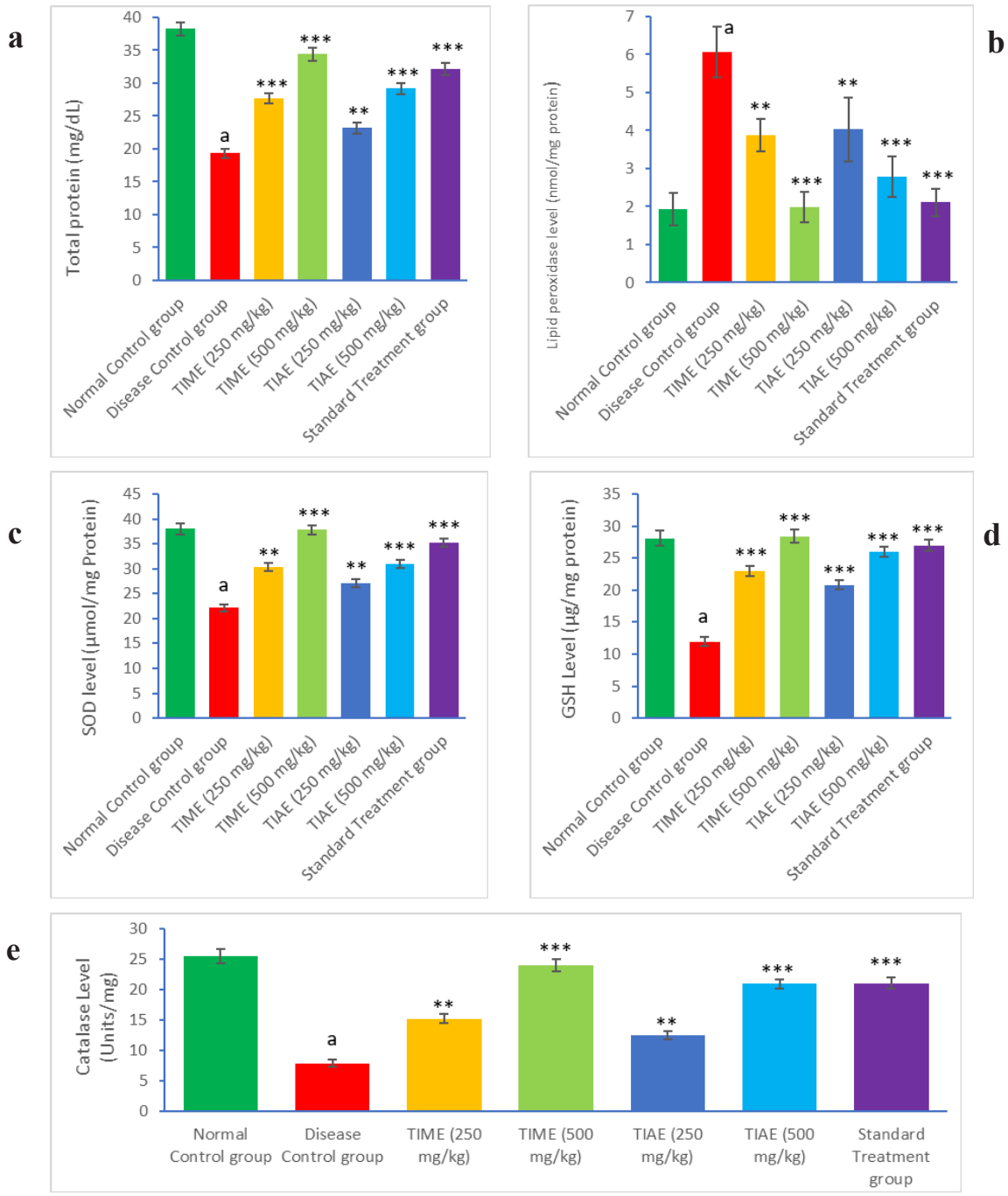

Figure 5. Effect of Different Aerial Extracts *T. indica* on Mice's Serum (a) total protein, (b) LPO, (c) SOD, (d) GSH, and (e) catalase level. All studies were done in triplicate. The graph represents the values as mean ± SEM. The level of significance is determined by ANOVA followed by Dunnet's t-test. *p <0.05, **p <0.01, and ***p <0.001 represented the level of significance between each group in comparison to the disease control group. [a]p <0.05 represented the significant difference between the disease and the normal control group.

was observed in TIME 500 mg/Kg dose and standard drug treatment (9 and 8 respectively).

The onset of skin cancer incidences was observed in the 5th week with 16.67% incidences in the disease-control mice which increased to 100 % up to the 11th week of DMBA/croton oil treatment and the tumor count continuously increased till the end of the study. The onset of tumor incidences was also found to be delayed in both extract treatment groups in a dose-dependent manner. The maximum delay in the onset of tumor incidences was observed in TIME 500 mg/Kg dose and standard (methotrexate) treatment group to the 10th and the 11th week, respectively.

Percentage inhibition of tumor multiplicity and Latency Period

Table 1 showed the highest percentage of tumor multiplicity and least average latency period (10.53 weeks) in the disease control group mice. The dose-dependent reduction in percentage tumor multiplicity was observed in both extract treatment groups. The highest percentage inhibition of tumor multiplicity of 76.32% and 78.95% was observed in TIME (500 mg/Kg dose) and standard drug treatment groups respectively. Both methanol and

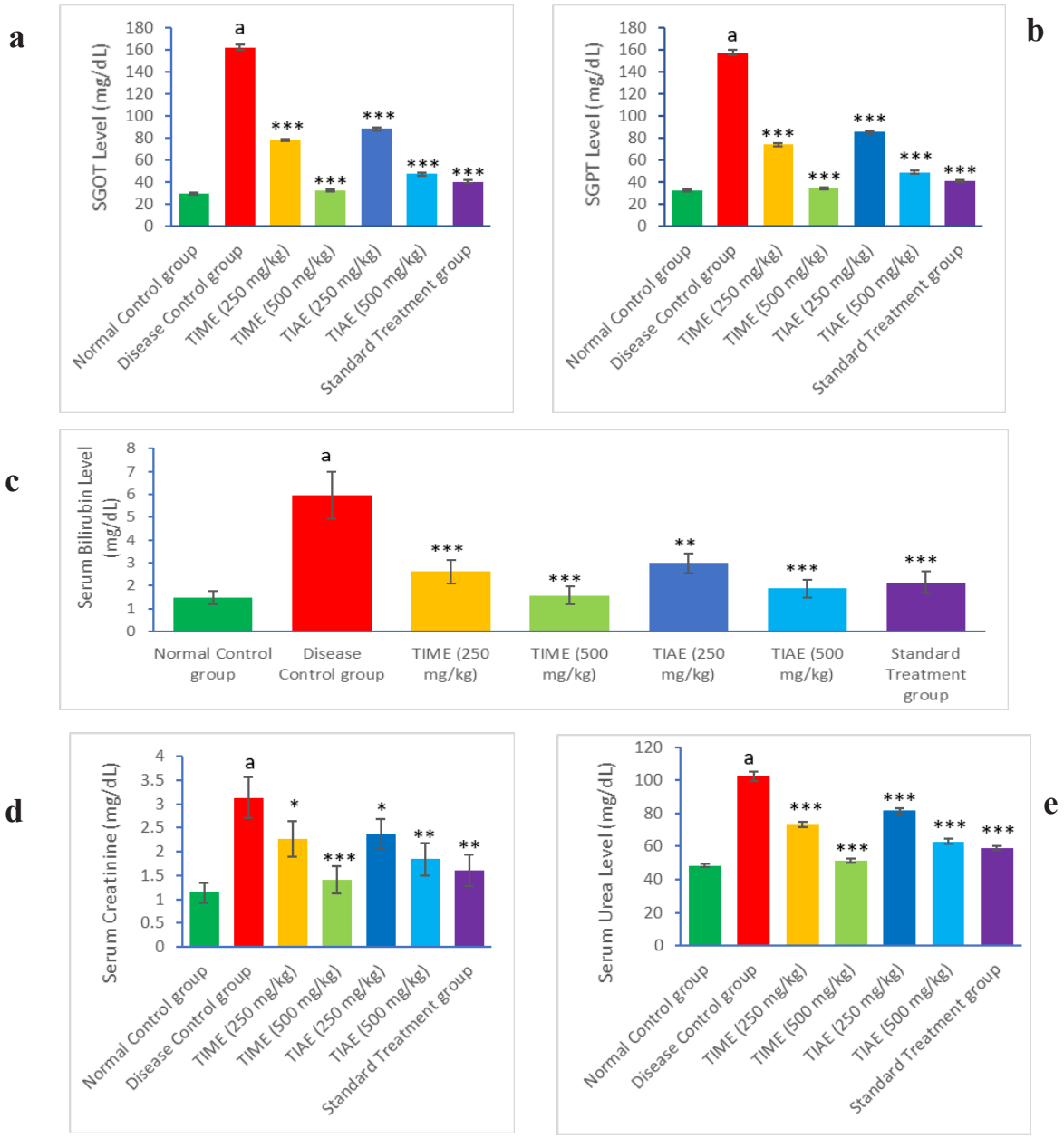

Figure 6. Effect of Different Aerial Extracts *T. indica* on Mice's Serum (a) SGOT, (b) SGPT, (c) bilirubin, (d) creatinine, and (e) urea level. All studies were done in triplicate. The graph represents the values as mean ± SEM. The level of significance is determined by ANOVA followed by Dunnet's t-test. *p <0.05, **p <0.01, and ***p <0.001 represented the level of significance between each group in comparison to the disease control group. ap <0.05 represented the significant difference between the disease and the normal control group.

aqueous extract of *T. indica* also showed a dose-dependent increase in the average tumor latency period. The maximum increase in the average latency period was found to be in TIME 500 mg/Kg dose and standard drug treatment group i.e. 12.78 and 13.38 weeks, respectively.

Effect of T. indica Aerial Extract on Serum Biochemical Parameters of mice

Total Protein and Lipid peroxide (LPO)Level

Figures 5a and 5b showed a significant decrease in serum protein level and an increase in LPO level of the disease control group (19.33±0.68 mg/dL and 6.07±0.66 nmol/mg respectively) compared to the normal control group mice (38.24±1.01 mg/dL and 1.93±0.43 nmol/mg respectively). Both methanol and aqueous extract treatment groups of *T. indica* showed a reversal of the protein level and LPO level toward normal in a dose-dependent manner. Methanol extract group (250 and 500 mg/Kg), aqueous extract (500 mg/Kg), and standard drug treatment with methotrexate showed the most significant improvement in the serum protein level (27.69±0.83, 34.4±0.96, 29.18±0.89 and 32.15±0.93 mg/dL respectively) in comparison to the disease control group (19.33±0.68 mg/dL) mice. The methanol extract (500 mg/Kg) group, aqueous extract (500 mg/Kg) group, and standard drug treatment showed the most significant reduction in the serum LPO level (1.99±0.41, 2.79±0.54, and 2.11±0.37 nmol/mg respectively) of mice when compared to the disease control group (6.07±0.66 nmol/mg) mice.

Superoxide Dismutase (SOD) and Glutathione Reductase (GSH) Level

The results in Figures 5c and 5d showed that SOD and GSH levels have been significantly reduced (22.12±0.79 µmol/mg and 11.92±0.72 µg/mg respectively) in the disease control group compared to that of the normal control group (38.01±1.1 µmol/mg and 28.1±1.15 µg/mg respectively). All the extract and standard drug treatment groups showed a dose-dependent increase in the SOD and GSH levels. The most significant improvement in SOD and GSH levels was observed in the methanol extract (500 mg/Kg) treatment group (37.87±0.92 µmol/mg and 28.4±1.02 µg/mg respectively) when compared to the disease control group.

Catalase Level

Figure 5e showed that treatment with DMBA/croton oil in the disease control group caused a significant reduction in serum catalase levels (7.82±0.55 units/mg) compared to the normal control group (25.5±1.17 units/mg). Both aqueous and methanol extracts, along with standard treatment, resulted in a dose-dependent improvement in catalase levels towards those of normal control group. This increase in catalase level was found to be most significant in the methanol extract (500 mg/Kg) and standard treatment group (23.96±0.97 and 21.01±0.88 units/mg).

Serum Glutamate Oxaloacetate Transaminase (SGOT) & Serum Glutamate Pyruvate Transaminase (SGPT) Level

Figure 6a and 6b results showed a significant increase in serum glutamate oxaloacetate transaminase (SGOT) and serum glutamate pyruvate transaminase (SGPT) levels (162.23±2.17 and 157.62±1.95 mg/dL respectively) in the disease control group treated in comparison to the normal control group (29.72±0.98 and 32.18±0.96 mg/dL respectively). All treatment groups showed a reduction in SGOT and SGPT levels in a dose-dependent manner. The most significant reduction, compared to the disease control group, was observed in the *T. indica* methanol extract 500 mg/Kg, (32.5±1.03 and 34.58±1.06 mg/dL respectively) and standard treatment group (40.57±1.14 and 41.05±1.17 mg/dL respectively).

Serum Bilirubin Level

Figure 6c showed that the serum bilirubin level was significantly increased in the disease-control group mice (5.97±1.02 mg/dL) compared to normal control group mice (1.48±0.3 mg/dL). The aqueous and methanol extract treatment groups showed a dose-dependent reduction in serum bilirubin levels. The most significant decrease in bilirubin levels was observed in the methanol extract (500 mg/Kg) treatment group and standard drug treatment group (1.58±0.39 and 2.15±0.46 mg/dL) compared to disease group mice.

Serum Creatinine and Urea Level

The results (Figure 6d and 6e) showed a significant increase in serum creatinine and urea levels in the disease-control group mice (3.13±0.42 and 102.34±2.96 mg/dL respectively) compared to normal group mice (1.14±0.21 and 48.42±1.01 mg/dL respectively). A dose-dependent reduction in creatinine and urea levels was observed with both, extract treatments and standard drug treatment. *T. indica* methanol extract in 500 mg/kg dose showed the most significant reduction in the creatinine and urea levels (1.41±0.28 and 51.24±1.13 mg/dL respectively) compared to the disease group.

Discussion

T. indica is a well-known herb traditionally recommended for many ailments. The preliminary phytochemical screening results indicated the presence of potential phytoconstituents such as alkaloids, flavonoids, tannins, steroids, phenols, terpenoids, flavanones, and isoflavones. Previous studies have established anticancer properties due to tannins, flavonoids, terpenoids, and polyphenols generally obtained from routine dietary sources [24]. These plant secondary metabolites produce their anticancer activity by modulating phase I or phase II enzymes of carcinogenesis [25, 26]. Most of these plant secondary metabolites having anticancer activity initiate apoptosis in cancer cells due to antioxidant and anti-inflammatory compounds [27, 28].

Skin the outer layer of the body, is continuously exposed to various stressors like pollution, radiation, chemicals, etc, resulting in increased generation of free radicals and reactive oxygen species (ROS). The excess production of ROS and lack of antioxidant defense are linked to cancer development. This imbalance leads to lipid peroxidation and DNA strand breaks, affecting various biochemical pathways and gene expression [27]. The results showed that methanol extract of aerial parts of *T. indica* has a potent antioxidant activity with an IC_{50} value of 9.17±0.51 µg/mL.

The most common method for assessing biochemical and genetic changes in skin cancer is two-stage carcinogenesis using DMBA and Croton oil. When applied topically, DMBA is absorbed systemically and converted in the liver by detoxification enzymes into the active metabolite 3,4-diol-1,2-epoxide. This metabolite binds covalently to the DNA, forming DNA adducts, which can lead to mutations. Croton oil promotes skin carcinogenesis by increasing the generation of hydroperoxides and reactive oxygen species (ROS) in skin cells [29]. Literature indicates that tumor initiation is a brief, irreversible event, while tumor promotion is a prolonged, cumulative process. Therefore, in cancer chemoprevention, focusing on inhibiting tumor promotion is an effective strategy than targeting tumor initiation. This study demonstrated the anticancer potential of extracts from the aerial parts of *Tamarix indica* in a skin carcinogenic mouse model. The results showed that methanol and aqueous extract treatment decreased the tumor burden, tumor yield, cumulative number of papillomas, tumor incidences, and tumor multiplicity compared to the carcinogen control group and increased the average latency period. The methanol extract *T. indica* in 500 mg/Kg dose showed the most potent activity. This effect is likely attributable to the potent antioxidant activity of the methanol extract, which reduces ROS

levels, thereby supporting the rationale for *T. indica*'s chemopreventive activity. The methanol extract from the aerial parts of *T. indica* was found to contain polyphenols, flavonoids, and tannins, all of which are known antioxidants. The primary chemical constituents of *T. indica* include 4-methyl coumarin, 3,3-di-O-methylellagic acid, tamarixin, tannin, and troupin [30]. Various tannins demonstrate in-vivo anti-cancer activity against a variety of cancers in animals, either by inhibiting cancer initiation or by inhibiting cancer promotion [31, 32].

Reactive oxygen species (ROS) significantly contribute to various chronic diseases, including cancer, by causing an imbalance between cellular proliferation and apoptosis. When antioxidant mechanisms are depleted or overwhelmed, oxidative stress increases, potentially damaging proteins, lipids, and nucleic acids. In cancer, elevated ROS levels, due to increased production of hydrogen peroxide (H2O2), hydroxyl radical (OH), and superoxide anion (O2), play a crucial role in activating signaling cascades and lipid peroxidation [29]. Lipid peroxidase (LPO) levels indicate oxidative stress in carcinogenesis and drive cancer progression. In DMBA-induced skin cancer, mice showed increased LPO levels while decreasing non-enzymatic antioxidants like GSH and enzymatic antioxidants like SOD and CAT compared to normal mice. Consuming plant extracts improved biochemical parameters, restoring them to near-normal levels. Carcinogen-generated free radicals deteriorate membranes and proteins, but total protein concentration, which decreased in the carcinogenic control group, improved with plant extract administration. SOD and GSH levels increased significantly in methanol extract-treated groups (500 mg/Kg) compared to the carcinogenic control group. SOD, a metalloprotein and chain-breaking antioxidant, converts superoxide radicals into hydrogen peroxide, preventing reactive oxygen species cascade. Reduced GSH is the richest cytosolic thiol and a crucial antioxidant, scavenging free radicals and acting as an essential cofactor for GSH S-transferases and GSH peroxidases in detoxifying nonradical oxidants. The increased SOD and GSH levels highlighted the significant antioxidant and anti-cancer potential of *T. indica* aerial methanol extract by elevating the detoxification system.

The liver and kidneys are vital for our body's functions. When they are impaired, the metabolism of chemotherapeutic drugs is disrupted, increasing body toxicity. The liver metabolizes xenobiotic compounds and can be damaged by chemical agents. Carcinogenic metabolites and ROS from DMBA metabolism cause liver and kidney degeneration [33]. In this study, DMBA-treated groups had higher SGOT, SGPT, and bilirubin levels, indicating liver damage, which was reduced after treatment with *T. indica* aerial extracts, showing hepatoprotective effects. The kidneys, responsible for excreting toxic metabolic waste, are also affected by DMBA. DMBA-treated groups showed elevated urea and creatinine levels, indicating renal damage. *T. indica* extracts significantly reduced these levels also, demonstrating renal protective effects.

In conclusion, different parts of Tamarix species exhibit antinociceptive, cytotoxicity, diuretic, wound healing, hepatoprotective, antirheumatic, antihypertensive, anti-fisher, and skin diseases. Previous studies have proven the anticancer activity of various derivatives of coumarin and ellagic acid which are also present in *T. indica*. The present study concludes that aerial extracts of *T. indica* have potent chemopreventive activity due to its anti-lipid peroxidative, antioxidant potential, modulating effect on phase I and II detoxification enzymes, as well as anti-proliferative in DMBA/croton oil-induced mice. This study calls for further investigation to uncover the phytoconstituent responsible for the activity, precise mechanism of action and potential clinical use of aerial parts of *Tamarix indica* as a chemopreventive agent.

Author Contribution Statement

Jai Parkash Kadian: Conceptualization, Methodology, Investigation, Writing, Editing, Data curation and Formal analysis; Madan L. Kaushik: Visualization, Supervision, Review, and Formal Analysis; Aniruddh Dev Singh: Formal analysis, Data curation, Resources and Editing.

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Conflicts of Interest

All the authors of the submitted manuscript declared that there is no conflict of interest.

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