Cytotoxic, Antitumor, and Chemopreventive Effects of Pointed Gourd (*Trichosanthes dioica*) Root

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Involvement of Antioxidant

Sanjib Bhattacharya

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Abstract

Pointed gourd, botanical name *Trichosanthes dioica* Roxb. (family, Cucurbitaceae), commonly known as *Potol* in Bengali, is a climber vine grown wild all over the plains of India. It is cultivated widely in the Indian subcontinent for its fruits, consumed as a very popular culinary vegetable. All parts of this plant have several traditional medicinal uses in India. The present chapter attempts to collate and review the outcomes of the preclinical cytotoxic, antimitotic, antitumor, and cancer chemopreventive studies of its roots – mediated by virtue of multimodal amelioration of oxidative stress. The cytotoxic, antimitotic, and antioxidant effects together may provide the basis for its antitumor and chemopreventive effects.

S. Bhattacharya (✉)
West Bengal Medical Services Corporation Ltd., Kolkata, West Bengal, India

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Trichosanthes dioica · Antimitotic · Antitumor · Chemopreventive · Root · Antioxidant

Introduction

Cancer is regarded as one of the most frightening diseases imposing global morbidity and mortality. Cancer, otherwise termed as "malignant neoplasm," is a group of diseases in which a class of cells undergo division beyond normal ceiling (aberrant growth), intrusion and destruction of adjacent tissues (invasion), and sometimes spreading to other organs via blood or lymph (metastasis). These three features differentiate malignant neoplasms from benign tumors, which do not proliferate abnormally and neither invade nor metastasize. Metastasis is the major cause of cancer-related mortality (Anonymous 2006). Carcinogens are the physical, chemical, and biological agents that can induce cancer. Carcinogenesis is the complex multistage process caused by different types of carcinogens that eventually lead to cancer (Bhattacharya 2012). A material that is toxic to tumor cells in vitro is called cytotoxic, and if the toxicity is demonstrated in tumor cells in vivo, the candidate is called antitumor agent. The materials which are toxic to tumor cells in clinical trials (humans) are specifically called as anticancer agents (Evans 2009).

In the last five decades, there has been enough advancement in the battle against cancer. Several arts of war have been utilized to treat cancer, viz., surgery, chemotherapy, radiotherapy, and immunotherapy. Among them, chemotherapy is the most widely used with or without other strategies. Different anticancer drugs or cancer chemotherapeutic agents have been developed in the last decades. Other current strategies like hormonal and gene therapy have been introduced to supplement or replace conventional antineoplastic therapy, with certain extent of success (Ayesh et al. 2003; Anonymous 2006). All of these conventional strategies have untoward effects, for example, after cancer surgery, the immune system is compromised, which increases the likelihood of cancer relapse. By radiotherapy, blood lymphocytes are immediately affected, resulting in prolonged T-cell suppression leading to immunosuppression (Cooper 1993).

Cancer chemotherapeutic agents can often afford transient symptomatic relief, increase the life span, and sometimes cure at the early stages. Nevertheless, the current use of chemotherapy is frequently accompanied with serious adverse effects. Regrettably, the conventional cancer chemotherapeutic agents immensely strike the normal body cells especially blood and hematopoietic system, epithelial tissues, and reticuloendothelial and reproductive systems. All the chemotherapeutic agents precipitate significant irreversible long-term toxicities in vital organs such as the liver, heart, kidney, and lungs, thus increasing morbidity (Tripathi 2013). Other adverse effects like anemia, bone necrosis, skin devascularization, lung fibrosis, ulceration, hair loss, nausea, and vomiting are also accompanied with all sorts of extant cancer treatments (Bennett and Brown 2018).

The usage of medicinal plants and their preparations to heal cancerous diseases is an age-old practice worldwide. Medicinal plants and natural products, thereof (phytochemicals), have contributed significantly to the evolution of contemporary anticancer chemotherapeutic agents. A multitude of plant or other (marine) natural products were screened for antitumor potential producing several clinically beneficial anticancer candidates (da-Rocha et al. 2001). According to a review, among the 79 US Food and Drug Administration (US FDA)-approved anticancer agents from 1983 to 2002, 9 of them were isolated natural products, and 21 of them were derivatives of natural products. Among the 39 synthetic anticancer drugs, 13 of them were synthesized based on a pharmacophore or lead obtained from the natural products (Cragg et al. 2003). The way to minimize the undesirable toxicity is using current bioactive natural products that could exert effect by divergent mode(s), thus imposing less serious adverse effects. Therefore, medicinal plants and constituents thereof have now been considered of tremendous merit in the discovery of effective anticancer agents with minimal host toxicity.

Different interacting intrinsic and extrinsic factors influence the carcinogenesis process. Most of the human cancers are owing to environmental influence, chiefly chemicals (Anonymous 1995). Diverse chemicals, viz., polycyclic aromatic hydrocarbons (PAH), alkylating agents, nitrosamines, and certain inorganic (heavy metals) and organic compounds of the environment, are carcinogenic (Haldar et al. 2010a). Most carcinogens are electrophiles, which react with nucleophilic groups of the DNA and RNA and proteins, thus resulting in genotoxicity (Levin et al. 1979).

Prevention of cancer can be attained by relinquishing exposure to putative carcinogens, by the consumption of fortifying agents, and by strengthening physiological defense systems. Several biological and molecular events have been found to be modulated by natural products leading to inhibition of carcinogenesis at the different phases. Moreover, there are increasing studies showing that certain naturally occurring principles have shielding effects against obnoxious carcinogens (Bhattacharya 2011). The term "chemoprevention" can be defined as the use of specific natural or synthetic agents to counteract or suppress carcinogenesis and thus avert the blooming of cancers (Greenwald and Kellof 1996; Surh 2003). Therefore, investigation on chemopreventive agents is getting considerable attention. Many of these agents demonstrate, apart from their cancer chemopreventive properties, additional beneficial effects such as antioxidant, hepatoprotective, cardioprotective, and other health beneficiary properties (Bhattacharya 2012).

The systematic scientific study of higher plants for detecting anticancer property in pursuit of antineoplastic constituents is of comparatively recent origin (Pezzuto 1997). The untapped wealth of plant world is being investigated for advanced and effective cancer chemotherapeutic as well as chemopreventive agents. Here, the plant under discussion, pointed gourd, botanical name *Trichosanthes dioica* Roxb. (family, Cucurbitaceae), commonly known as *Potol* in Bengali, is a climber vine grown wild all over the plains of India. It is widely cultivated commercially in the Indian subcontinent (India and Bangladesh) for its fruits, consumed as a very popular wholesome culinary vegetable. All parts of this plant have several traditional medicinal uses in India (discussed below). The fruit, leaf, and root of pointed gourd

Fig. 1 Roots of pointed gourd (*Trichosanthes dioica*)

plant have been explored phytochemically and pharmacologically by a plethora of preclinical studies with several beneficial outcomes (Khandaker et al. 2018; Islam 2018). However, the preclinical cytotoxic, antimitotic, antitumor, and chemopreventive activities have been reported only for its root (Figs. 1 and 2). The objective of the present chapter is therefore to critically review the outcome of these studies performed on the root of pointed gourd and to explore the antioxidant role underlying.

Plant Profile: Pointed Gourd

Systematics

According to the most recently developed Takhtajan's system (Takhtajan 1980), systematic position of pointed gourd plant is as arranged below:

Kingdom: Plantae.
Division: Magnoliophyta.
Class: Magnoliospida.
Subclass: Dilleniidae.
Superorder: Dillenianae.
Order: Violaes.
Suborder: Cucurbitales.
Family: Cucurbitaceae.
Genus: *Trichosanthes.*
Species: *dioica* (Roxb.)

Fig. 2 Dried roots of pointed gourd (*Trichosanthes dioica*)

Common Names

Bengali, Assamese, and Oriya: *Potol*
Hindi: *Parwal or Parval.*
Sanskrit: *Patola, Patulika.*
English: Pointed gourd.

Habitat

Trichosanthes is a large genus containing approximately 40 species, half or more of which occur in India (Som et al. 1993). Pointed gourd (*T. dioica*) is indigenous to India and grows in the warmer regions of the Indian subcontinent. It is flourished wild all around the plains of North and Northeast India from the states of Punjab to Assam and Tripura of India and in Bangladesh. It is widely cultivated in the Eastern

part of India, particularly in Orissa, West Bengal, Assam, and Bihar. It is also found to a lesser extent in other parts of Southeast Asia (Chakravarty 1990).

Commercial Importance

The unripe fruits are used as common culinary vegetable in the Indian subcontinent. It is used as constituents of soup, stew, pickle, curry, sweets, or fried dishes and as *potoler dorma* or *dolma* with fish, roe, mixed spice, or meat stuffing. It is available in the market abundantly from June to October. In Eastern India, its leaves (*Palta*) are also consumed as vegetable. Therefore, the plant is commercially cultivated in India. It is cultivated mostly for local market consumption and seldom enters large commercial channels. However, it is one of the most important vegetable crops in India and Bangladesh (Janick and Paul 2008).

Medicinal Uses

The root of pointed gourd has been reported as a strong purgative in Ayurveda, the traditional medicine system of India. It is employed as hydragogue cathartic, in the treatment of jaundice, antipyretic, tonic, anasarca, and ascites (Kirtikar and Basu 1935; Chopra et al. 1958; Anonymous 1976; Nadkarni 1976; Sharma et al. 2002; Khare 2007).

Relevant Activities

In Vitro Cytotoxic Effect

The cytotoxic activity of hydroalcoholic (water-alcohol) extract from *T. dioica* root, i.e., TDA, was evaluated against murine Ehrlich ascites carcinoma (EAC) cells in vitro by trypan blue cell viability and MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] assays. In trypan blue assay, TDA showed marked increase in nonviable cells when compared to normal control; the percent nonviability was maximum (48.45%) at the concentration of 4 μg/ml and then decreased at the higher TDA concentrations. Likewise, in MTT assay, the percent cytotoxicity was maximum (34.58%) at the concentration of 2 μg/ml and subsequently declined at increased TDA concentrations. In both of the in vitro bioassays, at the concentrations below 1 μg/ml of TDA, no considerable response was found (Bhattacharya et al. 2011a).

Antimitotic Effect

The dichloromethane, methanol, and aqueous extracts of *T. dioica* root (DCTD, METD, and AQTD, respectively) were assessed for in vitro antimitotic activity by

using onion (*Allium cepa*) root meristems by keeping them at different serial dilutions of each solvent extract under legitimate test conditions, followed by evaluation of root growth parameters (root length and number) and mitotic index (Allium assay). All three extracts significantly demonstrated concentration-based inhibition of root length and number and decline in mitotic index, demonstrating antimitotic effect, indicating cytotoxicity. DCTD was found to be the most active (effective concentration for 50% inhibition, i.e., EC_{50}, 2.8 mg/ml), followed by METD and AQTD (Bhattacharya and Haldar 2010).

Similarly, the antimitotic effect of triterpenoid-enriched extract of *T. dioica* root (CETD) was also assessed in vitro by Allium assay. The CETD significantly and concentration-dependently retarded root length and number (EC_{50}, 2.3 mg/ml) and reduced mitotic index, indicating antimitotic and cytotoxic activity (Bhattacharya and Haldar 2012a). These outcomes demonstrate antimitotic and cytotoxic activity of pointed gourd root.

Antitumor Effect

The hydroalcoholic extract of *T. dioica* root (TDA) at 5 and 10 mg/kg oral doses exhibited antitumor and oxidative stress-alleviating activity in Ehrlich ascites carcinoma (EAC) cells in Swiss albino mice. One day after intraperitoneal tumor (EAC) induction in mice, TDA was given orally at 5 and 10 mg/kg body weight at every day for 9 successive days. On the next day, half of the treated mice were immolated for the determination of tumor proliferation parameters, viz., tumor volume, tumor weight, packed cell volume, and viable cell count and hematological and hepatic antioxidant parameters, namely, reduced glutathione (GSH), glutathione-*S*-transferase (GST), lipid peroxidation, superoxide dismutase (SOD), and catalase (CAT), and the remaining had been live for survival assessment. TDA demonstrated dose-related and significant decrease in tumor proliferation parameters and prolonged the life span of tumor-affected mice. Hematological profiles were considerably normalized in TDA-treated mice, and TDA treatment markedly harmonized the liver antioxidative parameters as compared to control. Therefore, TDA demonstrated good antitumor potential in mice, mediated by melioration of oxidative distress via diverse modes (Bhattacharya et al. 2011b). Similarly, the triterpenoid-enriched fraction of *T. dioica* root (CETD) was evaluated at 2 and 4 mg/kg doses orally for 9 days in the same EAC bearing mice model (same parameters as above), and its antitumor efficacy has also been reported with multimodal amelioration of oxidative stress (Bhattacharya and Haldar 2012b). These designate antitumor potency of pointed gourd root (Table 1).

The same extract (TDA) has been reported to instigate tumor proliferation in EAC-bearing Swiss albino mice. In this report, mice were intraperitoneally inoculated with EAC cells. TDA was administered orally after 24 h at 25 and 50 mg/kg body weight to these mice for 8 consecutive days. Evaluation of tumor proliferation parameters and hematological and hepatic biochemical studies showed notable increase in tumor weight as well as volume, packed cell volume, and reduced

Table 1 Summary of relevant pharmacological effects of pointed gourd (*T. dioica*) root

Sl. no.	Activity	Model/bioassay	Inducer	Extracts	Regimen	Effects	References
1	Cytotoxic	Trypan blue assay, MTT assay	EAC[a] cells	Hydroalcoholic (TDA)	1–10 μg/ml	↓ viable cells, ↑ nonviable cells	Bhattacharya et al. (2011a)
2	Antimitotic	*Allium cepa*	–	Dichloromethane (DCTD), methanol (METD), aqueous (AQTD)	1–16, 20–320, 100–1600 mg/ml for 4 days	↓ root length, ↓ root number, ↓ mitotic index	Bhattacharya and Haldar (2010)
		Allium cepa	–	Triterpenoid enriched (CETD)	0.75–12 mg/ml for 4 days		Bhattacharya and Haldar (2012a)
3	Antitumor	Mice	EAC[a] cells	Hydroalcoholic (TDA)	5, 10 mg/kg, oral, daily, 9 days	↓ tumor weight, ↓ tumor volume, ↓ packed cell volume, ↓ viable cell count, ↑ life span, restoration of hematological and antioxidant parameters	Bhattacharya et al. (2011b)
		Mice	EAC[a] cells	Triterpenoid enriched (CETD)	2, 4 mg/kg, oral, daily, 9 days		Bhattacharya and Haldar (2012b)
4	Tumor proliferation	Mice	EAC[a] cells	Hydroalcoholic (TDA)	25, 50 mg/kg, oral, daily, 8 days	Reversal of above parameters	Bhattacharya and Haldar (2011)
5	Chemopreventive	Mice	3-MC¶, 200 μg s.c., single dose	Hydroalcoholic (TDA)	2, 4 mg/kg, oral, 45 days	↓ tumor incidence, ↑ life span, normalization of hematological and antioxidant parameters	Bhattacharya and Haldar (2012c)
6	Antioxidant	DPPH, nitric oxide, hydroxyl, peroxynitrite, and superoxide radicals	–	Hydroalcoholic (TDA)	25, 50, 100, 150 μg/ml	In vitro free radical scavenging effect	Bhattacharya and Haldar (2020)

[a]EAC, Ehrlich ascites carcinoma; 3-MC, 3-methylcholanthrene; ↓, decrease; ↑, increase

nonviable cells and life span of tumor-carrying mice and exacerbated hematological and all foregoing hepatic enzymatic and non-enzymatic antioxidative parameters indicating pro-oxidant effect (Bhattacharya and Haldar 2011). Both studies used almost the same experimental protocol; the later used higher doses of extract (TDA) and exhibited inverse relationship with effect.

Cancer Chemopreventive Effect

The cancer chemopreventive property of TDA was evaluated in Swiss albino mice against 3-methylcholanthrene (3-MC)-invoked carcinogenesis (fibrosarcoma). After 24 h of subcutaneous administration of 3-MC (200 μg) in mice, TDA was given orally at 2 and 4 mg/kg body weight in alternative days for 45 days and noticed to account the occurrence of fibrosarcoma (tumor incidence) and survival for 15 weeks. After that, the mice were immolated for the evaluation of hematological and hepatic antioxidant parameters, namely, reduced glutathione (GSH), glutathione-*S*-transferase (GST), lipid peroxidation, superoxide dismutase (SOD), and catalase (CAT). Oral treatment with TDA diminished tumor incidence and extended life span of sarcoma-affected mice when compared with 3-MC control. TDA therapy considerably modulated the hematological and liver antioxidative parameters of sarcomatous mice (Bhattacharya and Haldar 2012c).

In Vitro Antioxidant Effect

T. dioica root hydroalcoholic extract (TDA) was assessed against 1,1-diphenyl-2-picryl-hydrazyl (DPPH), hydroxyl radical, nitric oxide, peroxynitrite, and superoxide radicals in vitro. TDA exhibited marked and concentration-dependent free radical scavenging effect in all five in vitro models (Bhattacharya and Haldar 2020). The results from this work demonstrated that *T. dioica* root possesses promising in vitro antioxidant effect, which may provide the basis of its several reported activities in vivo.

Discussion

Cytotoxic effect is one of the basic prerequisites of prospective antitumor agents. The cytotoxic property of *T. dioica* root extract (TDA) against EAC cells was evaluated in vitro by trypan blue and MTT bioassays. The cell growth or death is generally assessed by counting live cells after vital staining, which is based on the theory that live cells bear intact cell/plasma membranes that keep out specific dyes, like trypan blue, eosin, and propidium; however, the dead cells do not. Hence, these are known as vital stains/dyes. Trypan blue is such a vital diazo dye that selectively stains dead cells blue. Live or viable cells are not colored because in a viable cell, trypan blue is not absorbed through intact cell membranes; however, it crosses the

necrotic membrane into dead cells. So, under microscope, dead cells are seen in distinguishing blue hue. As the living cells are excluded from this vital staining, therefore, this staining technique is known as a dye exclusion method (Haldar et al. 2010b). Trypan blue staining is a straightforward and fast experiment to assess cell proliferation or death; however, for high-throughput screening (HTS), the procedure is not much sensitive and may not be utilized. Radionuclide uptake estimation method by tritium-labeled thymidine is accurate though time-taking and requires handling of radioactive materials.

MTT assay is a rather sensitive technique indicating cell proliferation. MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide], a yellow tetrazole compound, is reduced to the purple formazan product by NADH-dependent mitochondrial reductase in the mitochondria of living cells (Fig. 3). The absorbance of the resultant colored solution can be estimated spectrophotometrically. This reduction occurs only if mitochondrial reductase enzymes are working, and this event is directly proportional to the quantity of live (viable) cells present (Alley et al. 1988; Van de Loosdrecht et al. 1994). Here, it is evident that the TDA is a medium active cytotoxic agent in vitro; although it did not cause more than 50% death of EAC cells in vitro, it has significant effect at lower concentrations. The trypan blue cell viability assay demonstrated higher cytotoxicity (maximum 48.45%, at 4 μg/ml), whereas, the MTT cell proliferation assay exhibited comparatively lower cytotoxicity (maximum 34.58%, at 2 μg/ml). In higher concentrations, however, inverse relationships with concentrations were observed in both assays. This biphasic response requires further definitive studies. Nonetheless, the hydroalcoholic extract of pointed gourd root exhibited cytotoxic potential at lower concentrations in vitro against Ehrlich ascites carcinoma cells, thereby putting forward its vow as natural antitumor candidate.

Antimitotic effect, i.e., inhibition of mitosis (cell division process), is the cause of cytotoxicity elicited by putative plant-derived anticancer agents like paclitaxel, docetaxel, vincristine, vinblastine, eribulin, etc. Mitosis is crucial for proliferation and propagation of cancer cells. The extracts from pointed gourd root were evaluated by using onion (*Allium cepa*) root (Allium assay), where root growth inhibition and antimitotic effects furnished the indication of cytotoxicity (Fiskesjo 1993; Badria et al. 2001). The antimitotic activity was affirmed by microscopic studies involving

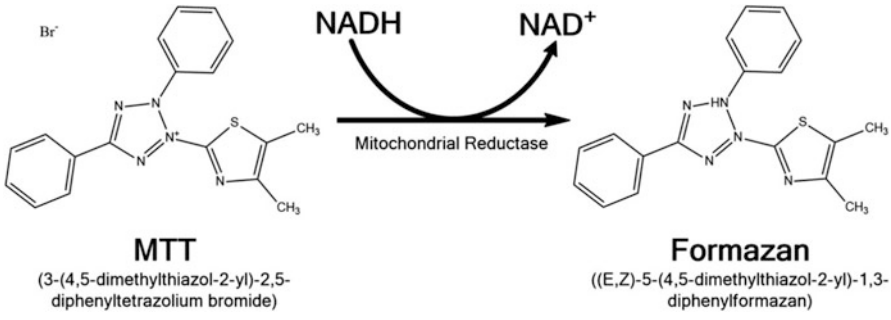

Fig. 3 MTT assay reaction

determination of mitotic index reduction which implied hindered mitosis cell division (antimitotic effect), thus yielding substantial evidence on the extent and mode of cytotoxic effect. With respect to present findings, it has been apparent that *T. dioica* root contains antimitotic constituents, plausibly triterpenoids, that can inhibit or cease the cell division (cell cycle arrest). These constituents plausibly affected the microtubule (cytoskeleton) functions or inhibited the activity of one or more elements of the cell cycle. Therefore, *T. dioica* root extracts had remarkable in vitro antimitotic and cytotoxic properties, which further suggest the feasibility of its prospect as natural antitumor agent.

The Ehrlich ascites carcinoma (EAC), which originally appeared as natural murine mammary adenocarcinoma, is a malignant cancer. It can easily be transplanted and is a highly proliferative carcinoma that can grow in both ascitic and solid forms in almost all strains of mice (Segura et al. 2000).

Polycyclic/polynuclear aromatic hydrocarbons (PAH) are environmental pollutants that are produced chiefly as a consequence of pyrolysis activities, particularly incomplete burning of organic substances like fossil fuel or biomass by industrial and other anthropogenic affairs. These are insidious pollutants as some PAHs are carcinogenic, mutagenic, and teratogenic. Among the various PAHs, benz[a]anthracenes are reported to be carcinogenic. The alkyl-substituted derivative of benz[a] anthracene, e.g., 3-methylcholanthrene (3-MC, Fig. 4), is a notorious carcinogen and teratogen (Anonymous 1995; Menzie et al. 1992). Studies under discussion report that *T. dioica* root extracts (TDA and CETD) have antitumor activity against EAC-bearing mice and TDA has chemopreventive effect against 3-MC-induced carcinogenesis in mice.

Cancer is a disease evoking unrestrained growth of malignant cells. In Ehrlich ascites carcinoma (EAC), the increased ascitic fluid volume was associated with a hike in total cell count (Bhattacharya and Haldar 2013). In mice, TDA and CETD therapy decreased intraperitoneal tumor load, thus decreasing tumor weight, tumor volume, packed cell volume, and viable cell count. The outcome of trypan blue viability assay revealed the reduction of viable tumor cells with higher count of nonviable cells with TDA and CETD treatments. This indicates in vivo cytotoxic effect of pointed gourd root extracts on tumor (EAC)-bearing animals.

The basic criteria for assessing the worthiness of an anticancer agent are the reduction of tumor incidence and prolongation of life span of the tumor bearing hosts (Haldar et al. 2011; Das et al. 2012). It was established that agents that can prolong

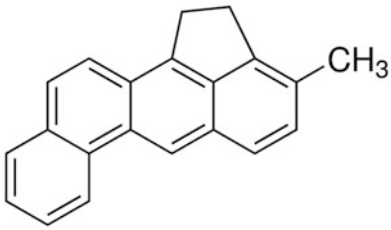

Fig. 4 3-methylcholanthrene (3-MC)

≥25% of lifetime of EAC-affected mice are regarded to possess considerable antitumor potential (Andreani and Galatulas 1983). TDA and CETD remarkably enhanced the life stretch of carcinomatous mice, which is owing to the inhibition of tumor progression implying their antitumorigenic effect. Another study demonstrates reduction in fibrosarcoma occurrence and increase in life span of TDA-treated sarcoma-bearing mice. The comparatively short life span of 3-MC control mice was certainly due to excessive tumor (sarcoma) incidence. Hence, TDA extended the life duration by slowing the tumor progression in sarcoma-bearing mice. The less tumor development and prolonged survival of TDA-treated 3-MC-induced mice indicated the retarding effect of TDA on carcinogenesis of sarcoma-carrying mice.

The usual adverse effects experienced in conventional cancer chemotherapy include myelosuppression and anemia (Tripathi 2013). The hematological perturbation including anemia observed in tumor-enduring mice is chiefly due to depletion of erythrocytes or hemoglobin content, which may happen either owing to iron deficit or hemolytic or myelopathic situations (Haldar et al. 2010b; Haldar et al. 2011). Pointed gourd root extracts, viz., TDA and CETD, normalized the erythrocyte count and hemoglobin content significantly with reduction in leucocyte count when compared to EAC control mice. Study also revealed that TDA treatment improved the erythrocyte count, leucocyte count, and hemoglobin content significantly when compared with 3-MC control mice. These indicative parameters demonstrated that TDA and CETD possessed less harmful effect to the blood and hematopoietic system and credibly had selective cytotoxicity toward carcinoma and sarcoma cells, and thus, they could sustain the typical hematological profile in both cases.

The cellular oxidation-reduction (redox) status is regarded to control its growth behavior (Bhattacharya et al. 2011c). Tissues have complex endogenous enzymatic and non-enzymatic antioxidant defense system, which has been found impaired during malignant growth (Oberley 2002). Endogenous antioxidant mechanism was observed weakened in cancer patients (Balasubramaniyan et al. 1994; Casado et al. 1995) and in experimentally induced cancers in animals (Yellin et al. 1994; Sharma et al. 1993). Preclinical studies reveal that EAC and 3-MC induce oxidative stress in mice (Haldar et al. 2010a, b; Das et al. 2012; Haldar et al. 2011). Oxidative stress is elicited through overgeneration of reactive oxygen species (ROS) in cells. ROS subsequently induce cellular lipid peroxidation and thereby increase lipid peroxides that are thiobarbituric acid reactive substances (TBARS) like malondialdehyde (MDA), which cause degradation of cellular bio-macromolecules like proteins, fatty acids, and nucleic acids (Bhattacharya et al. 2011c). Aggravated lipid peroxidation in several cancer cell lines and in chemical carcinogenesis is widely reported. Malondialdehyde (MDA), a biomarker of oxidative stress, was found to be higher in cancerous cells than in normal tissues (Neilson et al. 1997). Increase in TBARS (as MDA) in carcinoma control mice implied increased lipid peroxidation, imposing vital tissue damage and non-success of the endogenous antioxidant defense mechanisms to arrest overgeneration of ROS.

TDA and CETD treatments normalized MDA levels exhibiting inhibition of hepatic lipid peroxidation. This indicates the suppression of free radical (ROS)

production by TDA and CETD in carcinoma-carrying mice. Decline in lipid peroxidation by pointed gourd root extracts implies that their antitumor activity is effected by arresting or reducing the ROS-induced tissue toxicity. Research also demonstrated that TBARS content estimated as MDA in the fibrosarcoma-bearing hepatic tissues were higher than normal hepatic tissues. TDA application reduced MDA levels toward normal levels, indicating retarded hepatic lipid peroxidation, i.e., reduction in free radical (ROS) production by TDA in sarcomatous mice. Decline in hepatic lipid peroxidation by TDA suggests that the chemopreventive potential is effected by averting the tissue toxicity imposed by the ROS.

Glutathione, known as cellular antioxidant, in tissues takes part in different biochemical processes – detoxification of drugs and xenobiotics. Glutathione is the key component of non-enzymatic antioxidant system of the body. In reduced form (GSH), it occurs in peak levels in the liver and kidney, and it has crucial function as reducing agent (antioxidant) as it detoxifies hydrogen peroxide (ROS) by means of the enzyme glutathione peroxidase (Meister and Anderson 1983). Apart from the antioxidative detoxification, GSH also plays a pertinent role in normal lymphocyte functions, and diminished GSH level was reported with compromised immune system and higher cancer risks (Gmunder and Droge 1991). Depleted glutathione level was also found in human malignancies (Yellin et al. 1994; Sharma et al. 1993). In the studies under discussion, the lowered reduced glutathione (GSH) could be owing to reduced synthesis of GSH or depletion of GSH pool by oxidative impact in carcinoma- and sarcoma-bearing mice. Both TDA and CETD treatments considerably recuperated the hepatic GSH contents in carcinoma-bearing mice. Therefore, the antitumor effect of TDA and CETD was associated with the augmentation of endogenous non-enzymatic antioxidant defense through which the foregoing extracts might have exercised their antitumor action. TDA remarkably restored the hepatic GSH contents in sarcoma-bearing mice also. Hence, the chemopreventive property of TDA was associated with the improvement in non-enzymatic antioxidant shielding. These unveil that TDA may counteract carcinogenesis by the strengthening of tissue non-enzymatic antioxidative mechanisms, through which TDA could have exerted the chemopreventive activity.

Glutathione-*S*-transferases are a class of enzymes that catalyze the nucleophilic conjugation of the sulfhydryl group of reduced glutathione (GSH) on the electrophilic center of different pollutants, carcinogens, mutagens, and other xenobiotics (Devi et al. 2002). GSTs play pertinent role in detoxification by catalyzing the conjugation of GSH with the electrophilic noxious compounds for their disposition/elimination (Mulder et al. 1995). Human cancer tissues were found with low GST activity (Yellin et al. 1994; Coursin et al. 1996). Investigations revealed lower GST activity in carcinoma and sarcoma control mice. Pointed gourd root extracts, viz., TDA and CETD, normalized the dwindled hepatic GST activity in carcinoma-carrying animals. Fortified by TDA and CETD as well, GSH and GSTs played a cardinal part in prohibition of tumor progression in carcinoma-affected mice. Similarly, TDA treatment uplifted the low hepatic GST activity in fibrosarcoma-affected mice. Fortified by TDA therapy, GSH and GSTs played a

pivotal role in obviation of carcinogen-induced oxidative stress, resulting in retardation of tumor progression in carcinogenetic mice.

The enzymes are also simultaneously involved to save the cells from oxidative trauma by playing a key role to eliminate reactive free radicals (ROS), framing the enzymatic antioxidant system. These are considered as first line of endogenous antioxidant defense. Superoxide dismutase (SOD) and catalase (CAT) are the enzymes catalyzing the disposal of ROS, viz., superoxide and hydrogen peroxide radicals, respectively (Oberley 2002). It was reported that SOD and CAT activities are suppressed in malignancies (Marklund et al. 1982; Casado et al. 1995; Oberley and Oberley 1997). The reduction in hepatic SOD and CAT activities was also reported in EAC-affected and 3-MC-induced mice (Haldar et al. 2010a, b; Haldar et al. 2011), and the same was observed in the current context in carcinoma and sarcoma control mice. Treatments with TDA and CETD considerably restored the typical SOD and CAT activities. Recuperation of enzymatic activities, such as GSTs, SOD, and CAT, in TDA- and CETD-treated cancer-affected mice demonstrated the boosting of tissue enzymatic antioxidative defense operations through which TDA and CETD alleviated EAC- and 3-MC-induced oxidative stress.

Several putative antioxidant natural products have been reported to possess both pro-oxidant and antioxidant effects (Sotler et al. 2019). It is very interesting that the hydroalcoholic extract from *T. dioica* root (TDA) has been reported to exhibit pro-oxidant effect in vivo at higher doses in EAC-bearing mice, resulting in tumor growth promotion, i.e., completely opposite effect (see previous section). In the in vitro cytotoxic studies against EAC cells, TDA also demonstrated inverse relationship with increasing concentration (see above). This biphasic response both in vitro and in vivo against EAC requires thorough studies further.

The liver is the principal site for biotransformation of xenobiotics, including chemical carcinogens, e.g., PAHs. In the human body, 3-MC was reported to be metabolized in the liver to different oxygenated metabolites (dihydrodiols and epoxides) that are active electrophilic carcinogens attacking the vital cellular biomolecules with nucleophilic moieties, like DNA, RNA, and proteins, thus imposing tissue and genetic damages (Levin et al. 1979; Wall et al. 1991). Retardation of tissue necrosis, carcinogenesis, and mutagenesis can be mediated by deactivation of reactive metabolites in the body. The non-enzymatic and enzymatic detoxification machineries of the body hence protect the vital tissues from notorious environmental carcinogens/mutagens (Bhattacharya 2011, 2012). Modulation of non-enzymatic (GSH) and enzymatic (GSTs, SOD, and CAT) antioxidant elements in TDA-administered fibrosarcoma-suffering mice implied the augmentation of endogenous antioxidative and detoxification systems through which TDA has mediated the cancer chemopreventive action. Therefore, TDA possessed remarkable cancer chemopreventive efficacy against chemical carcinogenesis in mice via its multimodal antioxidative and detoxifying actions.

The in vitro antioxidant activity of hydroalcoholic extract of *T. dioica* root (TDA) may provide a basis for its observed antioxidant effect in vivo against carcinoma- and sarcoma-bearing mice. The cytotoxic and antimitotic effects may be responsible for its antitumor effect, and the cytotoxic and antitumor properties, in turn, may be

responsible for its cancer chemopreventive action, antioxidant effect being the common determinant role.

The occurrence of triterpenoids plausibly of cucurbitacin type was ascertained in *T. dioica* root in studies under discussion by qualitative phytochemical evaluation with high-performance thin-layer chromatography (HPTLC). Cucurbitacins (a group of tetracyclic triterpenoids) are reported to possess diverse pharmacological effects, including anticancer potential (Miro 2006). *T. dioica* root has already been reported to contain certain antitumor triterpenes like cucurbitadienol, euphol, α-amyrin, ß-amyrin, lupeol, taraxerol, etc. It also contains other antineoplastic principles, viz., glycoside colocynthin and protein trichosanthin (Khare 2007; Khandaker et al. 2018). The presence of these putative constituents could provide the phytochemical ground for the cytotoxic, antitumor, and chemopreventive efficacy of pointed gourd root.

Conclusion

Pointed gourd (*T. dioica*) is a well-studied traditional Indian medicinal plant. From the present array of preclinical studies discussed, it can be inferred that *T. dioica* root demonstrated marked in vitro cytotoxic effect against Ehrlich ascites carcinoma cells, antimitotic effect in *Allium cepa*, antitumor activity against Ehrlich ascites carcinoma-bearing mice, and cancer chemopreventive action against 3-methylcholanthrene-induced carcinogenesis in mice by virtue of its underlying antioxidative role mediated by multiple mechanisms. It may therefore be concluded that the cytotoxic, antimitotic, and antioxidant effects of pointed gourd root in vitro together may provide the basis for its antitumor and chemopreventive effects in vivo. Further, chemical and pharmacological studies on pointed gourd root may yield an effective antitumor lead/drug from this plant part for possible clinical applications in due course.

References

Alley MC, Scudiero DA, Monks A (1988) Feasibility of drug screening with panels of human tumor cell lines using a microculture tetrazolium assay. Cancer Res 48:589–601

Andreani AG, Galatulas SI (1983) Potential antitumor agents. IX: synthesis and antitumor activity of two analogues of ketocaine. J Pharm Sci 72:814–819

Anonymous (1976) The wealth of India: raw materials, vol X. Publication and Information Directorate, CSIR, New Delhi

Anonymous (1995) Polynuclear aromatic hydrocarbons. In: Maroni A (ed) Indoor air quality. A comprehensive reference book. Air quality monographs, vol III. Elsevier, Amsterdam, pp 79–84

Anonymous (2006) Cancer facts and figures. American Cancer Society Inc, New York

Ayesh B, Matouk I, Ohana P, Sughayer MA, Birman T (2003) Inhibition of tumor growth by DT-A expressed under the control of IGF2 P3 and P4 promoter sequences. Mol Ther 7:535–541

Badria FA, Houssein WE, Zaghloul MG, Halim AF (2001) Antimitotic activity of gossypol and gossypolone. Pharm Biol 39:120–126

Balasubramaniyan N, Subramanian S, Govindasamy S (1994) Status of antioxidant systems inhuman carcinoma of uterine cervix. Cancer Lett 87:187–192
Bennett PN, Brown MJ (2018) Clinical pharmacology. Churchill Livingstone, New Delhi
Bhattacharya S (2011) Natural antimutagens: a review. Res J Med Plants 5:116–126
Bhattacharya S (2012) Anticarcinogenic property of medicinal plants: involvement of antioxidant role. In: Capasso A (ed) Medicinal plants as antioxidant agents: understanding their mechanism of action and therapeutic efficacy. Research Signpost, Trivandrum, pp 83–96
Bhattacharya S, Haldar PK (2010) Evaluation of *in vitro* cytotoxic effect of *Trichosanthes dioica* root. Pharmacognosy Res 2:355–358
Bhattacharya S, Haldar PK (2011) *Trichosanthes dioica* root extract induces tumor proliferation and attenuation of antioxidant system in albino mice bearing Ehrlich ascites carcinoma. Interdiscip Toxicol 4:184–190
Bhattacharya S, Haldar PK (2012a) Evaluation of antimitotic and genotoxic effects of the triterpenoid enriched extract from *Trichosanthes dioica* root. Am Eurasian J Toxicol Sci 4:20–23
Bhattacharya S, Haldar PK (2012b) The triterpenoid fraction from *Trichosanthes dioica* root exhibits antiproliferative activity against Ehrlich ascites carcinoma in albino mice: involvement of possible antioxidant role. J Exp Therap Oncol 9:281–290
Bhattacharya S, Haldar PK (2012c) Chemopreventive property of *Trichosanthes dioica* root against 3-methylcholanthrene-induced carcinogenesis in albino mice. J Environ Pathol Toxicol Oncol 31:109–119
Bhattacharya S, Haldar PK (2013) The triterpenoid fraction from *Trichosanthes dioica* root suppresses experimentally induced inflammatory ascites in rats. Pharm Biol 51:1477–1479
Bhattacharya S, Haldar PK (2020) *In vitro* antioxidant activity of *Trichosanthes dioica* root. J Nat Ayurvedic Med 4:000279
Bhattacharya S, Prasanna A, Haldar PK (2011a) Evaluation of antiproliferative activity of *Trichosanthes dioica* root against Ehrlich ascites carcinoma cells. Acad J Cancer Res 4(2):38–42
Bhattacharya S, Prasanna A, Majumdar P, Kumar RBS, Haldar PK (2011b) Antitumor efficacy and amelioration of oxidative stress by *Trichosanthes dioica* root against Ehrlich ascites carcinoma in mice. Pharm Biol 49:927–935
Bhattacharya S, Ahmed KKM, Chakraborty S (2011c) Free radicals and cardiovascular diseases: an update. Free Radicals Antioxid 1:17–22
Casado A, Torre R, Lopez-Fernandez M, Carrascosa D, Casado M, Ramirez M (1995) Superoxide dismutase and catalase blood levels in patients with malignant diseases. Cancer Lett 93:187–192
Chakravarty HL (1990) Cucurbits of India and their role in development of vegetable crops. In: Bates DM, Robinson RW, Jeffery C (eds) Biology and utilization of the Cucurbitaceae. Cornell University Press, New York, pp 325–334
Chopra RN, Chopra IC, Handa KL, Kapur LD (1958) Chopra's indigenous drugs of India. Academic Publishers, New Delhi
Cooper GM (1993) The cancer book. Jones and Bartlett Publishers, Boston
Coursin DB, Cihla HP, Sempf JM, Oberley TD, Oberley LW (1996) An immunohistochemical analysis of antioxidant and glutathione *S*-transferase enzyme levels in normal and neoplastic human lung. Histol Histopathol 11:851–860
Cragg GM, Newman DJ, Snader KM (2003) Natural products as sources of new drugs over the period 1981-2002. J Nat Prod 66:1022–1037
da-Rocha AB, Lopes RM, Schwartsmann G (2001) Natural products in anticancer therapy. Curr Opin Pharmacol 1:364–369
Das S, Bhattacharya S, Pramanik G, Haldar PK (2012) Antitumour effect of *Diospyros cordifolia* bark on Ehrlich ascites carcinoma-bearing Swiss albino mice. Nat Prod Res 26:1631–1633
Devi KN, Reddy CC, Raveendra A, Thyagaraju K (2002) Effect of ß-methylcholanthrene on glutathione *S*-transferases of rat testis. Curr Sci 82:1282–1287
Evans WC (2009) Trease and Evans pharmacognosy. W.B. Saunders, Edinburgh
Fiskesjo G (1993) Allium test 1: a 2-3 day plant test for toxicity assessment by measuring the mean root growth of onions. Environ Toxicol Water Qual 8:461–470

Gmunder H, Droge W (1991) Differential effects of glutathione depletion on T-cell subsets. Cell Immunol 138:229–237

Greenwald P, Kellof GJ (1996) The role of chemoprevention in cancer control. IARC Sci Publ 139: 13–22

Haldar PK, Bhattacharya S, Bala A, Kar B, Mazumder UK, Dewanjee S (2010a) Chemopreventive role of *Indigofera aspalathoides* against 20-methylcholanthrene-induced carcinogenesis in mouse. Toxicol Environ Chem 92:1749–1763

Haldar PK, Kar B, Bala A, Bhattacharya S, Mazumder UK (2010b) Antitumor activity of *Sansevieria roxburghiana* rhizome against Ehrlich ascites carcinoma in mice. Pharm Biol 48:1337–1343

Haldar PK, Bhattacharya S, Dewanjee S, Mazumder UK (2011) Chemopreventive efficacy of *Wedelia calendulacea* against 20-methylcholanthrene-induced carcinogenesis in mice. Environ Toxicol Pharmacol 31:10–17

Islam MT (2018) A literature-based phytochemical evidence and biological activities of *Trichosanthes dioica* Roxb. Orient Pharm Exp Med 18:77–85

Janick J, Paul RE (2008) The encyclopedia of fruits and nuts. CABI International, Oxford

Khandaker M, Akter S, Imam MZ (2018) *Trichosanthes dioica* Roxb.: a vegetable with diverse pharmacological properties. Food Sci Human Wellness 7:34–48

Khare CP (2007) Indian medicinal plants: an illustrated dictionary. Springer Verlag, Berlin/Heidelberg

Kirtikar KR, Basu BD (1935) Indian medicinal plants, vol II. Bishen Singh Mahendra Pal Singh, New Delhi

Levin W, Buening MK, Wood AW, Chang RL, Thakker DR, Jerina DM, Conney AH (1979) Tumorigenic activity of 3-methylcholanthrene metabolites on mouse skin and in newborn mice. Cancer Res 39:3549–3553

Marklund SL, Westman NG, Lundgren E, Roos G (1982) Copper and zinc containing superoxide dismutase, manganese containing superoxide dismutase, catalase and glutathione peroxidase in normal and neoplastic human cell lines and normal human tissues. Cancer Res 42:1955–1961

Meister A, Anderson ME (1983) Glutathione. Annu Rev Biochem 52:711–760

Menzie CA, Potocki BB, Santodonato J (1992) Exposure to carcinogenic PAHs in the environment. Environ Sci Technol 26:1278–1284

Miro M (2006) Cucurbitacins and their pharmacological effects. Photother Res 9:159–168

Mulder TPJ, Manni JJ, Roelof HMJ, Peters WHM, Wiersma A (1995) Glutathione-*S*-transferase and glutathione in human head and neck cancer. Carcinogenesis 16:619–624

Nadkarni KM (1976) Indian materia medica, vol I. Popular Prakashan, Bombay

Neilson F, Mikkelson BB, Nielsen JB, Andersen HR, Grandjean P (1997) Plasma malondialdehyde as biomarker for oxidative stress, reference interval and effects of life-style factors. Clin Chem 47:1209–1214

Oberley TD (2002) Oxidative damage and cancer. Am J Pathol 160:403–408

Oberley TD, Oberley LW (1997) Antioxidant enzyme levels in cancer. Histol Histopathol 12:525–535

Pezzuto JM (1997) Plant-derived anticancer agents. Biochem Pharmacol 53:121–133

Segura JA, Barbero LG, Marquez J (2000) Ehrlich ascites tumor unbalances splenic cell populations and reduced responsiveness of T cells to *Staphylococcus aureus* enterotoxin B stimulation. Immunomol Lett 74:111–115

Sharma R, Singhal S, Srivastava S, Bajpai K, Frankel E, Awasthi S (1993) Glutathione and glutathione linked enzymes in human small lung cancer cell lines. Cancer Lett 75:111–119

Sharma PC, Yelne MB, Dennis TJ (2002) Database on medicinal plants used in Ayurveda, vol 5. Central Council for Research in Ayurveda and Siddha, New Delhi

Som MG, Maity TK, Hazra P (1993) Pointed gourd *Trichosanthes dioica* Roxb. In: Kallo G, Bergh BO (eds) Genetic improvement of vegetable crops. Pergamon Press, Oxford, pp 251–258

Sotler R, Poljšak B, Dahmane R, Jukić T, Jukić DP, Rotim C, Trebše P, Starc A (2019) Prooxidant activities of antioxidants and their impact on health. Acta Clin Croat 58:726–736

Surh YJ (2003) Cancer chemoprevention with dietary phytochemicals. Nat Rev Cancer 3:768–780
Takhtajan A (1980) Outline of the classification of flowering plants (Magnoliophyta). Bot Rev 46: 225–359
Tripathi KD (2013) Essentials of medical pharmacology. Jaypee Brothers Medical Publishers Pvt. Ltd., New Delhi
Van de Loosdrecht AA, Beelen RHJ, Ossenkoppele GJ, Broekhoven MG, Langenhuijsen MMAC (1994) A tetrazolium-based colorimetric MTT assay to quantitate human monocyte mediated cytotoxicity against leukemic cells from cell lines and patients with acute myeloid leukemia. J Immunol Methods 174:311–320
Wall KL, Wenshi G, Koppele JM, Kwei GY, Kauffman FC, Thurman RG (1991) The liver plays a central role in the mechanism of chemical carcinogenesis due to polycyclic aromatic hydrocarbons. Carcinogenesis 12:783–786
Yellin S, Davidson B, Pinto J, Sacks P, Qiao C, Schantz S (1994) Relationship of glutathione and glutathione-*S*-transferase to cisplatin sensitivity in human head and neck squamous carcinoma cell lines. Cancer Lett 85:223–232