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ANTIOXIDANT, IMMUNOMODULATORY AND ANTICANCER POTENTIAL OF *TINOSPORA CORDIFOLIA* - A REVIEW

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ABSTRACT

Tinospora cordifolia (Guduchi) is commonly found in tropical countries of the world like India, China, Myanmar, Sri Lanka and is a glabrous, woody and a succulent climbing shrub. The plant is well described for its therapeutic property in all the traditional systems of treatment like Ayurveda, Unani, Siddha, Folk etc. The extracts of the different parts of Tinospora cordifolia (bark, stem, and root) possess potential chemo-preventive, anti-inflammatory and anti-tumor effect against different cancerous cells. Phytochemical components involved are the alkaloids (Palmetin, berberine, derivatives of berberines like 9-o-alkyl- and 9-o-terphenyl berberine), phenolics, diterpenoids, lactones, glycosides (syringin, cordiol), steroids, sesquiterpenoids, aliphatic compounds and polysaccharides (α-D glucan), in which palmetin, berberine, G1-4A and α-D glucan are much effective as anti-inflammatory against cancerous cell lines. Antioxidant, immunomodulatory and anticancer mechanism of T. cordifolia is due to activation of enzymatic and non-enzymatic molecules in the cells/tissue. Immunomodulation is by regulating the activity of cytokines (like IL-2, IFN-Y, TNF-α etc), cytotoxicity is by increasing apoptosis and inhibition of cell cycle growth. This review focuses on a systematic analysis of existing knowledge of T.cordifolia on its anti-oxidant, anti-cancer, anti-inflammatory properties and further studies on the plant's other unexplored medicinal properties.

KEY WORDS

Antioxidant, Anticancer, Immunomodulatory, G1-4A, α-D glucan, cytokine.

INTRODUCTION

Cancer is the most dreaded disease, where there is an uncontrolled growth of abnormal cells in the affected region of body/tissue/organs. WHO (2012), reports that globally 14.1 million new cancer cases, 8.2 million cancer deaths and 32.6 million people living with cancer within 5 years of diagnosis exist. The five most common prevailing cancers in India are breast, cervical, colorectal, oral and stomach in women and oral, lung, stomach, colorectal and pharyngeal in men respectively. Out of five in each six breast cancers among females and mouth cancers in males are at the top of the list (GLOBOCAN 2012) (icmr.nic.in 2016). According to ICMR 2016 report, the number of new cancer cases reported around 14.5 lakh may increase to 17.3 lakh in 2020. Cancer is the second largest death causing disease after cardiovascular disorders in developing countries [1]. The diagnosis and treatment of this destructive disease becomes very challenging because all the conventional treatment methods like surgery, chemotherapy and radiotherapy have high treatment cost, multiple side effects and are effective only in early stages of cancer. The herbal formulation of drug is the most sustainable way to overcome these side effects and treatment. *T.cordifolia* commonly known as Guduchi or giloye belongs to family Menispermaceae, an Indian medicinal plant frequently used in Ayurvedic practices for fever, diabetes, dyspepsia, jaundice and skin diseases for a long time [2]. Active components derived from the

different parts of the plant are the alkaloids, flavonoids, saponins, phenolics, steroids, diterpenoids lactones, aliphatics, glycosides etc which have activity against different types of diseases [3]. Currently *T.cordifolia* is one among the most explored plants for its medicinal properties like anti-diabetic, anti-spasmodic, anti-inflammatory, anti-arthritic, anti-periodic anti-allergic, anti-stress, anti-leprotic, anti-malarial, hepatoprotective, immunomodulatory, anti-oxidant and anti-neoplastic activities.

Phytochemistry of *Tinospora cordifolia* (TC)

The most active constituents of TC are clerodane furano diterpene glycoside [4], N-formylannonain, 11-hydroxymustakone, N-methyl-2-pyrrolidone [5] arabinogalactone polysaccharide (G1-4A) [6], α-D-glucan [7], epoxy clerodane diterpene (ECD) [8], Immunomodulatory protein (ImP) [9], Policosanol [10], Jatrorrhizine [11], Berberin, Palmatine, Tembetarine, Mangoflorine, choline, Tinosporine, Isocolumbin, tetrahydropalmatine, [12, 13, 14] , Furanoid diterpene glucoside [15] Tinocordiside [16], Tinocordifolioside, Cordioside [17], Cordifolioside A, Cordifolioside B [18], Syringin [19], β sitosterol, and δ sitosterol [20,21], which are extracted using different organic solvents and mostly from stem of TC. Most of these active compounds are known as immunomodulatory (α-D-glucan, G1-4A, N-formylannonain etc), anticancer (berberine, palmatine, clerodane furano diterpene etc) and rest as antioxidants, all these compounds are effective against oxidative, inflammatory and cancerous disease directly or indirectly through series of chemical processes.

Antioxidant effects

Antioxidants are those phytochemicals that balance the oxidative stress condition by neutralizing the reactive oxygen species (ROS), reactive nitrogen species (RNS), reactive sulfur species (RSS) and other stress-producing oxidants, in animal body during different disease/syndrome conditions like cancers, inflammatory disease, ischaemic disease, atherosclerosis, hemochromatosis, emphysema, gastric ulcer, hypertension and other neurological disorders [22]. In the last two decades, plant-derived antioxidants are the most focused research fields by scientists because these antioxidants prove more efficient and safer in comparison to chemically derived antioxidants. In the present context the discussion is on plant-derived antioxidant property of *Tinospora cordifolia* (TC). Successive fraction of TC in different solvents has different amounts of phenolic and flavonoid content in which n-butanol has maximum phenolic content (5.1 ± 0.18 mg GAE/g of plant extract) followed by ethanol and dichloromethane fraction while petroleum ether has maximum flavonoid content (0.98 ± 0.01 mg QE/g of plant extract) followed by ethanol and n-butanol [23]. Aqueous root and stem extract of TC have the compound arabino galactane polysaccharide with dominant ascorbic acid activity which protects the protein and lipid damage in rat brain homogenate against iron-mediated and ɣ (gama) ray induction, due to its (arabino galactane polysaccharide and ascorbic acid) high re-activity towards superoxide radical, DPPH and hydroxyl radical [24, 6]. G1-4A also reduces oxidative stress by improving GSH and enzymatic antioxidant and thereby reduces the possibility of diseases like hyperinsulinemia, hypertriglyceridemia (due to high fructose diet in male albino Wistar rat, at 400 mg/kg/day) [25]. Ethanolic extract of TC regulates the level of serum glutamic - oxaloacetic transaminase (SGOT), serum glutamic pyruvic transaminase (SGPT) and alkaline phosphatase (ALP) with hematological variable in aflatoxin B1 treated mice [26]. Ethanolic extract is more potent in comparison to hexane, chloroform, methanol and water for scavenging oxidant from the target and has hypolipidaemic and hypoglycemic activity in Alloxan diabetics [27, 28]. Though cisplatin is another most effective chemotherapeutic anticancerous drug, cisplatin consumed animals excreated high amount of blood urea nitrogen (BUN - 60.10 mg/dl) and serum creatinine (SC-2.01 mg/dl) which could be recovered by 200 mg/Kg ethanolic extract of TC up to 53.11% (BUN - 28.12) and 92% (SC - 1.9) respectively compared to cisplatin alone induced rat group. Urinary total protein (UTP) level also decreased by 25.42% in rat excretion with the recovery of renal tissue injury [29]. TC ethanolic extract at 300 mg/kg body weight is also most effective against liver cancer in male Wistar albino rat [30]. Ethanolic extract of TC associated fungus *(Cladosporium velox*) also has significant oxidant scavenging and genoprotective activity in injured fish *Channa punctatus* [31]. Lipid peroxidation inhibition activity was maximum (53%) for ethyl alcohol extract of TC which is best seen in comparison to acetone and chloroform solvent as well as to the BHA (85%) standard [32]. Though methanolic stem extract of TC increases the quantity of catalase,

SOD and GSH but decreases the content of LPO in the liver and erythrocytic membrane of diabetic infected male Wistar albino rat [33]. Cyclophosphamide (CP) is a frequently used chemotherapeutic cancer drug which by itself is nontoxic but its metabolic derivative formed in liver microsome becomes toxic to the liver and urinary bladder of the patient. Methanolic extract of TC at 200 mg/kg for 5 days with CP (1.5 mmol/Kg/body wt) exhibits recovery of the size, morphology and histopathology of injured liver and urinary bladder in comparison to consumption of cyclophosphamide alone [34]. Male Wistar rat with Cd induced (5 mg/kg) cardiotoxicity indicates loss in body weight, kidney weight, decrease in enzymatic and non-enzymatic antioxidant, membrane bound ATPase activities (Na+ and K+ ATPase, Mg2+ ATPase and Ca2+ ATPase) and glycoprotein (hexose, hexosamine fucose and sialic acid) which get ameliorated by methanolic extract of TC at 100 mg/kg body weight after 28 days of treatment [35, 36]. TC modulates the level of GSH, decreases nitric oxide synthase (NOS) enzyme synthesis by enhancing glutamyl cysteinyl ligase gene expression and decreasing the inducible nitric oxide synthase (iNOS) gene expression respectively in oxygen-glucose deprivation (OGD) treated hippocampal slices with ROS and RNS scavenging activity, all this collective information proves TC extract as an effective therapeutic tool against ischemic brain damage [37]. Hydroalcohalic TC areal root extract treatment to swiss albino mice for two weeks, regulates liver weight, increases microsomal protein content, cytochrome P450 (1.5 fold), cytochrome b5 (1.28 fold), activity of NADPH - cytochrome P450 reductase and NADPH - cytochrome b5, glutathion S transferase (GST) (1.57 fold), DT-diaphorase (DTD) (1.46 fold), SOD (1.25 fold), CAT (1.21 fold), -SH, GPX (glutathion peroxidase), GSH reductase at 50 mg/kg body weight in dose dependent manner, it significantly inhibited lipid peroxidation and lactase dehydrogenase with increasing the level of GST activity in lung and fore-stomach, DTD activity in fore-stomach only, SOD activity in lung only and CAT activity in lung and kidney both [38]. Gama radiated (2.5 Gy) oxidative stress induced histopathological lesion, depletion in spermatogenic count and loss in body weight in swiss albino mice gets cured by TC root extract with increasing antioxidative parameter [39]. Oxidants play a major role as signaling molecules in the cells in a balanced state but in an unbalanced condition they become fatal to cell due to unstable nature becoming a potential barrier to protect the normal cell.

Immunomodulatory effect

A combination of TC herb (100mg/kg body wt. For 15 days) and cisplatin reduce the organ's alteration effectively, through a prominent increase in proliferation and differentiation of lymphocytes [40]. TC lotion modulates the IL level and strengthens its anti-scabies activity [41]. Aqueous extract of TC stem has given an immunogenic precipitate in acetone and its activity is enhanced on further GPC purification (stimulating index 17.4 to 66.8) but acid hydrolysis gave a mixture of monosaccharides viz, galactose (32%), arabinose (31%), and galacturonic acid [42]. Water soluble polysaccharide α-D glucan (RR1) activates the immune system through TLR-6 signaling, cytokine (IL-1β, IL-6, IL-12p70, IL-12p40, IL-18, IFN-ɾ, TNF-α) production, translocation, activation of macrophages and monocyte chemoattractant protein-1 (MCP-1), that help in reducing the intense inflammation and oxidative stress caused due to the disease concerned [43,7]. Aqueous extract of TC with LPS enhances production of NO molecule by macrophages [44]. Alcoholic extract of TC (ALTC) slows down the tumor growth in tumor bearing mice (Dalton's Lymphoma) by enhancing the proliferation of DC from TAM in response to micro-colony stimulation factor, IL-4, TNF and their antigen presenting ability, which prolong the survival of tumor bearing mice [45,46]. Administration of TC methanolic stem extract increases total WBC, bone marrow cellularity (18.16 x 106/femur) and α esterase positive cell (1423/4000 cells) in bone marrow with increasing humoral immune response [47]. Methanolic extract with 11 active components has the immunomodulatory potential which down-regulate the effect of pro-inflammatory cytokine in LPS treated dendritic cell suspension compared to positive standard for LOX/COX inhibitor with moderate NO radical scavenging activity without showing any cytotoxicity and the resulting activity is called free radical scavenging, an independent mechanism of immunomodulation [5]. Immunomodulatory protein (ImP) of TC increases 3-fold mitogenic activity in murine splenocytes and 5-7-fold in murine thymocytes respectively in comparison to control. ImP also induced nitric oxide production from macrophages of murine peritoneal exudate cells [9]. Cytokines regulate the immune response against various types of inflammations, both pro-inflammatory

and anti-inflammatory. Pro-inflammatory cytokines make the disease worse by producing endotoxic shock. In such instances arabinogalactan polysaccharide (G1-4A) from TC stem proved efficient as it reduces serum TNF-α and IL-1β level in a pretreated G1-4A mice compared to control, while LPS + G1-4A treatment increases serum IL-1β, IL-6, IFN-ɣ and decreases IL-10 which ultimately reduce the pro-inflammatory risk and also modify immune response by increasing the lysozyme secretion from activated macrophages [48,49]. G1-4A induces proliferation of B cell and RAW 264.7 macrophages, increases phagocytosis index in peritoneal exudate cell (PEC) and causes splenomegaly due to increased formation of lymphocytes [50]. G1-4A also induces maturation of killer bone marrow-derived dendritic cell (BMDC) on tumor cell and increases the tumoricidal activity many folds, the important principle behind the phenomena being increased secretion of nitric oxide by mBMDC(G1-4A) which generate peroxynitrite in tumor cells and ultimately lead to death of target cells [51]. TLR agonist is the effective therapeutic agent for the treatment of many fatal diseases like cancer, viral infection etc. In case of (*Mycobacterium tuberculosis*) MBT infected macrophages, G1-4A treatment induces proliferation of proinflammatory cytokine and nitric oxide production in TLRMyD88 dependent manner and reduces survival of both drug sensitive (DS) as well as drug-resistant strains (DRS) of MTB. In-vivo study of BALB/c mice has shown that G1-4A reduced lung bacillary burden in the presence of G1-4A by up-regulating the expression of TNF-α, INF-ɣ and NOS2. Further study revealed that cocktail of G1-4A and Isoniazid (INH) is more curative to MBT patient in comparison to individual dose [52]. Immunomodulatory properties and mechanism of action of TC plant parts could be beneficial in curing fatal diseases particularly cancer, diabetes and cordiovascular and can reduce chemical drug dependency.

Anticancer activity

TC methyl chloride extract has shown maximum cytotoxicity of HeLa cell with the IC_{50} value of 5 µg/ml followed by the methanolic and aqueous extract and the cytotoxicity might be due to inhibition of cell division by damaged DNA [53]. Alcoholic extract of TC increases tumor-associated macrophages (TAM) dendritic cell, IL-1 production, antigen-presenting ability, arginase activity (maximum at 100 mg/kg body weight), phagocytic activity, NO production at 100 and 200 mg/kg body weight in Dolton lymphoma (DL) mice compared to control mice. These molecules are associated with tumoricidal activity of macrophages and dendritic cell [54] and the proliferation ability of tumor cell decreases in a dose-dependent manner [46]. Dichloromethane extract of TC has shown dose-dependent survival rate in Ehrlich ascites carcinoma (EAC) administered mice, with the optimal neoplastic action dose of 50 mg/kg with average survival and median survival time by 56 and 55 days respectively, almost three times more compared to 19 days non-drug treated control. The effectiveness of drug decreases with increasing time intervals between tumor administration and the dose given. [55]. Hexane fraction of TC stems induces caspase 3 mediated apoptosis by activating the caspase-activated DNase (CAD) by increasing the expression of Bax and decreasing the expression of Bcl-2 resulting in decreased number and volume of Ehrlich ascites tumor cell in-vivo. This study shows that hexane fraction of TC stem extract also is important as much as methyl chloride and alcoholic extract of TC in which berberine is earlier evaluated as anticancer compound [10]. Diethyle nitrosamine (DEN) treated group shows decreased body weight, increase in liver weight (due to formation of tumor), increase in the activity of serum components, decrease in SOD and CAT levels, increase in the value of gamma-glutamyl transferase (GGT) and GST, decrease in GSH and GPX levels compared to normal treated mice group (control) but all the above mentioned parameters are restored in ECD treated mice group in both preventive (20 weeks) and curative (12th to 20 week) treatments. Epoxy clerodane diterpene (ECD) is an anticancer compound isolated and purified from the alcoholic extract of TC. The histopathological properties of the liver of mice, lost after DEN treatment could also be restored to their initial shape and size by the preventive and curative treatment of ECD [56]. TC extract induces cytotoxicity (maximum at 50µg/ml), cytostatic and antiproliferative (71.46% to 87% inhibition at 50µg/ml and 75µg/ml respectively) activity which significantly indicates a G1 phase arrest of cell cycle progression [57]. Methanolic extract of TC has shown antimutagenic activity (dose-dependent manner) and anticancerous activity at 750 mg/kg body weight in in-vivo study after 30 days of treatment against the induction of cyclophosphamide (CP) at 50 mg/kg body weight of mice [58]. In-vitro study

of methanolic extract of TC stem exhibited anticancer activity against MDA-MB-231 cell line at IC_{50} value of 50 µg/ml in .05% DMSO [59]. Ethanolic extract of TC (300 - 350 µg/ml) arrested the growth of rat C6 glioma, U87MG human glioma, PC3 prostate cancer and HeLa cell, with differentiation of C6 glioma cells at higher concentration (>450 µg/ml). The ethanolic extract also significantly decreases (40-53 %) cell migration in rat C6 glioblastoma, inhibited the expression of cyclin D1, bcl-xl and arrested cell cycle progression at G0/G1 and G2/M phase. The step by step study of antiproliferative, apoptosis-inducing and anti-metastatic potential of ethanolic extract of TC is a success to clear the signaling pathway of treatment of brain tumor [60]. TC stem extracted alkaloid in palmitin treated group of albino mice increases the content of GSH, SOD and catalase and decreases the level of lipid peroxidation, serum enzyme glutamate oxalate transaminase, glutamate pyruvate transaminase, alkaline phosphatase and bilirubin resulting in the reduction of number of papillomas besides tumor size in the skin of mice that is induced by 7,12- Dimethylbenz (a) anthracene (DMBA) in dose-dependent manner compared to positive control group ie. DMBA treated alone [61]. NBTC (n-butanol extract of TC) has shown cytoprotective activity in *Allium cepa* root meristem growth and radioprotective activity in Wistar albino mice against cyclophosphamide and 4Gy gamma radiation respectively. NBTC also has one known compound cordifolioside A which could be a major contributing compound against above mentioned activity [62]. Murine lymphoma cell line (EL4) treated with, Mature Bone Marrow Dendritic Cell (BMDC) obtained from C57BL/6 mice assisted with G1-4A isolated from the aqueous extract of TC enhances the expression of iNOS protein in continuation to iNOS mRNA which increases the concentration of NO in the target cell. The NO reacts with superoxide anion, produces peroxynitrite which ultimately causes the death of EL4 cancerous cell [51]. Clerodane furano diterpene glycoside (TC-2) purified from aqueous alcoholic extract of TC has shown anticancer activity against human colon cancer cell (HCT-116) by inducing the process of oxidative stress and apoptosis, which is confirmed by observed alteration in mitochondrial membrane potential, delocalization of cytochrome-c, at IC_{50} value of 8 µM, in concentration-dependent manner [4]. In-vitro and in-vivo study of different organic extract from different plant parts have shown potential anticancer activity against various cancerous cell line.

Table 1. Anticancer activity of TC against different cancer cell line(s) and animal models.

S. No.	Types of Cancer cell lines and cancer	Types of assay/study	Solvent(s)	IC_{50} value/optimum dose	Plant parts	Reference
1.	Hela cell	Pratt and Willis Test/In-vitro	Methylene chloride (99.5%)	IC_{50} - 5 µg/ml	stem	[53]
2.	BALB/c Mice	MTT/In-vivo	Ethanol (70%)	IC_{50} - 100 µl (aprox)	Whole plant	[45]
3.	Ehrlich ascites carcinoma model/Swiss albino mice	In-vivo	Dichloromethane (99.5%)	optimum dose - 50 mg/kg	Stem	[55]
4.	Ehrlich ascites tumor cell/Swiss albino female mice	In-vivo	Hexane (99%)	optimum dose - 100 µl	stem	[10]
5.	DLA cell/Swiss albino mice	In-vivo	Hydroethanolic (1:1)	200 mg/kg body weight	Crude powder	[63]
6.	DLA cell line	In-vitro/Trypan blue assay	Hydroethanolic (1:1)	IC_{50} (24.11 µg/ml)	Crude powder	[63]

7.	DLA cell line	LDH leakge assay	Hydroethanolic (1:1)	IC_{50} (25.76 µg/ml)	Crude powder	[63]
8.	Male Wistar Albino strain rat	In-vivo	Chloroform & methanol (19:1)	optimum dose - 10 mg/kg body weight	Stem	[56]
9.	Oral squamous carcinoma cell line (KB)	In-vitro/Trypan blue assay	-	optimum dose - 50 µg/ml	Crude powder	[57]
10.	Male swiss albino mice	Micronucleous assay Protocol/In-vivo, antimutagenic	Methanol (50%)	optimum dose - 600 mg/kg	Stem	[58]
11.	Melanoma cell Line from C57Bl hybrid mice	Melanoma assay Protocol/ In-vivo, Anticarcinogenic	Methanol (50%)	optimum dose - 750mg/kg	Stem	[58]
12.	HeLa S3 cell line	In-vitro	Metylene chloride (99.5%)	10 µg extract + 0, 1, 2, 3 Gy ɣ - irradiation	Stem	[64]
13.	Rat glioma (C6) cell line, U87MG, HeLa Cell	In-vitro	Ethanol (50%)	IC_{50} - 200 µg/ml	Stem	[60]
14.	Swiss albino mice	In-vivo	Methanol: Acetone (7:3)	optimum dose - 200 mg/kg	Stem	[61]
15.	Breast cancer cell line (MCF-7)	In-vitro	Ethanol (absolute)	IC_{50} - 84.4 µg/ml	Stem	[65]
16.	MDA MB 231	In-vitro	Ethanol (absolute)	IC_{50} - 66.3 µg/ml	Stem	[65]
17.	HeLa cell	In-vitro	Ethanol (absolute)	IC_{50} - 155.3 µg/ml	Stem	[65]
18.	HaCaT (Immortal non-cancerous cell)	In-vitro	Ethanol (absolute)	IC_{50} - 194.1 µg/ml	Stem	[65]
19.	Wistar albino mice	In-vivo	n-butanol (99%)	Optimum dose - 120 mg/kg	Stem	[62]
20.	Murine lymphoma cell line	In-vitro	Aqueous	Optimum dose - 1 mg/ml	Stem	[51]
21.	MDA-MB-231 cell line	In-vitro/MTT assay & Tryphan blue assay	Methanol (50%)	IC_{50} - 50µg/ml	Stem	[59]
22.	HCT-116	In-vitro	Dichloromethane (99.5%)	IC_{50} - 54.24 µg/ml	Stem	[66]
23.	MCF-7	In-vitro	Ethanol (95%)	IC_{50} - 101.26 µg/ml	Stem	[66]
24.	HCT-116	In-vitro	Alcohal:water (1:1)	IC_{50} - 8µM	Stem	[4]

25.	MCF-7	In-vitro	Alcohal:water (1:1)	IC_{50} - 40µM	Stem	[4]

DISCUSSION

Antioxidant activity

There are various exogenous (electromagnetic radiation, cosmic radiation, cigarette smoke, car exhaust, ozone) and endogenous (mitochondrial electron transport chain, respiratory burst by phagocytes, β-oxidation of fat in peroxisome, auto-oxidation of amino acid, catecholamines, haemoglobin and ischaemia reperfusion injury) sources which cause an increase in the pro-oxidant and antioxidant ratio and this continuously leads to the progression of different diseases like atherosclerosis, neuro-degenerative and carcinogenesis in additions to inflammations [67]. TC having various naturally occurring polysaccharides, terpenoids and alkaloids has the capacity to improve antioxidant activity of the host and hence is better suited for treatment. For example, arabinogalactan polysaccharides protect the cell against, iron-mediated lipid peroxidation and gamma radiation [6]. Rapid loss of reducing equivalent (GSH) of the cell due to oxidative stress leads to up-regulation of a wide variety of pro-inflammatory and inflammatory cytokines, including additional molecules with mitochondrial and cell membrane destruction causing apoptosis/necrosis [68]. TC organic solvent exerts antioxidant activity against iNOS gene expression caused due to neuronal/ischemic disease, by decreasing the level of NOS enzyme which further reduces the synthesis of NO and $ONOO^-$ and immobilizes the level of GSH near infected tissue. The iNOS gene and iNOS enzyme activity increases due to raised oxidative stress during ischemic/hypoxic condition [37], leading to generation of NO which causes neuronal death and necrosis in addition to other biochemical aberration. Hydro-alcoholic extract of TC enhance the activity of microsomal cytochrome P450, that plays a key role in diluting the toxicity of carcinogens [69], by increasing the hepatic and extra-hepatic activity of GST, in conjugation with endogenous ligand (GSH) to eliminate the carcinogen from the organism [70]. The elevated level of DTD protects the cell against the toxicity of quinones and their metabolic precursor such as PAH and benzene [71] and maintain the level of GR, GPX, SOD and catalase which play central role in detoxification of peroxides, hydroperoxide and superoxide radicals [72]. In case of fructose fed Wistar rat, aqueous extract of TC stem downregulates the oxidative stress and help in preventing the mice from insulin resistance and thereby protect the organism from hyperglycemia, hyperinsulinemia and hyperglyceridemia [25]. The root extract of TC enhances antioxidant enzymes like SOD, CAT, GSH and GPX in necrosed tissue of mice near to normal level, exhibiting preventive effect against aflatoxin-induced carcinogenesis by reducing the level of oxidative injuries on DNA and other components of hepatocytes during early stages of carcinogenesis [26]. N-nitrosodiethylamine (DEN) induces liver cancer in male Wistar albino rats by increasing the highly reactive hydroxyl (OH-) free radical which reacts with cell membrane to cause inflammation, which results due to the formation of LPO [73]. Ethanolic extract of TC breaks the above-mentioned chain reaction by inducing enzymatic and non-enzymatic antioxidant and reduces the chances of liver cancer in male Wistar rat [30]. Cd intoxication causes oxidative stress in the kidney cells by increasing the level of lipid peroxidation and protein carbonyl content, decreasing tissue glycoproteins like hexose, hexosamine, fucose and sialic acid with decreasing the activity of Na^+ K^+ ATPase, Mg^{2+} ATPase and Ca^{2+} ATPase [74]. The methanolic extract of TC could be the best option for treating Cd toxicity due to the presence of the enzymatic (GSH, GPX etc) and nonenzymatic antioxidant (ascorbic acid etc) [35]. As we earlier described the importance of the enzyme GSH and GPX in the oxidative defense of the cell by detoxification of the oxidating and alkylating agent in stress condition, these enzymes also plays major role in preventing the mitochondrial damage by reducing their own endogenously generated free radical [75] and transform L-dehydroascorbate back to ascorbate which was produced by the oxidation of ascorbic acid in oxidative stress condition. From the above discussion it is clear that oxidative stress being the major internal factor in increasing susceptibility to cancer which may occur in any body part and this way of cancer development can be arrested by developing the drugs from different organic solvent extracts of TC plant parts. Thus, the alcoholic and aqueous extract of stem of TC plant is more efficient in countering cancer, however the specific effective compound or groups of

compounds from the TC organic/aqueous extracts need to be further explored through extensive focused research.

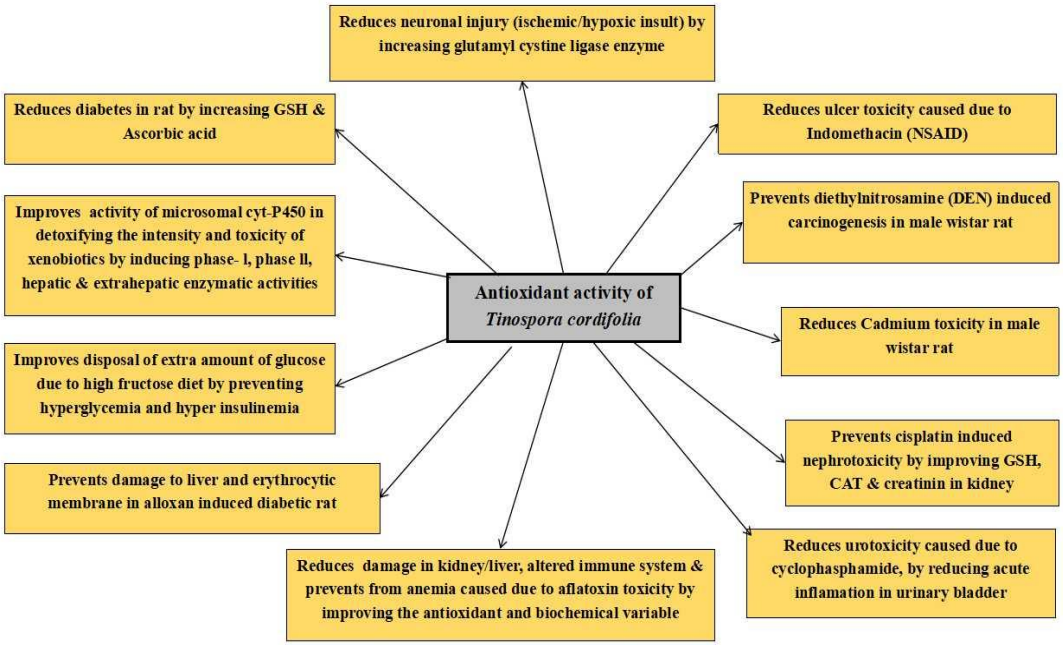

Fig. 1. A schematic presentation of anti-oxidant mechanism of *Tinospora cordifolia*

Immunomodulatory activity

TC belonging to the category of plants known as adaptogen which has the ability to maintain homeostasis, improve adaptation to noxious stimuli and provide endurance to attenuate disorder in human beings. The aqueous stem extract of TC enhances secretion of macrophagic NO in dose-dependent manner in the presence of LPS, that help in destroying tumor cell and immunomodulation with the secretion of IL-6 which plays a vital role in the stimulation of B-cell proliferation [76]. This induced proliferation activity of B-cell and degradation of IkB-α by G1-4A as well as LPS are blocked by TLR4-MD2 antibody which indicates that G1-4A attached to the TLR-4 receptor on the surface of B cell, activate the NF-kB and regulate gene expression, cytokine production and proliferation of B cell [50]. BRM are immunopotentiator that generally modifies the immune response against the pathogenic agent in the induced organism. TC extract has the ability to modify the activity of macrophages which regulate the secretion of lysozyme and NO at the sight of bacterial infection (bactericidal property) and improves the immunity of the organism [77]. Alcoholic extract of TC had immunomodulatory activity by enhancing bone marrow cellularity, α esterase activity, production of IL-1 from TAM and GM-CSF which differentiates macrophages into DC [78]. Adoptive transfer of TC treated TAM derived DC inhibites tumor progression in DL bearing mice by enhancing the amount of tumoricidal molecule like IL-1, TNF and NO, an important molecule in tumor destruction [54] and proves the modulatory immunotherapy property of the described plant TC [46]. RR1 could be a novel polysaccharide derived from aqueous extract of TC and can modulate the intensity and duration of immune response by regulating the cytokine IL-1β, IL-6, IL12p70, IL12p40, IL-18, IFN-ɾ, TNF-α and MCP-1 at 100µg/ml concentration. The dose-dependent synthesis of these cytokines clearly demonstrates the Th-1 pathway, which is essential for cellular immunity and killing of intracellular pathogen and malignant cell [7]. IL-12p70 is an efficient anti-tumor isoform of cytokine IL-12 [79] and differentiate native T cell in to effector T helper type 1 CD4+ lymphocyte which increase the secretion of IFN-ɾ, [80] while IL12p40 control the secretion of p70 form and also have stimulatory effect on NK cell [81]. MCP-1 and IL-18 are another potent inducer of CD8+and IFN-ɾ [82]. TNF-α is considered as a crucial endotoxic cytokine and is secreted in response to LPS (a polyclonal B cell mitogen). GI-4A mimic with the binding activity of LPS and significantly reduces the level of TNF-α, it also modulates the inflammatory effect of TNF-α by inducing

the shedding of TNFR-II. G1-4A administration induces the secretion of anti-inflammatory cytokine IL-10, pro-inflammatory cytokines like IL-1β, IL-6 and IFN-ɣ and NO production. IL-1β indirectly decrease the surface expression of TLR-4 which is a crucial effector for LPS [83]. IL-6 is known as TNF-α inhibitor cytokine in LPS administered pretreated G1-4A mice model [84] and key signaling molecule regarding immunomodulation potential. G1-4A sustaines the synthesis of NO and reduces the chances of endotoxic shock [48] because NO is considered as an important signaling molecule in the healthy cell at balanced concentration but it becomes destructive for tumor cell as well as MTB at an increased concentration. Arabinogalactan polysaccharide (G1-4A) induces the functional persistence cytotoxicity in BMDC and has shown as the killer phenotype. The series of reaction behind the phenomenon is that G1-4A induced mBMDC enhanced the secretion of TNF-α [50] which increases the secretion of NO with the expression of iNOS gene in the dose-dependent manner of G1-4A. NO is well known for the immunomodulatory and antitumor activity [85] because it easily diffuses inside the faster dividing tumor cell and reacts with ROS and RNS, resulting in peroxynitrite which is highly reactive and generate sulphydryl group, hydroxylate aromatic compound including tryptophan, tyrosine and guanosine [86]. These peroxynitrite modifications in structural protein with actin and neurofilaments can destroy whole cytoskeleton assembly with serious pathological consequences [87]. TC extracted G1-4A have the property to up-regulate the secretion of TNF-α, IL-6, IL-1β, and IL-12 with enhanced production of NO in the presence of NOS_2. The importance of NO in the destruction of MTB is confirmed by using the pharmacological inhibitor L-NAME which resulted in increase in MTB infection. G1-4A induced the TLR4-MyD88 dependent intracellular killing of MTB which is supported by Uszynki et al., 2001. TLR is the key pattern receptor protein which recognizes the invading pathogen and has great affinity for polysaccharides [88]. In certain disease conditions (like cancer, diabetes and other pathogenic activity) the wound healing property of organism is lost due to weak immune response towards disease [89]. To sort out this immunodeficient problem, TC immunomodulatory protein can be a best option because it has shown significant mitogenic activity towards murine as well as human lymphocytes and stimulates the secretion of NO from peritoneal exudate cells containing macrophages which become toxic to a spectrum of bacteria and tumor cells. Immunomodulatory protein also stimulates the phagocytic activity of murine macrophages [9]. The reduction in the toxicity of syngeneic and xenogeneic tumor cell in the presence of iNOS inhibitor ensures that mBMDC (G1-4A) accompanied the phagocytosis of the killed target cell by the dendritic cell [51].

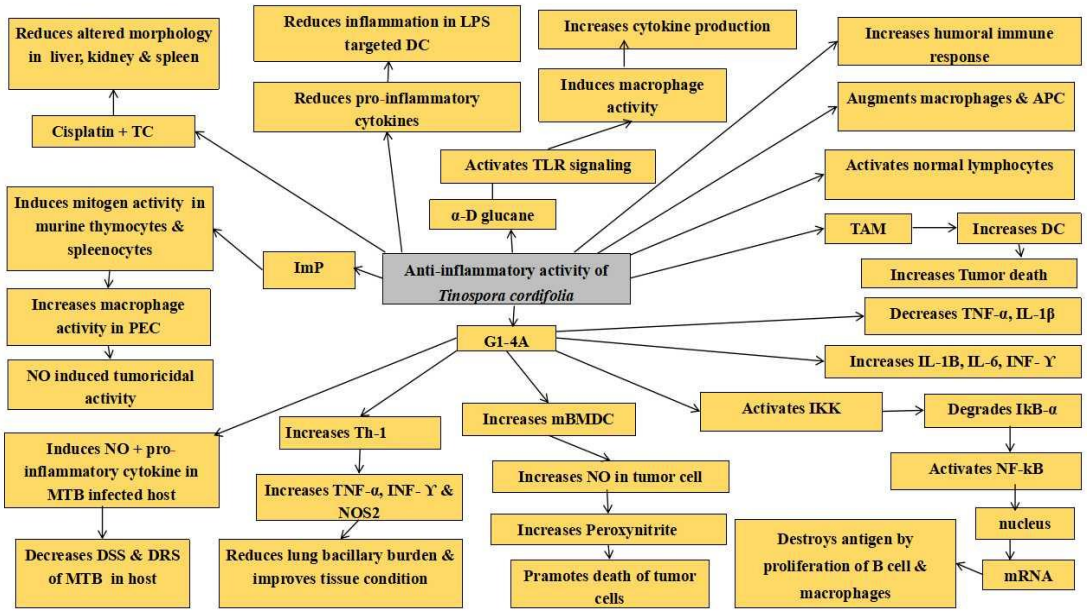

Fig. 2. A schematic presentation of anti-inflammatory mechanism of *Tinospora cordifolia*

Anticancer activity

Methyl chloride stem extract of TC has shown the 50% cytotoxicity in HeLa cell at 5 µg/ml and the mechanism is the formation of micronuclei in the cell and as the frequency of micronuclei increases the survival of the cell decreases due to loss of considerable part of genome which was used in proliferation of HeLa cell [53]. The tumor growth and rejection are governed by local up-regulation of arginine and NO pathway respectively, arginase metabolism of TAM plays an important role in determining the fate of tumor growth [90]. The Dalton lymphoma (DLMA) obtained from mid and late tumor bearing stages have shown a reduced level of Reactive Nitrogen Intermediate (RNI), TNF besides increased level of IL-1, and arginase activity in comparison to early stage DLMA [91]**.** It is reported that DLMA might shift their metabolic pathway from NO synthase in early tumor bearing stage to arginase in mid and late tumor bearing stage [92]. Alcoholic extract of TC enhanced the production of RNI by TAM and NO accumulation, which is regulated at the level of iNOS gene whereas it also downregulates the arginase pathway. The in-vitro study on the DL cell indicates the direct effect of alcoholic extract of TC on the fragility of tumor cell [45]. The imbalance in membrane permeability [93] and inhibition of topoisomerase ll enzyme by the compound berberine in dichloromethane extract of TC might be the main cause of tumor cell toxicity [94]. Cell cycle arrest is the important hallmark in inhibiting the proliferation of cancer cell. TC methanol stem extracted compound isoquinoline alkaloid, berberine cause G1 phase arrest in human epidermoid carcinoma (A431 cell) by the upregulation of Cdki protein (Cip1/p21 and Kip1/27) along the process of apoptosis [95]. Malignant tumor cells bypass the G1 phase and reduce the frequency of cell apoptosis. The activation of caspase-activated DNase (CAD) in programmed cell death is the key step, western blotting of CAD protein indicates that hexane fraction of TC activates CAD in Ehrlich ascites tumor (EAT) via a caspase -3 pathway [10]. The pathway involves break down of a specific intracellular substrate, such as poly ADP ribose polymerase (PARP), inhibitor caspase-activated DNase and lamins along the cascade resulting in programmed cell death [96]. Alcoholic extract of TC enhances the differentiation of TAM into DC in response to GM-CSF, IL-4 and TNF along the production of the tumoricidal soluble molecule like TNF, IL-1 and NO in DL bearing mice [46]. TC stem extracted compound epoxy clerodane diterpene (ECD) has a chemotherapeutic potential against diethylnitrosamine (DEN) induced hepatocellular carcinoma (HCC). The fast dividing neoplastic cell with faster glucose metabolism (due to high energy requirement), leads to abnormal increase in LDH activity. Gama glutamyltransferase (GGT) and Glutathione S transferase are important anti-hepatocarcinogenic enzymes because both are xenobiotics detoxifying enzymes and in the case of hepatocarcinoma cell, the level of both the enzymes gets elevated abnormally is brought under control after ECD administration, thus reducing the risk of hepatic carcinoma [56]. TC extract has been shown as an antiproliferative activity against the oral carcinoma (KB) cell line by arresting the simple G1 phase of cell cycle progression [57]. The moderate dose range (5-10 Gy) of gamma radiation lead a lot of tissue complexity which includes the destruction of mature blood cells, reduce the proliferation of new cells and increase the formation of micronuclei due to the breakdown of DNA and chromosomal aberration. [97]. Methyl chloride and Butanol stem extract of TC could be the best option to overcome the above mention complexities and might be enhanced the efficiency of radiotherapy without damaging the normal cell [67]. The main compound extracted from n-butanol extract of TC is cordifolioside-A and can be considered as the anti-radioactive compound for more detail further research is needed [64]. Earlier studies revealed the immunomodulatory & anticancer properties of TC but it also possesses antiproliferative, differentiation inducing (converting cancer cell into the respective normal cell) and anti-migratory activity in rat and human glioma cells. Antiproliferative property of TCE may be due to the combined effect of differentiation and senescence. TCE treatment, upregulated the GFAP expression coupled with morphological changes in C6 cell, which have shown significantly reduce tumor growth after transfected with GFAP cDNA. Mortalin is a heat shock protein, which in a cell is found in pancytoplasmic space while in tumor cell it is accumulated in perinuclear space but after TCE administration the tumor cell gets transformed to normal cell by activation of senescence pathway. TCE administration inhibits the expression of cyclin D1 which leads to the arrest of cell cycle at G0/G1 and G2/M phase and reduces the number of glioblastoma cell, thus indicating the differentiation property of TCE. TCE treatment down-regulates the

expression of anti-apoptotic gene bcl-XL. Neural cell addition molecule (NCAM) is a glycoprotein also denoted by CD56, widely expressed during embryogenesis while downregulating later, but it again activates during tumor progression [98, 99]**.** On administration of TCE, there is no repopulation of glioma cells in scratched area of culture, which indicate that the migration of glioma cell might be inhibited due to low expression of PAS-NCAM and the reduced rate of repopulation might be due to combined outcome of differentiation, apoptosis and cell cycle arrest which inhibit metastatic aggressiveness of tumor [60]. Carcinogenesis is a multistep complex process but two main steps are initiation and progression, DMBA treated swiss albino mice got oxidative stress, leading to lipid peroxidation of cell membrane and produces the reactive compounds such as 4-hydroxynoneal and malondialdehyde (MDA) which are known as mutagenic and carcinogenic [100]. As we know GSH is a tripeptide non-enzyme antioxidant whereas SOD and catalase are mutually supportive antioxidant and there is a significant increase in these antioxidants after administration of palmitin in albino mice which ultimately reduces the risk of skin cancer due to DMBA [61]. The process of internalization of exogenous antigen like bacteria, bacterial toxin and tumor etc, inside the Endoplasmic reticulum (ER) of APC in the presence of MHC 1 is called antigen cross-presentation, Nowadays DCs are the most focused APC and play important role in immunomodulation, TC extracted G1-4A exerted many phenotypic and functional changes which includes loss of phagocytic and endocytic receptor, chemokine secretion, upregulation of co-stimulatory molecules, translocation of MHC class II to the cell surface and cytokine secretion [101]. These morphological changes make the DCs more interactive to antigen specific T cell and in response to this interaction T cell improves the adaptive immunity, which is maturation of DCs, activation of naive allogeneic T cells, decrease in the ratio of T cell treated with G1-4A (1:128) compared to LPS (1:16), increase the secretion of IL-12, TNF-ɼ, CTL and NK cell in response of Th1 [102]. The secreted cytokines from iBMDC (G1-4A), like TNF- α upregulate the secretion of NO by increasing the activity of iNOS gene which leads to a series of reactions in the tumor cells and consequently produces a cytotoxic product like peroxynitrite (ONOO-) [87] etc. With the above mechanism the efficiency of killer DCs to kill tumor, could be increased by improving the range of uptake mechanism including receptor mediated uptake of soluble antigens released from killed tumor cells or phagocytosis of dead tumor cells to process and provide antigen more naturally [51]. So, all these improvements in the efficiency of DCs of EL4 murine lymphoma model, indicate that G1-4A is the most active natural adjuvant for cancer treatment after chemotherapy and surgery. Clerodane furano diterpene glycoside compound from aqueous alcohalic extract of TC has shown potent cytotoxic activity against human colon canver (HCT-116) and breast cancer (MCF-7) at IC_{50} of 8 µM and 40 µM respectively, the possible mechanism is the involvement of intrinsic apoptotic pathway [103] which proceeds with a series of reactions like imbalance of mitochondrial membrane permeabilization due to mitochondrial mediated generation of ROS, release of proapoptic protein (cytochrome c) from intermembrane space to cytosol, with the continuation of these reaction activation of caspase cascade series of reaction started that ultimately cause the death of treated cancerous cells [104, 4].

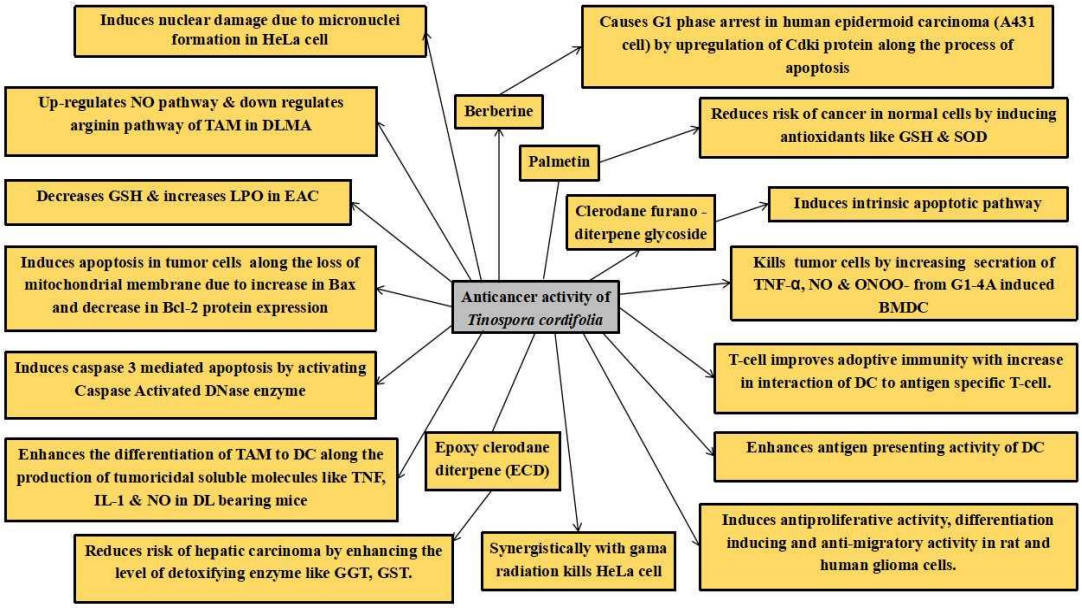

Fig. 3. A schematic presentation of the anti-cancer mechanism of *Tinospora cordifolia*

CONCLUSIONS

Tinospora cordifolia is a much-explored medicinal plant based on its potential effect against the various diseases since ancient time. The stem, root and bark parts of the plant have many active principles mainly in alcoholic and aqueous extracts that have shown prominent antioxidant, immunomodulatory and anticancer activity**,** with a basic nature of immunomodulatory followed by anticancer and antioxidant. The most active principles are berberine, palmetin, G1-4A, α-D glucan, clerodane furane diterpene glycoside, epoxy clerodane diterpene and immunomodulatory protein, which are effective against different diseases in either individual or synergistic manner. In general, there are two main principles of scavenging oxidant from the cell/tissue, the first is a direct quenching of oxidant induced by phytochemical and second is an indirect quenching of oxidant by the enzymatic and nonenzymatic pathway. TC component follows both the pathway either separately or simultaneously dependant on the site of toxicity, the source of toxicant, nature of toxicant and other environmental factors.

Immunomodulatory effect of TC is due to many active compounds which improve the innate and adaptive immune response, like the enhanced activity of mBMDC, TAM, cytokine and APC activity of DC that ultimately produces the toxic key molecule NO and $ONOO^-$ which is responsible to kill the cancer cell/pathogen. Antioxidant activity of TC is opposite for healthy and cancerous cells - antioxidants prevent the healthy cell by neutralizing oxidant but equal and opposite kill the tumor cell by enhancing the synthesis of oxidant inside the tumor cell. This behavior of antioxidant against cancerous cell and in favor of normal cells respectively has captured the focus of researchers to further explore the mechanism behind the phenomenon.

TC plant extract could be a best option as an auxiliary due to many active ingredients that target the cancerous cell without destroying the nearby healthy cell. The pathways through which series of reactions occur in the tissue/cell of organism to kill the cancerous cell by active component of the plant, include induction of antioxidant like GSH and SOD to protect the healthy cell from oxidative stress and kill the tumor cell by increasing oxidative stress, by inducing intrinsic apoptotic pathway, by increasing the secretion of TNF-α, NO and $ONOO^-$ in tumor cell alone with the down-regulation of arginin pathhway, by immunomodulation by inducing antiproliferative, differentiation and antimigratory activity, by enhancing the level of detoxifying enzyme (GGT, GST) and finally inducing apoptosis in tumor cell. The type of mechanism varies according to nature of the cancerous cell, nature of the source for the cancer cell, the potential of the immune system and local environmental conditions. TC is then the potential medicinal plant against cancer, and for further effective treatment, we need to discover the

unexplored active component(s) and their mechanism of action against cancerous cell as well as healthy cell.

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