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Tumor Anti-Initiation and Anti-Progression Properties of Sulphated-Extract of *Colocasia esculenta*

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Colocasia esculenta (Taro) is an edible tuberous plant; however, corms are its most worldwide consumed part while the corm powder is widely used in food industries. In this work, a sulphated polysaccharide extract of *C. esculenta* corm (SCE) was prepared and its cancer chemopreventive properties were explored. The amending of carcinogen metabolism and radical scavenging affinity revealed that SCE is a strong tumor anti-initiation agent *via* suppressing cytochrome P450–1A and enhancing glutathione and the carcinogen detoxification enzyme; glutathione *S*-transferase. SCE exhibited a strong scavenging affinity towards critical radicals (hydroxyl and peroxyl). It induced lymphocyte growth and modulated the macrophage functions into an anti-inflammatory profile, *via* elevating macrophage proliferation and its binding affinity of fluorescein isothiocyanate-lipopolysaccharide (FITC-LPS) and inhibiting nitric oxide and tumor necrosis factor-α generation. Furthermore, SCE showed a potent cytotoxicity against human breast MCF-7 carcinoma cells (IC_{50} 27.73 μg/mL), whereas SCE treatment inhibited the activity of histone deacetylase (HDAC IC_{50} 37.70 μg/mL) and disturbed the pattern of cell cycle phases. An arrest in both S- and G2/M-phases was linked with shifted cell populations towards late apoptosis and necrosis, as detected by flow cytometry. SCE is a promising cancer chemopreventive agent to be used in healthy food industries and for the high breast cancer-risk population.

INTRODUCTION

Colocasia esculenta (Liliatae, Araceae), traditionally called taro, is a tuberous plant classified as monocotyledonous and distributed in humid subtropics and tropics. All of the plant parts are edible; however, corms are its most worldwide consumed part [Lim, 2015]. The corms afford various nutrients, including proteins, carbohydrates, vitamins (niacin, riboflavin, and thiamine), minerals (iron, potassium, sodium, calcium, and phosphorus) and fibers [Temesgen & Retta, 2015]. Additionally, many bioactive compounds were extracted from *C. esculenta; e.g.*, phenolic compounds (including anthocyanins and tannins), sterols, organic acids, bioactive proteins, phytocystatin, alkaloids, terpenes, and saponins [Ferreres *et al*., 2012; Lim, 2015; Reyad-ul-Ferdous *et al*., 2015]. Preclinical studies reported that *C. esculenta* corm extracts exerted antitumoral and antimetastatic [Kundu *et al*., 2012; Park *et al*., 2013], antihyperlipidemic [Sakano *et al*., 2005], antioxidant [Lee *et al*., 2011], wound healing [Gonçalves *et al*., 2013], antidiabetic [Eleazu *et al*., 2013], and antiviral [Keyaerts *et al*., 2007] properties.

The widespread traditional usages of *C. esculenta* are for many health disorders including: gastrointestinal diseases, diabetes mellitus, alopecia, internal hemorrhages, anemia, body ache, snakebite, and additionally for immune system stimulation [Lim, 2015, Nwauzoma & Dappa, 2013]. *C. esculenta* corm powder is marketed as an ingredient and as a food supplement, worldwide. The flour constituents of *C. esculenta* are comparable to corn, potato, and soybean ones; it has a high fiber a low fat content, which makes the flour a satisfactory substitute for market flours as an economic alternative in developing countries [reviewed in Pereira *et al*., 2018]. *C. esculenta* flour may be utilized in numerous preparations, such as bread, noodle, cookies, paste, and infant formulations,

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especially for dietary restriction cases (*e.g*., gluten intolerance and allergenic disorders) [Kaushal *et al*., 2015; Noorfarahzilah *et al*., 2014].

The defensive mechanisms that provide prevention of the carcinogenesis cascade are defined as chemoprevention perception. Chemopreventive agents are capable to prevent, reverse or postpone carcinogenesis cascade. Daily consumption of these agents represents a hopeful approach to suppress or prevent carcinogenesis [Mollakhalili *et al*., 2017]. A variety of phytochemicals originated from dietary plants are proved to hinder specific carcinogenesis stages through the inhibition of tumor initiation, promotion, and progression, which among others, encompassed the modulation of the cancer cell cycle, proliferation inhibition, and initiation of apoptosis [Mollakhalili *et al*., 2017].

In our previous studies, we reported that the sulphated forms of natural polysaccharides showed promising tumor cancer chemopreventive potentials [Gamal-Eldeen *et al.*, 2006; 2007a,b 2009; 2021]. In continuation, the current study was planned to explore the cancer chemopreventive mechanisms of a sulphated extract of *C. esculenta* corms, targeting to function as a cancer chemopreventive alternative in the healthy food industries for high-risk populations.

MATERIAL AND METHODS

Preparation of the sulphated *C. esculenta* extract

C. esculenta tubers (2 kg) were purchased from the local market (Dokki, Giza). Corms were washed and cleaned from the foreign substances, peeled, and chopped into smaller pieces (~1 cm^3). Afterward, the pieces were macerated with distilled water in a kitchen blender, and then extracted for 1 h with hot water under reflux. A filtration was carried out to discard insoluble material, and the filtrate was dialyzed for 48 h against running distilled water, prior overnight incubation with cold ethanol (1:4; *v/v*). The precipitate was gathered by centrifugation before vacuum drying (crude polysaccharides). Sulphated *C. esculenta* extract (SCE) was prepared rendering to published methods [Mähner *et al*., 2001; Yang *et al*., 2003]. In brief, the sulphating agent was developed by dropping 20 mL of fuming sulphuric acid into 100 mL of formamide, in a cooling chamber. The crude polysaccharides (4 g) were mixed with formamide and then mixed with the sulphation solution (120 mL) under overnight stirring. After cooling, consecutive steps were carried out including neutralization by 1 N NaOH, dialysis against distilled water for 48 h, and finally lyophilization. All of the chemicals were purchased from Sigma-Aldrich (Saint Louis, MO, USA), unless otherwise mentioned.

Characterization of the sulphated *C. esculenta* extract

The total carbohydrate content of SCE was estimated by the phenol–H_2SO_4 protocol [DuBois *et al*., 1956]. The total protein was determined with the Lowry method [Lowry *et al*., 1951]. The sugar composition was estimated after a complete hydrolysis of polysaccharides with H_2SO_4 (2 M) at 100°C for 8 h. The hydrolysate was neutralized by $BaCO_3$ and then Dowex 50 resin (H^+ form) was used. The chromatography for 24 h on Whatmann no. 1 paper with butanol: acetone: water (4:5:1, *v/v/v*) as a mobile phase was applied to separate the individual sugars [Partridge *et al.*, 1949]. The spots were sprayed with aniline phthalate for visualization. The total sulphate content was determined after hydrolysis with HCl [Larsen *et al.*, 1966] and the liberated sulphate ions were estimated by $BaCl_2$ turbidimetric method [Hunt, 1980].

Cell culture

Various cell lines were utilized through the study, including human breast carcinoma (MCF-7), human hepatocellular carcinoma (Hep G2), human lymphoblastic leukemia (1301) and raw murine macrophages (RAW 264.7); purchased from the American Type Culture Collections (ATCC, Manassas, VA, USA). RAW 264.7 cells were cultured in Roswell Park Memorial Institute Medium-1640 (RPMI-1640), while the other cell lines were routinely cultured in Dulbecco's Modified Eagle Medium (DMEM). Media were supplemented with 10% fetal bovine serum (FBS), 2 mM L-glutamine, 100 U/mL streptomycin sulfate, 100 U/mL penicillin G sodium, and 250 ng/mL amphotericin B. Cells were maintained in humidified air containing 5% CO_2 at 37°C. Extracts were dissolved in the cell matching medium. The extract stocks were examined, before assay dilutions, for endotoxins by the Pyrogent® Ultra gel clot assay to confirm endotoxin-free status. Materials for cell culture were purchased from Lonza (Morristown, NJ, USA). All of the cellular experiments were repeated (n=8), except flow cytometry analysis (n=4).

Tumor anti-initiation activity

The total cellular capacity for scavenging the physiologically dangerous radicals; peroxyl ($ROO^{\bullet}$) and hydroxyl ($OH^{\bullet}$); was investigated by the oxygen radical absorbance capacity (ORAC) assay, which is an indication of the total antioxidant activity of the cells [Cao & Prior, 1999; Gamal-Eldeen *et al*., 2004]. Hep-G2 cells were treated with 10 μg/mL of SCE for 24 h. The protein content of the cell lysate was measured and only 1 μg protein/mL was subjected to ORAC assay. β-Naphthoflavone-treated Hep-G2 cells were used as cytochrome P450 1A1 (CYP1A1) source, which was further treated with SCE (1 μg/mL), and then CYP1A1 was assessed by the dealkylation rate of 3-cyano-7-ethoxycoumarin into 3-cyano-7-hydroxycoumarin [Crespi *et al*., 1997; Gerhäuser, *et al*., 2003]. Glutathione *S*-transferase (GST) activity was estimated in 1×10^6 Hep G2 cells after being incubated with SCE (10 and 20 μg/mL) for 48 h [Habig *et al*., 1974]. The kinetic analysis was traced at 340 nm, and then GST concentration was normalized to the protein content. The total thiol content was estimated by an enzymatic method [Griffith, 1980].

Tumor anti-promoting activity and macrophage functions

To select a safe dose, the macrophage proliferation index was calculated for RAW 264.7 cells (0.5×10^5 cells/well) after being seeded with SCE (0–40 μg/mL) for 48 h. Cell viability was assessed by MTT test. RAW 264.7 cells were cultured in phenol red-free RPMI, to estimate both of the secreted tumor necrosis factor-α (TNF-α) by ELISA kit (R&D Systems, Minneapolis, MN) and the generated nitric oxide (NO), as assayed by Griess reagent in Moorcroft *et al*. [2001]. Macrophages were treated with bacterial lipopolysaccharide

(LPS, 1 μg/mL) for 24 h and with/without SCE (10 μg/mL and 20 μg/mL). Additionally, the SCE influence on the binding affinity of FITC-conjugated LPS to macrophages was evaluated [Carracedo *et al*., 2002]. Cells were seeded with SCE (10 and 20 μg/mL) in phenol red-free RPMI with 10% FBS (source of CD14 and LPS-binding protein), then incubated for 1 h, and the FITC-LPS binding affinity was detected *via* microplate fluorometer (FluoStarOptima, BMG, USA).

Tumor anti-progression effect

Cytotoxicity of SCE against human cancer cells was evaluated by the 3-[4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H--tetrazolium bromide (MTT) assay after 48 h after the treatment of 0.5×10^5 cells/well with SCE (0–40 μg/mL) for 48 h. Thereafter, the media were discarded and 40 μL MTT solution/well were added and incubated for 4 h. MTT crystals were solubilized by acidified isopropanol [Hansen, *et al*., 1989]. Photometric readings were recorded at 570 nm using a microplate ELISA reader. The analysis of cell cycle phases in 5×10^5 MCF-7 cells/mL after being treated with SCE (IC_{50}) for 12 h was carried out by flow cytometry after cell staining with propidium iodide (PI), using a flow cytometer (Becton Dickinson, San Jose, CA, USA). The PI/FITC-anti-Annexin V Kit (Invitrogen, Waltham, MA, USA) was used to estimate apoptosis/necrosis by flow cytometry. MCF-7 cells were treated with SCE (0–40 μg/mL) for 48 h, and then the activity of histone deacetylase (HDAC) was measured in the cell lysate by a colorimetric kit (BioVision, Milpitas, CA, USA) according to the manufacturer's instructions.

Data analysis

Data were statistically analyzed by Student's unpaired t-test and one-way ANOVA test. The differences between mean values were considered insignificant at $p>0.05$.

RESULTS AND DISCUSSION

Halting, suppressing, retarding or reversing the sequence of carcinogenesis stages is regularly called "cancer chemoprevention", which is mostly achieved *via* using natural semi--natural, or synthetic chemicals to neutralize carcinogens [Tan *et al*., 2011]. Plant extracts are known to possess wide-range mechanistic chemopreventive activity, through blocking the enzymatic carcinogen-activation process in tumor initiation stage or inhibiting the growth of the pre-neoplastic and neoplastic cells [Tan *et al*., 2011]. The current study is an attempt to recognize whether SCE acts as a blocking or a suppressing agent.

Characterization of sulphated *C. esculenta* extract

The analysis of the chemical composition of SCE revealed that after the sulphation process, the sulphate substitution in SCE was 48% with a sulphation degree of 2.3 (molar ratio to monosaccharide unit) (Table 1) that indicated the accomplishment of the sulphation reaction. Chromatography analysis of SCE acid hydrolysates revealed the occurrence of a significant amount of glucose, and smaller amounts of mannose, galactose and uronic acids, as well as traces of arabinose, xylose, and rhamnose (Table 1).

Tumor anti-initiation activity

In oxidative stress status and inflammatory condition, extreme generation of reactive oxygen (ROS) and nitrogen (RNS) species occurs and causes DNA damage that ignites tumor initiation and promotion cascade [Sun *et al*., 2004]. Accordingly, eliminating the excess of physiologically-pertinent ROS, such as $ROO^{\bullet}$ and $OH^{\bullet}$, affords an effective approach to halt tumor initiation and promotion. Likewise, the augmentation of the non-enzymatic antioxidants; total thiols, supports the attenuation of ROS harmful effect. The total cell lysate capacity for scavenging the radicals; $OH^{\bullet}$ and $ROO^{\bullet}$; was assessed by ORAC assay in HepG2 cells. The results indicated that SCE remarkably enhanced the total cellular capacity to scavenge both radicals (Figure 1a). However, the affinity to scavenge $OH^{\bullet}$ was higher than $ROO^{\bullet}$, as concluded from their ORAC units in comparison with Trolox, where one ORAC unit is equivalent to the protection of the fluorescence decay of α-phycoerythrin (α-PE) achieved by Trolox (1.0 μM).

To antagonize xenobiotics and toxic agents, cells emerged a panel of responding genetic amendments that help them to repress the damaging effect of toxicants. Those amendments include upregulation of drug-metabolizing enzymes, cytoprotective proteins, and drug transporters, that facilitate clearance of toxicants from the body and restore normal homeostasis. Nuclear factor-erythroid 2–related factor 2 (Nrf2) as well as aryl hydrocarbon receptor (AhR) are transcription factors that mediate the enzymatic response towards xenobiotics [Hayes *et al*., 2009]. Among others, transcription factor AhR regulates CYP1A1 expression, which is triggered by its conjugation with polycyclic aromatic hydrocarbons. The metabolizing of exogenous and endogenous substrates is regulated by monooxygenase, which is primarily encoded by CYP1A1 gene. CYP1A1 is a pivotal player in the metabolism of benzo[α]pirene and linked polycyclic aromatic hydrocarbons, transferring both into strongly dangerous carcinogens. Accordingly, the enhanced expression of CYP1A1

TABLE 1. Chemical composition of a sulphated water-soluble extract of *C. esculenta* (SCE).

Chemical composition	Value
Carbohydrate (g/100 g)	35.4
Protein (g/100 g)	0.8
Sulphate (g/100 g)	48.0
Degree of sulphation	2.3
Relative monosaccharide contents (g/100 g)	
Uronic acid	1.15
Galactose	4.0
Glucose	83.5
Mannose	11.0
Arabinose	Traces
Xylose	Traces
Rhamnose	Traces

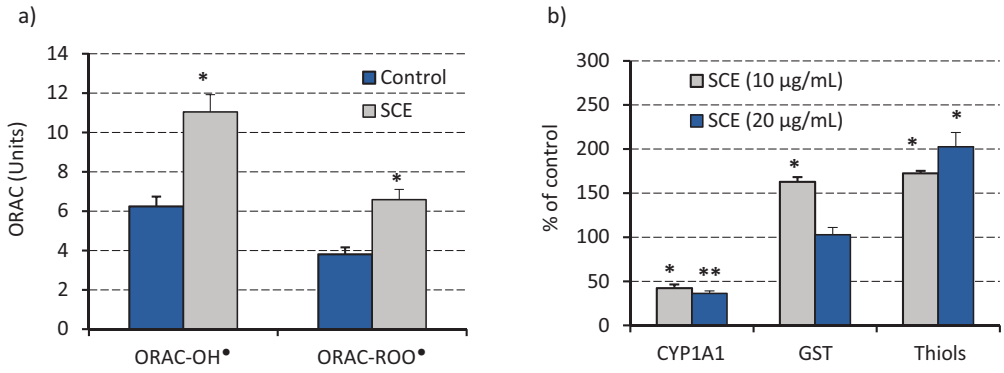

FIGURE 1. Tumor anti-initiating activity: (a) Oxygen radical absorbance capacity (ORAC) was used to investigate the radical scavenging (antioxidant) activity against OH• and ROO• in Hep-G2 cell lysate after being treated with a sulphated *C. esculenta* extract (SCE) in comparison with control cells. Data are expressed in ORAC units. (b) The modulation of the carcinogen metabolism: The effect of SCE (10 and 20 μg/mL) on the purified cytochrome P450 1A1 (CYP1A1; 1 μg protein/mL) and the cellular levels of glutathione *S*-transferase (GST) and total thiol content in Hep-G2 cells was investigated. Data was expressed as mean percentage ± standard error and control represents 100% of the scale. In control cells, GST and total thiol contents were 118 nmol/min/mg protein and 84 nmol/min/mg protein, respectively. *$p<0.05$ and **$p<0.01$.

gene is an indicator for AHR stimulation and is associated with the metabolism and toxicity of xenobiotics [Mescher & Haarmann-Stemmann, 2018]. Therefore, CYP1A1 is considered as a potential molecular target to modulate and prevent chemically-induced carcinogenesis.

Alteration of different enzymes that participate in the metabolic activation of carcinogens (phase I enzymes) and in the detoxification of carcinogens (phase II enzymes), is an effective strategy for recognizing cancer anti-tumor initiation agents. The estimation of the inhibitory effect of SCE on CYP1A1 enzyme, as one of the phase I-enzymes participating in the transformation of procarcinogens into active carcinogens, revealed that SCE (10 μg/mL and 20 μg/mL) can be recognized as a strong inhibitor of CYP1A1 activity ($p<0.01$) with inhibition of 57% and 64%, respectively (Figure 1b), in comparison to control.

In carcinogenesis, the initiation is the very early and crucial event, where it functionally promotes the clonal cell growth under the control of promoters and ends eventually with consequent preneoplastic cell generations [Bertram, 2000]. Retarding or stopping the initiation stage through suppressing the activators of carcinogen metabolism (*e.g.*, CYP1A), enhancing the carcinogen detoxification (*e.g.*, glutathione (GSH) and GSTs), and increasing cellular antioxidant activity are efficient operative strategies. A fundamental indicator of the cellular defense against the oxidative stress is the total thiol level, especially GSH, the key cytosolic thiol, that supports the elimination of peroxides and other free radicals [Aggarwal & Shishodia, 2006]. GSH homeostasis depends on its binding to GSTs, which is a panel of enzymes that regulates xenobiotic detoxification and defends the cells against carcinogens. GSTs generally amend the cellular GSH levels according to the ROS generation level [Prabhu & Guruvayoorappan, 2010]. In Hep-G2 cells, GST was explored (as one of phase II enzymes) after 48 h of cell seeding with SCE (10 μg/mL). Its activity was elevated up to 162.47% of the control ($p<0.05$), as shown in Figure 1b, while it remained unaffected by the higher SCE. The assessment of the total thiol level indicated that SCE dramatically increased the thiol content in cells in both of the tested doses (Figure 1b).

Our results revealed that SCE is an effective tumor anti-initiating agent, since it dramatically suppressed CYP1A activity. Cellular antioxidants guard the cells from the harm of dangerous physiological radicals, such as hydroxyl and peroxyl radicals, that can attack critical protein and DNA molecules. The cellular antioxidants maintain the homeostatic balance of cellular ROS [Bertram, 2000], whenever this balance is impaired, it directly disturbs cellular growth, apoptosis, and senescence. Our findings indicated that SCE strongly increased the total cellular scavenging activity, as estimated in the cell lysate of SCE-treated Hep-G2 cells, against OH• and ROO•, with higher affinity towards OH•, compared with the scavenging activity of cell lysate of untreated cells.

Tumor anti-promoting activity and modulation of macrophage function

As a key player in innate and adaptive immune responses, macrophages are regularly engulfing and then digesting pathogens *via* discharging mediators of inflammation including NO that is a potential RNS that in turn transforms into many oxidation products which are capable to trigger the carcinogenesis initiation and promotion [Prabhu & Guruvayoorappan, 2010]. The strong affinity of SCE to scavenge variable radicals including its suppressing of the LPS-induced NO may indicate that the suppression was because of a direct NO scavenging or *via* inhibiting iNOS pathway. The macrophage growth induced after SCE treatment may be associated with an elevation in the expression of the macrophage growth factor; IL-12. The outer membrane of Gram-negative bacteria enclosed LPS that is an essential molecule in septic shock pathogenesis.

LPS regularly conjugates the serum acute-phase reactant LPS-binding protein (LBP) that transports LPS to CD14 (a primary LPS receptor in serum) and as a glycophosphatidylinositol-linked agent on mononuclear phagocytes surface. LPS-CD14 activates the generation of the inflammatory cytokines [Kitchens, 2000]. Subsequently, the effective

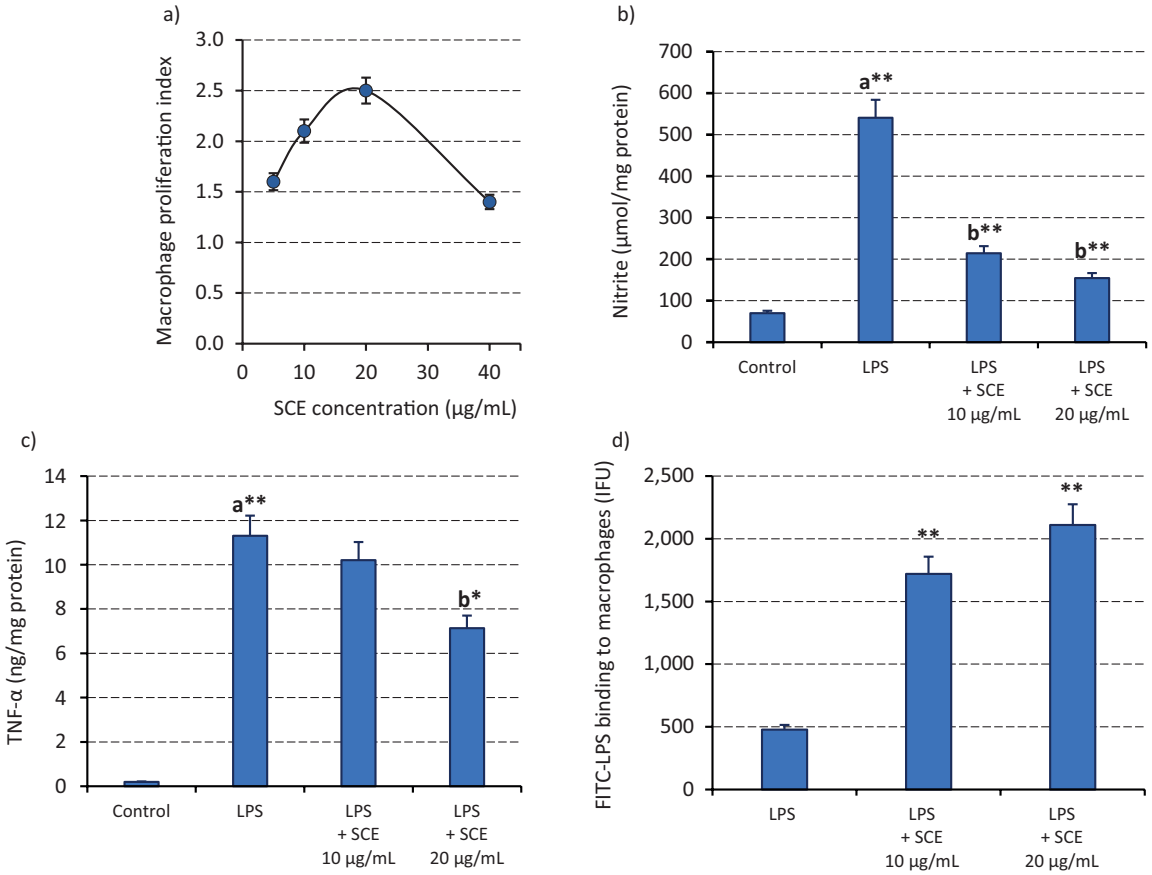

FIGURE 2. Modulation of macrophage function: (a) Macrophage proliferation index (folds of control) in sulphated *C. esculenta* extract (SCE) treated Raw 264.7 cells. (b) Nitric oxide (NO) production (μmol/mg protein). (c) Tumor necrosis factor (TNF-α) concentration (ng/mg protein) were estimated in the supernatants of Raw 264.7 after being stimulated by bacterial lipopolysaccharide (LPS) before being treated with/without 10 & 20 μg/mL SCE. (d) Analysis of fluorescein isothiocyanate-LPS (FITC-LPS) binding affinity to Raw 264.7 cells by flow cytometry. Data was expressed as mean percentage ± standard error. *$p<0.05$ and **$p<0.01$; [a]compared to control macrophages and [b]compared to LPS-treated macrophages.

enhancement of the macrophages/LPS binding affinity by SCE may ultimately result in enhanced LPS-LBP conjugation and/or LPS-CD14 conjugation. *C. esculenta* extract treatment for Her-2/neu negative murine mammary tumor cell line (410.4) showed antimetastatic activity, which suggested to be due to its inhibition of the inflammatory mediators including the suppression of prostaglandin E_2 (PGE_2) synthesis and downregulation of cyclooxygenase (COX) 1 and 2 expressions [Kundu *et al.*, 2012].

The influence of SCE on the macrophage proliferation and its functionality was explored. The results demonstrated that SCE displayed a gradual dose-dependent immunoproliferative outcome on macrophages (Figure 2a) to the highest level of 2.51-fold of control at 20 μg/mL ($p<0.05$), but not in the highest dose used. This high macrophage proliferation led to the interest of further investigations to check if this proliferation was concurrently accompanied with elevated macrophage functions. Bacterial LPS was used to induce inflammation cascade in the RAW 264.7 cells before being further treated with SCE. Interestingly, treating RAW 264.7 cells with SCE (10 μg/mL and 20 μg/mL) resulted in a significant inhibition ($p<0.01$) in the LPS-stimulated NO production, where it inhibited 60.32% and 71.43% of the LPS-generated NO, respectively (Figure 2b). While only the dose of 20 μg/mL of SCE significantly inhibited ($p<0.05$) the TNF-α release from LPS-treated macrophages (Figure 2c). The affinity of macrophages to bind a tumor surface antigen or a pathogen is their essential mechanistic activation function. That binding was traced after the seeding of macrophages with FITC-LPS with/without SCE (10 μg/mL and 20 μg/mL). The results revealed that both doses of SCE dramatically suppressed ($p<0.01$) the macrophage binding affinity to FITC--LPS (Figure 2d).

Anti-progression activity

Exploring the cytotoxicity of SCE against solid tumor cell lines showed a remarkable dose-dependent cytotoxicity in breast MCF-7 cells (IC_{50} 27.73 μg/mL) and a lower cytotoxicity extent in the case of Hep-G2 cells (IC_{50} 64.32 μg/mL), as shown in Figure 3a. However, the treatment of the hematopoietic tumor cells (1301 leukemia) with SCE resulted in a gradual increment in the lymphocyte proliferation, up to 1.5-fold at 40 μg/mL (Figure 3a).

Consequently, the effect of SCE on MCF-7 cell cycle phases was investigated, whereas the untreated MCF-7 cells exhibited an intact pattern of cell cycle stages, as 96.3% of the cell's population appeared in G0/G1 phase (Figure 3b). SCE treatment resulted in a significant arrest

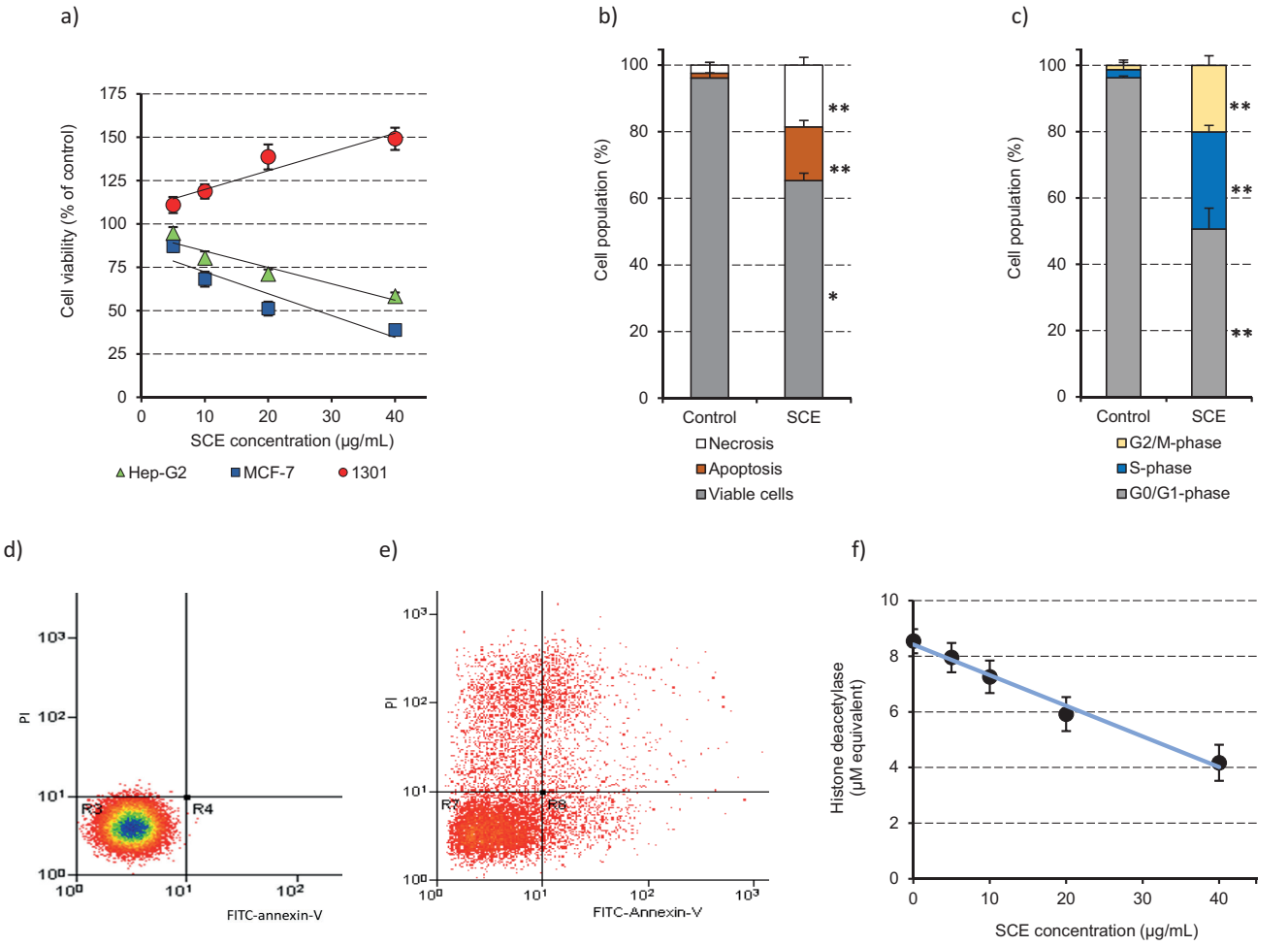

FIGURE 3. Anti-progression activity: (a) Cytotoxicity of a sulphated *C. esculenta* extract (SCE) against different human cancer cell lines. (b) Cell cycle analysis of breast MCF-7 cells after being treated with SCE (20% IC_{50}; 48 h), compared with control cells. (c-e) The analysis of apoptosis and necrosis (by anti-annexin V-FITC/PI) in MCF-7 cells that were seeded without/with 20% IC_{50} of SCE for 12 h. The data are presented as percentages of the cell population, while in (e) the flow cytometry dot plots for the cells are presented. (f) Histone deacetylase (HDAC) inhibition in SCE-treated MCF-7 cells. Data were presented as mean percentage ± standard error. $^{*}p<0.05$ and $^{**}p<0.01$.

($p<0.01$) in S-phase (29.2%), and G2/M phase (20.1%) and subsequently a concomitant significant decrease in cell population in G0/G1 phase, as shown in Figure 3b. Due to the noticed disturbance in cell cycle phases and SCE-induced cytotoxicity in MCF-7 cells, the meanwhile cell death mode stimulated by SCE was analyzed. The findings indicated that SCE encourages necrosis as much as apoptosis (Figure 3c, Figure 3e), as concluded from their total population percentages (18.5%, and 16.1%, respectively), compared to the control pattern (Figure 3c, Figure 3d).

Our results indicate the cytotoxicity of SCE against breast carcinoma MCF-7 cells and hepatocellular Hep-G2 carcinoma but not with lymphoblastic leukemia, signifying the SCE specificity to solid tumor cells. Rendering the polysaccharides high molecular weight, SCE IC_{50} of MCF-7 cells (27.73 μg/mL) provides a dramatic low molar concentration of SCE. In a parallel previous report, *C. esculenta* extract was reported to similarly reduce the proliferation of some breast as well as prostate cancer cell lines [Kundu *et al*., 2012]. Rounded cells with morphologic alterations were recorded, where cell migration was totally jammed by taro extract [Kundu *et al*., 2012]. Cell death may occur due to different death mechanisms, among them are the necrosis and apoptosis that are characterized by variable morphological and biochemical events, including cell swelling, disruption, and rapid cell membrane fragmentation in necrosis and elegant nuclear and cytoplasmic disintegration and formation of apoptotic bodies [Xu *et al*., 2019].

In the current study, SCE induced both necrosis and apoptosis in MCF-7 cells that was activated by a parallel disorder in cell cycle phases; arrested cell population in S- and G2/M phases. The rate of cell growth can interfere in the carcinogenesis stages by multiple mechanisms; among them the cell population *per se* may be carcinogenic through fixing of miscoding lesion in the freshly synthesized DNA [Lund, 2011]. Cells retort to cytotoxic stress and DNA impairment by arresting cell-cycle phases, repairing DNA or enduring apoptotic cell death. A wide array of cancer chemopreventive agents displayed their antitumor activity in association with disturbing cell cycle and arrested growth, with apoptosis [Tanaka & Ishigamori, 2011]. Subsequently, the ability of SCE to inhibit S-phase in breast cell cycle may diminish the frequency of DNA miscoding lesions. Anticancer therapeutics eliminate

cancer cells by targeted mechanisms including damaging cell membrane, interactions with DNA, suppressing DNA replication, and attacking cells by free radicals.

Since HDAC is one of the apoptosis-regulating factors, the acetylation of histone was estimated in the MCF-7 cell lysate after being treated with different concentrations of SCE. The results revealed that SCE is a potent inhibitor of HDAC, in dose--dependent linear profile, whereas the half maximal inhibitory concentration of SCE to HDAC (HDAC IC_{50}) was 37.7 μg/mL (Figure 3f). In the current study, the apoptosis was associated with a remarkable inhibition in HDAC (IC_{50} 37.7 μg/mL). It is known that the post-translational histone modification "histone acetylation" is regulated by histone acetyltransferases and HDACs. By eliminating the acetyl groups, HDACs opposite the acetylation of chromatin and amend tumor suppressor genes and oncogenes transcriptions [Li & Seto, 2016]. Interestingly, HDACs deacetylate other non-histone substrates that regulate a battery of biological pathways such as tumor initiation and progression. The therapeutic approach of HDAC inhibitors (HDACi) is an emerging cancer treatment [Li & Seto, 2016]. *Via* hyperacetylation of histone/non-histone, HDACi permit the restoration of cellular acetylation homeostasis and re-establish the normal expression of proteins and inverse the processes of tumor initiation and progression [Li &d Seto, 2016]. Accordingly, the strong inhibitory activity of SCE for HDAC is one of the mechanisms of SCE as a potential tumor anti-initiating agent.

CONCLUSION

Recently, due to their flexibility, cost-effectiveness, and desirable drug release and regulatory acceptance, many biotechnological approaches have been focused on hydrophilic polymers in pharmaceuticals. The current study findings propose SCE as a promising candidate for food industries as a functional and healthy food supplement and as an alternative of CE itself, to offer a cancer chemopreventive properties and evoke anti--inflammatory activity in targeted breast cancer-high risk communities. This study is an innovative trial to prepare a sulphated water extract of *C. esculenta*. Taken together, SCE is a strong tumor anti-initiation agent *via* suppressing cytochrome P450–1A and enhancing the total thiol content and the carcinogen detoxification enzyme (GST). SCE exhibited a strong scavenging affinity towards critical radicals ($OH^{\bullet}$ and $ROO^{\bullet}$). Also, it induced lymphocyte growth and modulated the macrophage functions into an anti-inflammatory profile, *via* elevating macrophage proliferation, its binding affinity of FITC-LPS and, to different extent, its inhibition of NO and TNF-α generation. Furthermore, it showed a potent cytotoxicity against MCF-7 cells, disturbed cell cycle phases (S- and G2/M-phases), and enhanced late apoptosis and necrosis. SCE is a promising cancer chemopreventive agent to be used in healthy food industries and for the high breast cancer-risk population, an assumption that needs to be validated in forthcoming *in vivo* studies.

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CONFLICT OF INTEREST

We declare that there is no conflict of interest.

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