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Original article

Cyclotrisiloxan and β-Sitosterol rich *Cassia alata* (L.) flower inhibit HT-115 human colon cancer cell growth via mitochondrial dependent apoptotic stimulation

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ABSTRACT

Cancer traits dependent chemo and radiotherapy display acute toxicity and long-term side effects. Since last two decades, researchers investigated a new anticancer agents derived from plants. *Cassia alata* (L.) is a medicinal herb distributed in the tropical and humid regions. In this study, *C. alata* flower methanol extract (CME) have been prepared using cold percolation method and the phytochemical components were identified using GC–MS analysis. CME have been used to study the antiproliferative and apoptosis properties against human colon cancer HT-115 colon cancer cells, its molecular mechanism have been explored. 0.2 mg/mL dose of CME, inhibited 50% of HT-115 colon cancer cell growth after 48hr was confirmed the significant antiproliferation effect. In normal cells such as Vero cells and hMSCs, 0.2 mg/mL dose of CME shown only 4% and 5% growth inhibition confirmed the HT-115 cell specific cytotoxic effect. This effect might be due to the availability of phytoactive biomolecules in CME such as, cyclotrisiloxan, beta-sitosterol and alpha-tocopherol have been confirmed by GC–MS. Most interestingly, PI and AO/ErBr staining of CME treated HT-115 cells shown early (25%), pro (17%) and late (8%) apoptotic and 3% necrotic cells after 48 hr. Treatment with CME extract showed potential effect on the inhibition of protumorigenic inflammatory and oxidative stress genes. Protumorigenic COX-2/PGE-2 and TNF-α/NF-κB immune axis were normalized after CME treatment. Amounts of both apoptosis related mRNA p53, Bax, caspase 3 and p21 genes were upregulated, whereas it resulted in significant reduction in the anti-apoptotic marker mdm2 and Bcl-2 genes. In conclusion, bioactive compounds present in CME potentially inhibit HT-115 colon cancer cell proliferation via an inhibition of protumorigenic immune axis and stimulation of mitochondria dependent apoptotic pathway without necrotic effect.

1. Introduction

Plants has been implicated in human and animals lives. It has been used as source enriched in nutrition including carbohydrates, lipids, proteins as well as minerals and vitamins. In addition, the availability of the abundance of bioactive compounds derived from plants are nowadays used as a pharmaceutical agents against various human diseases. Rich sources of bioactive compounds in plants protect and resist to environmental hazards such as pollution, stress, drought, UV exposure and pathogenic attack (Yoon et al., 2020; Mewis et al., 2012). In the developing countries people are using plants-derived products to cure various diseases such as rosin, gum or essential oils depending on their inherent traditional knowledge, so called traditional medicines (Zhao et al., 2016). They believe that, plants derived phytochemicals has no side effects compared to those which associated with synthetic drugs (Lim, 2014). Hence, in the present investigation, the plant ***Cassia alata (L.)*** have been tested for the potential antiproliferative and apoptotic activity against human colon cancer HCT-115 cells.

Cassia alata is one of the oldest known medicinal plant of Central America, worldwide, particularly in Asia Pacific countries. In

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traditional medicinal practice, ***Cassia alata (L.)*** have been used to treat various skin diseases caused by bacteria and fungal infection and by insect bite (Lim, 2014). *Cassia alata* is widely used as traditional medicine in India and Southeast Asia (Akinmoladun et al., 2010; Sagnia et al., 2014). This plant is reported to possess insecticidal, anti-inflammatory, hydragogue, sudorific, diuretic, pesticidal properties (Alalor et al., 2012). Fresh leaves juice is used for ring worm, snakebite, scorpion bite, skin diseases, impetigo, syphilis sores, itching, mycosis, herpes and eczema. The leaves have been reported to be useful in the treatment of convulsions, gonorrhea, heart failure, abdominal pains and oedema (Rekha et al., 2017).

Scientifically, *Cassia alata* has been reported to have very high medicinal values like antimicrobial property particularly against fungal dermatophytes and traditionally being used in the treatment of skin infections in humans (Sagnia et al., 2014; Lewis and Levy, 2011). Roots, leaves and flowers of this plant possess many biological properties such as antibacterial, antifungal, anti-inflammatory, antitumor, expectorant and also useful in urinary tract problems (Zhao et al., 2016). Moriyama et al., (2003) have identified kaempferol-3-O-gentiobioside in *Cassia alata* flowers and seeds. Fatmawati et al., (2020) have reviewed the chemical constituents identified from various parts of *Cassia alata* with their promising antidiabetic, anti-inflammatory, cardio protective and anticancer effect in animal and human models. Azman et al., (2020) have reported an inhibitory effect of acetylcholinesterase activity of *Cassia timorensis* DC. The bioactive compounds kampferol and rhein present in *Cassia alata* leaf which possess caspases 8 dependent cytotoxicity in A549 lung cancer cells (Fernand et al., 2011; Levy and Lewis, 2011). Phutsisen et al. (2019) have found a beneficial effect in gynecological cancer patients bowl function recovery. Olarte et al. (2013) have reported that the availability of polyunsaturated fatty acid in *Cassia alata (L.)* shown anticancer activity in breast cancer, bladder carcinoma and colorectal cancer cells.

The present study, we have selected *Cassia alata* (L.) flower to identify its photochemical components using GC–MS. Further, the effect of *Cassia alata* (L) flower on the regulation of protumorigenic (COX-2/PGE-2), immuno axis (TNF-α/NF-κB) and cytotoxic potential will be explored in HT-115 human colon cancer cells. In addition, to determine the mode of action the mechanistic effect associated with the caspases dependent apoptosis pathway will be explored.

2. Materials and method

2.1. Cell culture materials and fine chemicals

HT-115 cell (human colon cancer), human mesenchymal stem (hMSCs) and Vero (normal monkey kidney cell) cells have been purchased from American type culture collections (ATCC, USA). Roswell park memorial institute (RPMI-1640) medium, trypsin, EDTA and all other cell culture materials have been purchased from Gibco, Paisley, UK. Fetal bovine serum (FBS) was purchased from Hiclone, Germany. 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetra zolium bromide (MTT) were obtained from Sigma (St. Louis, MO, USA). cDNA synthesis kit and SYBR Green PCR master mix were purchased from Qiagen, Hilden, Germany. Deionized water was collected from Direct-QUV3 Multipore water purification system (Millipore, Burlington, MA, USA). Fine chemicals related to molecular biology experiment have been purchased from Sigma-Aldrich chemical company (St. Louis, MO, USA). Spectrophotometric measurements were performed with UV2010 spectrophotometer (Hitachi, Germany).

2.2. Cassia alata (L.) flower collection and extraction

The flowers of *Cassia alata* (L.) was collected from Tamilnadu, India. It was identified by a Taxonomist in Department of Botany, College of Science, King Saud University, Riyadh. The shade dried flower was ground and utilized for sequential extraction using cold percolation method. About 40 g flower powder was subjected to successive extraction with 200 ml of hexane for 48hr with shaking under dark condition, then the solution was filtered thought whatman No.1 filter paper. The filtrate were concentrated using rotary vacuum evaporator and the obtained extract have been stored in refrigerator until needed. The residue were left to air dry and extracted with 200 ml of ethyl acetate and methanol for 48 hr with shaking under a dark condition, respectively. The filtration and evaporation of solvents were performed as same like hexane extraction method.

2.3. GC–MS analysis

The extract dissolved in HPLC grade methanol, loaded in Agilent GCMS unit through using helium (99.99%) gas at a constant flow rate of 1 ml/min. The system was equipped with a J&W-5MS fused silica capillary column containing 5% diphenyl and 95% dimethylpolysiloxane (30 m 0.25 mm), injected Dose (ID) 1 ml (% ID/g), with an injection volume of 3 μl (split ratio of 10:1). GC injector temperature was 250 °C and ion source temperature were set at 250 °C. Also, oven temperature started initially at 50 °C for 4 min, then increased gradually to 300 °C. The ionization voltage was set to 70 eV with a scan interval of 0.5 s and fragments from 35 to 800 Da. 35 min was the total run time of the GC. Turbo Mass version 5.2 was used to analysis Mass spectra and chromatograms. National Institute of Standard Technology (NIST-11) database library was used to interpret the result.

2.4. HT-115, Vero and hMSCs cell culture conditions

HT-115 human colon carcinoma cells, Vero cells and hMSCs were subcultured in RPMI-1640 medium supplemented with 10% FBS, 100U/ml penicillin and 100 μg/ml penicillin/streptomycin at 37 °C, 5% CO_2 in T75 culture flask. For the in vitro experiments, HT-115 cells, Vero cells and hMSCs were harvested after trypsinization, the cell viability and cell count were tested by trypan blue using a hemocytometer. Then desired cells (1×10^4 cell/well in 100 μl of medium) were seeded into 96-well plate for cytotoxicity and in 24 well plate for nuclear staining or gene expression studies.

2.5. Cell viability assay

Cell viability was evaluated by MTT assay as described by Mosmann (1983). In brief, preplated HT-115 cells, Vero cells and hMSCs were cultured in growth media for 24hr, then the media was replaced with increasing concentration (0, 0.025, 0.05, 0.1, 0.2, 0.4 and 0.8 mg/dL) of *Cassia alata* methanol extract (CME) and cultured for 24hr and 48hr, respectively. The selection of incubation time such as, 24 hr have been used to determine the effect of CME on cell death during the cell doubling time (16–24 hr) [24hr cell death- represent necrosis]. Incubation for 48 hr, majorly represent the intracellular uptake of drug, mitochondrial dependent cell death have been determined [48 hr - represent apoptosis]. After incubation, 20 μl of MTT solution was added to all the wells in dark and incubated for 4hr at 37 °C. The purple formazan crystals were produced were dissolved using DMSO and absorbance were measured at 492 nm using a micro plate reader. The results were analyzed in triplicates and the viability percentage were calculated.

2.6. Analysis of cell and nuclear morphological changes

To determine the characteristic apoptotic and morphological changes in CME treated HT-115 cells, propidium iodide (PI) or acridine orange/ ethidium bromide (AO/ErBr) staining assay have been carried out according to the methods of Leite et al. (1999). Briefly, HT-115 cells (1×10^4) were cultured in 24-well plate and treated with control and CME (0.1 and 0.2 mg/dL) for 48hr. Further, CME treated cells were fixed with 4% paraformaldehyde and stained with 1 mg/mL of PI or AO/ErBr (BD Biosciences, USA) at 37 °C for 15 min in the dark. Randomly 300 stained cells were analyzed using inverted fluorescence microscope (200× magnification) and thee pathological changes of the cells were manually calculated and pictures were taken.

2.7. RNA preparation and quantitative real-time PCR analysis

To analyze the expression level of the oxidative stress, inflammatory, protumorigenic (CYP1A, GSK3β, TNF-α, NF-κB, COX-2, PGE-2) and apoptotic (Bcl-2, Bax, Caspase 3, p53, mdm2, PCNA & p21) genes were quantified. Primer sequences for the proinflammatory, antioxidant and apoptosis related genes are provided in Table 1. Total RNA was extracted (in triplicate) after 48hr treatment with 0.1 and 0.2 mg/dL dose of CME treated HT-115 cells. cDNA have been prepared directly using Fastlane® Cell cDNA kit (QIAGEN, Germany). The cDNA concentration was determined as 100 nm by diluted with water (1:10) and used for qRT-PCR analysis with primers specific to the desired genes. The qRT-PCR reactions were performed in 96 well optical plates in a thermocycler "7500 Real-Time PCR system" (Applied Biosystems) and the amplification data was analyzed by the 7500 software v2.0. PCR conditions were: 2 min at 50 °C, 10 min at 95 °C, and 40 cycles of 15 s at 95 °C and 30 s at 60 °C. $2^{-\Delta\Delta Ct}$ method were used to determine the relative mRNA expression level of specific gene. Such as, where ΔΔCt = (Ct, target gene of experimental group − Ct, β-actin of experimental group) − (Ct, target gene of control group − Ct, β-actin of control group) (Yuan et al., 2006; El-Hallouty et al., 2020).

2.8. Statistical analysis

SPSS/29.5 software were used for statistical significance evaluation. The values were analyzed using one-way analysis of variance (ANOVA) followed by Tukey's test (Kim, 2014). All results were four replicates in each group (mean ± SD) and the differences were presented statistically significant at $p \leq 0.01$ and $p \leq 0.05$.

3. Results

3.1. GC–MS analysis of Cassia alata methanol extract (CME)

GC–MS chromatogram of the Cassia alata flower methanol extract showed 97 hits indicating the presence of a wide range of phytochemicals with the retention time range between 3.786 and 34.828 (Table 2). The active principles in CME were identified through GC–MS analysis. GC–MS analysis result of C. alata flowers indicated that the most abundant identified compounds were cyclotrisiloxan, β-Sitosterol, tocopherol, eicosane, beta carotene, omega 3 & omega 6 derivatives and cyclotrisiloxan derivative compounds (Table 2) with an average of 38% of all identified phytochemicals.

3.2. In vitro cytotoxic effect of C. alata methanolic extract

The MTT assay revealed that increasing concentration of CME showed a significant growth inhibition of HT-115 cells. We found that the cytotoxicity was significantly lower in 24 hr even at the level of 0.1 mg/dL ($p \leq 0.01$), it reflect CME up taken by the cells and the secondary metabolite stimulate the cytotoxicity mechanism. After 48 h of CME treatment, yielded significantly ($p \leq 0.05$) higher values of growth inhibition. We found CME at a concentration of 0.1 mg/dL in 48 hr and 0.2 mg/dL in 24 hr inhibited 50% of HT-115 cell population (IC_{50}), respectively (Fig. 1A). Observed results reflected that the gradual decline in the cell viability with the higher doses of CME may be due to apoptosis stimulation not by necrosis. The cell proliferation inhibitory effect of CME found only in HT-115 colon cancer cells, same dosage of CME did not shown significant inhibitory effect in normal cells neither Vero cells nor hMSCs (Fig. 1B). In normal cells, 0.2 mg/mL dose of CME shown only 4% and 5% growth inhibition in Vero cells and hMSCs, respectively. The tested higher concentration of CME (8 mg/dL) shown 9% cell death in Vero cells and 7% of cell death in hMSCs, confirmed the HT-115 cell specific cytotoxicity. Therefore, 0.1 and 0.2 mg/dL of CME was selected as the optimum concentration for the subsequent in vitro cell morphology and gene expression analysis.

3.3. Effect of C. alata methanolic extract on cell and nuclear morphology

HT-115 cells treated with the 0.1 and 0.2 mg/dL of CME for 48hr showed morphological variations after PI or AO/ErBr staining. We found and distinguish an irregular shaped and horseshoe-shaped nuclei confirmed endoplasmic reticulum stressed cells and apop-

Table 1
Primers used in the Sybrgreen based real-time polymerase chain reaction (RT-PCR).

Primer	Forward Sequence (5′ to 3′)	Reverse Sequence (5′ to 3′)
CYP1A1	GCTGACTTCATCCCTATTCTTCG	TTTTGTAGTGCTCCTTGACCATCT
GSK3β	GGAACTCCAACAAGGGAGCA	TTCGGGGTCGGAAGACCTT
COX-2	TTCAAATGAGATTGTGGGAAAATTGCT	AGATCATCTCTGCCTGAGTATCTT
PGE-2	CTCCTTGTTCCACGTGCTG	GGCGAAGAGCATGAGCATC
TNF-α	CTCTTCTGCCTGCTGCACTTTG	ATGGGCTACAGGCTTGTCACTC
NF-κB	GCGCTTCTCTGCCTTCCTTA	TCTTCAGGTTTGATGCCCCC
Bax	TCAGGATGCGTCCACCAAGAAG	TGTGTCCACGGCGGCAATCATC
Bcl-2	GTGGATGACTGAGTACCT	CCAGGAGAAATCAAACAGAG
p53	CCTCAGCATCTTATCCGAGTGG	TGGATGGTGGTACAGTCAGAGC
mdm2	CCCAAGACAAAGAAGAGAGTGTGG	CTGGGCAGGGCTTATTCCTTTTCT
PCNA	CAAGTAATGTCGATAAAGAGGAGG	GTGTCACCGTTGAAGAGAGTGG
Caspase-3	ACATGGAAGCGAATCAATGGACTC	AAGGACTCAAATTCTGTTGCCACC
p21	AGGTGGACCTGGAGACTCTCAG	TCCTCTTGGAGAAGATCAGCCG
GAPDH	GTCTCCTCTGACTTCAACAGCG	ACCACCCTGTTGCTGTAGCCAA

Table 2
Cassia alata flower methanol extract (CME) composition analysis using GC–MS shown 99–95% similarity in the database.

Peak No.	List of compounds	Peak area (%)	Retention time	Reported Anticancer activity
1	2-Pentanol	3.28	4.321	–
2	4-methyl-3-pentanol	6.90	6.37	–
3	2-nonanone	0.12	9.78	–
4	Hexadecanoic acid	4.05	22.89	–
5	9,12-Octadecanoic acid	1.82	24.54	(Goupille et al., 2020)
		6.76	25.19	
6	9,12,15- Octadecatrienoic acid	3.11	24.59	(Zhang et al., 2020)
7	Cyclotrisiloxane	0.12	28.68	(Bouabdallah et al., 2016)
		0.67	30.28	
		0.49	30.64	
		0.43	30.73	
		0.89	31.06	
		1.08	31.51	
		1.01	34.32	
8	Eicosane	3.38	29.43	(Ignatenko et al., 2006)
		2.98	30.826	
9	13-Decosenamide	8.16	30.096	–
10	Cyclohexane	2.55	32.36	–
11	Alpha-tocopherol	1.94	32.78	(Jiang, 2019)
12	Campesterol	3.19	33.85	(Alvarez-Sala et al., 2019)
13	Stigmasterol	7.85	34.21	(Alvarez-Sala et al., 2019)
14	β-Sitosterol	10.42	34.88	(Tomou et al., 2020)

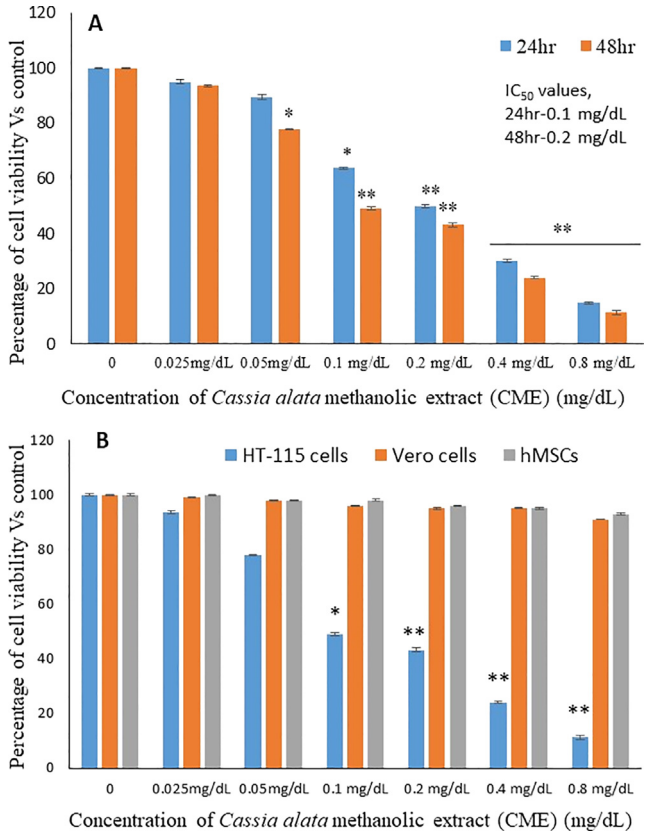

Fig. 1. Effect of increasing concentration of *C. alata* methanol extract on the percentage of viability inhibition of HT-115 cancer cell (1A) and percentage viability inhibitory effect on comparison between HT-115 cells, Vero cells and hMSCs (1B). Data are expressed as the mean ± SD (n = 6). * $p \leq 0.01$ vs. Control, ** $p \leq 0.05$ vs. control.

totic bodies (Fig. 2). There were no chromatin condensation, noticeable change or cell death in vehicle control cells. Indicating that, CME might play a definite molecular level role inducing at the earliest stages of cell death. In the result of AO/ErBr staining showed that, 85% of HT-115 cells were in apoptotic stage and 6% of the cells were in necrotic stage compared to vehicle control (Fig. 2A, B). Our findings confirmed that cells were treated with 0.2 mg/dL of CME for 48 hr mediate apoptosis in HT-115 cells.

3.4. Effect of CME on oxidative stress, inflammatory and protumorigenic gene expression

To determine the phytochemical present in CME have a potential against oxidative stress and inflammatory stimulating signaling cascade, we analyzed the dose responsive transcription ranges in protumorigenic inflammatory genes, such as CYP1A, GSK3β, TNF-α, NF-κB, COX-2 and PGE-2. The gene expressions levels were calculated in HT-115 cells after 48 hr of CME treatment at 0.1 mg/dL, 0.2 mg/dL against untreated control cells. As shown in Fig. 3A, we found that gene expression levels of COX-2, PGE-2, NF-κB, TNF-α were significantly decreased compared to control. On contrary, one-fold increase in the expression levels of CYP1A and GSK3β was observed a single fold after 48 hr in HT-115 cells. Observed results indicating that CME derived phytochemical has a potential activity as antioxidant and anti-inflammatory (see Fig. 4).

3.5. Effect of CME on apoptotic gene expression

We analyzed an alternation in the gene expression levels of tumor suppression related genes (p53 and mdm2) and apoptosis related genes (Bax, Bcl-2, Caspase 3, p21 and PCNA) after 48 h of CME treated HT-115 cells. As shown in Fig. 3B, the expression levels of p53, Bax, caspase 3, p21 were significantly increased by one-fold. While a significant reduction in the expression levels of mdm2, Bcl-2 and PCNA genes. These obtained results indicting that *C. alata* methanol extract derived phytochemicals has a potential to trigger apoptosis induction in HT-115 human colon cancer after 48 hr of treatment.

4. Discussion

Oxidative stress-induced damage to intestinal epithelial cells is a key event in the initiation and progression of pathologies associated with multiple intestinal inflammatory disorders including ulcerative colitis, colon cancer (Packiriswamy et al., 2017). Uncontrolled oxidative stress, deplete antioxidant defense mechanism against pro-oxidants, further immune cells or epithelial cells pro-

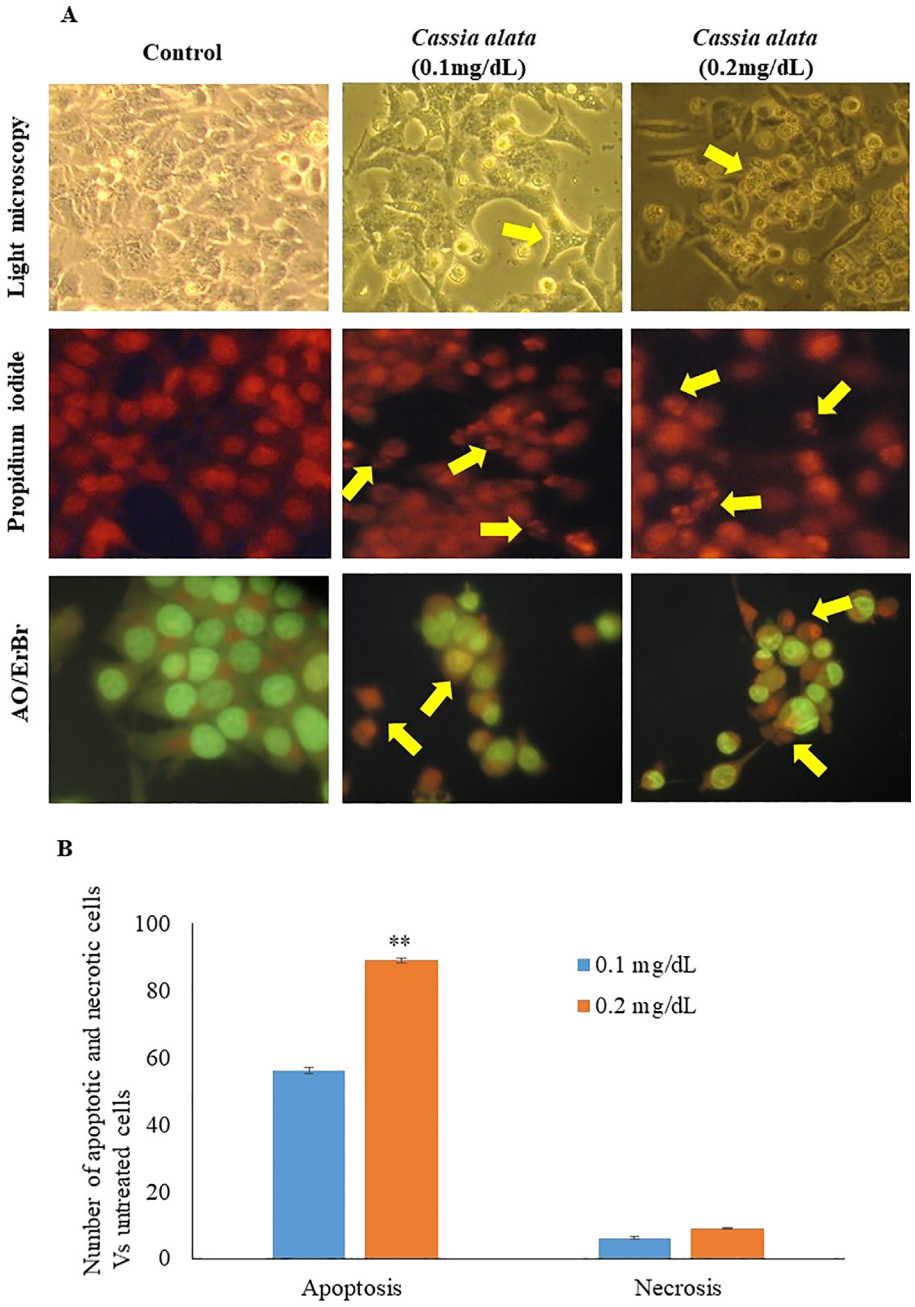

Fig. 2. Light microscopy, propidium iodide and AO/ErBr-stained images (40 X) of HT-115 cells treated with *Cassia alata* flower methanol extract (CME) after 48 hr (Fig. 2A). Fig. 2B, showing the percentage of apoptotic and necrotic cells after 48hr of 0.1 and 0.2 mg/dl of CME treated HT-115 cell. Data presented as the means ± SD are shown; n = 6; values sharing a common superscript as ** $p \leq 0.01$ compared with 0.1 mg/dL.

duced inflammatory mediators and reactive oxygen species which damage DNA and proteins (Raza et al., 2014). Intracellular ROS produced by mitochondrial oxidative phosphorylation, ROS primarily target epithelial cell's mitochondria and alter the tumor suppressor or DNA integrity associated protein synthesis causes colon cancer (Bhattacharyya et al., 2014). Plant-derived phytochemicals are an alternative and valuable new strategies as pharmaceutical treatments against different diseases. Plant has the potential to overcome certain environmental conditions, therefore it can generate new bioactive, secondary metabolites such as phenolic, alkaloids and flavonoid compounds. These compounds have been reported to be biologically safe and having wide margins of safety to normal cells compared to traditional synthetic chemo-therapeutic drugs. In cancer treatments, several plant-derived compounds found to fight against a wide range of cancer models, such as colon, breast, liver and prostate cancer (Seca and Pinto, 2018; Solowey et al., 2014). In this study, we aim to explore the anticancer properties of Cassia alata flower as well as their inherent active compounds and its mode-of-actions.

Methanol extract of *Cassia alata* flower exhibit potent antiproliferative activities against HT-115 cells (Fig. 1A), the MTT cytotoxicity assay shown that the concentration that kill 50% (IC_{50}) of the cells was around 0.2 and 0.1 mg/dL at 24 and 48 hr, respectively. Most notably, CME's cell proliferation inhibitory potential have been more specific to tested colon cancer cells only. In this context, Cassia alata leaves have been reported for the cytotoxic activity against breast carcinoma (MCF-7), bladder carcinoma (T24) and colorectal carcinoma (Col 2) (Olarte et al., 2013). GC–MS analyses

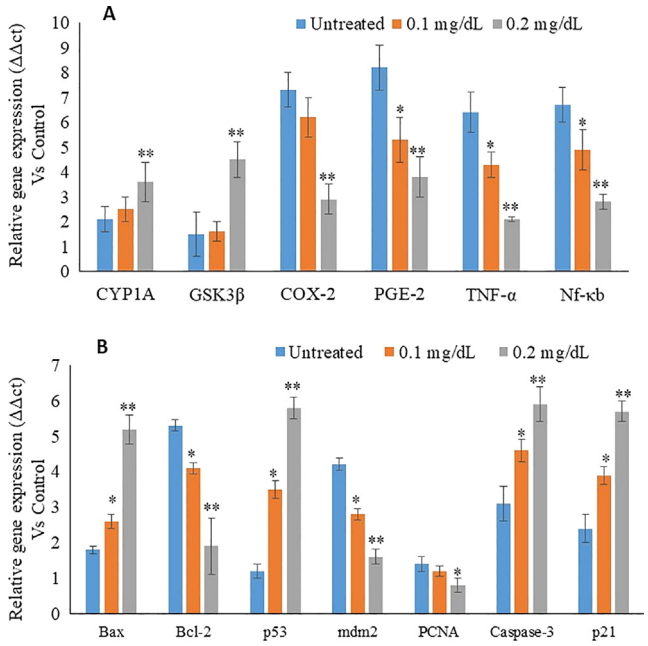

Fig. 3. Effect of *C. alata* methanol extract on oxidative stress, protumorigenic (3A) and apoptosis related (3B) gene expression levels in HT-115 cells after 48hr. Data presented as the means ± SD (n = 6). Values sharing a common superscript as * $p \leq 0.01$ & ** $p \leq 0.05$ compared with untreated HT-115 cells.

of methanol extract tentatively identified 97 compounds, predominantly phenolic and flavonoid compounds (Table 2). However, the major identified compounds were cyclotrisiloxan, β-Sitosterol, tocopherol, eicosane, beta carotene and cyclotrisiloxan derivative compounds, omega 3 and omega 6 compounds represented with 38% of the identified compounds. Thiophene is a heterocyclic compound that display a potential anticancer agent based on its ability to inhibit tumor cell growth as it has been reported by many researchers (Cai et al., 2019; Mendoza et al., 2009). It was previously reported, that the molecular mechanisms underlying the anticancer chemotherapeutic potential of plant-derived phytochemicals are mediated via cell cycle arrest and apoptosis induction (Lu et al., 2015; El-Shemy et al., 2010). To evaluate the potential anticancer activity mediated neither apoptosis nor necrosis the observations of chromatin condensation, nuclear damage or morphologic abnormalities shape and size of the nucleus are the key determinants in cancer cells. In this study, we observed CME induced cellular and nuclear damage, the results revealed that the observed potent anticancer activities of are mediated by early and late apoptotic events. In this context, a significance alteration in nuclear morphology in the HT-115 treated cells, where nuclear fragmentation, chromatin condensation and shrunken nuclear morphology were observed compared to untreated control group of cells showed intact nuclear morphology no indicating for apoptosis (Fig. 2A).

Physiology of intestinal epithelial cell tend to underestimate the cellular oxidative responses because of their inherent resistance to oxidative stress, changes in endogenous antioxidant levels, altered

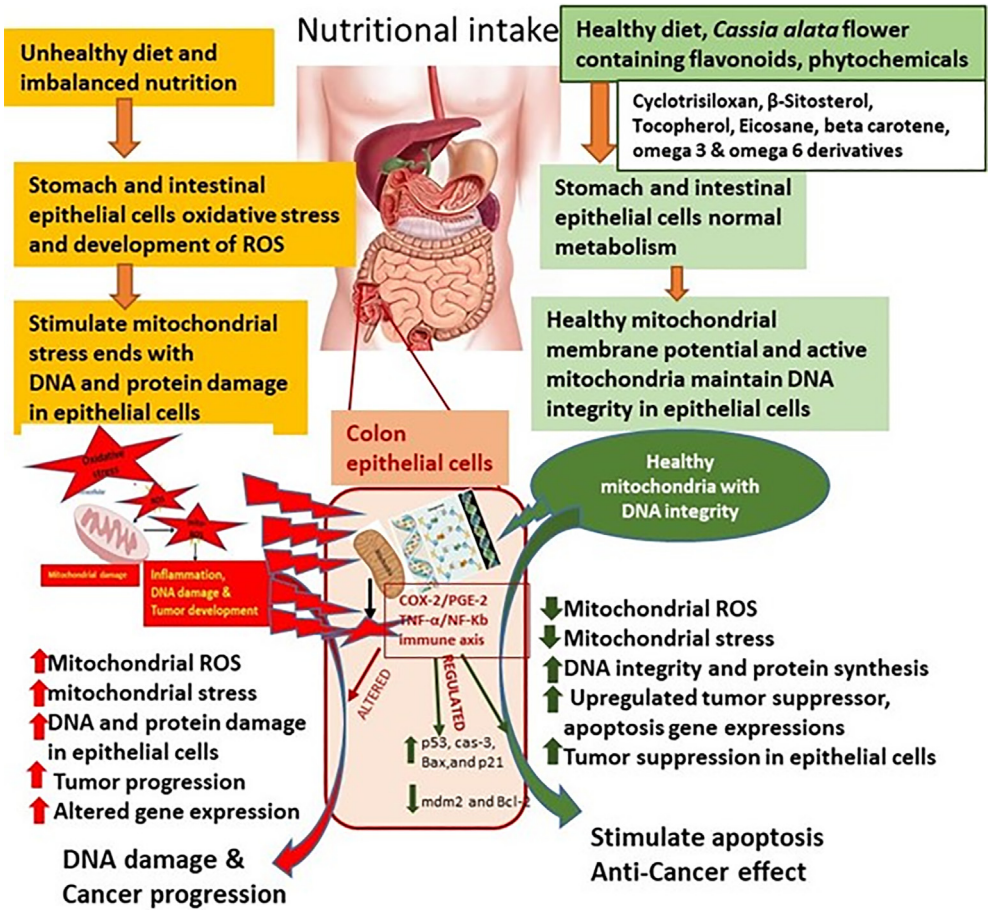

Fig. 4. Diagrammatic representation for the antiproliferative effect of *Cassia alata* flower against human colon cancer HT-115 cells.

expression or activation of detoxifying systems, and changes in the protective capacity of mitochondria and genetic components to inhibit ROS development (Koh et al., 2015; Zhao et al., 2016). Present study, the obtained RT-qPCR data revealed that incubation of HT-115 cells with CME display a potential activity against oxidative stress and anti-inflammatory gene signaling pathways. As shown in Fig. 3A, a significant reduction in the gene expression of COX-2, PGE-2, NF-κB and TNF-α; while an increase in the expression levels of CYP1A and GSK3β levels was observed in HT-115. Phytochemical, such as cyclotrisiloxan and β-Sitosterol present in CME have the potential to behave as an antioxidant, anti-inflammatory and anticancer agents (Bouabdallah et al., 2016). In this context, El-Desoky et al. (2017) have reported that curcumin down regulated NF-κB control COX-2 expression in cancer cells. In addition, Xu et al (2014) have confirmed that inhibition of COX-2 prevent metastasis and support in antiangiogenic cancer therapy in preclinical models. COX-2 is induced by inflammatory stimuli cytokines, and is the molecular target for analgesic and anti-inflammatory drugs. Mechanistically, increased COX-2 and decreases the intracellular levels of free arachidonic acid, thereby preventing apoptosis and facilitating the growth of cancer cells (Ashour et al., 2016). Meanwhile, a significant induction of pro-apoptotic proteins and mRNA markers for Cas-3, p53 and Bax, p21, whereas, a significant reduction in the mRNA expression of the anti-apoptotic marker Bcl-2, mdm2 and PCNA were observed (Fig. 3B). Caspases 3 is a key effector protein that are implanted in apoptosis induction pathway. Upregulation of Bax-mediated mitochondrial cytochrome *c* release, therefore activation of caspase 9 leading to the subsequent engagement of caspase 3 (Lombardo et al., 2004). Up-regulation of cas-3, p53 and Bax, together with reduction of Bcl-2, expression induced by Cassia alata extract trigger apoptosis induction. Upregulation of p53 further stimulate expression of Bax, which, in turn, will induce cytochrome *C* release, followed by cas-9 and cas-3 activation (Saquib et al., 2012a,b). In this context, Lee et al (2021) reported that indole −3-carbinol inhibit colon cancer cell proliferation via inhibition of p35 and caspase dependent apoptotic pathway. In addition, crude methanol extract of rosin gum has been reported to exhibit an anticancer activity against breast cancer, which mediated via the upregulation of pro-apoptotic (p53, caspase 3 and bax) and downregulation of the anti-apoptotic marker Bcl-2 in both protein and mRNA levels (Saquib et al., 2012a,b).

5. Conclusion

Observed results provided evidence that methanol extract of Cassia alata flowers exhibit potent anticancer properties mediated by suppression of oxidative stress induced protumorigenic and inflammatory signaling pathway causing DNA damage and epithelial cell stress. Phytochemical analysis also revealed that the availability of cyclotrisiloxan, β-Sitosterol, thiophene, tocopherol, beta carotene and cyclotrisiloxan derivative compounds from Cassia alata flowers are the source of natural phenolic and flavonoid compounds with unparalleled and unique antioxidant and anticancer properties. Previously, Sadek et al., (2017) plant sterol esters inhibited colon carcinogenesis via suppressing inflammation and stimulating apoptosis. The mechanistic action of CME treatment found to be an increased antioxidant capacity in HT-115 cells which arrested mitochondrial oxidative stress, further to maintain DNA integrity stimulate tumor suppressor and caspase dependent apoptosis mechanisms. The findings reported here warrant investigations of the active principles present in Cassia alata in preclinical and clinical cancer trials.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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