IJBPAS, September, 2018, 7(9): 1731-1745

ISSN: 2277–4998

International Journal of Biology, Pharmacy and Allied Sciences (IJBPAS)

'A Bridge Between Laboratory and Reader'

www.ijbpas.com

RUTIN: A POTENTIAL ANTICANCER DRUG AGAINST HUMAN COLON CANCER (HCT116) CELLS

JAYAMEENA P, SIVAKUMARI K*, ASHOK K. AND RAJESH S

Department of Zoology, Presidency College, Tamil Nadu, India

***Corresponding author: Dr. K. Sivakumari: Associate Professor of Zoology, Presidency College, Chennai, Email: dr.sivakumari@rediffmail.com, Mobile: 9790934900**

Received 17th March. 2018; Revised 4th April 2018; Accepted 9th May 2018; Available online 1st Sept. 2018

https://doi.org/10.31032/IJBPAS/2018/7.9.4532

ABSTRACT

Human colon cancer is one of the deadliest diseases worldwide. Natural products had shown potential anti-cancer effects. Rutin is one of the natural polyphenolic compounds with high medicinal properties. Thus, the present study was intended to explore the anti-tumour properties of rutin against human colon cancer (HCT116) cells. The MTT assay revealed the anti-proliferative activity of rutin in a concentration dependent manner and also promoted apoptosis in HCT116 cells when exposed to median inhibitory concentration (IC_{50}). The results also showed that the rutin was able to decrease HCT116 cells migration and arrest cell cycle in G0-G1 phase. The current study strongly proves that rutin inhibits proliferation of colon cancer cells by inducing apoptosis mediated through cell cycle arrest and activation of caspase protein. Moreover, the expression of caspase-3 and β-Actin in HCT116 were significantly enhanced after rutin treatment. These findings provide a promising method to treat the colon cancer. Hence, rutin could be considered as a good natural drug for Human Colon Cancer (HCT 116), and a good replacement for chemical therapeutic drugs.

Keywords: Rutin, HCT 116, MTT, Annexin V-FITC and PI, Comet, Cell cycle, Western blotting

1. INDRODUCTION

Medicinal plants are integral part of traditional medicine since ancient era. Drug discovery process has witnessed phytochemicals for discovery of new leads [1,2,3]. Flavonoids are polyphenolic compounds, which are one of the important classes of plant derived chemicals that contain benzopyrone moiety. About 4000 types of flavonoids have been reported to be present in plants [4]. Flavonoids, act as major nutritional constituents of plant-based food as habitual and folklore medicine worldwide [5,6]. Rutin, also called as rutoside, quercetin-3-rutinoside, and sophorin is a citrus flavonoid glycoside found in buckwheat [7]. Rutin, a common dietary flavonoid with a wide range of pharmacological activities is present in many plants, fruits, vegetables and red wine [8, 9, 10, 11]. Different studies have represented the biological effects of rutin, such as anti-oxidative, anti-inflammatory, antihypertensive, anti-carcinogenic, cytoprotective, anti-platelet, anti-thrombic, anti-diabetic, anti-adipogenic, neuroprotective, hormone therapy and cardio protective activities [12, 13, 14, 15].

Cancer as a second cause of death after heart disease in the world poses a great challenge to the field of medicine and immunology [16, 17]. There has been a recent upsurge in the use of natural products to supersede current treatment in patients that develop multidrug resistance. Scientific studies of plants used in various types of ethnic medicine have led to the discovery of many valuable drugs, including taxol, camptothecin, vincristine and vinblastine [18, 19]. Colon cancer (HCT 116) is the most common cancer in the world [20]. More than 80% of colon cancer deaths occur in developing countries, and it is the fourth most common cause of all cancer deaths and accounts for most of colon cancer deaths of the world [21]. In view of this it was aimed to carry out work on the anticancer and anti-apoptotic properties of rutin against human colon cancer (HCT 116) cell line.

2. MATERIALS AND METHODS:

Anticancer Activity

Human Colorectal Cancer (HCT 116) and Aneuploid immortal Keratinocyte (HaCaT) Cell Lines

HCT 116 and HaCaT cell lines were used for the present study, cell lines were purchased from National Centre for Cell Science (NCCS), Pune, India.

Standardization of Rutin

Standardization of rutin against HCT 116 and HaCaT cell lines were done by MTT assay.

Cell Viability

Cell viability was assessed by the MTT method as described by Mosmann (1983) [22].

HCT 116 and HaCaT cells at a concentration of 1×10^4 cells/mL were plated in 96 well plates with DMEM containing 10% FBS. The cells were incubated for 24 h under 5% CO_2 and 95% O_2 at 37°C. The medium was removed, washed with PBS and fresh serum free medium was added, and kept in incubator for 1 h. After starvation, the cells were treated with different concentrations of rutin compound such as 62.5, 125, 250, 500 and 1000 µM and incubated for 48 h. After incubation, 10.0 µL of MTT solution was added to each well and incubated for 4 h. After incubation, the supernatant was aspirated and 100 µL of DMSO was added to solubilize the crystals. A microplate reader was used to measure the absorbance at 570 nm for each well and the per cent viability of cell was calculated as per the following formula:

$$\text{Cell viability (\%)} = \frac{\text{Absorbance of Sample}}{\text{Absorbance of Control}} \times 100$$

Based on the above calculation, the cell viability was calculated. The IC_{50} concentration of rutin was calculated and it was found to be 679.858 µM for HCT 116 cells and 1070.445 µM for HaCaT cells.

Cytomorphological Changes

To observe cytomorphological changes, 1×10^6 HCT 116 and HaCaT cells/mL were plated in 100 mm dishes and incubated at controlled environment for 24 h. Following this, medium was removed and fresh medium with rutin (IC_{50} concentration and maximum concentration) added and incubated for 48 h. Then, the incubated cells were visualized by inverted light microscope at 20X magnification.

Nuclear Morphological Changes

Changes in the nuclear morphology were detected following Koopman et al. (1994) [23] method by using Annexin V-FITC/PI staining using apoptosis detection kit (Sigma St. Louis, USA) according to the manufacturer's protocol. About $1x10^6$/HCT 116 cells/well were plated in six-well plates and kept overnight for attachment. Cells treated with IC_{50} concentration and maximum concentration of rutin along with a control. Then, cells were harvested and washed with PBS twice and re-suspended in 500 µL of 1X binding buffer. The cells were again incubated for 15 min. at room temperature in dark condition, after staining them with 5.0 µL of Annexin V- FITC conjugate and 10.0 µL of propidium iodide, the cells were observed by fluorescent microscope. If the cells stained negative for both Annexin V-FITC and PI, they were considered as viable, and if the cells stained positive for Annexin V-FITC and negative for PI, they were considered as at early apoptotic stage. In contrary, if the cells stained positive for both Annexin V-FITC and PI, then they were considered to be at late apoptotic stage.

Comet Assay

Comet assay was carried out according to Singh et al. (1988) [24] method by single cell gel electrophoresis to determine the damage of DNA in HCT 116 cells. The HCT 116 cells were exposed to IC_{50} concentration and maximum concentration of rutin along with a control, which was treated with 0.1% DMSO. This was followed by isolation of DNA using agarose gel electrophoresis.

Cell Cycle Analysis

The phase's of distribution in cell cycle and their measurements were analysed using flow cytometry [25]. HCT 116 cells were incubated in serum free DMEM media for 36 h to synchronize cells in G0/G1 Phase. Then, the medium was replaced by 10% serum-supplemented medium with 0.1% DMSO (as control) and IC_{50} concentration of rutin was added to the wells and kept for 48 h. After treatment, floating cells in the medium were combined with attached cells and collected by trypsinization. Cells were washed with cold PBS and fixed in 80% ethanol in PBS at -20^{o}C. The fixed cells were pelleted and stained with PI (50 µg/mL) in the presence of RNase A (20 µg/mL) for 30 min. at 37^{o}C. About ≥20,000 cells were analyzed using flow cytometer. Cell cycle histograms were analyzed using 'Cell Quest' software.

Western Blot Analysis

Western blot analysis was carried out following the method of Towbin et al. (1979) [26]. Electrophoresed gel with separated proteins was transferred into nitrocellulose membrane. Prior to transfer, the membrane was placed for 30 sec. in 100% methanol by immersion and then kept on transfer buffer. As per the instructions of the manufacturer, using pads, cushions, filter papers, gel and nitrocellulose membranes, a sandwich was prepared. Trapping of air bubbles between the gel and membrane was carefully avoided. Western blot was carried out for 90 min. at a constant current of 100 V and 4°C. Then, the membrane was incubated with blocking buffer for 2 h at 37°C or overnight at 4°C using a shaker. Then, primary antibodies *viz*., Caspase-3 and β-Actin were diluted with antibody dilution buffer at required concentrations and incubated at 37°C for 2 h, using the shaker. Following this, the membrane was washed with washing buffer thrice for 5-10 min. each time with the help of the shaker. Similarly, secondary antibodies (skimmed milk powder) were diluted with antibody dilution buffer at the ratio of 1:1000 and the membrane was incubated with diluted secondary antibody for 1 h at 37°C using the shaker. The membrane was then washed with washing buffer for five times taking 5-10 min. for each time using the

shaker. The protein-antibody interactions were detected, using DAB.

Statistical Analysis

Data obtained from MTT assay were subjected to statistical analysis and the mean value along with the standard error for five individual observations was calculated for each parameter and presented in appropriate tables in the text. The significance of the sample mean between various extracts and the concentrations of each extract was tested using Two Way ANOVA [27].

RESULTS

Cell Viability

The cell viability of HCT 116 and HaCaT cells was assessed using anti-proliferation activity by MTT assay for 24 h and 48 h. Rutin showed a dose-dependent decrease in cell viability. The IC_{50} value of rutin treated cells for 24 h could not be reached. The cell viability of rutin treated HCT 116 cells for 48 h at 62.5 µM concentration was -8.30% and at 1000 µM, it recorded at per cent decrease of -67.56%. On the other hand, cell viability of rutin treated HaCaT cells recorded -9.60% at 62.5 µM and -46.86% at 1000 µM at 48 h incubation. The present investigation showed an indirect relationship between the incubation period and cell viability. Statistical analysis of the data by two-way ANOVA revealed that the values were found to be significant. The 50% inhibitory concentration was 679.858 µM for HCT 116 cells and 1070.445 µM for HaCaT cells at the end of 48 h incubation (Table 1). The graphical data of cell viability of HCT 116 and HaCaT are presented in Fig. 1. Based on the preliminary studies, it can be concluded that rutin acts well in controlling proliferation of HCT 116 cells. Therefore, the present study focused only on the effect of IC_{50} concentration and maximum concentration of rutin on HCT 116 treated cells for further studies.

Cytomorphological Changes

HCT 116 cells were treated with 679.858 µM and 1000 µM concentrations of rutin for 48 h, and the cytomorphological changes were observed under the inverted microscope. The cells treated with rutin showed distinct cellular morphological changes indicating unhealthy cells, whereas the control cells were irregular confluent aggregates with rounded and polygonal cells. Changes in cell morphology of rutin treated cells were 679.858 µM than 1000 µM. Most of the treated cells became round instead of elongated shape and at 1000 µM the cells underwent maximum destruction of shape (Fig. 2). Based on the above results, it can be concluded that rutin causes changes in cell morphology, thus resulting in decreased cell viability.

Nuclear Morphological Changes

For observing nuclear morphological changes, HCT 116 cells

were stained with Annexin V-FITC, PI and Annexin V-FITC/PI. The changes observed by confocal microscope. The control cells stained negative for Annexin V-FITC, PI and Annexin V-FITC/PI, while rutin treated cells (679.858 µM and 1000 µM) stained positive with Annexin V-FITC, PI and Annexin V-FITC/PI. When compared among rutin treatment, the later treatment revealed high intense positive staining than the former treatment. These results clearly depict that rutin induced cell death (Fig. 3).

Comet Assay

Comet assay was carried out for control as well as rutin treated HCT 116 cells by stained with propidium iodide and visualized under fluorescence microscope. The results indicated the cells treated with 679.858 µM of rutin recorded higher DNA fragmentation and damage, whereas the control cells showed no fragmentation and damage (Fig. 4). The fragmented DNA migrated out of the nucleus and appeared as a comet like tail in HCT 116 rutin treated cells. When, DNA damage was analyzed by CASP software, more DNA damage was observed at 679.858 µM as well as in 1000 µM, as depicted by the DNA content at head and tail (Fig. 5 and Table 2).

Cell Cycle Analysis

The effect of rutin on cell cycle distribution was determined using flow cytometry analysis and is presented in Table 3 and Fig. 6. The graph indicates that control HCT 116 cells showed 3.73% gating of cells in sub G0-G1 phase, 55.51% gating of cells in G0-G1 phase, 29.22% gating of cells in S phase and 11.85% gating of cells in G2-M phase. In contrary, the HCT 116 cells treated with 679.858 µM of rutin showed 25.9% gating of cells in sub G0-G1 phase, 51.29% gating of cells in G0-G1 phase, 14.37% gating of cells in S phase and 5.75% gating of cells in M phase. Likewise, HCT 116 cells treated with 1000 µM of rutin showed, 43.81% gating of cells in sub G0-G1 phase, 28.96% gating of cells in G0-G1 phase, 15.76% gating of cells in S phase and 8.89% gating of cells in M phase. The rutin treated cells showed significant reduction in DNA content with consequent loss of cells in sub G0/G1 phase itself, which appeared as an indication of apoptosis. Cell cycle analysis in the present study revealed that rutin arrested all stages of the cells and caused severe damage to the DNA than compared to control.

Apoptotic Analysis

The expression of apoptotic protein Caspase-3 and β-Actin (loading control) are presented in Fig. 8. On the treatment of HCT 116 cells with rutin (679.858 µM and 1000 µM) showed that the apoptotic protein expression of effecter Caspase-3 increased in a dose-dependent manner. The active fragment (17 kDa) levels of Caspase-3 significantly elevated than that of the control β-Actin.

Table 1: Cell viability of HCT 116 and HaCaT cells are treated with rutin for 48 h

Concentration (µM)	Cell Viability (%)	
	HCT 116	HaCaT
Control	100 ± 0	100 ± 0
62.5	91.701 ± 1.729* (-8.30)	90.399 ± 1.652* (-9.60)
125	91.186 ± 1.988* (-8.81)	80.720 ± 1.960* (-19.28)
250	91.118 ± 0.589* (-8.88)	77.467 ± 1.097* (-22.53)
500	59.867 ± 2.839* (-40.13)	75.458 ± 1.310* (-24.54)
1000	32.437 ± 1.787* (-67.56)	53.144 ± 2.975* (-46.86)
IC50	679.858	1070.445

Values are mean ± SE of six individual observations; Values in parentheses are per cent change over control; - Denotes per cent decrease over control; *Values are significant at P<0.001.

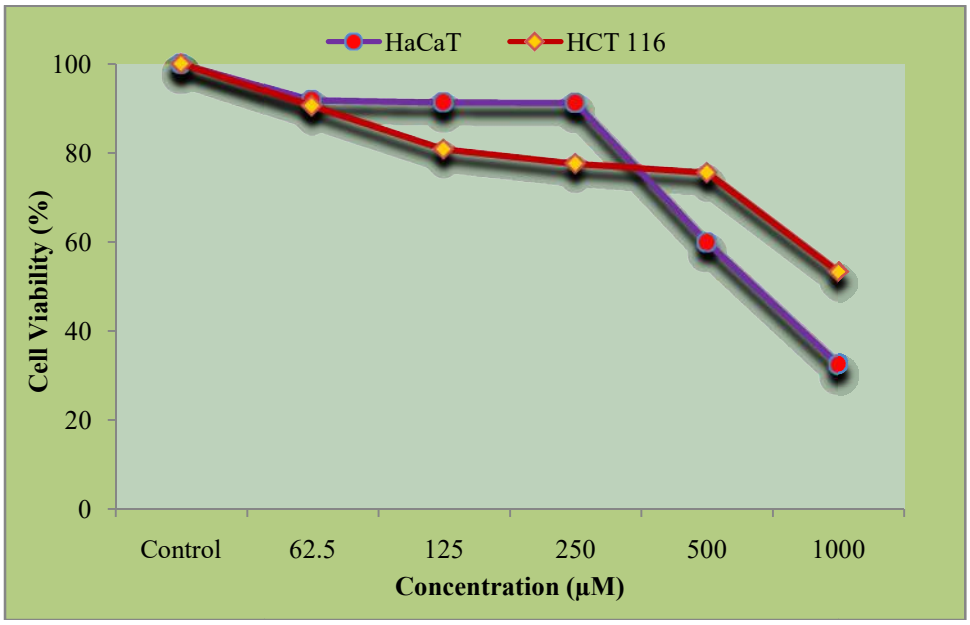

Fig. 1: Per cent cell viability of HCT 116 and HaCaT cells when treated with rutin

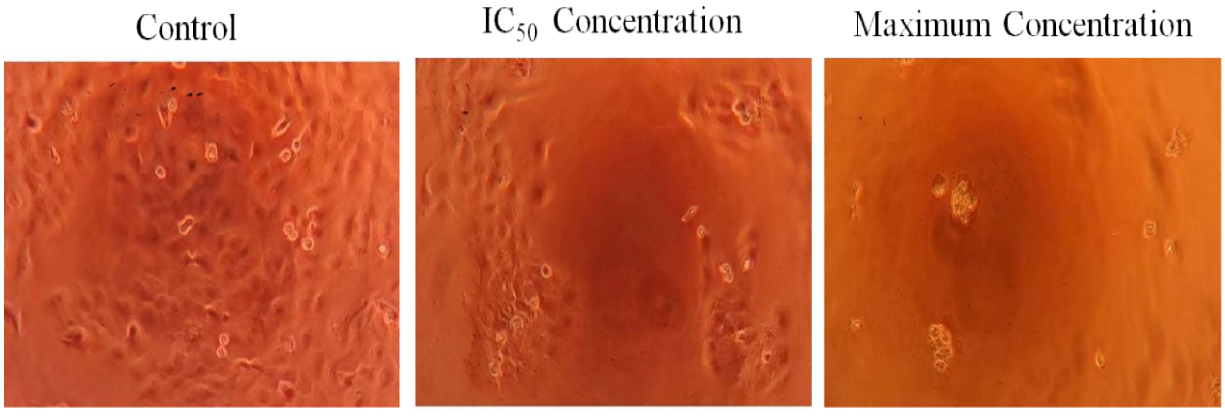

Fig. 2: Cytomorphological changes of HCT 116 cells treated with IC_{50} and Maximum concentrations of rutin for 48 h when compared Control cells

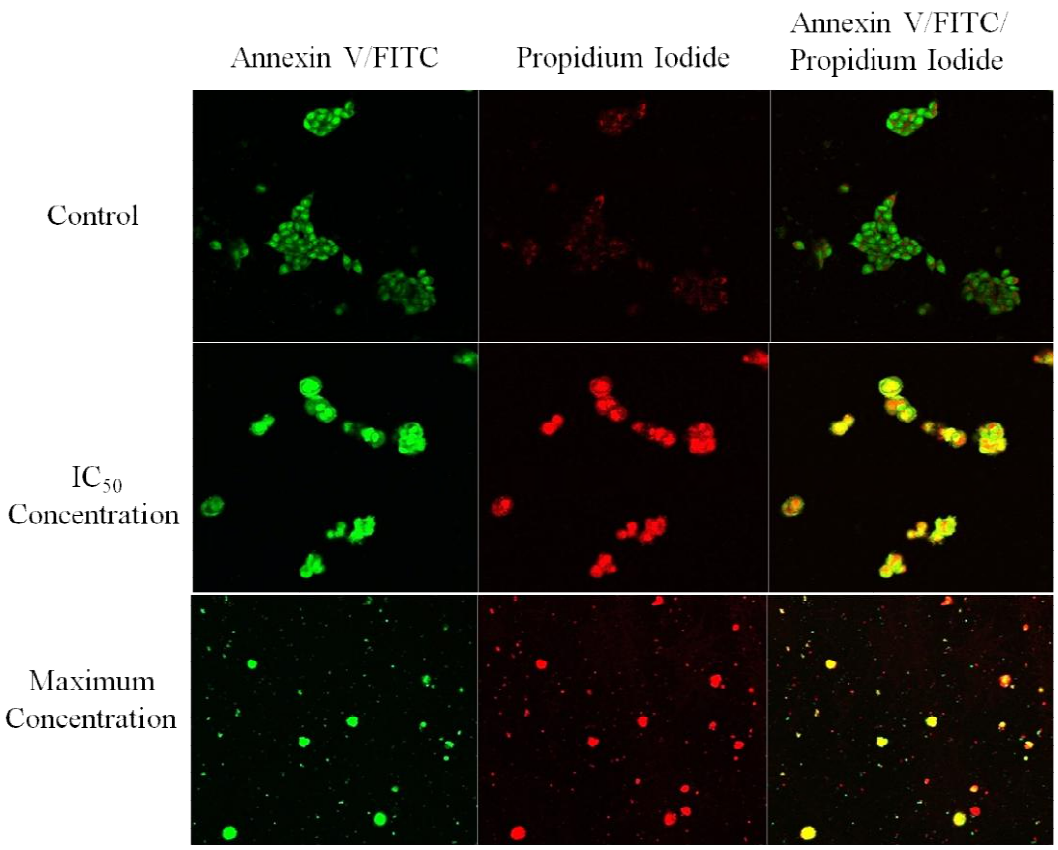

Fig. 3: Nuclear morphological changes in control and rutin treated HCT 116 cells

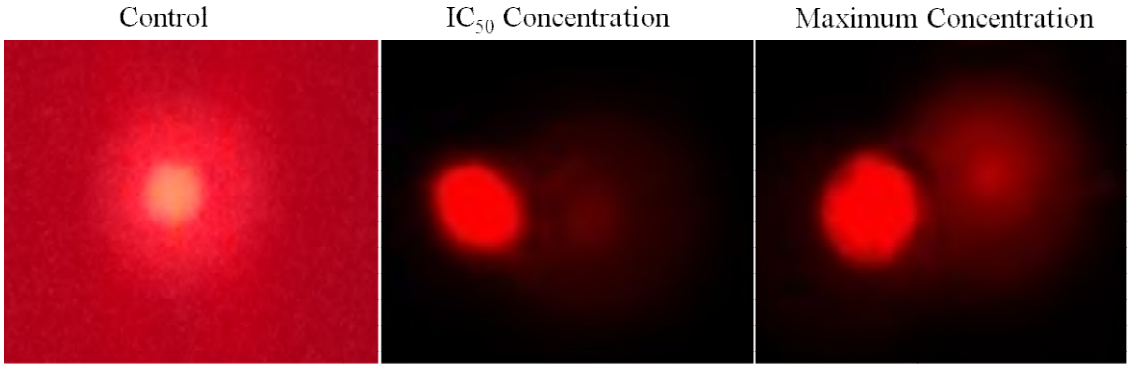

Fig. 4: DNA damage of control and rutin treated HCT 116 cells

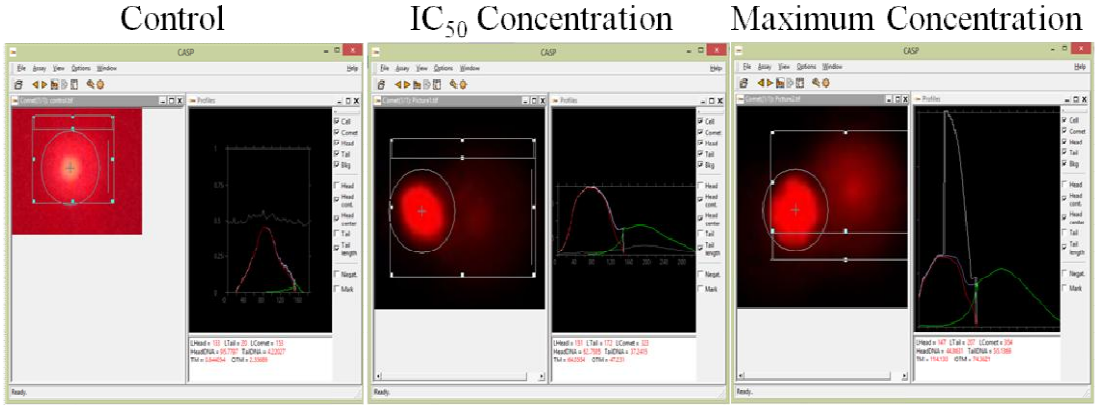

Fig. 5: DNA damage was analyzed by CASP software

Table 2: Quantitative DNA fragmentation analysis by CASP software

Samples (HCT 116 cells)	Head	Tail	Comet	Head DNA	Tail DNA	Tail movement	Olive tail movement
Control	133	20	153	95.78	4.22	0.84	2.36
IC_{50} 48 h concentration	151	172	323	62.76	37.24	64.06	47.23
Maximum concentration	147	207	354	44.86	55.14	114.13	74.36

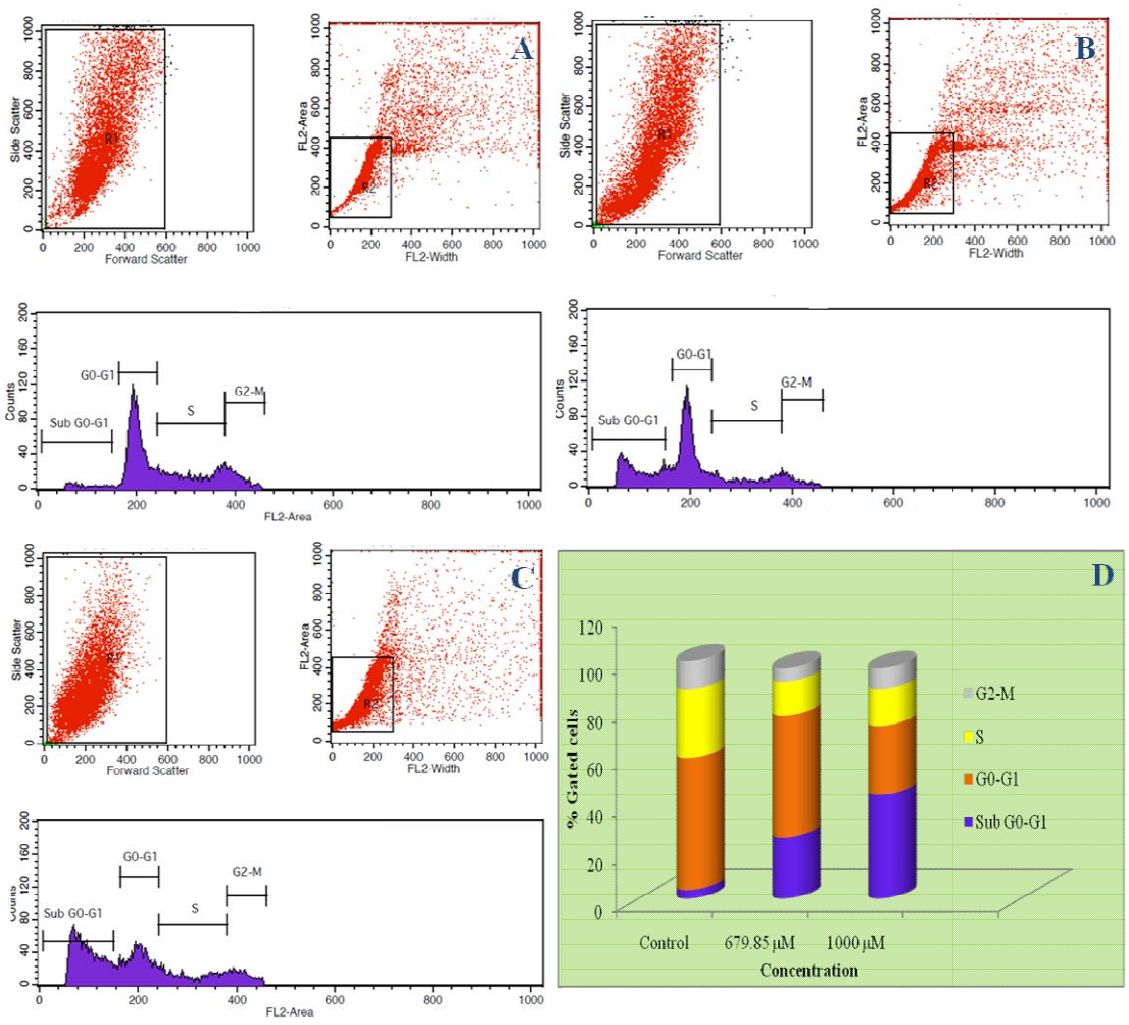

Fig. 6: Cell cycle analysis of HCT 116 cells induced by rutin
A. Control **B. IC_{50} Concentration**
C. Maximum Concentration **D. Graphical data of cell cycle analysis**

Table 3: % of cells arrest in cell cycle analysis of HCT 116 cells when treated with rutin compound at 48 h.

Concentration	% of Gated cells in phase			
	Sub G0-G1	G0-G1	S	G2-M
Control	3.73	55.51	29.22	11.85
679.85 µM	25.9	51.29	14.37	5.75
1000 µM	43.81	28.96	15.76	8.89

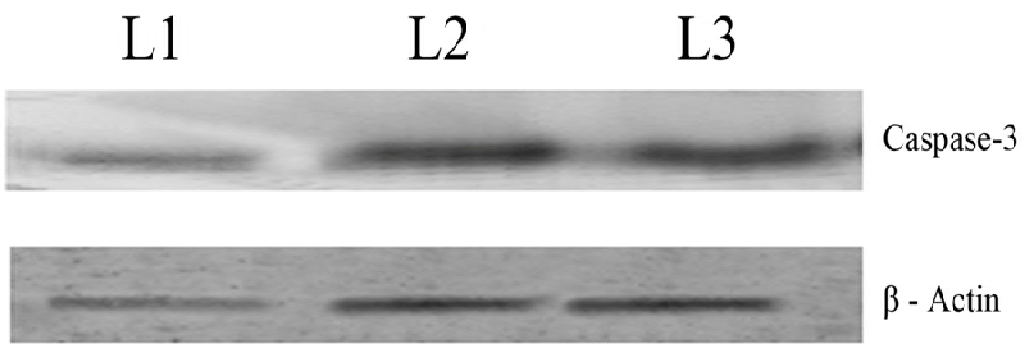

Fig. 8: Expression of apoptotic protein in control and rutin treated HCT 116 cells (L1) – Control, (L2) – IC_{50} concentration, (L3) – Maximum concentration

DISCUSSION

Rutin, a polyphenolic bioflavonoid has shown wide range of pharmacological applications due to its significant antioxidant properties. Conventionally, it is used as antimicrobial, antifungal, and anti-allergic agent. However, current researchers have been shown its multispectral pharmacological benefits for the treatment of various chronic diseases such as cancer, diabetes, hypertension and hypercholesterolemia [28]. Rutin use is advantageous over other flavonoids as it is a nontoxic and nonoxidizable molecule [29]. Polyphenols are known to an extensive assortment of biological effects, comprising of antioxidant and radical scavenging activities.

Rutin has been extensively studied for anticancer/antineoplastic effects; human leukemia HL-60 cells were implanted in a murine model, and rutin (dose 120 mg/kg) caused a significant reduction in tumour size justifying anti-leukemic potential as reported by Lin et al. (2012) [30]. According to Alonso-Castro et al. (2013) [31], rutin when administered to SW480 tumor cell lines (human colon cancer cell lines), caused less detrimental effects on the body and relative organ weight in mice along with increase of mean survival time of 50 days. Anti-neuroblastoma effect of rutin, significantly inhibited the growth of LAN-5 cells and chemotactic ability [32]. A similar mechanism of rutin might have occurred in thw present case also leading to growth inhibition of HCT 116 cells, thus findings support from the above authors.

Annexin V-FITC apoptosis detection kit is the combination of fluorescein isothiocyanate (FITC) and Annexin V with propidium iodide to distinguish living cells in early and late apoptosis [33]. Annexin is a group of homologous proteins which bind phospholipids in the presence of calcium. During early apoptosis, phosphatidylserine which is usually located in the inner membrane of cells is transported into the outer portion of the membrane, which can

be detected by its strong affinity for Annexin V-FITC, whereas the dead cells can be detected by the binding of propidium iodide to the cellular DNA in cell [34, 35]. In our study also, we observed apoptosis in rutin treated HCT 116 cells, which resulted positive cells increased in Annexin-V and PI staining when compared to control. Abu Bakar et al. (2015) [33] also recorded similar results in Hep-G2 cells, when treated with *Garcinia dulcis*, thus supporting our findings.

The cell cycle is the series of events that take place in a cell leading to its division and duplication (replication); regulation of the cell cycle involves processes crucial to the survival of a cell. The previous studies reported by Majewska-Wierzbicka and Czeczot (2012) [36], that flavonoids promote cell arrest in distinct phases is main effect on anticancer. Cell cycle regulation is also important in mediating radio sensitivity, and cells are most sensitive to radiation during the G2/M phase, less sensitive during G1, and least sensitive near the end of the S phase [37]. These results support the observations of the present study, which indicated that rutin treated HCT 116 cells showed significant reduction of the DNA content with consequent loss of cells in sub G0/G1 phase itself, which appeared as an indication of apoptosis.

Rutin is also known to inhibit cancer cell growth by cell cycle arrest and/or apoptosis, along with inhibition of proliferation, angiogenesis, and/or metastasis in colorectal cell lines [38]. According to Chen et al. (2013) [32], it has been demonstrated that rutin could decrease BCL2 expression and BCL2/BAX ratio along with a reduction in levels of MYCN mRNA level and the secretion of TNF-a. Rutin analog and quercetin when tested for anti-proliferative effect of the ovarian cancer cell line OVCA 433, resulted in dose-related inhibition [39]. Rutin also seems to be useful as an adjuvant in radioiodine therapy [40].

In conclusion, our study demonstrated that rutin plays an important role on the anti-proliferation of human colon cancer (HCT 116) cells line through induction of apoptosis, triggering apoptosis and cell cycle arrest. Moreover, rutin also inhibited Caspase-3 expression. Hence rutin is a potential therapeutic drug for the treatment of human colon cancer (HCT 116) cells.

CONCLUSION

In the present investigation it is demonstrated that rutin has anticancer properties against Human Colon Cancer (HCT 116) cell lines by *in vitro* method. Hence, rutin could be considered as a good natural drug for Human Colon Cancer

(HCT 116), and a good replacement for chemical therapeutic drugs.

ACKNOWLEDGEMENT

The authors are thankful to V Clin Bio Labs (P) Ltd, Sri Ramachandra Medical University, Porur, Chennai, for the technical support.

CONFLICT OF INTEREST

None.

REFERENCES

[1] Grdina DJ, Murley JS, Kataoka Y. Radioprotectants: current status and new directions. Oncology, 2002; 63(2): 2–10.

[2] Weiss JF, Landauer MR. Protection against ionizing radiation by antioxidant nutrients and phytochemicals. Toxicology, 2003; 189: 1–20.

[3] Arora R, Gupta D, Chawla R, Sagar R, Sharma A, Kumar R, et al. Radioprotection by plant products: present status and future prospects. Phytother Res, 2005; 19: 1–22.

[4] Guardia T, Rotelli AE, Juarez AO, Pelzer LE. Anti-inflammatory properties of plant flavonoids. Effects of rutin, quercetin and hesperidin on adjuvant arthritis in rat. Farmaco, 2001; 56(9): 683–687.

[5] Carnat AP, Carnat A, Fraisse D, Lamaison JL, Heitz A, Wylde R. Violarvensin, a new flavone di-C-glycoside from viola arvensis. J Nat Prod, 1998; 61: 272–274.

[6] Goncalves AFK, Friedrich RB, Boligon AA, Piana M, Beck RCR, Athayde ML. Antioxidant capacity, total phenolic contents, and HPLC determination of rutin in Viola tricolor (L) flowers. Free Radicals Antioxid, 2012; 2: 32–37.

[7] Kreft S, Knapp M, Kreft I. Extraction of rutin from buckwheat (*Fagopyrum esculentum* Moench) seeds and determination by capillary electrophoresis. J. Agric. Food Chem, 1999; 47(11): 4649–4652.

[8] Buszewski B, Kawka S, Suprynowicz Z, Wolski T. Simultaneous isolation of rutin and esculin from plant material and drugs using solid-phase extraction. J Pharm Biomed Anal, 1993; 11: 211-215.

[9] Tang DQ, Wei YQ, Gao YY, Yin XX. Protective effects of rutin on rat glomerular mesangial cells cultured in high glucose conditions. Phytother Res, 2011; 25: 1640–1647.

[10] Wu CH, Lin MC, Wang HC, Yang MY, Jou MJ, Wang CJ. Rutin inhibits oleic acid induced lipid accumulation via reducing lipogenesis and oxidative stress in

hepatocarcinoma cells. J Food Sci, 2011; 76: 65-72.

[11] Jayameena P, Sivakumari K, Ashok K, Rajesh S. *In Silico* Molecular Docking Studies of Rutin Compound against Apoptotic Proteins (Tumor Necrosis Factor, Caspase-3, NF-Kappa-B, P53, Collagenase, Nitric Oxide Synthase and Cytochrome C). Journal of Cancer Research and Treatment, 2018; 6(2): 28-33. doi: 10.12691/jcrt-6-2-1.

[12] Chen S, Gong J, Liu F, Mohammed U. Naturally occurring polyphenolic antioxidants modulate IgE-mediated mast cell activation. Immunology, 2000; 100: 471-480.

[13] Jayameena P, Sivakumari K, Ashok K, Rajesh S. *In vitro* anti-inflammatory (membrane stabilization) and antioxidant potential of rutin. RJLBPCS, 2018; 4(3): 265-274. doi: 10.26479/2018.0403.24

[14] Lee S, Suh S, Kim S. Protective effects of the green tea polyphenol (-)-epigallocatechin gallate against hippocampal neuronal damage after transient global ischemia in gerbils. Neurosci Lett, 2000; 287: 191-194.

[15] Novakovic A, Gojkovic-Bukarica L, Peric M, Nezic D, Djukanovic B, Markovic-Lipkovski J. The mechanism of endothelium-independent relaxation induced by the wine polyphenol resveratrol in the human internal mammary artery. J Pharmacol Sci, 2006; 101: 85–90.

[16] Jemal A, Siegel R, Ward E, Murray T, Xu J, Thun MJ. CA Cancer J. Clin, 2007; 57: 43.

[17] Rajesh S, Sivakumari K, Ashok K, Abitha AR. Anti-cancer activity of *Cardiospermum halicacabum* L. leaf extracts against human breast cancer cell line (MCF-7). European J. Biomed. Pharmaceu. Sci, 2016b; 3(10): 213-222.

[18] Heinrich M, Bremner P. Ethnobotany and ethnopharmacy--their role for anti-cancer drug development. Curr Drug Targets, 2006; 7: 239-45.

[19] Newman DJ, Cragg GM. Natural products as sources of new drugs over the last 25 years. J Nat Prod, 2007; 70: 461-77.

[20] Siegel R, Naishadham D, Jemal A. Cancer statistics, 2013. CA Cancer J Clin, 2013; 63: 11-30.

[21] Chen W, Zheng R, Zeng H, Zhang S and He J. Annual report on

status of cancer in China, 2011. Chin J Cancer Res, 2015; 27: 2-12.

[22] Mosmann T. Rapid colorimetric assay for cellular growth and survival: Application to proliferation and cytotoxicity assays. J. Immunol. Methods, 1983; 65(1-2): 55-63.

[23] Koopman G, Reutelingsperger CP, Kuijten GA, Keehnen RM, Pals ST, Van Oers MH. Annexin V for flow cytometric detection of phosphatidylserine expression on B cells undergoing apoptosis. Blood, 1994; 84(5): 1415-1420.

[24] Singh NP, MeCoy MT, Tice RR, Schneider EL. A simple technique for quantification of low levels of DNA damage in individual cells. Exp. Cell Res, 1988; 175(1): 184-191.

[25] Pozarowski P, Darzynkiewicz Z. Cell cycle analysis of *in vitro* cancer cell lines. Current Protocols in Biology, 2004; 5: 832-836.

[26] Towbin H, Staehelin T, Gordon J. Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: Procedure and some applications. Proc. Natl. Acad. Sci. USA, 1979; 76(9): 4350-4354.

[27] Rajesh S, Sivakumari K, Ashok K, Abitha AR. Anticancer activity of *Cardiospermum halicacabum* L. leaf extracts against hepatocellular carcinoma cell line (HepG-2). W. J. Pharm. Pharmaceu. Sci, 2016a; 5(3): 1133-1154.

[28] Hunyadi A, Martins A, Hsieh TJ, Seres A, Zupk´o I. Chlorogenic acid and rutin play amajor role in the in vivo antidiabetic activity of Morus alba leaf extract on type II diabetic rats. PLoS ONE, 2012; 7(11): Article ID e50619.

[29] Sharma S, Ali A, Ali J, Sahni JK, Baboota S. Rutin: therapeutic potential and recent advances in drug delivery. Expert Opinion on Investigational Drugs, 2013; 22(8): 1063–1079.

[30] Lin JP, Yang JS, Lin JJ, Lai KC, Lu HF, Ma CY, Sai-Chuen Wu R, Wu KC, Chueh FS, Gibson Wood W, Chung JG. Rutin inhibits human leukemia tumor growth in a murine xenograft model *in vivo*. Environ. Toxicol, 2012; 27(8): 480–484.

[31] Alonso-Castro AJ, Domı´nguez F, Garcı´a-Carranca´ A. Rutin exerts antitumor effects on nude mice bearing SW480 tumor. Arch. Med. Res, 2013; 44(5): 346–351.

[32] Chen H, Miao Q, Geng M, Liu J, Hu Y, Tian L, Pan J, Yang Y. Anti-tumor effect of rutin on

human neuroblastoma cell lines through inducing G2/M cell cycle arrest and promoting apoptosis. Sci. World J. 2013; 20 269165.

[33] Abu Bakar MF, Ahmad NE, Suleiman M, Rahmat A, Isha A. *Garcinia dulcis* fruit extract induced cytotoxicity and apoptosis in HeP-G2 liver cancer cell line. BioMed Res Int, 2015; 10(1): 1-10.

[34] Reed JC. Balancing cell life and death: Bax, apoptosis, and breast cancer. J Clin Invest, 1996; 97(11): 2403-2404.

[35] Vijayababu MR, Arunkumar A, Kanagaraj P, Venkataraman P, Krishnamoorthy G, Arunakaran J. Quercetin downregulates matrix metalloproteinases 2 and 9 proteins expression in prostate cancer cells (PC-3). Mol. Cell. Biochem, 2006; 287(1-2): 109-116.

[36] Majewska-Wierzbicka M, Czeczot H. Anticancer activity of flavonoids. Polski Merkuriusz Lekarski, 2012; 33(198): 364–369.

[37] Zhao Y, Jiang W, Li B, et al. Artesunate enhances radio-sensitivity of human non-small cell lung cancer A549 cells via increasing NO production to induce cell cycle arrest at G2/M phase. International Immuno-pharmacology, 2011; 11(12): 2039–2046.

[38] Araujo JR, Goncalves P, Martel F. Chemopreventive effect of dietary polyphenols in colorectal cancer cell lines. Nutr. Res, 2011; 31(2): 77–87.

[39] Scambia G, Ranelletti FO, Benedetti Panici P, Bonanno G, De Vincenzo R, Piantelli M, Mancuso S. Synergistic antiproliferative activity of quercetin and cisplatin on ovarian cancer cell growth. Anticancer Drugs, 1990; 1(1): 45–48.

[40] Goncalves CF, Santos MC, Ginabreda MG, Fortunato RS, Carvalho DP, Freitas Ferreira AC. Flavonoid rutin increases thyroid iodide uptake in rats. PLoS One, 2013; 8(9), e73908.