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Original Article

Rutin Inhibits Hepatic and Pancreatic Cancer Cell Proliferation by Inhibiting CYP3A4 and GST

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ABSTRACT

Background: Digestive cancer is among the major causes of mortality and morbidity worldwide. Rutin, a bioactive secondary metabolite belonging to flavonoids and distributed in many fruits and vegetables has shown anti-proliferative, anti-cancer, and neuroprotective activities. In this study, the antiproliferative, antioxidant and apoptotic activities of rutin on hepatic and pancreatic cancer cell lines were investigated. **Materials and Methods:** The effect on cellular viability was monitored by SRB assay. Increasing activity of caspases (3/7) was used as an indicator of apoptosis. Additionally, the anti-inflammatory and antioxidant activities of rutin were evaluated after measuring amount of prostaglandin E2 (PGE2) produced and through DPPH free radical scavenging assays, respectively. Moreover, the inhibitory effect on both CYP3A4 and GST enzymes has also been evaluated. **Results:** According to the data presented here, rutin has anti-proliferative effect and raises the number of caspases 3/7 in investigated cell lines. **Conclusion:** HepG-2 cells showed the highest cellular growth inhibition, followed by BxPC-3, Huh-7, MiaPaCa-2, Suit-2, and the normal cell line HPDE. Rutin also inhibited the cyctochrome P450 enzyme (CYP450 3A4) and glutatihione-S-transferase activity, with dose-dependent inhibition. Furthermore, suppression of PGE2 synthesis in BxPC-3 cells supported rutin's anti-inflammatory action (high COX-2 expression).

Keywords: Rutin, Pancreatic and Liver cancer, Apoptosis and Anti-inflammatory, Antioxidant.

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INTRODUCTION

Major digestive cancers include liver, esophageal, pancreatic, gastric, and colorectal cancers, which are among the leading causes of cancer mortality and morbidity globally.[1] GIT tumors account for about one-fifth of cancer cases and one-fourth of tumor-related fatalities in the US.[2] The most common digestive cancers worldwide are those of colorectal and gastric cancers, and account for more than 2 million new cases and nearly 1.3 million deaths yearly.[3] The early detection of colorectal and gastric cancers, will be accompanied by 5-year survival about 90% and 63%, respectively.[3,4] Among digestive cancers, pancreatic cancer is one of the most lethal malignancies for humans, with nearly identical incidence and mortality rates and a very poor 5-year survival rate.[5] The above mentioned facts pinpoint that early detection of gastrointestinal cancers is vital in reducing the number of deaths, and thereby, identification of suitable biomarkers for this purpose is an urgent need.[4,6]

Natural products, particularly dietary sources, and their semi-synthetic derivatives have recently received a lot of attention for treating a variety of human disorders, including malignancies.[7] Vegetable and fruit consumption has been linked to a lower risk of a variety of chronic conditions, including malignancies. According to a recent study, several bioactive compounds in plants can either activate or suppress numerous antiapoptotic, angiogenic, and metastatic pathways in cancer cells by targeting small molecules, showing that natural products and derivatives may have a large influence.[8,9]

Rutin is a naturally occurring bioflavonoid polyphenolic compound, widely found in fruits (apples, lemons, grapes), and vegetables (Potatoes and carrots) and beverages (tea) has shown multiple pharmacological activities including anticarcinogenic,

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antidiabetics, antimicrobial, anti-inflammatory, and cardioprotective activities.[10-12]

Despite the wide range of pharmacological activities of the natural compound rutin, its effect on hepatic and pancreatic tumors, needs to be investigated. Our results showed that rutin, extracted from the dried and powdered leaves and flowers of *Nicotiana glauca* was able to inhibit cell growth and proliferation and induce apoptosis in tumor cell lines including Suit-2 (the resistant pancreatic cancer cells), through activation of caspases 3/7 and inhibition of CYP3A4, GST enzymes and PGE2 activities. Moreover, rutin showed a powerful antioxidant activity.

MATERIALS AND METHODS

Plant Material

Nicotiana glauca (Figure 1) dried plant was collected from Asir region (Abha district 2019). The plant specimen was identified by Dr. Hany Gouda Department of Pharmacognosy, College of Pharmacy, Najran University, Saudi Arabia.

Extraction and Isolation of Rutin

The dried and powdered leaves and flowers of *Nicotiana glauca* (1000 g) were extracted by maceration for 2 weeks with 80% ethanol at room temperature followed by filtration and evaporation under reduced pressure. The aqueous layer was fractionated with saturated n-butanol (500 ml x 3 times). A yellow precipitate separated out of the two phases (aqueous and n-butanol).

The precipitate was filtrated and washed three times with dichloromethan: ethyl acetate (2:1) to give 1000 mg of a yellow amorphos powder. Which appear as dark purple spot of flavonoidal nature on TLC Silica plates. The resulted compound was identified by UV and NMR spectroscopic analysis (^{1}H NMR and ^{13}C NMR). The resulted data was compared with the previously published data.[13]

Identification of Rutin by NMR spectroscopy

^{1}H and ^{13}C-NMR data suggest the isolated compound could be rutin (Figure 2). It also added further evidence to the assumption made from the UV-vis data that the obtained compound could be rutin derivatives. The UV-spectra showed bands at 255 and 365 that corresponded to flavonol moieties Band I and II. Two protons at chemical shift 7.55 (dd, J=2.1 Hz, 7,5 Hz), one proton at chem shift 6.8 (dd, J=8.7 Hz), and two protons at 6.3 and 6.1 each are doublet with J= 2.1 Hz were identified by ^{1}H-NMR (DMSO-d6, 600 MHz). The presence of two sugar moieties was further confirmed by the ^{1}HNMR spectra, with anomeric proton signals at H 5.29 (1H, d, J=7.5 Hz, Glc-H-1″) and H 4.39 (1H, s, Ram-H-1‴) corresponding to glucose and rhamnose, respectively (Table 1). Also, 27 carbon signals detected by C13 NMR (DMSO-d6, 100 MHz). Nine oxygenated carbon atoms (C76.9–67.5), two anomeric carbon signals (C 101.6 and 101.2), and one methyl group (C 18.2) of rhamnose were also visible in the spectrum.

Cell lines and culture

The BxPC-3, Suit-2 and MiaPaCa-2 human pancreatic cancer cell lines, in addition to the HPDE normal immortalized pancreatic cells and the hepatocellular carcinoma HepG-2 and Huh-7 cells were used in our study. Cells were grown and treated as reported earlier.[5,14,15] Cancer cells were maintained in DMEM medium and supplemented with antibiotics (penicillin/streptomycin) and Fetal Bovine Serum (FCS) (Invitrogen, Carlsbad, CA). Mycoplasma detection kits were used to check that (Minerva Biolabs, Germany) all cell lines were mycoplasma-free, as previously stated.[16]

Effect of rutin on cell growth and viability

To determine the inhibitory activity of extracted rutin on different cancer cell lines, as previously described, SRB colorimetric technique (Sulforhodamine B assay) was utilized.[17,18] Briefly, cell lines were cultured after addition of increasing doses of rutin

Figure 1: *Nicotiana glauca* leaves and flowers.

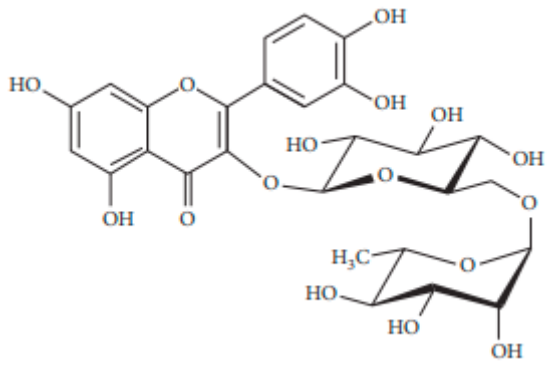

Figure 2: Rutin flavonoid isolated from *Nicotiana glauca* leaves and flowers.

for 48 hr or DMSO. After removal of the media, cellular fixation was done with acetic acid, staining and incubation at room temperature for 30 min have been done. After washing steps, 10 mM tris-base solution was added to dissolve the dyes that had remained. Finally, the absorbance of the samples was determined using a plate reader (Molecular Devices, CA).

Caspase-Glo 3/7 assay

A caspase 3/7 activity assay was used to investigate the influence of rutin on apoptosis induction.[14,18,19] Cell lines were treated with the flavonol rutin at various concentrations. Various doses of the flavonol rutin (2 × IC_{50}; IC_{50} and ½ × IC_{50}) or DMSO were used to treat cell lines (control). After 6 hr of incubation, the caspase reagent was added to each well, which was then mixed and incubated for 1 hr at room temperature. After that, the amount of light produced was measured, and caspase 3/7 activity was reported as a percentage of the untreated control.

Quantitative determination of prostaglandin E2

The effect of rutin on production of prostaglandin E2 (PGE2), has been tested using the PGE2 Kit (Cayman Chemicals, Michigan, USA).[5,20] Cell lines were given arachidonic acid then drug treatment for twenty-four hours. The levels of prostaglandin E2 were then measured using an enzyme immunoassay in comparison to the untreated control.

Cytochrome P450 (CYP3A4) assay

The CYP450-GloTM assay (Promega, Germany) was used to determine the effect of rutin on recombinant human CYP3A4 in this study.[21] Equal volumes of varied chemical concentrations were incubated at room temperature for 10 min with a reaction mixture including CYP3A4 substrate (luciferin 6-benzyl ether in phosphate buffer) and CYP3A4. After adding a regeneration system (NADPH) to citrate buffer, the reaction began. After half an hour, 50 liters of luciferin were introduced. The generated luminescence was measured using a precise TecanTMSafire II reader after twenty minutes. Each experiment was replicated at least three times using ketoconazole as a positive control.

Inhibition of Glutathione-S-transferase assay[22]

The GST enzyme activity assay kit was used to analyze the effect of our product rutin on GST (Glutathione-S-transferase enzyme). The procedure was followed as before.[23] GST substrate (1-chloro-2,4-dinitrobenzene (CDNB)) was used. In a nutshell, this experiment used untreated and rutin-treated cell lysates prepared with a standard assay combination containing CDNB, GSH (reduced glutathione), and PBS buffer. At 340 nm, the reaction was observed using spectrophotometry.

Antioxidant activity

The diphenylpicrylhydrazyl DPPH assay was used in three separate assays to determine rutin's free radical scavenging capacity. After mixing equal volumes of various sample solutions with a 0.2 mm methanolic DPPH solution, the absorbance was measured using a Tecan II Reader after 30 min of incubation at room temperature against a blank. As a positive control, Vitamin C was used.

RESULTS

Rutin inhibits cellular proliferation and viability of hepatic, and pancreatic cancer cell lines

Here we investigated the cellular anti-proliferative effect of rutin on different cancer cell types. HepG-2, Huh-7, MiaPaca-2, BxPc-3, Suit-2, and HPDE cells were cultured and treated with increasing concentrations of rutin for 48hr. After that we measured cellular viability and growth using SRB assay. Rutin inhibited growth of cell lines in a concentration dependent manner (Table 1). The most affected cell line was the human liver cancer cells (HepG-2) (IC_{50} 13.95±1.41) while the least affected cancer cell line was the pancreatic suit-2 cells (30.50 ± 4.63). Cellular sensitivity to rutin were in the following descending order: HepG-2 > Huh-7 > MiaPaca-2 > BxPc-3 > Suit-2. The normal immortalized HPDE cells were the least affected cells (Table 2).

For two days, exponentially developing cells were cultivated and treated with rutin. After that, the SRB test was used to determine cell viability and proliferation. The results are reported as a mean value (M) ± S.E.M.

Apoptotic induction by rutin is dose-dependent

Induction of apoptosis is among main mechanisms of chemotherapy-induced cell death.[24] Our aim was to show whether the antiproliferative effect of rutin was a result of its ability to induce the apoptosis; therefore, HepG-2, cells were treated with or without rutin or etoposide (apoptotic positive control) for 6 hr then the activity of caspases 3/7 was measured using the Caspase-Glo assay kit. Results showed that rutin increased activation of caspase 3/7 compared to untreated DMSO-treated control but the apoptotic effect was less than that of etoposide, hence we concluded that rutin induced apoptosis was through activation of members in theapoptotic cascade (Figure 3).

Table 1: ^{1}H and ^{13}C NMR data for Rutin (at 600 MHz in DMSO-d6,J in Hz).

Position	^{1}H-NMR	^{13}C-NMR
Aglycone		
2	-	164.5
3	-	133.7
4	-	177.8
5	-	157.0
6	6.2 (1H, *d, J* = 2.1)	99.14
7		164.5
8	6.4 (1H, *d, J* = 2.1)	94.05
9	-	161.6
10	-	104.4
1`	-	121.05
2'	7.56 (1H, *d, J* = 2.1)	115.8
3`	-	145.21
4`	-	148.8
5'	6.86 (1H, *d, J* = 8.7)	116.1
6'	7.54 (1H, *dd, J* = 2.1, 8.4)	122.05
Glucose moiety		
1"	5.29 (1H, *d, J* = 7.7)	104.4
2"	3.49*	72.3
3"	3.31*	76.3
4"	3.32*	71.02
5"	3.43*	76.9
6"	3.41* 3.70 (1H, *m*)	67.4
Rhamonse moiety		
1"'	4.39 (1H, *d, J* = 1.5)	101.6
2"'	3.39*	70.4
3"'	3.55*	70.48
4"'	3.27*	74.5
5"'	3.46*	68.1
6"'	0.99 (3H, *d, J* = 6.2)	18.8

Table 2: Rutin inhibits growth of BxPC-3, Suit-2, MiaPaCa-2, HepG-2, Huh-7, HepG-2, and Suit-2 cancer cells.

Cell line	IC_{50} (µg/mL)
Miapaca-2 cells	25.71± 2.62
Suit-2 cells	30.50 ± 4.63
BxPC-3 cells	15.03 ± 1.61
HepG2 cells	13.95 ± 1.41
Huh-7 cells	16.05 ± 1.86
HPDE cells	> 50

(B)

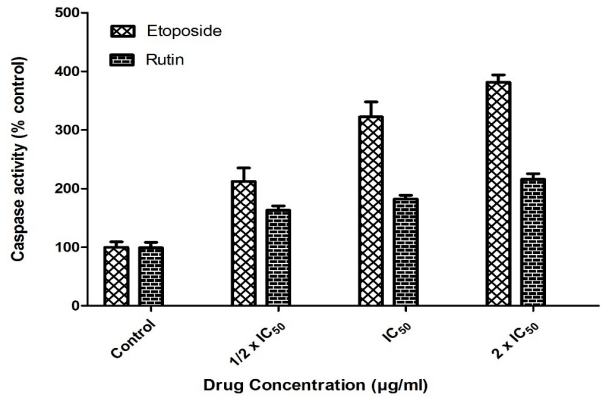

Figure 3: Apoptotic effect of rutin as shown by measuring caspase 3/7 activatioin.

Cells were treated with increasing concentrations of rutin and caspase 3/7 assay was carried out. Results are presented as mean value (M) ± S.E.M

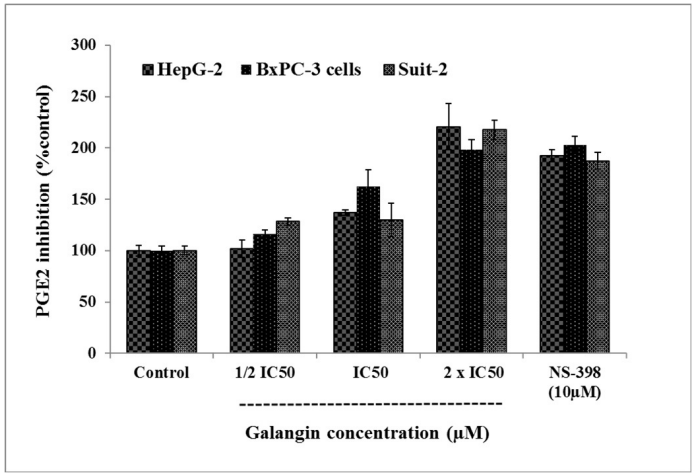

Figure 4: Inhibition of prostaglandin E2 after rutin treatment of Bx-PC3, HepG-2 and Suit-2 cells, results are represented as mean value (M) ± S.E.M of at least three independent experiments.

Anti-inflammatory effect of rutin (inhibition of PGE2 production)

Here, HepG-2, BxPC-3 and Suit-2 cancer cell lines were used for PGE2 assay.[19] To evaluate the anti-inflammatory effect of rutin compared to NS-398 (positive control) through inhibition of PGE2 production, the PGE2 levels produced by cancer cells after rutin treatment were determined. Our results showed that rutin significantly inhibited prostaglandin E2 production in a dose-dependent manner as shown in Figure 4.

Effect of rutin on cellular metabolism (CYP3A4 assay)

The CYP450-GloTM assay was used to determine the inhibitory effect of rutin on the cytochrome CYP3A4 enzyme (Figure 5). Rutin inhibited CYP3A4 in a dose-dependent manner, therefore we concluded that rutin-induced cell death was partly due to cellular metabolism suppression.

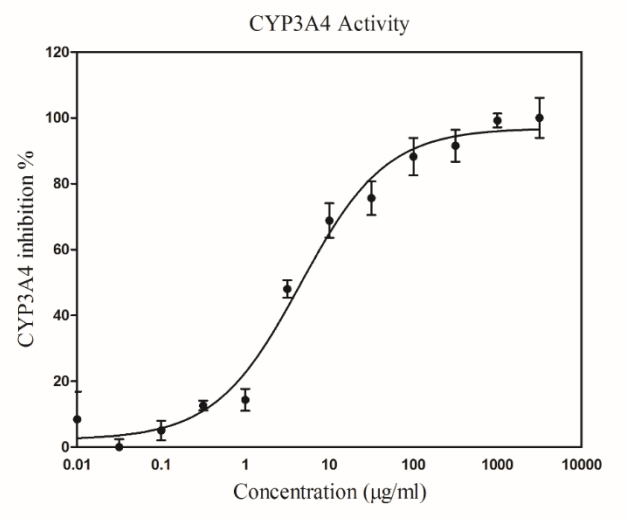

Figure 5: Effect of rutin on CYP3A4 enzyme activity.

Rutin inhibits CYP3A4 activity in a dose dependent manner. Results are presented as mean value (M) ± S.E.M.

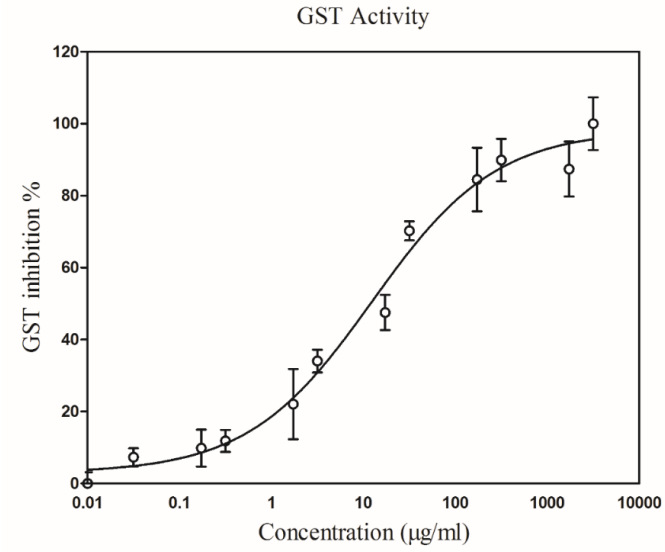

Figure 6: Effect of rutin on GST enzyme activity.

Rutin inhibits GST activity in a dose dependent manner. Results are presented as mean value (M) ± S.E.M.

Effect of rutin on Glutathione-S-transferase enzyme (GST assay)

The GST enzyme activity assay was used to assess the inhibitory effect of rutin on GST (Glutathione-S-transferase enzyme). The results demonstrated that rutin inhibited the GST enzyme in a dose-dependent manner (Figure 6).

Antioxidant activity

Antioxidants and free radical scavengers are used to combat reactive oxygen species (ROS), which can cause a variety of human malignancies. Here our aim was to investigate the free radical scavenging capacity of extracted rutin. As shown in Figure 7, the antioxidant effect of rutin was dosage dependent and comparable to that of the positive control Vitamin C. Rutin decreased the purple-colored DPPH radical to the yellow-colored diphenylpicrylhydrazine.

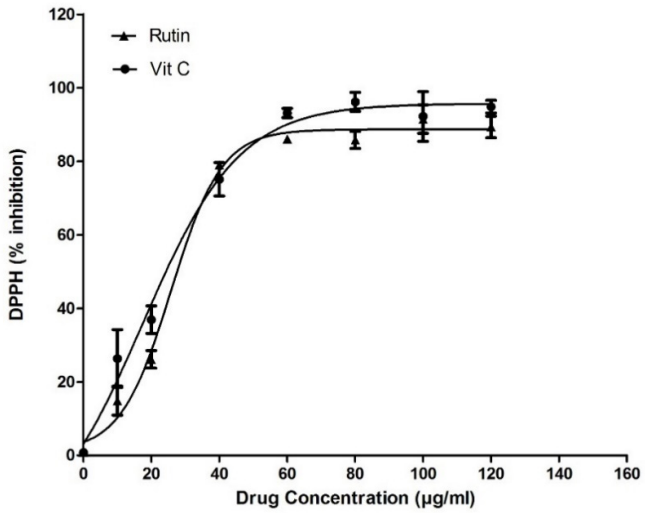

Figure 7: Antioxidant activity of rutin.

Rutin was able to reduce the purple-colored DPPH radical in a dose dependent manner. Results are presented as mean value (M) ± S.E.M.

DISCUSSION

There is a serious need for more effective and safer medications to overcome the severe side effects of the present clinically available anti-cancer agents. Long-term dietary intake of fruits and vegetables, with its proven safety, favors their use in cancer chemoprevention and can lead to significant variations in the risks and occurrences of numerous malignancies. Many scientists have backed up the idea of using safe and non-toxic innovative plant-derived compounds and tiny molecules to prevent tumors. Natural materials and their synthetic or semisynthetic derivatives account for a large portion of all approved anticancer medication candidates to date. Numerous researches has been carried out to investigate natural products for their use as anti-cancers among them, rutin is a highly promising candidate. Rutin is one of the phenolic compounds that have aroused interest due to the beneficial effect in reducing the risk of degenerative diseases.[7,25-27]

Previous results showed that rutin exhibits wide range of activities, including anti-angiogenic, neuroprotective, neurotrophic, and antioxidant effects.[11] In addition, several reports showed that rutin protects against several tumor types, including lung, colorectal, cervical, prostate, breast, bladder, and breast cancer.[27-34] Rutin also causes cell cycle arrest, apoptosis, and inhibits the growth of human colon cancer cells, according to other studies.[27,30,32,35-37] Rutin also affects cisplatin's anticancer efficacy and genotoxicity in tumor-bearing animals.[33] Furthermore, rutin

has been shown to promote autophagy mediated by p53 in human hepatoma Hep-G2 cells, while others have shown that rutin inhibits Sorafenib-induced Chemoresistance and Autophagy in hepatocellular carcinoma through modulating the BANCR/miRNA-590-5P/OLR1 axis in hepatocellular carcinoma.[25,38]

In spite of great efforts done, the detailed pharmacological actions of rutin on tumor cells is still needs to be further clarified. Here, the inhibitory effect of rutin on five cell lines representing two different tumor types (hepatic, and pancreatic) were investigated in addition to normal pancreatic cell line. Cellular inhibition was measured by SRB cell viability assay. Apoptotic induction was investigated by measuring the amount of caspases 3/7 produced after treatment of cells with rutin. Effect of rutin on metabolic enzymes like CYP 3A4 and GST (Cytochrome P450 (3A4) and Glutathione-S-transferase) and prostaglandin production (PGE2) was also evaluated. Additionally, the antioxidant activity of rutin was also evaluated

Rutin decreased cellular growth and proliferation while also inducing apoptosis in all examined cell lines, according to our findings. The human liver cancer cells (HepG-2) were the most affected (IC_{50} 13.951.41), while the pancreatic suit-2 cells (30.50 4.63), were the least affected. Rutin also inhibited the activity of cyctochrome P450 (CYP450 3A4) and glutatihione-S-transferase, with a dose-dependent impact. Furthermore, suppression of PGE2 synthesis in BxPC-3 cells with strong COX-2 expression validated rutin's anti-inflammatory action. Our results showed that rutin is able to induce apoptosis in cancer cells, is in complete agreement with previous studies in human cancer cells.[11]

Cytochromes (P450) are group of enzymes that play an important role drugs metabolism. It was noted that determining a compound's ability to reversibly block cytochrome P450 enzymes is critical since co-administration of drugs might hinder one another's metabolism, affecting plasma levels *in vivo* and potentially causing adverse drug responses or toxicity.[39-42]

In cancer patients, overdose or underdosing as a result of a drug-drug interaction will have unexpected and/or severe clinical implications.

Orally delivered natural ingredients may also impact the bioavailability of orally co-administered medications. Furthermore, numerous plants have the potential to cause adverse interactions with targeted drugs.[43] The goal of our research was to see if rutin had any inhibitory effects on CYP3A4 (the enzyme involved in the hepatic metabolism of most drugs). Our findings revealed that rutin can inhibit the CYP3A4 enzyme in a dose-dependent manner, indicating a vital sign and/or alarm during the development of rutin-based anticancer methods and/or combinations.

GSTs are a group of enzymes that catalyze the reaction of electrophilic hydrophobic molecules being conjugated to reduced Glutathione (GSH). As a result, by removing electrophilic endogenous and foreign substances from the body, you can help to avoid carcinogenesis.[44] These enzymes have previously been proven to detoxify and remove many carcinogens and/or their metabolites from the body. Furthermore, treatment with GST enzyme inhibitors potentiated the cytotoxicity of numerous anticancer medicines *in vitro*, and higher levels of GST enzyme activity were related with resistance to chemotherapy *in vivo*.[44-47] Overexpression of GST (glutathione S-transferases) and efflux pumps in tumor cells may impair chemotherapy drugs' anticancer efficacy.[48-50] Recently, it has become evident that glutathione-S-transferases are also involved in the control of apoptosis through involvement of the JNK signaling pathway.[51] Here, we reported that rutin was able to inhibit GST in a dose-dependent manner indicating a rational for its combination-based therapies and that rutin could prevent resistance to chemotherapy in combination therapies.

Reactive Oxygen Species (ROS) have been linked to a variety of human malignancies in the past. Antioxidants and scavengers were also utilized to combat these harmful radicals. We've raised a question about the relationship between rutin's cytotoxicity and its ability to scavenge free radicals. The DPPH assay was used to measure free radical scavenging. Rutin has been shown to have potent free radical scavenging properties, making it a good candidate for use in tumor combination therapy.

CONCLUSION

The present analysis clearly demonstrated that rutin inhibited hepatic and pancreatic cancer cellular growth and proliferation. The most affected cell line was the human liver cancer cells (HepG-2) (IC_{50} 13.95±1.41) while the least affected cancer cell line was the pancreatic suit-2 cells (30.50 ± 4.63). The growth inhibitory effect was mainly mediated through activation of caspases 3/7, inhibition of PGE2 and antioxidant activities. Additionally, we proved that rutin was able to inhibit GST enzyme responsible for reducing the antitumor activity of various chemotherapeutics agents. Moreover, rutin inhibited CYP3A4 enzyme involved in cellular metabolism indicating a critical alarm during development of combinations–based therapeutics.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

CYP450 3A4: Cytochrome P450; **HepG-2:** Human liver cancer cells; **GSH:** Reduced glutathione; **GST:** Glutathione S-transferases; **ROS:** Reactive oxygen species; **Hep-G2:** Human hepatoma cells; **DPPH:** Diphenylpicrylhydrazyl; **CDNB:** 1-chloro-2,4-dinitrobenzene; **PGE2:** Prostaglandin E2; **Suit-2:** The resistant pancreatic cancer cells.

SUMMARY

The bioactive secondary metabolite rutin has been investigated for antiproliferative, antioxidant and apoptotic activities on hepatic and pancreatic cancer cell lines. The effect on cellular viability was monitored by SRB assay. Increasing activity of caspases (3/7) was used as an indicator of apoptosis. The anti-inflammatory and antioxidant activities of rutin were evaluated after measuring the amount of Prostaglandin E2 (PGE2) produced and through DPPH free radical scavenging assays, respectively. Moreover, the inhibitory effect on both CYP3A4 and GST enzymes has also been evaluated. Accordingly rutin has an anti-proliferative effect and raises the number of caspases 3/7 in investigated cell lines. HepG-2 cells showed the highest cellular growth inhibition, followed by BxPC-3, Huh-7, MiaPaCa-2, Suit-2, and the normal cell line HPDE. Rutin also inhibited cyctochrome P450 enzyme (CYP450 3A4) and glutatihione-S-transferase activity, with dose-dependent inhibition. Furthermore, suppression of PGE2 synthesis in BxPC-3 cells supported rutin's anti-inflammatory action (high COX-2 expression).

REFERENCES

1. Tang S, Wu WK, Li X, Wong SH, Wong N, Chan MT, *et al*. Stratification of digestive cancers with different pathological features and survival outcomes by microRNA expression. Sci Rep. 2016;6:24466. doi: 10.1038/srep24466, PMID 27080237.
2. Ahmed YH, El-Naggar ME, Rashad MM, M Youssef A, Galal MK, Bashir DW. Screening for polystyrene nanoparticle toxicity on kidneys of adult male albino rats using histopathological, biochemical, and molecular examination results. Cell Tissue Res. 2022;388(1):149-65. doi: 10.1007/s00441-022-03581-5, PMID 35088181.
3. Ferlay J, Shin HR, Bray F, Forman D, Mathers C, Parkin DM. Estimates of worldwide burden of cancer in 2008: GLOBOCAN 2008. Int J Cancer. 2010;127(12):2893-917. doi: 10.1002/ijc.25516, PMID 21351269.
4. Vatandoost N, Ghanbari J, Mojaver M, Avan A, Ghayour-Mobarhan M, Nedaeinia R, *et al*. Early detection of colorectal cancer: from conventional methods to novel biomarkers. J Cancer Res Clin Oncol. 2016;142(2):341-51. doi: 10.1007/s00432-015-1928-z, PMID 25687380.
5. Youns M, Fathy GM. Upregulation of extrinsic apoptotic pathway in curcumin-mediated antiproliferative effect on human pancreatic carcinogenesis. J Cell Biochem. 2013;114(12):2654-65. doi: 10.1002/jcb.24612, PMID 23794119.
6. Vedeld HM, Andresen K, Eilertsen IA, Nesbakken A, Seruca R, Gladhaug IP, *et al*. The novel colorectal cancer biomarkers CDO1, ZSCAN18 and ZNF331 are frequently methylated across gastrointestinal cancers. Int J Cancer. 2015;136(4):844-53. doi: 10.1002/ijc.29039, PMID 24948044.
7. Khan N, Syed DN, Ahmad N, Mukhtar H. Fisetin: a dietary antioxidant for health promotion. Antioxid Redox Signal. 2013;19(2):151-62. doi: 10.1089/ars.2012.4901, PMID 23121441.
8. Chung MY, Lim TG, Lee KW. Molecular mechanisms of chemopreventive phytochemicals against gastroenterological cancer development. World J Gastroenterol. 2013;19(7):984-93. doi: 10.3748/wjg.v19.i7.984, PMID 23467658.
9. Lee KW, Bode AM, Dong Z. Molecular targets of phytochemicals for cancer prevention. Nat Rev Cancer. 2011;11(3):211-8. doi: 10.1038/nrc3017, PMID 21326325.
10. Rakhit RD, Matter C, Windecker S, Garachemani AR, Seiler C, Eberli FR. Five French versus 6 French PCI: A case control study of efficacy, safety and outcome. J Invasive Cardiol. 2002;14(11):670-4. PMID 12403895.
11. Saleh A, ElFayoumi HM, Youns M, Barakat W. Rutin and orlistat produce antitumor effects via antioxidant and apoptotic actions. Naunyn Schmiedebergs Arch Pharmacol. 2019;392(2):165-75. doi: 10.1007/s00210-018-1579-0, PMID 30465055.
12. Caparica R, Júlio A, Araújo MEM, Baby AR, Fonte P, Costa JG, *et al*. Anticancer activity of Rutin and its combination with ionic liquids on renal cells. Biomolecules. 2020;10(2). doi: 10.3390/biom10020233, PMID 32033222.
13. Abdelfattah MS, Badr SEA, Lotfy SA, Attia GH, Aref AM, Abdel Moneim AE, *et al*. Rutin and selenium co-administration reverse 3-nitropropionic acid-induced neurochemical and molecular impairments in a mouse model of Huntington's disease. Neurotox Res. 2020;37(1):77-92. doi: 10.1007/s12640-019-00086-y, PMID 31332714.
14. Youns M, Efferth T, Reichling J, Fellenberg K, Bauer A, Hoheisel JD. Gene expression profiling identifies novel key players involved in the cytotoxic effect of artesunate on pancreatic cancer cells. Biochem Pharmacol. 2009;78(3):273-83. doi: 10.1016/j.bcp.2009.04.014, PMID 19393226.
15. Nasr T, Bondock S, Youns M. Anticancer activity of new coumarin substituted hydrazide-hydrazone derivatives. Eur J Med Chem. 2014;76:539-48. doi: 10.1016/j.ejmech.2014.02.026, PMID 24607878.
16. Alhamdani MS, Youns M, Buchholz M, Gress TM, Beckers MC, Maréchal D, et al. Immunoassay-based proteome profiling of 24 pancreatic cancer cell lines. J Proteomics. 2012;75(12):3747-59. doi: 10.1016/j.jprot.2012.04.042, PMID 22579748.
17. Torres Salazar A, Hoheisel J, Youns M, Wink M. Anti-inflammatory and anti-cancer activities of essential oils and their biological constituents. Int J Clin Pharmacol Ther. 2011;49(1):93-5. PMID 21176743.
18. Ahmed MF, Youns M. Synthesis and biological evaluation of a novel series of 6,8-dibromo-4(3H)quinazolinone derivatives as anticancer agents. Arch Pharm. 2013;346(8):610-7. doi: 10.1002/ardp.201300158, PMID 23873839.
19. Youns M, Efferth T, Hoheisel JD. Transcript profiling identifies novel key players mediating the growth inhibitory effect of NS-398 on human pancreatic cancer cells. Eur J Pharmacol. 2011;650(1):170-7. doi: 10.1016/j.ejphar.2010.10.026, PMID 20969859.
20. Mulyaningsih S, Youns M, El-Readi MZ, Ashour ML, Nibret E, Sporer F, *et al*. Biological activity of the essential oil of *Kadsura longipedunculata* (Schisandraceae) and its major components. J Pharm Pharmacol. 2010;62(8):1037-44. doi: 10.1111/j.2042-7158.2010.01119.x, PMID 20663038.
21. Cali JJ, Ma D, Sobol M, Simpson DJ, Frackman S, Good TD, *et al*. Luminogenic cytochrome P450 assays. Expert Opin Drug Metab Toxicol. 2006;2(4):629-45. doi: 10.1517/17425255.2.4.629, PMID 16859410.
22. Alberts AWC, J. Kuron, G.; Hunt, V.; Huff, J.; Hoffman, C; Rothrock, J.; Lopez, M.; Joshua, H.; Harris, E.; Patchett, A.; Monaghan, R.; Currie, S, Stapley E A-S, G.; Hensens, O.; Hirshfield, J.; Hoogsteen, K.; Liesch, J. J Proc Natl Acad Sci. 1980;77:3957-61.
23. Habig WH, Pabst MJ, Jakoby WB. Glutathione S-transferases. The first enzymatic step in mercapturic acid formation. J Biol Chem. 1974;249(22):7130-9. doi: 10.1016/S0021-9258(19)42083-8, PMID 4436300.
24. Igney FH, Krammer PH. PH. Death and anti-death: tumour resistance to apoptosis. Nat Rev Cancer. 2002;2(4):277-88. doi: 10.1038/nrc776, PMID 12001989.
25. Zhou M, Zhang G, Hu J, Zhu Y, Lan H, Shen X, *et al*. Rutin attenuates sorafenib-induced Chemoresistance and Autophagy in Hepatocellular Carcinoma by regulating BANCR/miRNA-590-5P/OLR1 Axis. Int J Biol Sci. 2021;17(13):3595-607. doi: 10.7150/ijbs.62471, PMID 34512168.
26. Qu Q, Cui W, Xing X, Zou R, Huang X, Wang X, *et al*. Rutin, A natural inhibitor of IGPD protein, partially inhibits biofilm formation in Staphylococcus xylosus ATCC700404 *in vitro* and *in vivo*. Front Pharmacol. 2021;12:728354. doi: 10.3389/fphar.2021.728354, PMID 34456739.
27. Chen X, Yu M, Xu W, Zou L, Ye J, Liu Y, *et al*. Rutin inhibited the advanced glycation end products-stimulated inflammatory response and extra-cellular matrix degeneration via targeting TRAF-6 and BCL-2 proteins in mouse model of osteoarthritis. Aging (Albany, NY). 2021;13(18):22134-47. doi: 10.18632/aging.203470, PMID 34550907.
28. Abarikwu SO, Njoku RC, John IG, Amadi BA, Mgbudom-Okah CJ, Onuah CL. Antioxidant and anti-inflammatory protective effects of rutin and kolaviron against busulfan-induced testicular injuries in rats. Syst Biol Reprod Med. 2022;68(2):151-61. doi: 10.1080/19396368.2021.1989727. PMID 34753368.
29. Ghanbari-Movahed M, Mondal A, Farzaei MH, Bishayee A. Quercetin-and rutin-based nano-formulations for cancer treatment: A systematic review of improved efficacy and molecular mechanisms. Phytomedicine. 2022;97:153909. doi: 10.1016/j.phymed.2021.153909, PMID 35092896.
30. Imani A, Maleki N, Bohlouli S, Kouhsoltani M, Sharifi S, Maleki Dizaj S. Molecular mechanisms of anticancer effect of rutin. Phytother Res. 2020. doi: 10.1002/ptr.6977, PMID 33295678.
31. Khan F, Pandey P, Jha NK, Khalid M, Ojha S. Rutin mediated apoptotic cell Death in Caski cervical cancer cells via Notch-1 and Hes-1 downregulation. Life (Basel). 2021;11(8). doi: 10.3390/life11080761, PMID 34440505.
32. Pinzaru I, Chioibas R, Marcovici I, Coricovac D, Susan R, Predut D, *et al*. Rutin exerts cytotoxic and senescence-inducing properties in human melanoma cells. Toxics. 2021;9(9). doi: 10.3390/toxics9090226, PMID 34564377.

33. Prasad R, Prasad SB. Modulatory effect of Rutin on the antitumor activity and genotoxicity of cisplatin in tumor-bearing mice. Adv Pharm Bull. 2021;11(4):746-54. doi: 10.34172/apb.2021.084, PMID 34888222.
34. Satari A, Ghasemi S, Habtemariam S, Asgharian S, Lorigooini Z. Rutin: A flavonoid as an effective sensitizer for anticancer therapy; insights into multifaceted mechanisms and applicability for combination therapy. Evid Based Complement Alternat Med. 2021;2021:9913179. doi: 10.1155/2021/9913179, PMID 34484407.
35. Azevedo MI, Pereira AF, Nogueira RB, Rolim FE, Brito GA, Wong DV, *et al*. The antioxidant effects of the flavonoids rutin and quercetin inhibit oxaliplatin-induced chronic painful peripheral neuropathy. Mol Pain. 2013;9:53. doi: 10.1186/1744-8069-9-53, PMID 24152430.
36. Ben Sghaier M, Pagano A, Mousslim M, Ammari Y, Kovacic H, Luis J. Rutin inhibits proliferation, attenuates superoxide production and decreases adhesion and migration of human cancerous cells. Biomed Pharmacother. 2016;84:1972-8. doi: 10.1016/j.biopha.2016.11.001, PMID 27829548.
37. Farha AK, Gan RY, Li HB, Wu DT, Atanasov AG, Gul K, *et al*. The anticancer potential of the dietary polyphenol rutin: Current status, challenges, and perspectives. Crit Rev Food Sci Nutr. 2022;62(3):832-59. doi: 10.1080/10408398.2020.1829541, PMID 33054344.
38. Yu MH, Tsai MC, Wang CC, Wu SW, Chang YJ, Wu CH, *et al*. Mulberry leaf polyphenol extract and Rutin induces autophagy regulated by p53 in human hepatoma HepG2 cells. Pharmaceuticals (Basel). 2021;14(12). doi: 10.3390/ph14121310, PMID 34959709.
39. Smith G. European Medicines Agency guideline on bioanalytical method validation: what more is there to say? Bioanalysis. 2012;4(8):865-8. doi: 10.4155/bio.12.44, PMID 22533559.
40. Pean E, Demolis P, Moreau A, Hemmings RJ, O'Connor D, Brown D, *et al*. The European Medicines Agency review of cabazitaxel (Jevtana®) for the treatment of hormone-refractory metastatic prostate cancer: summary of the scientific assessment of the committee for medicinal products for human use. Oncologist. 2012;17(4):543-9. doi: 10.1634/theoncologist.2011-0364, PMID 22477727.
41. Hanaizi Z, van Zwieten-Boot B, Calvo G, Lopez AS, van Dartel M, Camarero J, *et al*. The European Medicines Agency review of ipilimumab (Yervoy) for the treatment of advanced (unresectable or metastatic) melanoma in adults who have received prior therapy: summary of the scientific assessment of the Committee for Medicinal Products for Human Use. Eur J Cancer. 2012;48(2):237-42. doi: 10.1016/j.ejca.2011.09.018, PMID 22030452.
42. Cohen D. European Medicines Agency is to tighten up on advisers' conflicts of interest. BMJ. 2012;344:e2388. doi: 10.1136/bmj.e2388, PMID 22457472.
43. Kandagatla SK, Mack T, Simpson S, Sollenberger J, Helton E, Raner GM. Inhibition of human cytochrome P450 2E1 and 2A6 by aldehydes: structure and activity relationships. Chem Biol Interact. 2014;219:195-202. doi: 10.1016/j.cbi.2014.05.014, PMID 24924949.
44. Hatono S, Jimenez A, Wargovich MJ. Chemopreventive effect of S-allylcysteine and its relationship to the detoxification enzyme glutathione S-transferase. Carcinogenesis. 1996;17(5):1041-4. doi: 10.1093/carcin/17.5.1041, PMID 8640910.
45. Katiyar T, Yadav V, Maurya SS, Ruwali M, Singh M, Hasan F, *et al*. Interaction of glutathione-s-transferase genotypes with environmental risk factors in determining susceptibility to head and neck cancer and treatment response and survival outcome. Environ Mol Mutagen. 2020;61(5):574-84. doi: 10.1002/em.22362, PMID 32067264.
46. Mandal RK, Mittal RD. Glutathione S-Transferase P1 313 (A>G) Ile105Val Polymorphism Contributes to Cancer Susceptibility in Indian Population: a Meta-analysis of 39 Case–Control Studies. Ind J Clin Biochem. 2020;35(1):8-19. doi: 10.1007/s12291-018-0787-1.
47. Wang S, Yang J, Ding C, Li J, You L, Dai M, *et al*. Glutathione S-transferase Mu-3 predicts a better prognosis and inhibits malignant behavior and glycolysis in pancreatic cancer. Front Oncol. 2020;10:1539. doi: 10.3389/fonc.2020.01539, PMID 32984010.
48. Ajaz S, Zaidi SE, Ali SM, Siddiqa A, Memon MA, Firasat S, *et al*. Absence of glutathione S-transferase Theta 1 gene is significantly associated with breast cancer susceptibility in Pakistani population and poor overall survival in breast cancer patients: A case-control and case series analysis. Front Oncol. 2021;11:678705. doi: 10.3389/fonc.2021.678705, PMID 34938652.
49. Jou YC, Wang SC, Dia YC, Wang ST, Yu MH, Yang HY, *et al*. Anti-cancer effects and tumor marker role of glutathione S-transferase mu 5 in human bladder cancer. Int J Mol Sci. 2021;22(6). doi: 10.3390/ijms22063056, PMID 33802702.
50. Kogawa T, Sato Y, Shimoyama R, Kurata W, Tashiro Y, Niitsu Y. Chemoprevention of pancreatic cancer by inhibition of glutathione-S transferase P1. Invest New Drugs. 2021;39(6):1484-92. doi: 10.1007/s10637-021-01129-y, PMID 34031785.
51. Sau A, Pellizzari Tregno F, Valentino F, Federici G, Caccuri AM. Glutathione transferases and development of new principles to overcome drug resistance. Arch Biochem Biophys. 2010;500(2):116-22. doi: 10.1016/j.abb.2010.05.012, PMID 20494652.

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