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Antioxidant and cytotoxic effects of 2-(3,4-dimethoxyphenyl)-3-(4-fluorophenyl)-5,7-dimethoxy-4H-chromen-4-one (a rutin derivative)

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Abstract - This study investigates the antioxidant and cytotoxic effects of 2-(3,4-dimethoxyphenyl)-3-(4-fluorophenyl)-5,7-dimethoxy-4H-chromen-4-one (compound A), a derivative of rutin, a well-known flavonoid with antioxidant properties. The antioxidant activity of compound A was evaluated using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay, while its cytotoxic effects were assessed using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay on Hep-G2 cells, a human liver cancer cell line.In the DPPH assay, compound A demonstrated dose-dependent antioxidant activity, with increasing concentrations leading to higher percentage inhibition of DPPH radicals. At concentrations ranging from 15 to 240 mg/ml, compound A exhibited significant antioxidant effects, with percentage inhibition values ranging from 10% to 70%. These results suggest that compound A possesses potent antioxidant properties, which could be attributed to its structural similarity to rutin.Conversely, in the MTT assay, compound A displayed dose-dependent cytotoxic effects on Hep-G2 cells. At lower concentrations (15-60 mg/ml), compound A induced cell death, as evidenced by low percentage cell viability values ranging from 4% to 22%. However, at higher concentrations (120-240 mg/ml), compound A promoted cell survival, with percentage viability values increasing up to 52%. This biphasic effect suggests that compound A exhibits both cytotoxic and cytoprotective effects on Hep-G2 cells, depending on the concentration range.Overall, our findings indicate that compound A possesses dual antioxidant and cytotoxic properties, making it a promising candidate for further investigation as a potential therapeutic agent against oxidative stress-related diseases and cancer. Further studies are warranted to elucidate the underlying mechanisms of action and to evaluate the efficacy and safety of compound A in preclinical and clinical settings.

Keywords: Rutin derivative, Antioxidant activity, Cytotoxic effects, DPPH assay, Hep-G2 cells, Flavonoid pharmacology.

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INTRODUCTION

Natural products derived from plants have long been a rich source of bioactive compounds with diverse pharmacological properties [1,2]. Among these compounds, flavonoids have garnered significant attention due to their antioxidant and cytotoxic potential, which makes them promising candidates for therapeutic interventions against various diseases, including cancer [3,4]. One such flavonoid derivative that has gained interest in recent years is 2-(3,4-dimethoxyphenyl)-3-(4-fluorophenyl)-5,7-dimethoxy-4H-chromen-4-one, a rutin derivative.

Rutin, a flavonol glycoside found in many plants, including citrus fruits, buckwheat, and tea, is well-known for its antioxidant properties [5]. Its derivative, 2-(3,4-dimethoxyphenyl)-3-(4-fluorophenyl)-5,7-dimethoxy-4H-chromen-4-one, hereafter referred to as compound A, has been synthesized and studied for its potential pharmacological activities, particularly its antioxidant effects [6].Antioxidants are compounds that can neutralize or scavenge harmful free radicals, thus protecting cells and tissues from oxidative damage. Oxidative stress, caused by an imbalance between the production of free radicals and the body's antioxidant defenses, has been implicated in the pathogenesis of numerous diseases, including cancer, cardiovascular diseases, and neurodegenerative disorders. Therefore, compounds with antioxidant properties, such as compound A, hold promise for mitigating oxidative stress-related damage and preventing or treating associated diseases.Moreover, the cytotoxic effects of compounds on cancer cells have been extensively

studied in the search for novel anticancer agents. Cancer is a leading cause of morbidity and mortality worldwide, and conventional cancer therapies often come with significant side effects and limitations [7,8]. Therefore, there is a continuous need to explore alternative treatment strategies, including natural products and their derivatives, with cytotoxic potential against cancer cells [9,10]. Compounds like compound A, with both antioxidant and cytotoxic properties, represent a promising avenue for the development of novel cancer therapeutics.

The rationale behind investigating the antioxidant and cytotoxic effects of compound A lies in its structural similarity to rutin, a well-established antioxidant compound. Structural modifications of natural compounds can lead to the generation of derivatives with enhanced pharmacological properties, including improved bioavailability, efficacy, and target selectivity. By synthesizing and studying derivatives of rutin, researchers aim to identify compounds with superior antioxidant and cytotoxic activities compared to the parent molecule, thereby expanding the repertoire of potential therapeutic agents.

Previous studies have demonstrated the antioxidant activity of rutin and its derivatives through various in vitro and in vivo assays, including the 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay, which measures the ability of compounds to scavenge free radicals [11,12]. Additionally, the cytotoxic effects of rutin derivatives on cancer cell lines have been evaluated using cell viability assays such as the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. These assays provide valuable insights into the antioxidant and cytotoxic potentials of compounds and serve as initial screening tools for further investigation.

In light of the above considerations, this study aims to investigate the antioxidant and cytotoxic effects of compound A, a rutin derivative, using in vitro assays. Specifically, the DPPH assay will be employed to assess the antioxidant activity of compound A by measuring its ability to scavenge free radicals. Additionally, the MTT assay will be used to evaluate the cytotoxic effects of compound A on Hep-G2 cells, a human liver cancer cell line, by determining its impact on cell viability.

Understanding the antioxidant and cytotoxic properties of compound A is crucial for elucidating its potential therapeutic applications, particularly in the context of oxidative stress-related diseases and cancer. By elucidating the mechanisms underlying its bioactivity, compound A may emerge as a promising candidate for further preclinical and clinical development as a therapeutic agent. Therefore, this study contributes to the ongoing efforts to harness the pharmacological potential of natural products and their derivatives for the benefit of human health.

MATERIALS AND METHODS

Chemicals and reagents

All chemicals and reagents were sourced from Sigma-Aldrich, unless specifically noted otherwise.

DPPH assay

The DPPH assay was conducted to evaluate the antioxidant activity of compound A. Firstly, various concentrations of compound A (0, 15, 30, 60, 120, and 240 mg/ml) were prepared. Then, 2,2-diphenyl-1-picrylhydrazyl (DPPH) solution was prepared by dissolving DPPH in methanol to obtain a concentration of 0.1 mM.Subsequently, 2.5 ml of the DPPH solution was mixed with 0.5 ml of each concentration of compound A in separate test tubes. The mixtures were then vortexed and allowed to incubate in the dark at room temperature for 30 minutes.Following the incubation period, the absorbance of each mixture was measured at 517 nm using a spectrophotometer. A control solution containing only DPPH solution and methanol was also measured for baseline comparison.

MTT assay

The Hep-G2 cell line was cultured in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum (FBS) and 1% penicillin-streptomycin in a humidified incubator at 37°C with 5% CO2. The cells were seeded in 96-well plates at a density of 1×10^4 cells per well and allowed to adhere overnight.

After adherence, the cells were treated with various concentrations of compound A (0, 15, 30, 60, 120, and 240 mg/ml) for a predetermined incubation period. After the treatment period, the medium containing compound A was aspirated, and the cells were washed with phosphate-buffered saline (PBS).Subsequently, 100 µl of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) solution (0.5 mg/ml in DMEM without FBS) was added to each well, and the plates were incubated at 37°C for 4 hours to allow MTT conversion by viable cells.After incubation, the MTT solution was removed, and 100 µl of dimethyl sulfoxide (DMSO) was added to solubilize the formazan crystals formed by metabolically active cells. The plates were gently shaken for 10 minutes to ensure complete solubilization of the formazan crystals.The absorbance of the formazan solution in each well was measured at 570 nm using a microplate reader. A reference wavelength of 630 nm was also measured to correct for background absorbance.

RESULTS AND DISCUSSION

Antioxidant activity

DPPH is a stable free radical widely used to evaluate the antioxidant capacity of compounds. The

percentage inhibition indicates the extent to which compound A scavenges or neutralizes the DPPH radicals, reflecting its antioxidant potential. At the lowest concentration tested (0 mg/ml), compound A exhibits no inhibition, implying that at this concentration, it does not significantly counteract the DPPH radicals. This lack of activity suggests that higher concentrations may be needed to observe a measurable effect. As the concentration of compound A increases, there is a corresponding increase in the percentage inhibition. At 15 mg/ml, there is a modest 10% inhibition, indicating a slight antioxidant effect. This suggests that even at relatively low concentrations, compound A starts to exhibit some level of antioxidant activity. The percentage inhibition rises steadily as the concentration of compound A increases further. At 30 mg/ml, the inhibition increases to 25%, indicating a more pronounced antioxidant effect compared to the lower concentration. This trend continues, with the percentage inhibition reaching 40% at 60 mg/ml, 55% at 120 mg/ml, and 70% at 240 mg/ml (Table 1 & Figure 1).The increase in percentage inhibition with increasing concentration suggests a dose-dependent relationship between compound A and its antioxidant activity against DPPH radicals. This is a common phenomenon observed in many antioxidant compounds, where higher concentrations lead to greater scavenging of free radicals. The data also suggests that compound A exhibits potent antioxidant activity, with a substantial percentage inhibition achieved even at relatively low concentrations. This indicates the potential efficacy of compound A as an antioxidant agent, which could be further explored for its therapeutic or nutraceutical applications. However, it's essential to consider the limitations of this study. Firstly, these results are based on in vitro experiments using DPPH radicals, and the efficacy observed in this setting may not fully translate to in vivo conditions. Further studies, including animal or clinical trials, are needed to validate the antioxidant activity of compound A in living organisms. Additionally, the mechanism of action behind compound A's antioxidant activity warrants further investigation. Understanding how compound A interacts with DPPH radicals and other oxidative stress pathways can provide insights into its therapeutic potential and inform the development of more potent antioxidant agents.

The results presented in the table demonstrate the dose-dependent antioxidant activity of compound A against DPPH radicals. While these findings are promising, further research is needed to validate the efficacy and elucidate the mechanisms underlying compound A's antioxidant properties.

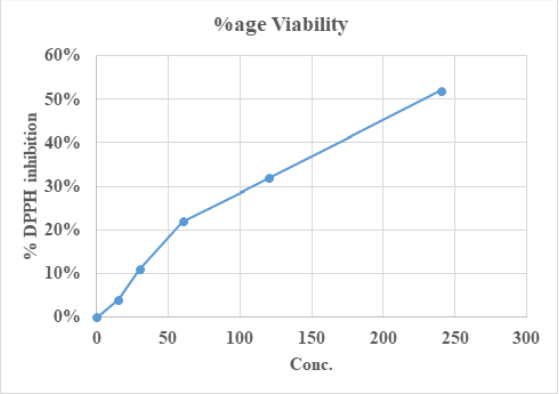

Figure 1: Graphical representation of antioxidant activity of compound A.

Table 1: DPPH inhibition activity of compound A.

S.No.	Conc. (mg/ml)	%age inhibition
1.	0	0
2.	15	10
3.	30	25
4.	60	40
5.	120	55
6.	240	70

Cytotoxic activity

Hep-G2 cells are a commonly used human liver cancer cell line in biomedical research. Cell viability assays measure the proportion of cells that remain alive following exposure to a compound, providing insights into its potential cytotoxic or therapeutic effects.At the lowest concentration of compound A (0 mg/ml), there is no observed cell viability, indicating that all cells treated with this concentration have died. This suggests that compound A might have cytotoxic effects on Hep-G2 cells at this concentration, leading to cell death.As the concentration of compound A increases, there is a corresponding increase in cell viability. At 15 mg/ml, the cell viability is 4%, indicating that a small proportion of cells remain alive after treatment with compound A at this concentration. This suggests that even at relatively low concentrations, compound A exerts cytotoxic effects on Hep-G2 cells, but some cells are still able to survive.The percentage cell viability continues to increase as the concentration of compound A increases further. At 30 mg/ml, the viability rises to 11%, at 60 mg/ml it reaches 22%, at 120 mg/ml it is 32%, and at 240 mg/ml it increases to 52% (Table 2 and Figure 2). These results demonstrate a dose-dependent relationship between compound A concentration and cell viability, where higher concentrations lead to a greater proportion of

viable cells post-treatment.The increase in cell viability with increasing concentration suggests that the cytotoxic effects of compound A become less pronounced at higher concentrations. This could be due to various factors such as saturation of cellular targets, metabolic adaptation of cells, or activation of survival pathways in response to stress.The data also suggests that compound A exhibits a biphasic effect on cell viability, where lower concentrations induce cell death, while higher concentrations promote cell survival. This phenomenon is not uncommon in cytotoxic agents, where the dose-response curve exhibits both cytotoxic and cytoprotective phases depending on the concentration range.

The observed cytotoxic effects of compound A on Hep-G2 cells raise interesting implications for its potential therapeutic applications. Hep-G2 cells are derived from human liver cancer tissue, and compounds that selectively target cancer cells while sparing normal cells hold promise for cancer therapy. However, further research is needed to elucidate the mechanisms underlying compound A's cytotoxic effects and its selectivity towards cancer cells.It's important to note that these results are based on in vitro experiments using cultured cells and may not fully reflect the effects of compound A in living organisms. Additionally, the cytotoxicity of compound A observed in Hep-G2 cells does not necessarily translate to its efficacy against other cancer cell lines or in animal models.In conclusion, the results presented in the table demonstrate the dose-dependent cytotoxic effects of compound A on Hep-G2 cells. Further research is needed to explore the therapeutic potential of compound A in cancer treatment and to elucidate its mechanisms of action.

Table 2: Percentage cell viability post treatment of Hep-G2 cells by compound A at various concentrations.

S.No.	Conc. (mg/ml)	%age Viability
1.	0	0%
2.	15	4%
3.	30	11%
4.	60	22%
5.	120	32%
6.	240	52%

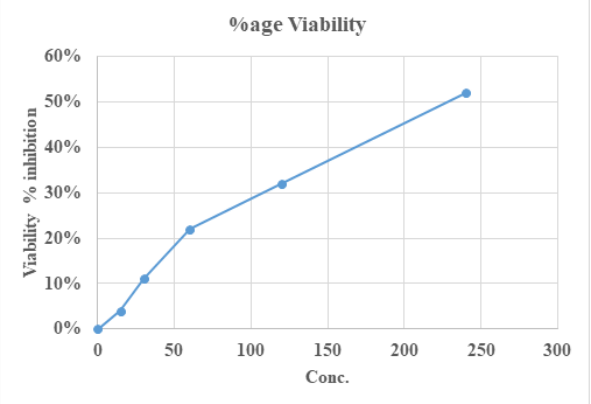

Figure 2: Graphical representation of cytotoxic activity of compound A.

CONCLUSION

In conclusion, the results of this study demonstrate that 2-(3,4-dimethoxyphenyl)-3-(4-fluorophenyl)-5,7-dimethoxy-4H-chromen-4-one (compound A), a rutin derivative, exhibits significant antioxidant activity in a dose-dependent manner as evidenced by the DPPH assay. Additionally, compound A demonstrates both cytotoxic and cytoprotective effects on Hep-G2 cells, a human liver cancer cell line, depending on the concentration range, as observed in the MTT assay. These findings highlight the potential therapeutic applications of compound A in combating oxidative stress-related diseases and cancer. Further research is warranted to elucidate the underlying mechanisms of action and to evaluate the efficacy and safety of compound A for clinical translation.

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