Original Article

Synthesis of Kaempferol-Cr (III) Complex and study its Effect on Bax and Bcl-2 Genes Expression in SW480 Cell Lines

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ABSTRACT

Objective: Impact of a kaempferol-Cr(III) complex on the expression of Bax and Bcl-2 genes in the SW480 after 24 hours of treatment and to highlight the complex's potential role in promotion.

Study Design: Experimental study

Place and Duration of Study: This study was conducted at the Department of Pharmacognosy and Medicinal Plants, College of Pharmacy, University of Basrah, Iraq from 1st June 2022 to 30th November 2022.

Methods: Kaempferol and its Cr(III) complex were successfully synthesized and examined through FT-IR, UV-Vis spectroscopy, EI-MS, and HPLC analyses. Their anti-proliferative effects were tested using the MTT assay on SW480 and normal HDFn, with IC50 values calculated to determine cytotoxic activity.

Results: Treatment with the kaempferol–Cr(III) complex significantly suppressed the viability of SW480 colorectal cancer cells more effectively than kaempferol alone, with negligible impact on healthy cells. Gene expression profiling via real-time PCR demonstrated an elevation in Bax levels alongside a reduction in Bcl-2 expression after 24 hours, indicating the induction of apoptosis. These findings point to the complex's ability to trigger programmed cell death through modulation of apoptotic gene expression, supporting its potential as a promising chemotherapeutic agent

Conclusion: Kaempferol-Cr(III) complex exhibits anticancer properties. When compared to ligand alone, this compound has demonstrated a noticeably greater lethal effect in experiments using the SW480 cancer cell line.

Key Words: Kaempferol-Cr(III) complex, Apoptosis, Bax, Bcl-2, SW480 cell line

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INTRODUCTION

Theorists are particularly interested in the development of novel materials. Day by day, the percentage of deaths attributable to cancer illnesses rises.[1] Since the first metal-based chemotherapy medication, cisplatin, was discovered, metal complexes have been employed as antitumor agents.[2] With the development of a multitude of platinum relatives, cisplatin or its derivatives, such as carboplatin or oxaliplatin, are utilized for treating nearly fifty percent of all people with cancer receiving chemotherapy.[3] Flavonoids are secondary metabolites composed of a benzopyrone ring with polyphenolic groups.[4] Their poor absorption and low water solubility have limited biological evaluation for drug development.[5] However, their hydroxyl and carbonyl groups allow interaction with biomolecules and chelation of metal ions, enabling the formation of metal complexes with improved and distinct biological properties.[6]

Kaempferol is a member of the tetrahydroxy flavonoid group because it has four hydroxy groups at positions 3, 5, 7, and 4′.[7] New research has focused on its possible application in cancer treatment since higher consumption was shown to reduce the risk of several cancers, including skin, ovarian, and stomach cancer.[8] Several investigations have shown that kaempferol play a significant part in the apoptosis of carcinoma of the breast.[9] Bcl-2 gene promotes survival of cancer cells by inhibiting apoptosis. On the other hand, cancer cells undergo apoptosis and die when the Bax gene is expressed.[10] Diantini et al[11] found that Kaempferol-3-O-rhamnoside induces death in MCF-7 breast cancer cells by activating the caspase cascade involving caspase-9, caspase-3, and PARP, along with reduced Bcl-2 expression. Nandi et al[12] demonstrated that kaempferol may be effective in treating in vitro triple-negative breast cancer cells. The present study aims to synthesize the kaempferol–Cr(III) complex and evaluate its effect on SW480 cancer cell inactivation by

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assessing the expression of apoptotic genes (BAX and BCL-2) in these cells.

METHODS

The synthesis of the kaempferol complex was performed based with slight modifications. Kaempferol (0.3 g, 1 mmol) was dissolved in 20 mL methanol, followed by the addition of NaOH (0.02 g, 0.5 mmol) in 10 mL methanol. $CrCl_3 \cdot 6H_2O$ (0.1 g, 0.5 mmol) dissolved in 10 mL methanol was then added with stirring.[13] The mixture was stirred at room temperature for 1 hour, then refluxed at 60°C for 4 hours. It was dried at room temperature, washed, and the filtrate was left to dry.

The wavelength (λmax) of kaempferol and kaempferol-Cr(III) complex was determined by preparing standard solutions (1000 µg/mL). Methanol was used to dilute 1 mL of standard solutions to 10 mL, creating the working solution (100 µg/mL).

FTIR spectroscopy was used to kaempferol, and Kaempferol-Cr (III) complex. The KBr sample discs were scanned to obtain infrared spectra in the wavelength range of 4000 to 400 cm^{-1} at a resolution of 4 cm^{-1}.

EI-MSS spectrometry is a potent instrument for metal-flavonoid complex investigation.[14] ACN (0.1% formic acid) and H_2O (0.1% formic acid) was used as mobile phase. EI/MS was performed using a Shimadzu LC/MS 2010 A system in the positive ion mode.

Using HPLC-grade methanol, 1mg.mL^{-1} kaempferol initial solution was made. We then prepared working solutions with concentrations of 100, 90, 70, 40, and 20 µg/mL at the ambient temperature. A calibration plot was then made to illustrate the correlation between peak area and concentration (µg.mL^{-1}). The concentration of kaempferol in the experimental samples was ascertained using the equation for linearity derived from the standard plot.

Dissolved 0.0005 mg of complex in 1mL methanol and the taken 250 µL from solution and added 750 mL from methanol to it. HPLC analysis was then performed on 100 µL of this solution.

HPLC analysis was performed using the German S600 Sykam system with a UV/VIS detector and specimen injector. The column was maintained at 30°C, using methanol (A), acetonitrile (B), and water (C) as eluents. The mobile phase consisted of 20% methanol, 60% acetonitrile, and 20% water (v/v/v) at a flow rate of 1.0 mL/min. An autosampler injected 100 µL of kaempferol and complex solutions, and spectra were recorded at 280 nm.

The Rwafid Alelom Company in Iraq provided the SW480 and HDFn cell lines. 10% FBS was added to RPMI-1640 while the cells were being grown. Every cell was cultivated at 37°C with 5% CO_2 and all media included100U mL of streptomycin and penicillin.

The viabilities of SW480 and HDFn cells were assessed in the presence of kaempferol and its Cr(III) complex. Cells were cultured in triplicate in 96-well plates and incubated for 24 hours at 37 °C with 5% CO_2. After washing with 1X PBS, cells were treated with various concentrations (100, 50, 25, 12.5, 6.25, 3.12 µg/mL) of kaempferol and the metal complex. Untreated cells served as controls. After 24 hours, 200 µL of MTT solution was added, and absorbance at 570 nm was measured after 3 hours. The values were calculated using Excel 2016

The overall RNA Mini Kit (Blood/Cultured Cell) (geneaid) was used to extract total RNA from untreated control cells in accordance with the manufacturer's recommendations, cells (SW480) treated with IC_{50} kaempferol-Cr(III) complex, or kaempferol alone. Total RNA was then treated with RNase-free DNase Sets (Qiagen) and 2 mg of RNA was used for (cDNA) synthesis utilizing the TransScript® Green One-Step qRT- PCR SuperMix, Cat. No. AQ211), as directed by manufacturer.

qRT-PCR was used to measure BAX and BCL-2 gene transcription. A 20 µL reaction mixture contained 6 µL distilled water, 2 µL of each gene-specific primer (Table 1), and 10 µL of green master mix. The reaction was performed using the Cycler96-Roche instrument (Table 2). Gene expression changes were quantified using the $2^{-\Delta\Delta Ct}$ method, with Bax and Bcl-2 expression levels compared to the β-actin reference gene. Three distinct tests are used to calculate the mean standard deviation of the results. The following differences are indicated by asterisks: for $p< 0.0$, two way-ANOVA is utilized.

RESULTS

Kaempferol-Cr (III) complex was created using a straightforward process. The way that compound react, kaempferol, $CrCl_3.6H_2O$, and NaOH in methanol led to the formation crystal-like particle (Fig. 1), Kaempferol, has two unique levels in its electronic spectra. peak I, which represents the cinammoyl component, shows at 365.5 nm, while band II, which represents the benzoyl part arises at 267.0 nm. As a result, both bands underwent a considerable shift due to the chelation of the Cr(III) ion (from 267.0 nm to 350.0 nm for band II and from 365.5 nm to 424.0 nm for band I). As seen in (Fig. 2), band I exhibits a very slight red shift of 58.5 nm, but band II shows a red shift of 83 nm due to the coordination of Cr(III) with kaempferol.

FTIR spectra were recorded in the range of 4000–400 cm^{-1} for both kaempferol and its Cr(III) complex. In the kaempferol spectrum, a broad absorption band was observed between 3400–3000 cm^{-1}, corresponding to O–H stretching vibrations. A sharp peak at 1658 cm^{-1} was attributed to the C=O stretching vibration at position 4 of the kaempferol molecule. In the Cr(III) complex, this peak shifted to 1632 cm^{-1}, indicating a frequency decrease of 26 cm^{-1}.

Table No. 1: Primers sequence utilized

Gene	Sequences of Primer pair	Acquisition number	Reference
Bcl-2 (F)	5'-TCGCCCTGTGGATGACTGA-3'	NM-000633.3	15
Bcl-2 (R)	5'-CAGAGACAGCCAGGAGAAATCA-3'		
Bax (F)	5′GAGCTGCAGAGGATGATTGC-3′	NM-138764.5	16
Bax (R)	5′-AAGTTGCCGTCAGAAAACATG-3′		
β-actin (F)	5′-TCCTCCTGAGCGCAAGTAC-3′	NM-011001.5	16
β-actin (R)	5′-CCTGCTTGCTGATCCACATCT-3′		

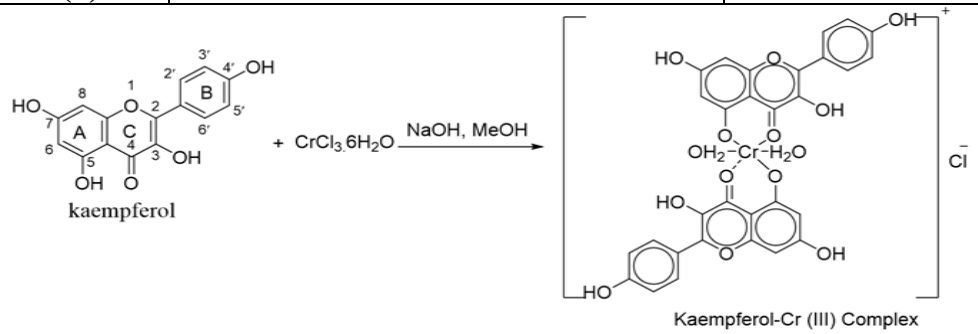

Figure No. 1: Overall reaction

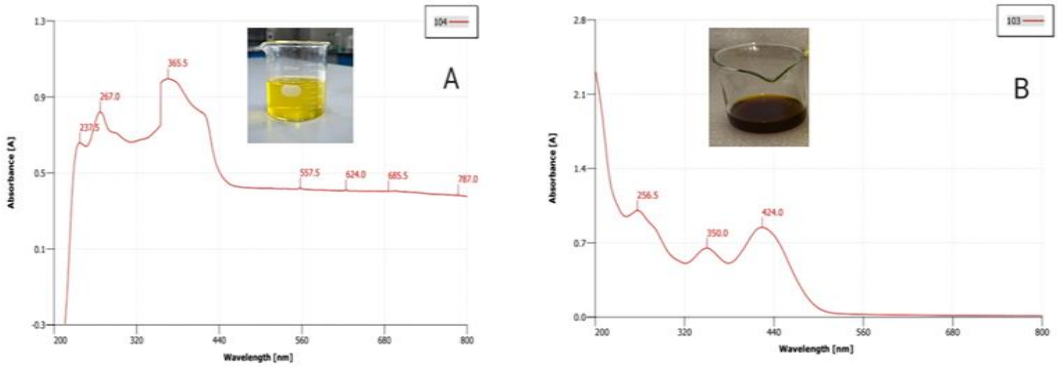

Figure No. 2: A: Uv spectrum of Kaempferol, B: Uv spectrum of kaempferol- Cr(III) complex

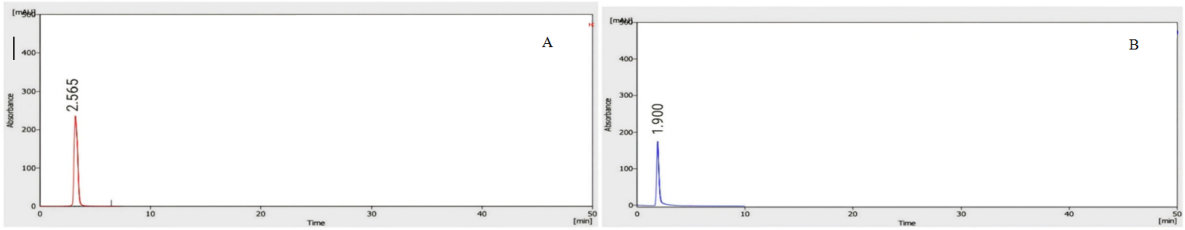

Figure No. 3: HPLC chromatogram A:Kaempferol B:kaempferolCr(III) Complex

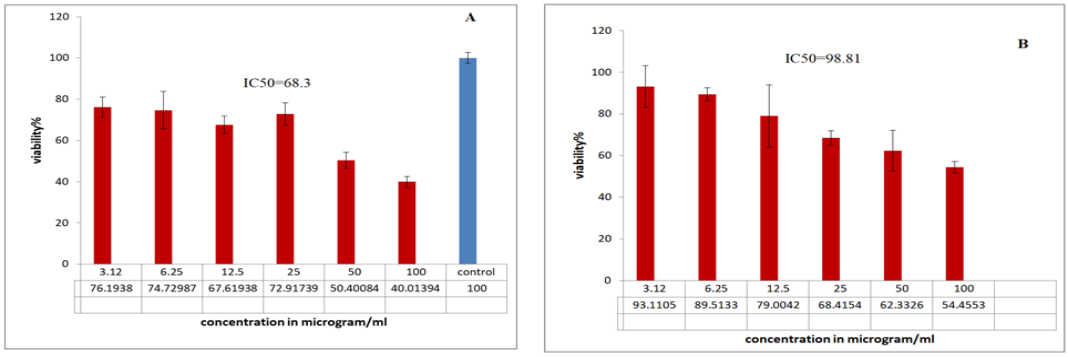

Figure No. 4: Percentage of SW480cells (A) HDFn cells (B) that survived for 24 hour at various complex concentrations

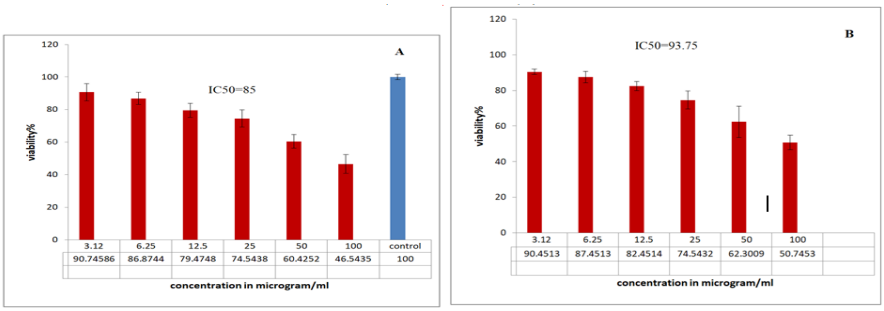

Figure No. 5: Percentage of SW480cells (A) HDFn cells (B) that survived for 24 hour at various kaempferol concentrations

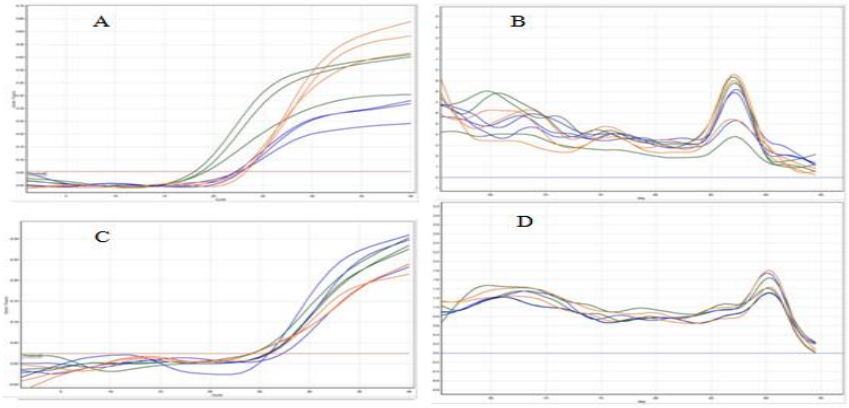

Figure No. 6 :Amplification of Genes in RT-qPCR:Data for β-actin and Bcl genes: A. Fragment duplication, B. Melting curve; Data for β-actin and Bax genes: C. Fragment duplication, D. Melting curve control colored green, blue and orange for kaempferol-Cr(III)complex and kaempferol respectively

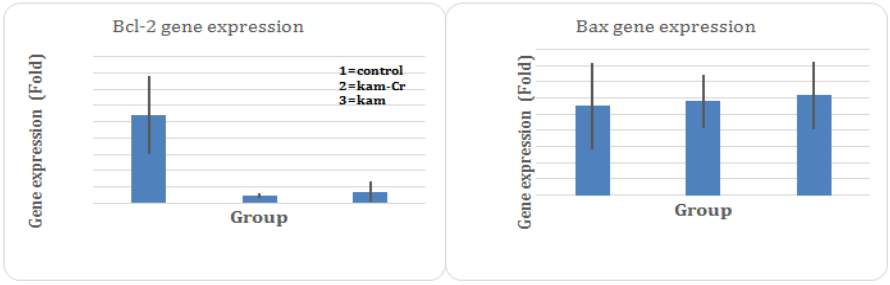

Figure No. 7: Expression rate of Bcl-2 and Bax genes in 24h treatment (p value < 0.05)

Table No.2: PCR steps and repeat cycle

Stage	Temperature (°C)	Time (s)	Number of cycles
Denaturation	94	20	40
Annealing	60	30	
Extension	60	30	

Table No. 3: The main EI/MS data of kaempferol Cr(III) complex

Nominal m/z	Structure
658.5	2 Kaempferol+ Cr(III) +2H_2O
622.47	2 Kaempferol+ Cr(III)
336.23	Kaempferol+ Cr(III)
153 and 165	Fragmentation of kaempferol ring system

The C=C and C–O–C stretching frequencies were observed at 1610 cm^{-1} and 1224 cm^{-1} in kaempferol, and at 1598 cm^{-1} and 1224 cm^{-1} in the complex, respectively. A new peak appeared at 462 cm^{-1} in the complex, which was not present in the free ligand spectrum (Supp. Fig. 1).

EI-MSS spectrometry has been employed to identify chemical structures. (Table 3), displayed the complex's fingerprint. In this case, the ions of m/z 658.5, 622.47, 336.23, 153, and 165 were obtained(Supp. Fig. 2). HPLC was used to analyze both kaempferol and its Cr(III) complex. The chromatogram of kaempferol showed a retention time of 2.565 minutes, while the kaempferol–Cr(III) complex showed a distinct peak at 1.900 minutes as seen in (Fig.3).

MTT assays were employed to assess the impact of 24-hour exposure to kaempferol and the kaempferol-Cr(III) complex on the SW480 cell line's vitality. The IC values were determined to be 85 µg/mL for kaempferol and 68.3 µg/mL for the kaempferol-Cr(III) complex, indicating a stronger cytotoxic effect of the metal

complex. Both compounds significantly reduced the viability of SW480 cells at all tested concentrations (100, 50, 25, 12.5, 6.25, and 3.12 µg/mL). In contrast, neither kaempferol nor the kaempferol-Cr(III) complex caused significant cytotoxicity in normal HDFn cells (Figs. 4-5)

Tables 4-5 present the statistical analyses of the 24-hour administrations with the complex and kampferol with p value < 0.05. This tables show that SW480 cells have the best survival rate at a concentration of 3.12µg/mL. As the concentration rises, SW480 cells become less viable.

Real time PCR: The curve of melting (Fig. 6) demonstrates that the targeted gene fragments were amplified specifically. The control was thought to be β-actin. Primers for the Bcl-2 and Bax genes were used in a real-time PCR experiment following cDNA synthesis. Ct values were calculated using the amplification plot as a guide. Modifications in gene expression were then computed using the 2-ΔΔCT technique (Table 6).

Expression of the genes under investigation in tumer cells subjected to kaempferol-Cr(III) complex and Kampferol: The 2-ΔΔCT method was used to analyze gene expression using Ct values from real-time PCR and the β-actin gene. Treatment with the kaempferol–Cr(III) complex and kaempferol resulted in a notable increase in Bax expression (1.160715 and 1.22837) and a significant decrease in Bcl-2 expression (0.096 and 0.139) with $p<0.05$. Figure 6 shows Bax and Bcl-2 gene expression after 24 hours in SW480 cell lines treated with the kaempferol–Cr(III) complex and kaempferol (Fig. 7).

Table No.4: Average bioavailability of SW480 line using the MTT test for kaempferol-Cr(III) complex and kampferol at varying doses throughout a 24-hour

Compound	Concentration µg/ml					
	3.12	6.24	12.5	25	50	100
Kam-Cr	76.194±2.65	74.7298±2.6	67.6194±3.86	72.91739±5.37	50.400836±4.23	40.01394±9.01
Kam	90.74586±1.55	86.8744±5.72665	79.4748±4.174257	79.4748±4.174257	60.4252±4.357739	46.5435±3.817748

Table No. 5: Average bioavailability of healthy cells using the MTT test for kaempferol-Cr(III) complex and kampferol at varying doses throughout a 24-hour

Com-pound	Concentration µg/ml					
	3.12	6.24	12.5	25	50	100
Kam-Cr	93.1105±2.7082	89.5133±9.7024	79.0042±3.4001	68.4154±14.90102	62.3326±3.024612	54.4553±10.03418
Kam	90.4513±4.025	87.4513±8.8	82.4514±5.079	74.5432±2.5252	62.4009±3.1286	50.7453±1.5316

Table No. 6: Ct value and fold changes in gene expression

	Ct-Bactin	***Ct-Bcl2***	**Fold change**	***Ct-Bactin***	***Ct-Bax***	**Fold change**
Control	19.98666667	18.71	1.08	19.98666667	22.55333333	1.097168333
Kam-Cr	20.17666667	20.37285714	0.096	20.17666667	22.81666667	1.160715333
Kam	20.39121212	21.05207792	0.139	20.39121212	23.29	1.228371333

DISCUSSION

The successful formation of the Kaempferol–Cr(III) complex indicates a strong interaction between the metal ion and the functional groups of kaempferol, which contains several hydroxyl groups. Notably, the A and C rings include 5-hydroxy-4-keto and 3-hydroxy-4-keto groups, while the B ring has its own hydroxyl group. Since the 3-hydroxy and 5-hydroxy sites compete for metal binding, the hydroxy-keto positions on rings A and C are of particular significance. Ligand molecules form more stable compounds by using their most suitable chelation sites. Hydroxy-keto sites, in particular, create strong compounds through additional ring formation and extended conjugation. In contrast, chelation between the 4'-hydroxyl group of ring B and the 3-hydroxyl group of ring C, likely due to the easy ionization of the 4'-hydroxyl group, appears much less certain.[15] As a result, Cr(III) shows notable bathochromic shifts, likely due to its high charge density and strong ligand binding. Band II shows a greater red shift than band I, indicating stronger conjugation at the benzoyl system in ring A compared to the cinnamonoyl system in ring B. The study concludes that, during the formation of the kaempferol–Cr(III) complex, deprotonation occurs at the 5-OH site rather than the 3-OH.[16]

In FTIR, the observed shift of the carbonyl stretching frequency from 1658 cm^{-1} to 1632 cm^{-1} upon complexation suggests coordination of the Cr(III) ion through the carbonyl oxygen of kaempferol.[17] The unaltered frequencies of the C=C and C–O–C bonds imply that the ring oxygen is not involved in metal coordination. The appearance of a new band at 462 cm^{-1} is attributed to the formation of a Cr–O bond, which confirms the complexation between kaempferol and Cr(III).[18]

According to EI-MASS spectrometry, two kaempferol molecules coordinate with one Cr(III) atom through their 5-OH and 4-oxo groups, donating two hydrogen atoms and often binding with two water molecules. The difference in HPLC retention times between kaempferol and its Cr(III) complex indicates successful complex formation (Fig. 3). The shorter retention time of the complex may result from changes in polarity or interactions with the stationary phase due to metal coordination. MTT assay results show that the kaempferol–Cr(III) complex has greater anticancer potential than kaempferol, with a lower IC_{50} and stronger cytotoxic effects at all concentrations. Its selective toxicity toward cancer cells and minimal impact on normal cells suggest it as a promising and safer therapeutic agent.[19]

Apoptosis is a technique of deliberate destruction of cells that preserves homeostasis.[20] The two main mechanisms that mediate apoptosis are the mitochondrial-mediated pathways and the death receptor. They gather at killing pathway, which includes caspase-3 cleavage, nuclear fragmentation, and

the creation of an apoptotic body that is ultimately phagocytosed.[21]

CONCLUSION

Kaempferol-Cr(III) complex exhibits anticancer properties. When compared to ligand alone, this compound has demonstrated a noticeably greater lethal effect in experiments using the SW480 cancer cell line.

Author's Contribution:

Concept & Design or acquisition of analysis or interpretation of data:	Ghadeer Sadeq Jayed, Zainab Tuama Al-Dallee
Drafting or Revising Critically:	Ghadeer Sadeq Jayed , Hiba Najeh Al-Saad
Final Approval of version:	All the above authors
Agreement to accountable for all aspects of work:	All the above authors

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REFERENCES

1. Chhikara BS, Parang. Global Cancer Statistics 2022: the trends projection analysis. Chem Biol Letters 2023; 10(1): 451-51.
2. Casini A, Pöthig A. Metals in cancer research: beyond platinum metallodrugs. ACS Cent Sci 2024;10(2):242-50.
3. Forgie BN, Prakash R, Telleria CM. Revisiting the anti-cancer toxicity of clinically approved platinating derivatives. Int J Mol Sci 2022; 23(23):15410.
4. Ullah A, Munir S, Badshah SL, Khan N, Ghani L, Poulson BG, Emwas AH, Jaremko M. Important flavonoids and their role as a therapeutic agent. Molecules 2020;25(22):5243.
5. Chen L, Cao H, Huang Q, Xiao J, Teng H. Absorption, metabolism and bioavailability of flavonoids: a review. Crit Rev Food Sci Nutr 2022;62(28):7730-42.
6. Rodríguez-Arce E, Saldías M. Antioxidant properties of flavonoid metal complexes and their potential inclusion in the development of novel strategies for the treatment against neurodegenerative diseases. Biomed Pharmacother 2021;143:112236.
7 Imran M, Salehi B, Sharifi-Rad J, Aslam Gondal T, Saeed F, Imran A, et al. Kaempferol: a key emphasis to its anticancer potential. Molecules 2019 19;24(12):2277.
8. Mir SA, Dar A, Hamid L, Nisar N, Malik JA, Ali T, et al. Flavonoids as promising molecules in the cancer therapy: an insight. Curr Res Pharmacol Drug Discov 2023 8;6:100167.
9. Kaur S, Mendonca P, Soliman KFA. The anticancer effects and therapeutic potential of kaempferol in triple-negative breast cancer. Nutrients 2024 23;16(15):2392.
10 Qian S, Wei Z, Yang W, Huang J, Yang Y, Wang J. The role of BCL-2 family proteins in regulating apoptosis and cancer therapy. Front Oncol 2022; 12:985363.
11. Diantini A, Subarnas A, Lestari K, Halimah E, Susilawati Y, Supriyatna, et al. Kaempferol-3-O-rhamnoside isolated from the leaves of Schima wallichii Korth. inhibits MCF-7 breast cancer cell proliferation through activation of the caspase cascade pathway. Oncol Lett 2012;3(5):1069-1072.
12. Nandi SK, Pradhan A, Das B, Das B, Basu S, Mallick B, et al. Kaempferol attenuates viability of ex-vivo cultured post-NACT breast tumor explants through downregulation of p53 induced stemness, inflammation and apoptosis evasion pathways. Pathol Res Pract 2022;237:154029.
13. Panhwar QK, Memon S. Synthesis of Cr(III)-morin complex: characterization and antioxidant study. Scientific World J 2014;2014:845208.
14. Jiang C, Gates PJ. Systematic Characterisation of the Fragmentation of Flavonoids Using High-Resolution Accurate Mass Electrospray Tandem Mass Spectrometry. Molecules 2024;29(22):5246
16. Simunkova M, Barbierikova Z, Jomova K, Hudecova L, Lauro P, Alwasel SH, et al. Antioxidant vs. Prooxidant Properties of the Flavonoid, Kaempferol, in the Presence of Cu(II) Ions: A ROS-Scavenging Activity, Fenton Reaction and DNA Damage Study. Int J Mol Sci 2021;22(4):1619.
17. Mohsen EMA, Saeed AAM, Yahia ARAB. Synthesis, characterization, and bioactivity evaluation of several divalent transition metal ion-bioflavonoid complexes. Results Chem 2024,7: 101535
18. Matsia S, Tsave O, Hatzidimitriou A, Salifoglou A. Chromium flavonoid complexation in an antioxidant capacity role. Int J Mol Sci 2022;23(13):7171.
19. Venkatachalam H, Jayashree BS, Nigam S. A preliminary cytotoxic study on the zinc complexes of flavonols. Indian J Pharmaceutical Sci 2017; 79: 5.
20. Chen H, Tang T, Xue C, Liu X, Xi Z, Xie L, et al. Exploration and breakthrough in the mode of intervertebral disc cell death may lead to significant advances in treatments for intervertebral disc degeneration. J Orthop Surg Res 2024;19(1):825.
21. Zhang S, Rao S, Yang M, Ma C, Hong F, Yang S. Role of Mitochondrial Pathways in Cell Apoptosis during He-Patic Ischemia/Reperfusion Injury. Int J Mol Sci. 2022 Feb 21;23(4):2357.
22. Tao S, Gu J, Wang Q, Zheng L. Translational control of Bcl-2 promotes apoptosis of gastric carcinoma cells. BMC Cancer 2021;21:1-10.