EGYPTIAN DENTAL JOURNAL
www.eda-egypt.org
VOL. 71, 3097:3107, OCTOBER, 2025
PRINT ISSN 0070-9484 • ONLINE ISSN 2090-2360
ORAL MEDICINE, X-RAY, ORAL BIOLOGY AND ORAL PATHOLOGY
Submit Date : 04-08-2025 • Accept Date : 28-08-2025 • Available online: 01-10-2025 • DOI : 10.21608/edj.2025.410257.3578

THERAPEUTIC SYNERGY BETWEEN GRAVIOLA (ANNONA MURICATA) EXTRACT AND CISPLATIN IN HEAD AND NECK SQUAMOUS CELL CARCINOMA: AN IN VITRO STUDY

Safaa Baz*, Abeer Abd El Maksoud Mohammed **, *and* Ali AW Kotb ***

ABSTRACT

Background: Patients with head and neck squamous cell carcinoma (HNSCC) suffer from poor prognosis despite advancements, primarily due to toxicity and resistance associated with conventional chemotherapeutics such as cisplatin (CDDP). Graviola extract (GE), also known as *Annona muricata*, is a natural compound with anticancer properties and favorable safety profile. This *in vitro* study aimed to investigate the cytotoxic, antiproliferative, and pro-apoptotic potential of GE, CDDP, and their combination (CDDP+GE) on HEp-2 cells derived from HNSCC.

Methods: Cytotoxicity was assessed using MTT assay, and IC50 values were determined for the GE, CDDP, and CDDP+GE treatment groups compared to the untreated control. HEp-2 cells were subsequently treated with the predetermined IC50 doses for further investigations. Immunocytochemistry and RT-qPCR were conducted to evaluate the expression of Ki-67 and Bcl-2. Statistical analysis was performed using one-way ANOVA followed by Tukey's post-hoc test, with significance set at $p < 0.05$.

Results: All treatment groups significantly reduced HEp-2 cells viability compared to the control ($p < 0.0001$). The combination group (CDDP+GE) exhibited enhanced cytotoxicity (IC50 = 28.47 ± 0.03 μg/mL) compared to GE alone (73.99 ± 0.29 μg/mL) or CDDP alone (2.88 ± 0.30 μg/mL). Immunocytochemical and gene expression analyses revealed decreased Ki-67 and Bcl-2 expression across all treated groups, with the lowest levels observed in the CDDP+GE group ($p < 0.0001$), indicating synergistic and antiproliferative and pro-apoptotic effects.

Conclusions: GE combined with CDDP offers strong antiproliferative and apoptotic effects on HEp-2 cells, indicating its potential as an adjunct or alternative treatment for HNSCC.

KEYWORDS: Graviola, Annona muricata, Cisplatin, Head and Neck Squamous Cell Carcinoma, Synergy

* Lecturer, Oral Pathology Department, Faculty of Dentistry, The British University in Egypt,
** Lecturer of Oral Biology, Faculty of Dentistry, Fayoum University, El Fayoum, Egypt,
*** Lecturer of oral and Maxillofacial pathology, Faculty of Dentistry, Cairo University, Giza, Egypt

INTRODUCTION

Head and neck squamous cell carcinoma (HNSCC) is a diverse group of human epithelial malignancies that remain associated with high rate of recurrence and poor prognosis. Its treatment remains challenging due to the limited efficacy, high toxicity, and resistance of the traditional chemotherapeutic agents [1,2]. Cisplatin (*cis-diamminedichloroplatinum II*, CDDP) is a frequently utilized conventional chemotherapeutic agent in the treatment of HNSCC, owing to its effectiveness to target and damage dividing cancerous cells. Nonetheless, its clinical utility is frequently restricted by the development of dose-dependent systemic toxicities, along with the risk of acquired drug resistance [3].

Naturally occurring compounds have demonstrated promising anticancer activities, characterized by favorable safety profiles and selective toxicity towards cancer cells, while exerting minimal effects on normal tissues [4, 5]. *Annona muricata L.*, a medicinal plant commonly known as Graviola extract (GE), belongs to the *Annonaceae* family. GE has been used for its antimicrobial, anti-inflammatory, and anticancer properties [6]. Globally, this plant is used traditionally in herbal medicine for traditionally used for its anti-inflammatory, anti-diabetic, and anticancer properties [7-9]. According to ethnobotanical reports, GE is frequently used in anticancer therapy; this may be due to its specific cytotoxicity [10]. Several *in vitro* and *in vivo* studies have investigated its anticancer effects and the underlying mechanisms across a range of cell types and tissues [11-15].

This study aims to evaluate the cytotoxic, antiproliferative, and pro-apoptotic therapeutic potential of GE (*Annona muricata*) extract versus CDDP, either individually or in combination, on HEp-2 cells, an *in vitro* model of HNSCC, providing a basis for its potential use as an adjunct or alternative therapeutic agent in its management.

MATERIALS AND METHODS

This *in vitro* study was conducted at the Faculty of Medicine, Al-Azhar University, Cairo, Egypt. The current study does not include human participants therefore it was exempt from ongoing review; it was not subject to ethical approval by the British University in Egypt's Ethical Committee, Faculty of Dentistry, The British University in Egypt. Three separate replicates were used for the studies, and the data is the mean of the three.

Cell Line Culturing

Human epithelial cell line of HNSCC (HEp-2 cells) were obtained from the International Center for Advanced Research (ICTAR), Cairo, Egypt and cultured in DMEM [Catalog # 11995065, ThermoFisher-Scientific, MA, USA] with 10% heat-inactivated FBS, [Catalog # 10082147] and 1% penicillin-streptomycin [Catalog # 15140122]. Cells were incubated at 37°C with 5% CO_2 and subcultured at about 80% confluence using 0.25% trypsin- EDTA [Catalog # 25200056]. Cell status was repeatedly monitored via Olympus CKX53 inverted-phase microscopy [Olympus Corporation, Tokyo, Japan].

MTT Cytotoxicity and IC50 Value

HEp-2 cells were seeded with growth media in 96-well-plates with density of 3×10^3 cells/well in incubator for 48 hours of treatment at 37°C. The effect of different treatments including GE [*Annona muricata*; Nutrics®, UK], CDDP [*cis-diamminedichloroplatinum II*; Cisplatin®; Saint-Priest, France], and mixed GE and CDDP (CDDP+GE) on cell viability of HEp-2 cells were assessed compared to negative control untreated cells. Wells received 2.5 mg/ml MTT reagent [Catalog # M5655, Sigma-Aldrich, MO, USA] for 4 to 6 hours of incubation. The medium was washed, and the formazan crystals were subsequently dispersed in DMSO [Catalog # D2650, Sigma-Aldrich].

GraphPad Prism [Software 10.0 for Windows, MA, USA] was used to determine each treatment's half-maximal inhibitory concentration (IC50). The subsequent tests were then conducted using the IC50 values of each group.

Real Time-Quantitative Polymerase Chain Reaction (RT-qPCR)

For total RNA extraction, the kit of RNA extraction [Catalog # R6934, Sigma-Aldrich] was used, meanwhile for cDNA.synthesis, a kit for reverse transcriptase [Catalog # 200412, Sigma-Aldrich] was used. SYBR® Green PCR Master Mix [Catalog # S4438, Sigma-Aldrich] was applied for qPCR. A real-time Rotor-Gene® Corbett analyzer [RG6000-Qiagen, Hilden, Germany] was used following the manufacturer's instructions. The ACTB was the used housekeeping gene for standardizing gene expression levels. The ΔΔCT method was applied to determine expression levels of Bcl-2 and Ki-67. The sequences of primers used were listed in **Table 1**.

TABLE (1) The sequences of primers used in RT-qPCR analysis.

Gene	Sequences of the primers
Ki-67	Forward: 5'-GAAAGAGTGGCAACCTGCCTTC-3' Reverse: 5'-GCACCAAGTTTTACTACATCTGCC-3'
Bcl-2	Forward: 5'-CCTGTGGATGACTGAGTACC-3' Reverse: 5'-GAGACAGCCAGGAGAAATCA-3'
ACTB	Forward: 5'-GTGACATCCACACCCAGAGG-3' Reverse: 5'-ACAGGATGTCAAAACTGCCC-3'

Quantitative Immunocytochemistry (ICC)

HEp-2-treated cells with GE, CDDP, and mixed CDDP+GE and HEp-2 untreated cells were subjected to cold centrifugation at 1500 rpm for 10 minutes. Either detached or adherent HEp-2-cells were gathered to be distributed on positively charged slides, they were allowed to air dry. Following fixation, primary antibodies against Ki-67 [Catalog # AB9260, Sigma-Aldrich] and Bcl-2 [Catalog # B3170, Sigma-Aldrich] were incubated at room temperature for 1 hour. Phosphate buffer solution (PBS; pH 7.2) [Catalog # P4417, Sigma-Aldrich] was used to wash the samples. To examine immunoreactivity, ten representative fields for each group were captured at 40x magnification by a Canon-EOS-650D camera attached to a BX60-Olympus light microscope [Olympus, Japan]. Image analysis was conducted using ImageJ software [NIH, Bethesda, MD, USA] to calculate the percentage of positively stained cells to total number per slide.

Statistical Analysis

SPSS software version 27.0 [IBM Corp., Armonk, NY, USA] was used to analyze all data. The results were presented as mean ± standard deviation (SD) based on 3 independent experiments per group, with 95% CI. The data were normally distributed, and group differences were evaluated using one-way analysis of variance (ANOVA). Post-hoc tests were performed using Tukey's multiple comparison test following one-way ANOVA to identify significant differences between groups. A p-value of less than 0.05 was considered statistically significant.

RESULTS

MTT Cytotoxicity and IC50 Results

The cytotoxic effects of different interventions on HEp-2 cell line were evaluated using the MTT assay, with IC50 values being calculated after 48 hours of treatment. All treated groups demonstrated significantly reduced cell viability compared to the untreated control group. The highest IC50 value was exhibited in the GE-treated group (73.99 ± 0.29 μg/mL), while the lowest value was observed in the CDDP-treated group (2.88 ± 0.30 μg/mL). The combined CDDP+GE group exhibited an intermediate IC50 value (28.47 ± 0.03 μg/mL). One-way ANOVA analysis revealed a highly statistically significant difference among the groups ($p < 0.0001$) (**Table 2, Figure 1**).

TABLE (2). IC50 values (μg/mL) with one-way ANOVA comparisons among the study groups.

	Mean	SD	95% CI of Difference	F value	*p*-value
GE	73.99	0.29	73.69 – 74.29	F=114499.37	$p < 0.0001$
CDDP	2.88	0.30	2.59 – 3.18		
CDDP + GE	28.47	0.03	28.17 – 28.77		
Control	99.88	0.16	99.58 –100.18		

Data are expressed as mean ± standard deviation (SD). Confidence interval (CI) represents 95%. Significance level was set at $p < 0.05$.

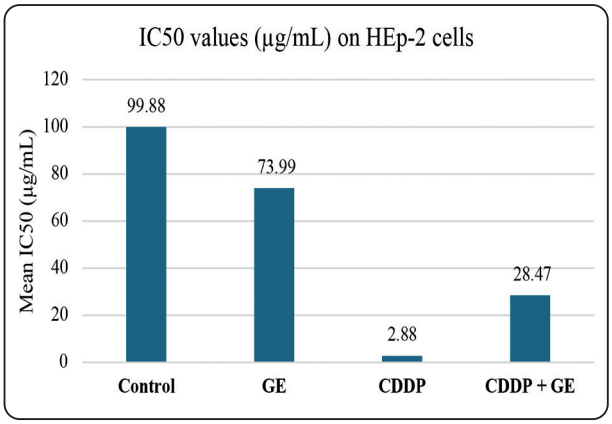

Fig. (1). Bar chart represents the mean IC50 values (μg/mL) on HEp-2 cells across the study groups.

Immunocytochemical Expression

The immunocytochemical results showed reduced nuclear Ki-67 and cytoplasmic Bcl-2 immunoreactivity across all treated groups. The lowest expression levels for both markers were detected in the CDDP and CDDP+GE groups, with minimal staining evident in the combined CDD+-GE group (**Figure 2**).

Statistical analysis of Ki-67 immunoexpression revealed that GE-treated HEp-2 cells exhibited the highest mean expression (71.20 ± 4.60), followed by the CDDP -treated group (51.57 ± 15.77), while the combination CDDP+GE group showed the lowest expression level (29.33 ± 2.92). One-way ANOVA showed a statistically significant difference in Ki-67 expression among the groups ($p < 0.0001$). Post hoc Tukey's multiple comparison test revealed significant differences between all pairs of groups ($p < 0.0001$) (**Table 3 and 4, Figure 3**).

Regarding Bcl-2, the GE-treated group exhibited lower Bcl-2 expression (82.46 ± 4.53) compared to control (92.77 ± 1.40). Meanwhile, the combination group (CDDP+GE) showed the lowest expression (41.71 ± 1.92), even when compared to the CDDP group (51.07 ± 4.62). One-way ANOVA revealed these differences to be statistically significant ($p<0.0001$). Then, Post hoc Tukey's test revealed significant differences between all paired groups ($p<0.001$) (**Tables 5 and 6, Figure 3**).

Ki-67 Gene Expression Results by RT-qPCR

The quantitative PCR analysis revealed a statistically significant downregulation of Ki-67 gene expression in all treated groups compared to the untreated control group. Among the treatment groups, the GE -treated group exhibited the highest relative gene expression (fold change: 0.63 ± 0.02), followed by the CDDP-treated group (fold change: 0.44 ± 0.01), meanwhile the combined CDDP+GE group revealed the lowest gene expression level (fold change: 0.37 ± 0.03). Statistically, one-way ANOVA analysis showed a highly significant difference among the study groups ($p < 0.0001$). Furthermore, Tukey's post hoc multiple comparison test confirmed that all intergroup differences were statistically significant ($p < 0.001$, $p < 0.01$) (**Tables 7 and 8**).

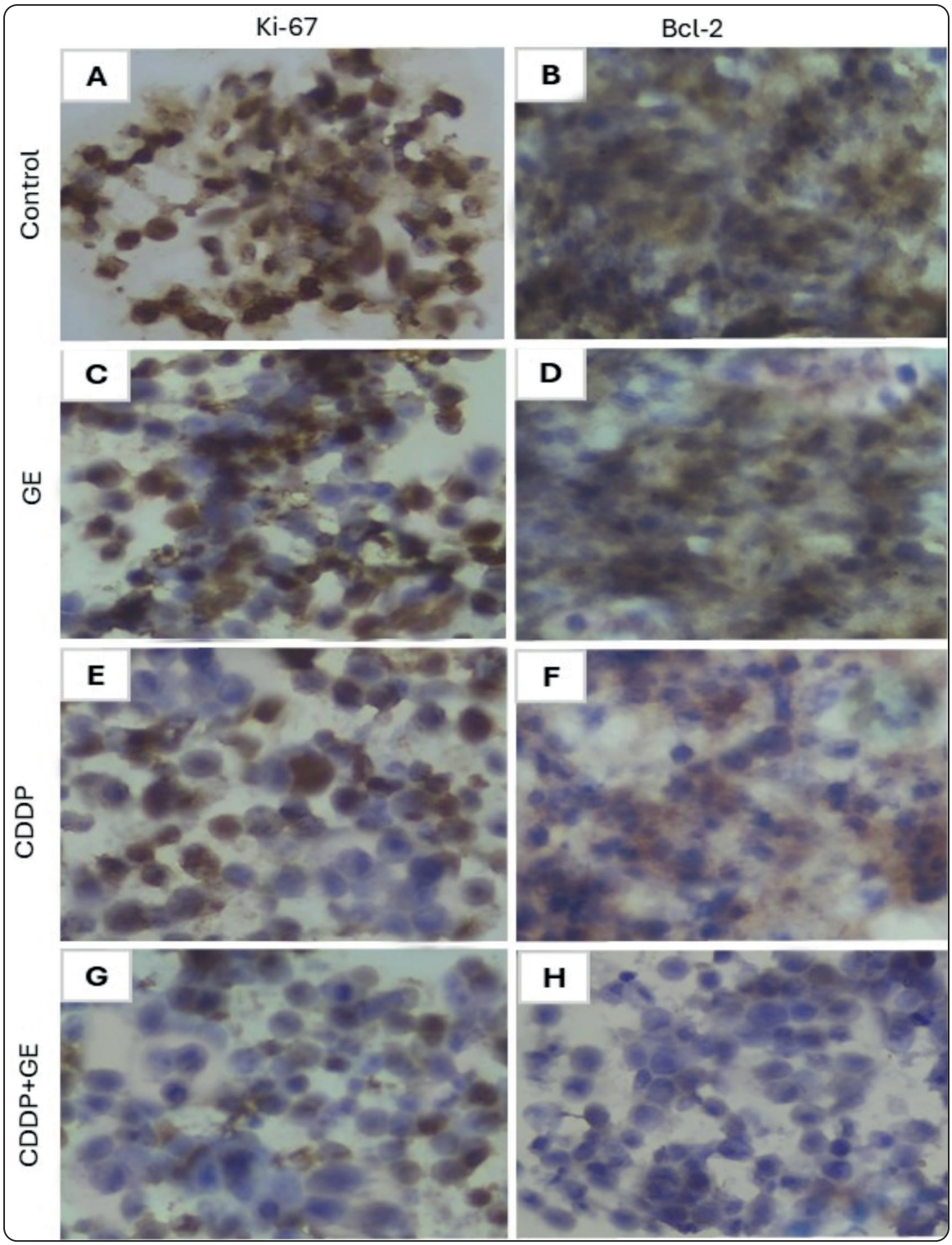

Fig. (2) Photomicrographs of immunocytochemical analysis of HEp-2 cells in the Control (untreated) group (A, B), GE-treated group (C, D), CDDP-treated group (E, F), and CDDP+GE-treated group (G, H). Nuclear staining of Ki-67 and cytoplasmic staining of Bcl-2 are demonstrated with decreased expression levels in the treated groups compared to the control group, with the lowest expression observed in the CDDP+GE-treated group **(Diaminobenzidine (DAB), original magnification ×400)**.

For Bcl-2, the gene expression levels showed significant downregulation in all treated groups compared to control, with the highest fold changes recorded in the GE-treated group (0.58 ± 0.01), while the lowest expression in the CDDP+GE treatment group (0.25 ± 0.01). One-way ANOVA analysis revealed significant statistical differences in all treatment groups compared to control ($p < 0.0001$). Tukey's post hoc test confirmed a significant difference in all treated groups versus control ($p < 0.01$, $p < 0.001$), with a significant difference between the GE-treated group (**Tables 9 and 10**).

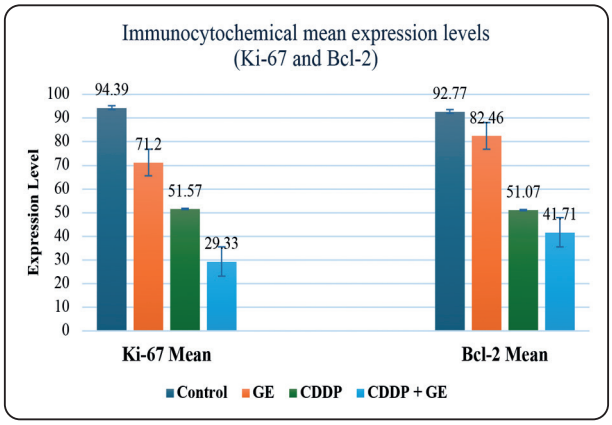

Fig. (3). Bar chart representing the mean immunocytochemical expression levels of Ki-67 and Bcl-2 across the study groups.

TABLE (3). Descriptive statistics and one-way ANOVA analysis of Ki-67 immunocytochemical expression among the study groups.

	Mean	SD	95% CI of Difference	F value	*p*-value
GE	71.20	4.60	65.38 – 77.01	F=69.63	$p < 0.0001$
CDDP	51.57	15.77	45.76 – 57.38		
CDDP + GE	29.33	2.92	23.51 – 35.14		
Control	94.39	1.57	86.17 –102.61		

Data are expressed as mean ± standard deviation (SD). Confidence interval (CI) represents 95%. Significance level was set at $p < 0.05$.

TABLE (4). Tukey's multiple comparison test of Ki-67 immunocytochemical expression between study groups.

	Mean Difference	95% CI of Difference	*p*-value	Significance
Control vs. **GE**	23.20	9.80 –36.59	0.0003	***
Control vs. **CDDP**	42.82	29.43 –56.21	< 0.0001	****
Control vs. **CDDP + GE**	65.07	51.67 –78.46	< 0.0001	****
GE vs. **CDDP**	19.62	8.69 –30.56	0.0002	***
GE vs. **CDDP + GE**	41.87	30.93 –52.80	< 0.0001	****
CDDP vs. **CDDP + GE**	22.25	11.31 –33.18	< 0.0001	***

Data are expressed as mean differences with 95% confidence interval (CI). Tukey's multiple comparison test was used following one-way ANOVA. Significance level was set at $p < 0.05$. Asterisks denote highly significance: * = $p < 0.05$; ** = $p < 0.01$; *** = $p < 0.001$; **** = $p < 0.0001$.

TABLE (5). Descriptive statistics and one-way ANOVA analysis of Bcl-2 immunocytochemical expression among the study groups.

	Mean	SD	95% CI of difference	F value	*p-value*
GE	82.46	4.53	80.10–84.83	F=350.73	p < 0.001*
CDDP	51.07	4.62	48.71–53.44		
CDDP + GE	41.71	1.92	39.34–44.08		
Control	92.77	1.40	89.42–96.11		

Data are expressed as mean ± standard deviation (SD). Confidence interval (CI) represents 95%. Significance level was set at $p < 0.05$.

TABLE (6). Tukey's multiple comparison test of Bcl-2 immunocytochemical expression between study groups.

	Mean Difference	95% CI of Difference	*p*-value	Significance
Control vs. **GE**	10.31	4.85 –15.76	< 0.001	***
Control vs. **CDDP**	41.69	36.24 –47.15	< 0.001	***
Control vs. **CDDP + GE**	51.06	45.60 –56.51	< 0.001	***
GE vs. **CDDP**	31.39	26.93 –35.84	< 0.001	***
GE vs. **CDDP + GE**	40.75	36.30 –45.21	< 0.001	***
CDDP vs. **CDDP + GE**	9.36	4.91 –13.82	< 0.001	***

Data are expressed as mean differences with 95% confidence interval (CI). Tukey's multiple comparison test was used following one-way ANOVA. Significance level was set at $p < 0.05$. Asterisks denote highly significance.

TABLE (7). Descriptive statistics and one-way ANOVA analysis of Ki-67 gene expression among the study groups.

	Mean	SD	95% CI of Difference	F value	*p*-value
GE	0.63	0.02	0.61 – 0.66	F=721.87	$p < 0.0001$
CDDP	0.44	0.01	0.41 – 0.46		
CDDP + GE	0.37	0.03	0.34 – 0.39		
Control	1.00	0.00	0.97–1.02		

Data represent mean ± standard deviation (SD). Confidence interval (CI) represents 95%. Significance level was set at $p < 0.05$.

TABLE (8) Tukey's multiple comparison test of Ki-67 gene expression between study groups.

	Mean Difference	95% CI of Difference	*p*-value	Significance
Control vs. **GE**	0.37	0.32 –0.41	< 0.001	***
Control vs. **CDDP**	0.56	0.51 –0.61	< 0.001	***
Control vs. **CDDP + GE**	0.63	0.59 –0.68	< 0.001	***
GE vs. **CDDP**	0.20	0.15 –0.24	< 0.001	***
GE vs. **CDDP + GE**	0.27	0.22 –0.32	< 0.001	***
CDDP vs. **CDDP + GE**	0.07	0.02 –0.12	0.006	**

Data represent mean differences with 95% confidence interval (CI). Tukey's multiple comparison test was used following one-way ANOVA. Significance level was set at $p < 0.05$. ***$p < 0.001$ indicates a highly significant difference, **$p < 0.01$ indicates a significant difference.

TABLE (9). Descriptive statistics and one-way ANOVA analysis of Bcl-2 gene expression among the study groups.

	Mean	SD	95% CI of Difference	F value	*p*-value
GE	0.58	0.01	0.47 – 0.70	F=39.54	$p < 0.0001$
CDDP	0.46	0.17	0.35 – 0.58		
CDDP + GE	0.25	0.01	0.13 – 0.36		
Control	1.00	0.00	0.88–1.12		

Data represent mean ± standard deviation (SD). Confidence interval (CI) represents 95%. Significance level was set at $p < 0.05$.

TABLE (10). Tukey's multiple comparison test of Bcl-2 gene expression between study groups.

	Mean Difference	95% CI of Difference	*p*-value	Significance
Control vs. **GE**	0.42	0.19 to 0.65	0.0017	**
Control vs. **CDDP**	0.54	0.31 to 0.76	0.0003	***
Control vs. **CDDP + GE**	0.75	0.53 to 0.98	< 0.0001	***
GE vs. **CDDP**	0.12	–0.11 to 0.34	0.4113	ns
GE vs. **CDDP + GE**	0.33	0.11 to 0.56	0.0067	**
CDDP vs. **CDDP + GE**	0.22	– 0.01 to 0.45	0.0612	ns

Data represent mean differences with 95% confidence interval (CI). Tukey's multiple comparison test was used following one-way ANOVA. Significance level was set at $p < 0.05$. ***$p < 0.001$ indicates a highly significant difference, **$p < 0.01$ indicates a significant difference, ns: non-significant ($p \geq 0.05$).

DISCUSSION

The treatment of head and neck squamous cell carcinoma (HNSCC) with conventional chemotherapeutic agents remains challenging [2]. CDDP, widely used in HNSCC treatment, exerts its cytotoxic effects by DNA inducing damage in rapidly dividing cancerous cells. However, its use is often limited by severe systemic toxicity and the development of chemoresistance [3]. In response to these limitations, there has been increasing interest in the use of natural extracts and plant-derived compounds with safer toxicity profiles and potential synergistic efficacy when used in conjunction with established chemotherapeutics [5]. *Annona muricata* L., commonly referred to as GE, is a medicinal plant from the Annonaceae family that has been traditionally used in anticancer therapies [10]. In this context, the current study investigated the anticancer effects of GE, both alone and in combination with CDDP, on the HEp-2 human cancer cell line.

The IC50 findings revealed that treatment with GE and CDDP, both individually and in combination, significantly reduced HEp-2 cell viability compared to the untreated control. The IC50 values were 73.99 ± 0.29 μg/mL for GE, 2.88 ± 0.30 μg/mL for CDDP, and 28.47 ± 0.03 μg/mL for the CDDP+GE group, with statistically significant differences when compared to the untreated control group ($p < 0.0001$). The marked reduction in IC50 in the combination group suggests an enhanced cytotoxic effect, indicating a possible synergistic interaction between GE and CDDP in targeting HNSCC cells.

These findings align with previous studies demonstrating cytotoxic potential of GE across various cancer cell lines. For instance, Abu Soukhon

et al. reported that GE exhibited cytotoxicity against PC3 and MCF-7 cell lines with IC50 values of 62 μg/mL and 79 μg/mL, respectively [16]. Similarly, Hashem *et al*. reported strong anticancer activity of GE in MCF-7 and DU-145 cell lines, where IC50 values reached 20 mg/mL for MCF-7 and 5mg/mL for DU-145 cells [17]. Kuete *et al*. further supported the potent cytotoxicity of GE against colon carcinoma cell lines HCT-116 and HT-29, reporting IC50 values of approximately 11.43 and 8.98, respectively [18].

At the molecular level, the present study demonstrated a significant downregulation of Ki-67 mRNA in all the treated groups versus the control ($p < 0.0001$). GE-treated cells showed a Ki-67-fold change of 0.63 ± 0.02, whereas the CDDP+GE group exhibited the lowest expression levels, with a fold change of 0.37 ± 0.03. These findings further support enhanced suppression of cell proliferation when GE is combined with CDDP. Parallel to these results, Bcl-2 gene expression was downregulated in all treated groups with significant differences ($p<0.0001$). Among them, the GE-treated group showed the highest Bcl-2 expression (fold change: 0.58 ± 0.01), while the lowest expression was observed in the combination group (fold change: 0.25±0.01). These results strongly indicate a synergistic enhancement of apoptosis via the intrinsic pathway. Post-hoc analysis further confirmed significant differences between the control and each treated group ($p<0.0001$), and between the GE group and the combined GE and CDDP group ($p < 0.01$), supporting the superior efficacy of the combination regimen in inhibiting the anti-apoptotic signaling. In alignment with these findings, Hadisaputri *et al*. reported that the GE of leaf significantly reduced Ki-67 expression and inhibited proliferation in MCF-7 breast cancer cell line through induction of apoptosis and mitochondrial pathway activation [19].

In terms of its anticancer activity, several recent studies have shown that GE could exert its effects through multiple molecular mechanisms. These include inhibition of NADH oxidase activity on the plasma membrane of cancer cells, induction of cell cycle arrest, and modulation of apoptotic pathways by upregulating pro-apoptotic markers including Bax and caspases while downregulating anti-apoptotic proteins such as Bcl-2. Moreover, GE could induce mitochondrial dysfunction and oxidative stress, leading to activation of caspase-mediated apoptosis and regulation of Bcl-2 family proteins. Furthermore, GE was shown to cause G1 and G2/M cell cycle arrest in various cancer cell lines, enhancing intrinsic apoptosis [11, 19-23].

Immunocytochemical findings further confirmed the apoptotic and antiproliferative activities of GE. Ki-67 nuclear expression was significantly reduced in all treated groups compared to the control levels ($p<0.0001$). Among the treated groups, GE-treated HEp-2 cells exhibited the highest Ki-67 expression (71.20±4.60), followed by CDDP-treated cells (51.57±15.77), while the lowest Ki-67 levels were observed in the CDDP+GE group (29.33 ± 2.92). Post hoc Tukey's test confirmed significant differences between all group pairs ($p<0.0001$). These results reflect a synergistic interaction between GE and CDDP in suppressing tumor cell proliferation. Similarly, immunocytochemical analysis of Bcl-2 revealed variations in cytoplasmic expression levels among the study groups with statistically significant differences ($p<0.0001$). The control group exhibited the highest Bcl-2 expression (92.77±1.40), followed by the GE-treated group (82.46±4.53), then the CDDP-treated group showed a marked reduction (51.07±4.62), and the lowest Bcl-2 level was in the CDDP+GE group (41.71± 1.92). These results indicate that the GE treatment may enhance the apoptotic effects in HEp-2 cells when combined with CDDP, potentially allowing for the use of lower doses of the chemotherapeutic agent while maintaining efficacy and minimizing associated side effects.

Based on the findings of the current study, it is recommended that future research should extend to *in vivo* models of HNSCC to validate the synergistic efficacy of GE combined with CDDP,

further elucidate the underlying molecular signaling pathways, and optimize dosing strategies to support potential clinical application.

CONCLUSIONS

Taken together, the findings demonstrate that GE, particularly when combined with CDDP, have considerable antiproliferative and apoptotic properties against HNSCC cancer cells, offering enhanced therapeutic advantages with safety profile. However, further research should be conducted to fully elucidate the involved mechanistic interactions between the two agents and their combined potential clinical application.

REFERENCES

1. Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin. 2018; 68(6): 394-424. doi:10.3322/caac.21492

2. Ferlay J, Colombet M, Soerjomataram I, et al. Estimating the global cancer incidence and mortality in 2018: GLOBOCAN sources and methods. Int J Cancer. 2019;144(8):1941-1953. doi:10.1002/ijc.31937

3. Waissbluth S, Daniel SJ. Cisplatin-induced ototoxicity: transporters playing a role in cisplatin toxicity. Hear Res. 2013;299:37-45. doi:10.1016/j.heares.2013.02.002

4. Tuli HS, Garg VK, Bhushan S, et al. Natural flavonoids exhibit potent anticancer activity by targeting microRNAs in cancer: A signature step hinting towards clinical perfection. Transl Oncol. 2023;27:101596. doi:10.1016/j.tranon.2022.101596

5. George BP, Chandran R, Abrahamse H. Role of Phytochemicals in Cancer Chemoprevention: Insights. Antioxidants (Basel). 2021;10(9):1455. Published 2021 Sep 14. doi:10.3390/antiox10091455

6. Kim GT, Tran NK, Choi EH, et al. Immunomodulatory Efficacy of Standardized Annona muricata (Graviola) Leaf Extract via Activation of Mitogen-Activated Protein Kinase Pathways in RAW 264.7 Macrophages. Evid Based Complement Alternat Med. 2016;2016:2905127. doi:10.1155/2016/2905127

7. de Sousa OV, Vieira GD, de Jesus R G de Pinho J, Yamamoto CH, Alves MS. Antinociceptive and anti-inflammatory activities of the ethanol extract of Annona muricata L. leaves in animal models. Int J Mol Sci. 2010;11(5):2067-2078. Published 2010 May 6. doi:10.3390/ijms11052067

8. Florence NT, Benoit MZ, Jonas K, et al. Antidiabetic and antioxidant effects of Annona muricata (Annonaceae), aqueous extract on streptozotocin-induced diabetic rats. J Ethnopharmacol. 2014;151(2):784-790. doi:10.1016/j.jep.2013.09.021

9. Moghadamtousi SZ, Fadaeinasab M, Nikzad S, Mohan G, Ali HM, Kadir HA. Annona muricata (Annonaceae): A Review of Its Traditional Uses, Isolated Acetogenins and Biological Activities. Int J Mol Sci. 2015;16(7):15625-15658. Published 2015 Jul 10. doi:10.3390/ijms160715625

10. George VC, Kumar DR, Rajkumar V, Suresh PK, Kumar RA. Quantitative assessment of the relative antineoplastic potential of the n-butanolic leaf extract of Annona muricata Linn. in normal and immortalized human cell lines. Asian Pac J Cancer Prev. 2012;13(2):699-704. doi:10.7314/apjcp.2012.13.2.699

11. Ilango S, Sahoo DK, Paital B, et al. A Review on Annona muricata and Its Anticancer Activity. Cancers (Basel). 2022;14(18):4539. Published 2022 Sep 19. doi:10.3390/cancers14184539

12. Silihe KK, Mbou WD, Ngo Pambe JC, et al. Comparative anticancer effects of Annona muricata Linn (Annonaceae) leaves and fruits on DMBA-induced breast cancer in female rats. BMC Complement Med Ther. 2023;23(1):234. Published 2023 Jul 15. doi:10.1186/s12906-023-04073-x

13. Daddiouaissa D, Amid A, Abdullah Sani MS, Elnour AAM. Evaluation of metabolomics behavior of human colon cancer HT29 cell lines treated with ionic liquid graviola fruit pulp extract. J Ethnopharmacol. 2021;270:113813. doi:10.1016/j.jep.2021.113813

14. Daddiouaissa D, Amid A, Kabbashi NA, Fuad FAA, Elnour AM, Epandy MAKMS. Antiproliferative activity of ionic liquid-graviola fruit extract against human breast cancer (MCF-7) cell lines using flow cytometry techniques. J Ethnopharmacol. 2019;236:466-473. doi:10.1016/j.jep.2019.03.003

15. Prasad SK, Veeresh PM, Ramesh PS, Natraj SM, Madhunapantula SV, Devegowda D. Phytochemical fractions from Annona muricata seeds and fruit pulp inhibited the growth of breast cancer cells through cell cycle arrest at

G_0/G_1 phase. J Cancer Res Ther. 2020;16(6):1235-1249. doi:10.4103/jcrt.JCRT_494_19

16. Abu Soukhon AA, Abu-Qatouseh L, Mansoor K, et al. Cytotoxicity Activity of Graviola Fruit Extract with Carbamazepine and Valproic Acid Show Antagonistic and Indifferent Effects. Asian Pac J Cancer Prev. 2023;24(6):1869-1875. Published 2023 Jun 1. doi:10.31557/APJCP.2023.24.6.1869

17. Hashem S. A, Mallah E, Mansoor K, El-Hajji F. D, Malkawy M, Bustami M, Idkaidek N, Al-Masalmeh A. M, Qatouseh L. A. The Effect of Graviola Leaves Extract (Annona muricata L.) on Pharmacokinetic of Metformin in Rats' Plasma and Pharmacological Activity of their Combination on Breast and Prostate Cancer Cell Lines. Biomed Pharmacol J 2023;16(1). doi: https://dx.doi.org/10.13005/bpj/2613

18. Kuete V, Dzotam JK, Voukeng IK, Fankam AG, Efferth T. Cytotoxicity of methanol extracts of Annona muricata, Passiflora edulis and nine other Cameroonian medicinal plants towards multi-factorial drug-resistant cancer cell lines. Springerplus. 2016;5(1):1666. Published 2016 Sep 27. doi:10.1186/s40064-016-3361-4

19. Hadisaputri YE, Habibah U, Abdullah FF, et al. Antiproliferation Activity and Apoptotic Mechanism of Soursop (Annona muricata L.) Leaves Extract and Fractions on MCF7 Breast Cancer Cells. Breast Cancer (Dove Med Press). 2021;13:447-457. Published 2021 Jul 16. doi:10.2147/BCTT.S317682

20. Rady I, Bloch MB, Chamcheu RN, et al. Anticancer Properties of Graviola (Annona muricata): A Comprehensive Mechanistic Review. Oxid Med Cell Longev. 2018;2018:1826170. Published 2018 Jul 30. doi:10.1155/2018/1826170

21. Gavamukulya Y, Abou-Elella F, Wamunyokoli F, AEl-Shemy H. Phytochemical screening, anti-oxidant activity and in vitro anticancer potential of ethanolic and water leaves extracts of Annona muricata (Graviola). Asian Pac J Trop Med. 2014;7S1:S355-S363. doi:10.1016/S1995-7645(14)60258-3

22. Yang C, Gundala SR, Mukkavilli R, Vangala S, Reid MD, Aneja R. Synergistic interactions among flavonoids and acetogenins in Graviola (Annona muricata) leaves confer protection against prostate cancer. Carcinogenesis. 2015;36(6):656-665. doi:10.1093/carcin/bgv046

23. Abdallah RH, Al-Saleem MSM, Abdel-Mageed WM, et al. LCMS/MS Phytochemical Profiling, Molecular, Pathological, and Immune-Histochemical Studies on the Anticancer Properties of Annona muricata. Molecules. 2023;28(15):5744. Published 2023 Jul 29. doi:10.3390/molecules28155744