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Original Article

Molecular Mechanism of Apoptotic Cell Death in Cyanidin-3-glucoside-induced Cytotoxic Potential on Human Liver Carcinoma (HepG2) Cell Line

Suresh Mickymaray[1,2,*]

[1]Department of Biology, College of Science- Al-Zulfi, Majmaah, University, Majmaah, Riyadh Region, SAUDI ARABIA.
[2]Centre of Molecular Medicine and Diagnostics (COMManD), Saveetha Dental College and Hospitals, Saveetha Institute of Medical and Technical Sciences, Saveetha University, Chennai, Tamil Nadu, INDIA.

ABSTRACT

Background: Liver cancer is one of the most prevalent cancers around the world and the leading cause of cancer-related deaths. Because liver cancer prevalence has increased significantly, there are no established therapies, and there are serious adverse reactions associated with the use of medications already available, new and more potent anticancer agents must be investigated. **Aim:** Using *in vitro* methods, we have assessed Cyanidin-3-glucoside (C3gc)'s anticancer activities and the underlying molecular mechanisms. **Materials and Methods:** In the current investigation, the cytotoxicity and apoptotic activity of C3gc against liver cancer cell line HepG2, has been investigated using MTT assay, LDH release assay, AO/EtBr dual staining, DAPI staining, ROS estimation, Mitochondrial ATPase assay, and oxidative stress parameters has been examined. **Results:** Assays of MTT and LDH release showed that C3gc exhibited cytotoxic potential and cell membrane distribution by reducing cell viability dose-dependently. The AO/EB and DAPI staining techniques were used to assess morphological changes associated with apoptosis, confirming its apoptotic nature. In HepG2 cell lines, C3gc increased ROS generation and ATPase activity. Furthermore, C3gc-treated cells showed a significant increase in MDA levels while decreasing SOD and GSH levels. This reflects increased oxidative stress and decreased antioxidant activity in HepG2 cells. **Conclusion:** C3gc inhibits cancer cell proliferation by enhancing ROS levels, disrupting cell membrane integrity, and causing DNA fragmentation and apoptosis through oxidative stress-mediated pathways. Thus, C3gc could be effectively utilized as an effective anti-cancer alternative for liver cancer.

Keywords: Liver cancer, Cyanidin-3-glucoside, HepG2 cells, Reactive oxygen species, Apoptosis.

Correspondence:
Dr. Suresh Mickymaray
Department of Biology, College of Science- Al-Zulfi, Majmaah, University, Majmaah, 11952, Riyadh Region, SAUDI ARABIA.
Email: s.maray@mu.edu.sa

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INTRODUCTION

The third-leading cause of cancer-associated mortality globally, following lung and stomach cancers, liver cancer ranks among the five major malignancies in terms of prevalence and death across all different ages and genders.[1,2] During the recent decades, liver cancer incidence has climbed worldwide, and modeling studies indicate that some countries will see an increase in prevalence going forward.[3] The most prevalent histological subgroup of primary liver cancer, Hepatocellular Carcinoma (HCC), contributes to 70-85% of global liver cancer cases.[4] Although there are surgical and medicinal therapies available, surgery has limited therapeutic options, especially for individuals with advanced HCC, and drugs can have serious adverse effects.[5] It has been demonstrated that several molecular processes and signaling pathways contribute to the development and progression of HCC. The PI3K/Akt/mTOR signaling route, the Wnt/ß-catenin pathway, the MAPK pathway, the receptor tyrosine kinase pathways, the proteasome degradation pathway, and the hedgehog signaling network are a few of the most common mechanisms implicated in HCC. It may be possible to develop new HCC treatments by targeting this important signaling pathways.[6]

The discovery of novel medications for the management of illnesses is successful when using natural substances extracted from plants, animals, or other natural materials. More than 60% of cancer-treatment drugs are comprised of natural components.[7] Phytochemicals have long been intriguing prospects for the formulation of novel anticancer drugs. Among these phytochemicals that have gained the most attention in the battle against cancer is the anthocyanins family of flavonoids. They can affect various biological processes related to the onset and spread of cancer; including mutation, proliferation, differentiation,

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angiogenesis, and apoptosis.[8] They also possess antioxidant as well as anti-inflammatory properties. Moreover, anthocyanins may modify signaling pathways that are dysregulated in malignancies.[9] There is confirmation that certain anthocyanin-rich plant extracts, such as those from *Ribes nigrum* and *Morus alba*, suppress the development of HCC in animal models.[10]

The most prevalent anthocyanin, Cyanidin-3-glucoside (C3gc), is found in dark-colored fruits, vegetables, and cereals including red cabbages, red raspberries, blueberries, *Morus alba* (mulberry), and purple rice bran. C3gc has been shown in multiple investigations to have positive benefits, including action against UV-B radiation, antioxidation, anti-cancer, and anti-inflammation properties.[11,12] Moreover, it has been documented that C3gc reduces CCl_4-induced liver damage in mice by acting as an antioxidant and an anti-inflammatory.[13] Moreover, oxidative stress-induced apoptosis of liver cells was prevented by C3gc by scavenging ROS and boosting the antioxidant response.[14] Its neuroprotective nature has been established in previous studies conducted with rat models, where it reportedly preserved mitochondrial membrane damage of neurons.[15]

Chemotherapy is regarded as a promising method of treating cancer. Furthermore, modulating ROS production to selectively target cancer cells has been proposed as an excellent therapeutic option. Apoptosis, increased ROS, and decreased GSH levels are all caused by chemotherapeutic drugs like amino benzenesulfonamide.[16] Novel drugs that increase ROS levels and modulate mitochondrial membrane potential have been identified, making tumor cells susceptible to cell death. Many studies have found that anti-tumor agents work by inducing ROS, but the exact mechanism of ROS generation is unknown.[17] GSH levels are associated with cancer-related multidrug resistance. One of the primary criteria for potential anti-cancer drugs is the maximum effect on cancer cells while causing the least amount of damage to neighboring normal cells.[18] Furthermore, there has been an increase in demand in recent years for the development of new and effective anti-tumor drugs at reasonable prices.[19] The use of oxidative anti-tumor compounds does not harm normal cells because these drugs increase the levels of reactive oxygen species, but the production of ROS in normal cells is efficiently regulated by the antioxidant defence system.[20] Endogenous ROS production in cells is regulated by enzymatic reactions, primarily in mitochondria. Anthocyanins have emerged as promising cancer treatment agents due to their diverse mechanisms of action and low toxicity.[21] Although some anthocyanins have antioxidant properties and others cause oxidative stress, anthocyanins are less toxic than traditional therapies.[22,23]

The novelty and contribution of studying the effects of Cyanidin-3-glucoside (C3gc) on cancer lies in their multifaceted properties that encompass antioxidant, anti-inflammatory, anti-proliferative, and signalling modulation effects. Furthermore, the exploration of their epigenetic regulation and potential for synergistic combination therapies adds depth to their therapeutic potential. A natural compound with fewer side effects compared to synthetic drugs, and understanding the mechanisms underlying these effects and their application to various cancer types, will advance cancer research and potentially improve patient care.

The research objectives for studying the effects of Cyanidin-3-glucoside (C3gc) on the prevention and treatment of cancer encompass elucidating mechanisms of action, evaluating anti-proliferative and anti-inflammatory properties, exploring synergies with existing therapies, and investigating source-specific effects. These objectives collectively contribute to advancing our understanding of the potential therapeutic role of Cyanidin-3-glucoside (C3gc) in cancer and can inform future research, clinical trials, and healthcare strategies. The major purpose of the present investigation is to analyze the anticancer potential of anthocyanin, cyanidin-3-glucoside, in HepG2 liver cancer cells. Further, its LDH activity, apoptotic mechanism, ROS production, and oxidative stress parameters were analyzed to evaluate its anticancer mechanism.

MATERIALS AND METHODS

Materials

Chemicals including Cyanidin-3-glucoside (C3gc) were obtained from Sigma Aldrich, USA. The culture consumables and media were procured from Sigma Aldrich. The ELISA kits were provided by Abcam.

In vitro anticancer activity of C3gc

Cytotoxicity analysis by WST-8 assay

HepG2 cells (3×10^3 cells/mL) were seeded into each well of 96-well plates to assess the cytotoxicity of C3gc against human liver cancer cells (HepG2) were maintained in DMEM medium with additives. C3gc and doxorubicin were introduced at different concentrations (3, 9, 18, 36, 72, and 144 µg/mL). Using a Cell Counting kit, the mitochondrial activity in converting tetrazolium salt (WST-8) to formazan was assessed to determine the viability of the cells 24 hr after incubation. As directed by the manufacturer, cells were treated with a reagent. With the help of a spectrometer, plates were analysed at an absorbance of 450 nm. The concentration which resulted in 25 and 50% (IC_{25} and IC_{50}, respectively) growth decline was noted.

Estimation of lactate dehydrogenase (LDH) activity

In 96-well plates, HepG2 cells were cultured at $1X10^5$ cells/mL in an effort to determine if the LDH enzyme is liberated from the cytosol when cells are disrupted. A commercial kit was employed to measure the LDH activity. The number of live cells was then correlated with the absorbance readings to determine the cytotoxic action. Doxorubicin was utilized as the positive control.

Examination of apoptotic cells by AO/EtBr staining approach

To evaluate the apoptotic efficacy of C3gc against HepG2 cell lines, the dual staining method was employed. The cells were seeded into a 6-well plate and treated with C3gc at IC_{25} and IC_{50} concentrations for 24 hr. Following treatment, the cells underwent a 5 min incubation period in a solution containing an equal amount of acridine orange and ethidium bromide (AO/EBr). PBS was then used to rinse the cells. The cells were studied using a fluorescence microscope after being rinsed with PBS.

Investigation of cell morphology by DAPI staining

Using fluorescence microscopy, cell nuclear morphology was evaluated after DAPI labeling. The cancer cells were exposed to the compound C3gc (IC_{25} and IC_{50}) and incubated for 24 hr. The cells were then rinsed with PBS, fixed with ice-cold ethanol (70%) and resuspended in DAPI, and left for 15 min while being covered in aluminium foil. After being washed with PBS, the cells were afterward examined under a fluorescent microscope.

Analysis of reactive oxygen species (ROS) by Dichloro-dihydro-fluorescein diacetate (DCFH–DA) staining

HepG2 cells were inoculated and subjected to C3gc treatment (IC_{25} and IC_{50}). The cells were collected after 24 hr and three PBS washes were carried out. Cells were extracted at a concentration of 1-20 × 10^6 cells/mL, combined with DCFH–DA (10 mmol/L), and then incubated for 20 min at 37°C. The cells were rinsed thrice with cell culture medium to entirely neutralize DCFH-DA. After that, fluorescent microscopy was employed to quantify the levels of cellular ROS.

Mitochondrial ATPase assay

By correlating the NADH oxidation and the generation of ADP through the previously mentioned lactate dehydrogenase process, it was possible to assess the ATPase activity of the solubilized mitochondrial fraction of C3gc by measuring the absorbance at 340 nm. The following ingredients comprised the reaction buffer and Oligomycin, an inhibitor of F0-targeting, or efrapeptin, an effective and selective inhibitor of F1-targeting, were employed to measure the F-type ATPase activity.

Analysis of oxidative stress parameters

The C3gc treatment was carried out on the cells in line with the instructor's guidelines, and then SOD activity was assessed using a commercial kit. A plate reader was employed to calculate the samples' absorbance at 450 nm. The CAT test kit was used by the instructions to assess the CAT enzyme's activity. The kit measures the enzyme based on how hydrogen peroxide breaks down. Moreover, a kit was employed to assess the levels of MDA activity. The absorbance of the samples was determined at 450 nm. MDA and thiobarbituric acid mix to form a complex, which is then tested for absorbance at 532 nm to determine the degree of lipid peroxidation. Furthermore, a kit was used to assess the levels of GSH activity. The quantity of GSH is determined by continually converting 5, 5′-dithiobis (2-nitrobenzoic acid) in the presence of catalytic amounts (nmoles) of GSH using a kinetic assay.

Statistical analysis

In addition to performing each investigation thrice, we conducted a one-way ANOVA and a *post hoc* Tukey post-test to analyze the findings. Data were reported as mean+SD and $p<0.05$ and $p<0.01$, respectively, were used for statistical significance tests.

RESULTS

Anticancer activity of C3gc by cytotoxicity assay

The cytotoxic potential of C3gc toward HepG2 cells was evaluated via WST-8 assay, and the findings are presented in Figure 1A our results demonstrated that the C3gc treatment considerably reduced the growth of liver cancer cells dose-dependently. At the highest concentration of C3gc, the viability of cells was reduced to 20%. This finding demonstrates C3gc's remarkable cytotoxicity toward HepG2 cells. The IC_{50} value for the cells was determined to be 18 µg/mL, therefore this concentration of C3gc was applied for further experiments.

Estimation of LDH activity in C3gc treated cells

The amount of the LDH enzyme produced from the cytosol rises when cells are harmed under stress. The activity of the enzyme was analyzed to determine whether treating the cells with C3gc caused an increase in LDH release, and the findings are depicted in Figure 1B. It was revealed that with increasing C3gc concentration, the LDH activity also elevated, with the doxorubicin drug displaying the highest LDH activity, indicating the damaging effect of C3gc on HepG2 cells.

Assessment of apoptosis by AO/EtBr staining

Cell membrane alterations associated with apoptosis are mainly caused by ROS build-up within the cells. Dual AO/EtBr labelling was employed to examine the membrane deformation associated with apoptosis in HepG2 cells exposed to C3gc (Figure 2). Cell membrane changes associated with apoptosis have been identified using dual labelling. In contrast to EB dye, which can only stain cells whose membranes have been lost, AO dye can stain the nuclear DNA of cells that retain their entire membranes. AO-positive, yellow, and greenish-coloured cells were also observed in the test groups, indicating early apoptosis during C3gc treatment. This was in contrast with EtBr-positive cells, which showed late apoptosis or dead cells during treatment with C3gc.

DAPI staining

It is crucial to evaluate apoptosis to differentiate between apoptosis and necrosis. Nuclear fragmentation is one of the standout features of the apoptotic process of cell death. Cell morphological changes and apoptosis-related cell death were examined using the fluorescent DNA-binding agent DAPI. It was evident from Figure 3 that C3gc could induce apoptosis by fragmenting apoptotic bodies and shrinking nuclei in the treated cells, in contrast to a healthy and expanded nucleus in control cells. Consequently, C3gc caused apoptosis through an increase in ROS levels in cells (Figure 3).

Detection of ROS by DCFH–DA staining

The ROS has an impact on both cell growth and apoptosis and can cause cell death in a variety of ways. To identify the reason for the cytotoxicity observed in HepG2 cells, we thus investigated the ROS-inducing potential of C3gc using DCFH-DA staining. Enhanced green fluorescence was detected with increasing C3gc concentrations, which suggests that the treated cells produced higher ROS (Figure 4). Thus, it was noticed that C3G produced ROS in HepG2 cells, indicating that C3G triggered these cells to undergo oxidative stress-mediated cell death.

Mitochondrial ATPase assay

The mitochondrial ATPase activity of the liver cancer cells treated with C3gc IC_{25} and IC_{50} is presented in Figure 5. In the control cells, the ATPase activity was minimum, whereas when the cells were treated with IC_{25} and IC_{50} concentration C3gc, their activity was elevated, suggesting their tumor-promoting potential.

Estimation of lipid peroxidation and SOD, CAT, and GSH levels

In the HepG2 cancer cells, the influence of C3gc administration on the Figure 6 MDA, SOD, CAT, and GSH levels was assessed, and the findings are illustrated in Figure 6. The C3gc-treated cells demonstrated a significant enhancement in the MDA levels. On the other hand, C3gc administration decreased the activity of SOD, CAT, and GSH levels significantly. These results indicated that administering C3gc to HepG2 cells significantly reduced the antioxidant levels and increased oxidative stress. Through the production of ROS in human liver tumor cells, oxidative stress has been shown to contribute to C3gc-induced cytotoxicity.

DISCUSSION

One of the most frequent cancerous tumors in the world, hepatocellular carcinomas account for 90% of all primary liver malignancies. 90% of all first liver cancers are hepatocellular carcinomas.[24] Even though several cytotoxic drugs are introduced to the market each year, the prevalence of liver cancer is increasing due to the unexpected side effects or low efficacy of these therapies. As a consequence, there is an increasing demand for therapeutic strategies that address malignancy while also being efficient and having fewer side effects. HepG2, a cell line from human hepatocellular carcinoma, has been widely utilized to investigate liver cancer.[25] One of the most prevalent anthocyanins is C3gc, which has been found to combat malignancies of the liver, colon, stomach, prostate, ovary, cervix, and breast.[18] Consequently, the current study's goal is to provide a better understanding of cell death and the molecular mechanism by which C3gc inhibits apoptosis utilizing HepG2 cells as an *in vitro* model.

Cell viability has been extensively assessed using WST-8, a tetrazolium salt. It is a water-soluble disulfonated tetrazolium salt that, upon evaluation, transforms into a water-soluble product formazan and has thus been used to assess cell viability with the least amount of error possibilities.[26] It was discovered that C3gc declined the viability of HepG2 cells in a concentration-based trend. A similar dose-based growth reduction has been reported when C3gc was tested against H661 lung cancer cells and MDA-MB-453 breast cancer cells.[27] Following the disruption of the cytoplasmic membrane and cell necrosis, damaged or stressed cells might leak cytoplasmic LDH and other molecules into the medium.[28] Consequently, it's probable that the C3gc cytotoxic action wasn't dependent on the process of necrosis-induced cell death. These findings suggest that apoptosis-inducing pathways may be involved in the cytotoxic action of C3gc. Apoptosis is

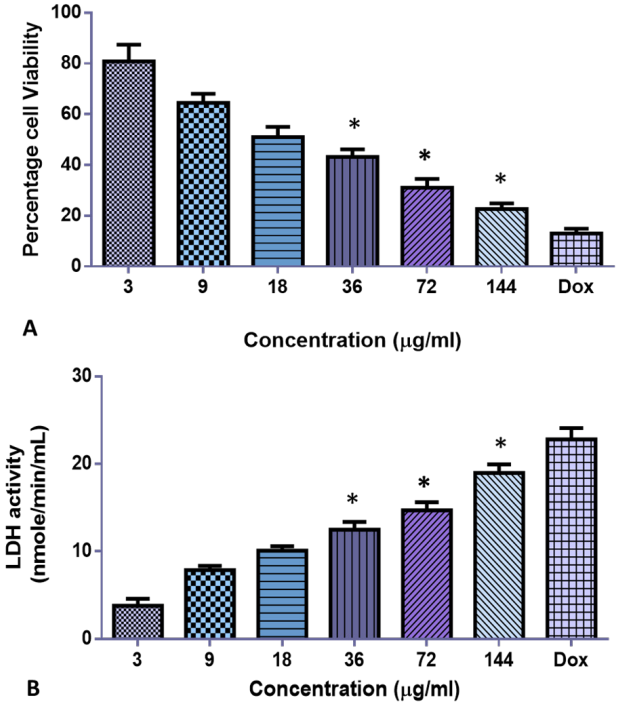

Figure 1: Analyses of the cytotoxic potential of cyanidin-3-glucoside on HepG2 cells. The WST-8 assay (A) and Lactate Dehydrogenase (LDH) enzyme release assay (B) assessed Cyanidin-3-glucoside at different concentrations in HeLa cells. Doxorubicin was employed as a positive control. Results were computed using the mean±standard deviation of three distinct investigations. '*' denotes a significant difference $p<0.05$ compared with the DOX.

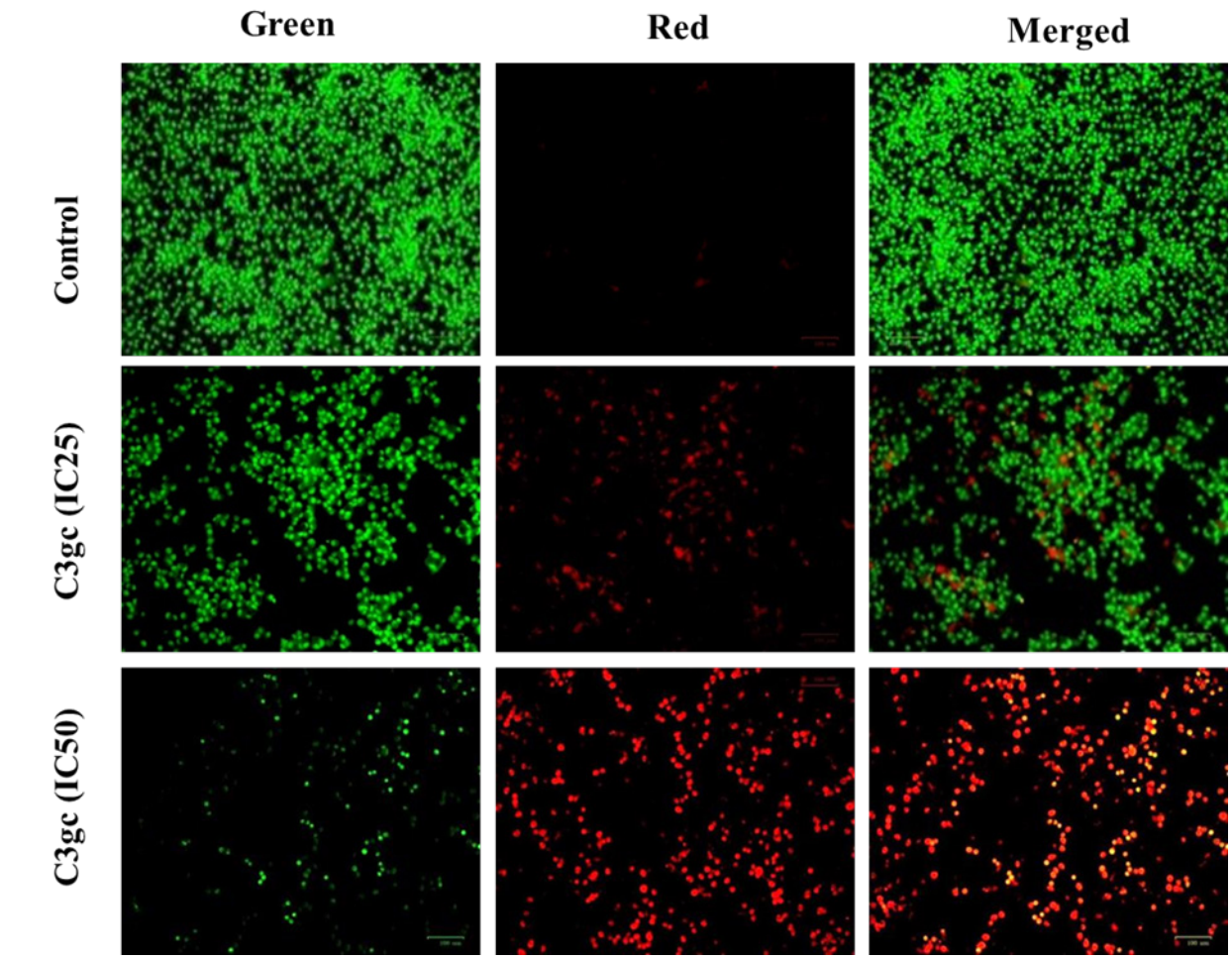

Figure 2: HepG2 cells were stained with AO/EtBr to determine the effect of cyanidin-3-glucoside. The AO/EB-stained images reveal that the C3G-treated cells display more orange and yellowish colour due to an increase in apoptotic cells when compared to the control cells. Control, C3gc (IC_{25}), and C3gc (IC_{50}) fluorescence microscopic studies with dual staining [AO (100 µg/mL): EtBr (100 µg/mL) in a 1:1 ratio]. After 24 hr of drug treatment, three channels (green, red, and merged) were measured with 20X magnification to document morphological changes associated with apoptosis.

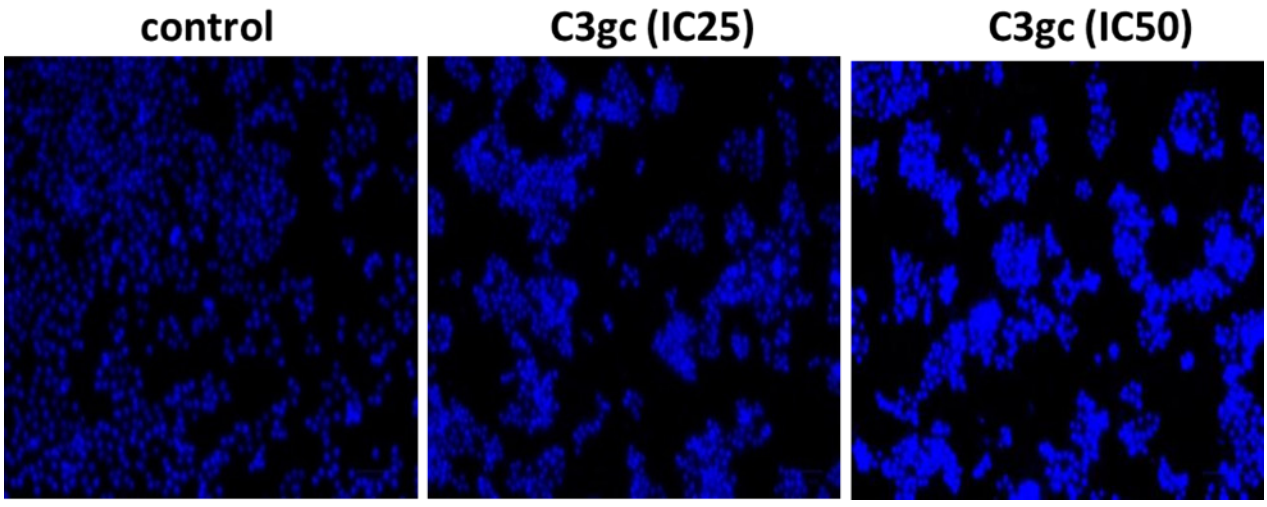

Figure 3: Cyanidin-3-glucoside-induced apoptotic cell death in HepG2 cells. DAPI staining shows the nuclear morphology of C3gc-treated HepG2 cells. The experiments were performed in triplicate. C3gc led to cell apoptosis as indicated by its tendency to induce cell shrinkage, chromatin condensation, and nuclear fragmentation.

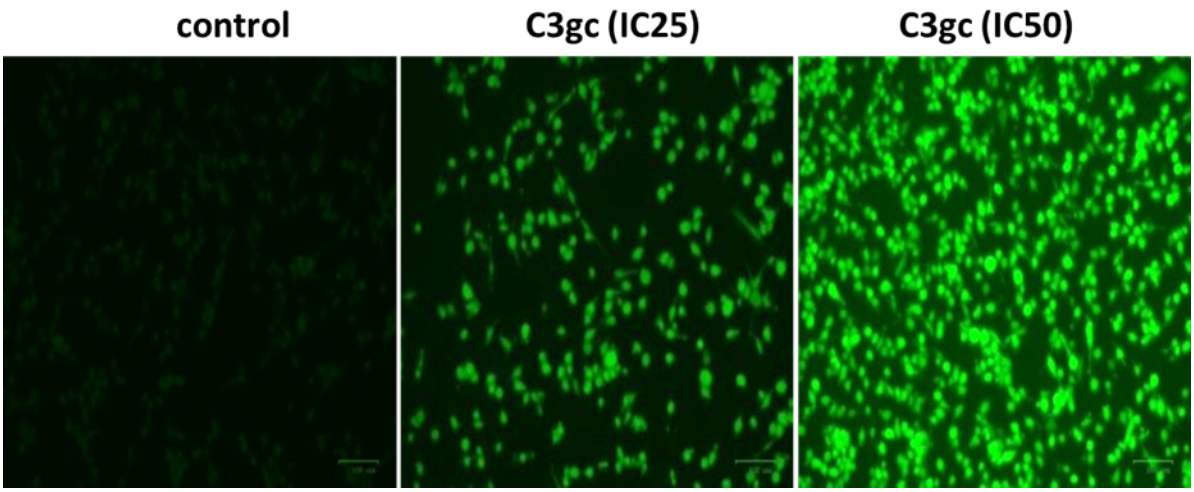

Figure 4: Induction of oxidative stress by cyanidin-3-glucoside in HepG2 cells through ROS production. Analysis of ROS formation using DCFH-DA staining on HepG2 cells treated with C3gc (IC_{25} and IC_{50}) after 24 hr of treatment. The images were taken using light (magnification 20×). Compared with control cells, C3gc (IC_{25}) and C3gc (IC_{50})-treated cells showed greater green fluorescence due to ROS formation. Three separate experiments are represented in the picture.

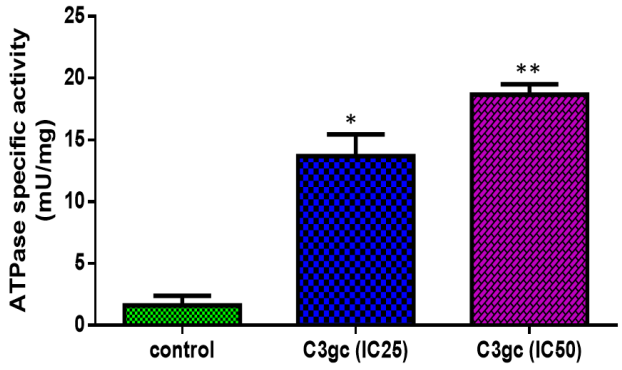

Figure 5: Effect of cyanidin-3-glucoside on mitochondrial ATP synthase enzyme activity in HepG2 cell lines. We measured the mitochondrial ATP synthase enzyme activity using cyanidin-3-glucoside at two different concentrations (IC_{25} and IC_{50}). Results were computed using the mean±standard deviation of three distinct investigations. '*' denotes a significant difference from the control in the data ($p<0.05$). '**' denotes a significant difference from the control in the data ($p<0.01$).

thought to play a significant role in several processes, including immune system establishment, normal cell homeostasis, and drug-induced cell death.[29] There is a possibility for novel drugs that might help treat cancer as a result of research showing that certain chemo-preventive treatments work to prevent cancer by controlling apoptosis.[30]

By analysing morphological alterations about apoptosis, the AO/EB staining method was employed to increase our understanding of the process of cell death induced by C3gc. The acridine orange's penetration into the cell membranes caused the live cells to fluoresce green, while the apoptotic cells fluoresced orange as a result of nuclear shrinkage, confirming the activation of the apoptotic process. Cell death caused by apoptosis is manifested by nuclear fragmentation. A DAPI-binding fluorescent DNA-binding agent was used to evaluate the morphological changes and apoptosis-associated cell death.[31] Among the characteristics that signal the beginning of apoptosis in cancer cells are fragmentation of DNA and aberrant cell size.[32] Cells with IC_{25} and IC_{50} concentrations of C3gc displayed aberrant morphology, including shrinkage and an unexplained nucleus shape, as compared to untreated cancer cells. This is due to DNA fragmentation in the cancer cells, which caused apoptosis. In previous studies, C3gc has been documented to induce apoptotic cell death in numerous cancerous cell lines including breast and lung adenocarcinoma.[33,34]

The death of tumor cells is mostly triggered by ROS.[35] To ascertain if ROS are implicated in the C3gc-induced cell death, the amount of ROS generated within the cells was estimated using the ROS-sensitive fluorimetric probe DCFDA. Elevated green fluorescence in the cells treated with anthocyanin C3gc indicated an augmented intracellular production of ROS. A similar augmented ROS production was induced by secondary metabolites from *Morus alba* leaf extract.[36] The majority of the ATP needed to power cells is produced by mitochondrial ATP synthase or ATPase which is located in the inner membranes of mitochondrial cells. ATP synthase has the potential to be a target in the treatment of cancer and is crucial for the development of tumors.[37]

The mitochondrial ATPase activity of C3gc-treated cells was found to be higher than those of normal cells, indicating higher tumor progression of liver cells. An increase in ROS, which restricts the production of antioxidants, causes oxidative stress in cancer cells.[38] SOD offers protection from lipid peroxidation while enabling the catalysis of superoxide anions to oxygen and hydrogen peroxide conversion. Membrane lipid peroxidation produces MDA, which has been utilized as a biological indicator

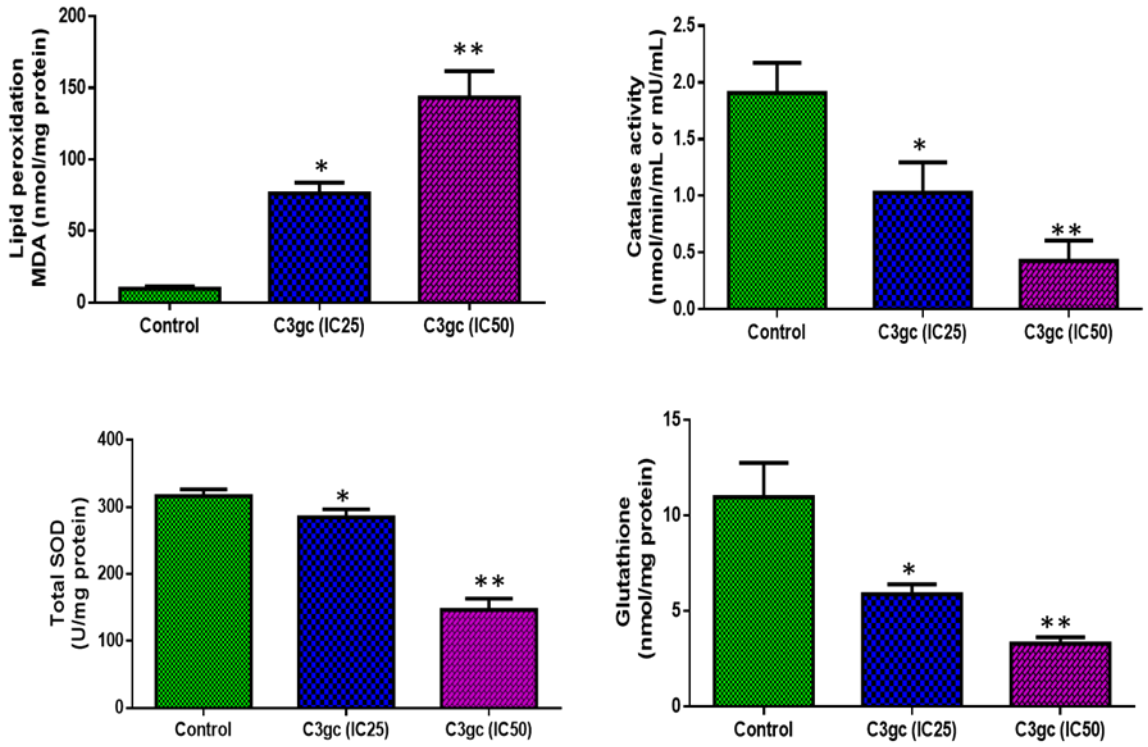

Figure 6: Effect of cyanidin-3-glucoside on Lipid peroxidation (MDA), and antioxidant enzymes such as SOD, CAT, and GSH activities in HepG2 cell lines at two different concentrations (IC_{25} and IC_{50}). The antioxidant levels were measured by the ELISA technique. Results were computed using the mean±standard deviation of three distinct investigations. '*' denotes a significant difference from the control in the data ($p<0.05$). '**' denotes a significant difference from the control in the data ($p<0.01$).

of oxidative stress.[39] The generation of ROS causes lipid peroxidation, which commonly leads to the overproduction of malondialdehyde, which disrupts normal cell function and causes cancer.[40]

ROS are produced by cancer cells as a result of their increased ATP requirements; oxidative stress causes cell death when an imbalance between antioxidants and prooxidants occurs. The dysregulated redox balance in cancer cells prevents cancer cells from escaping programmed cell death, while increased levels of reduced glutathione promote tumor cell survival. Furthermore, anticancer drugs have been shown to induce apoptosis in response to GSH depletion.[41] Our findings show that treating C3gc and HepG2 cells increases ROS levels while decreasing GSH levels. The GSH/GSSH index is influenced more by the combination of maleic anhydride derivatives and C3gc. *In vitro* analysis of maleic anhydride derivatives revealed a clear selective reaction with glutathione' C3gc reduces the levels of reduced glutathione in HepG2 cells. Furthermore, C3gc increases the depletion of ROS and reduced glutathione is limited when administered before maleic anhydride derivatives. The combination of antioxidant derivatives and C3gc, on the other hand, resulted in a greater decrease in the level of reduced glutathione. Surprisingly, despite the presence of C3gc, high ROS levels were observed with this combination and order of administration.

According to the findings, changes in glutathione and ROS levels may account for the greater antitumor effect of C3gc administration, and its potentially toxic oxidation products exert prooxidant effects within cells as a result of persistent high ROS levels, and these radicals, with high reactivity towards thiols, react with GSH.[42] C3gc may also act as a prooxidant by changing ROS metabolism due to a decrease in intracellular GSH, or by downregulating heat shock protein (Hsp)-90 and inhibiting TRX reductase.[43] A high glutathione index indicates redox balance and appropriate intracellular redox homeostasis because GSH is one of the main cellular free radical scavengers in addition to thioredoxin family members.[44] The combination of antioxidants also has been shown to induce apoptosis while suppressing cell proliferation.[45]

Understanding the molecular and cellular pathways through which these compounds exert their anticancer properties requires mechanistic insights into the effects of Cyanidin-3-glucoside (C3gc) on cancer prevention and treatment. While the precise mechanisms differ depending on the type of cancer and the Cyanidin-3-glucoside (C3gc) compound. These

complex mechanisms can be unraveled by studying the effects of Cyanidin-3-glucoside (C3gc) on HepG2 cells, which cause an increase in the levels of oxidative stress enzymes, ROS, mitochondrial ATPase enzymes, DNA damage, and oxidative stress-mediated cell death. Understanding these mechanisms can help guide the development of future targeted therapies, combination treatments, and personalized interventions for Hepatic cancer prevention and treatment.

Our findings indicated that the activities of antioxidant enzymes SOD, CAT, and GSH were decreased upon C3gc treatment, whereas the MDA levels were elevated. Collectively, the current work investigated the cytotoxic potential of C3G in HepG2 cells and discovered that it significantly increased ROS levels, disrupted cell membrane integrity, caused DNA fragmentation, and induced apoptosis, leading to cell death.

CONCLUSION

This investigation intended to elucidate the anti-tumor potential of an anthocyanin, Cyanidin-3-glucoside, against HepG2 liver cancer cells. The impact of C3gc on cell viability, apoptotic induction, ROS generation, and oxidative stress marker levels has been examined. Our findings suggest that C3gc cytotoxic potency in cells is mediated via triggering apoptosis, which is confirmed by high ROS and mitochondrial ATPase levels, as well as the AO/EB staining results. Elevated ROS concentration causes the cell membranes to get disrupted, resulting in apoptosis. Overall, our findings support C3gc anticancer properties, and more research into this framework might contribute to the progress of a powerful anticancer medicine to effectively manage liver cancer.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

C3gc: Cyanidin-3-glucoside; **HCC:** Hepatocellular carcinoma; **LDH:** Lactate Dehydrogenase; **AO/EtBr:** Acridine Orange and Ethidium Bromide; **DAPI:** 4',6-diamidino-2-phenylindole; **DCFH-DA:** Dichloro-dihydro-fluorescein diacetate; **LPO:** Lipid peroxidation; **SOD:** Superoxide dismutase; **CAT:** Catalase.

SUMMARY

Our findings suggest that cyanidin-3-glucoside (C3gc) cytotoxic potency in cells is mediated via triggering apoptosis, which is confirmed by high ROS and mitochondrial ATPase levels, as well as the AO/EB staining results. Elevated ROS concentration causes the cell membranes to get disrupted, resulting in apoptosis. Overall, our findings support C3gc anticancer properties, and more research into this framework might contribute to the progress of a powerful anticancer medicine to effectively manage liver cancer.

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