

Monacolin X Modulates Cellular Antioxidant Defense by Disrupting Reactive oxygen species in HepG2 Liver Cancer Cells

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Abstract. Hepatocellular carcinoma (HCC) is one of the leading aggressive malignancies worldwide, characterized by metabolic reprogramming and redox imbalance that support tumor progression and survival. Monacolin X, a bioactive secondary metabolite, has recently gained attention for its potential anticancer properties; however, its underlying molecular mechanisms remain poorly understood. The present study investigates the effect of Monacolin X on cellular antioxidant defense in HepG2 (liver cancer cells). HepG2 (liver cancer) cells were treated with Monacolin X, and alterations were assessed through key metabolic indicators, along with evaluation of oxidative stress markers and antioxidant enzyme levels. Our findings demonstrate that Monacolin X significantly disrupts energy homeostasis in HepG2 cells. This metabolic disruption was accompanied by increased intracellular reactive oxygen species (ROS) levels and modulation of antioxidant defense systems, including changes in enzymatic antioxidants. The combined effects resulted in increased oxidative stress, contributing to reduced cell viability and enhanced susceptibility to cell death. Overall, this study highlights Monacolin X as a promising metabolic modulator that targets redox balance in liver cancer cells, providing mechanistic insights into its potential therapeutic application against hepatocellular carcinoma.

Keywords: Monacolin X; HepG2 cells; Hepatocellular carcinoma; Antioxidant defense; Oxidative stress; Liver cells; Redox homeostasis

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1. Introduction

One of the most common and deadly types of liver cancer in the world, hepatocellular carcinoma (HCC) has few treatment options and a high recurrence rate. The disruption of redox homeostasis is a characteristic of cancer cells, including HCC [1]. Reactive oxygen species (ROS) are frequently raised in cancer cells because of increased metabolic activity, mitochondrial malfunction, and oncogenic signaling [2]. Excessive ROS production can overwhelm antioxidant defenses, resulting in oxidative damage and cell death, whereas moderate ROS levels support tumor growth and survival. As a result, focusing on redox balance has become a viable cancer treatment approach [2].

To maintain intracellular ROS equilibrium, cellular antioxidant defense systems which include enzymatic antioxidants like glutathione peroxidase (GPx), catalase (CAT), superoxide dismutase (SOD) and glutathione S-transferase (GST) are essential [3]. These enzymes are constantly overexpressed in liver cancer cells in order to combat oxidative damage and promote the spread of the disease. Cancer cells may remain more vulnerable to oxidative damage and tumor growth may be inhibited if this antioxidant network is disrupted [4].

A naturally occurring bioactive substance obtained from microbial sources, monacolin X has drawn interest because of its possible pharmacological qualities, such as anticancer action [5-6]. Monacolin X may have lethal effects on cancer cells via interfering with metabolic and redox-regulated pathways, despite the fact that monacolins are well known for their ability to decrease cholesterol. However, it is still unclear exactly how Monacolin X affects antioxidant defense and oxidative stress in liver cancer cells. In this context, the present study aimed to investigate the effect of Monacolin X on ROS-mediated antioxidant defense mechanisms in HepG2 liver cancer cells. By evaluating intracellular ROS levels and key antioxidant enzyme activities, this study seeks to elucidate whether Monacolin X disrupts redox homeostasis and weakens cellular antioxidant capacity. Understanding this mechanism may provide valuable insights into the therapeutic potential of Monacolin X as a redox-modulating anticancer agent against hepatocellular carcinoma.

2. Materials and Methods

2.1 Cell lines

The human hepatic cell line (HepG2) was procured from the National Centre for Cell Science (NCCS) in Pune, India. The cell lines were cultured in Dulbecco's Modified Eagle Medium (DMEM) (Sigma Aldrich, USA) augmented with 10% v/v heat-inactivated fetal bovine serum (FBS) (PAA, Austria), 100 U/mL-1 penicillin, and 100 mg/mL-1 streptomycin (Sigma Aldrich, USA). The cells were

cultivated at 37 °C in a humidified incubator with 90% humidity and 5% CO₂. Upon reaching 80% confluence, the cells were subcultured into fresh medium for subsequent studies [5].

2.2 Reactive oxygen species (ROS) Staining

The ROS generation was observed using DCFH-DA staining in HepG2 liver cancer cells post treatment with monacolin X at its IC₅₀ concentration for 24 hours. After treatment, the cells were washed in phosphate-buffered saline (PBS) to eliminate any remnants of the initial medium, and the cultures were incubated with DCFH-DA (1 μM) containing media for 30 min at 37°C in a standard incubator with 5% CO₂. After growth, DCFH-DA-containing media was removed, and PBS washes done. The cells were analysed and imaged quickly by using fluorescence microscope [6]. The generation of ROS were quantitatively measured using image J software [7].

2.3 Antioxidant activity

2.3.1. Superoxide Dismutase (SOD) Activity Assay

A spectrophotometric technique based on the inhibition of nitroblue tetrazolium (NBT) reduction was used to measure the activity of superoxide dismutase (SOD). After being exposed to Monacolin X for 24 hours at its IC₅₀ concentration of 72.4 μM, HepG2 cells were collected and cleaned with ice-cold phosphate-buffered saline (PBS). Cell lysis buffer was used to lyse the cells, and the supernatant was obtained by centrifuging the lysates at 10,000 × g for 15 minutes at 4 °C. After the cell supernatant was incubated with the reaction mixture containing sodium carbonate buffer, NBT, and hydroxylamine hydrochloride, absorbance was measured at 560 nm. Units per milligram of protein were used to express SOD activity [8].

2.3.2 Catalase (CAT) Activity Assay

Hydrogen peroxide (H₂O₂) breakdown was used to catalase (CAT) activity. HepG2 cells were collected, rinsed with PBS, and lysed after being treated with Monacolin X (72.4 μM) for 24 hours. After centrifuging the cell lysates, the supernatant was utilized for examination. A UV-visible spectrophotometer was used to measure the decrease in absorbance at 240 nm after the supernatant was added to phosphate buffer containing H₂O₂ to start the reaction. Micromoles of H₂O₂ broken down per minute per milligram of protein were determined and express catalase activity [9].

2.3.3. Glutathione Peroxidase (GPx) Activity Assay

The rate of reduced glutathione (GSH) oxidation was measured to check the activity of glutathione peroxidase

(GPx). HepG2 cells were collected and rinsed with PBS, and lysed following a 24-hour exposure to Monacolin X at its IC₅₀ concentration. Following centrifugation, the supernatant was treated with PBS (phosphate buffer) that contained hydrogen peroxide, sodium azide, and GSH. Trichloroacetic acid was used to stop the reaction, and spectrophotometric analysis was used to determine how much GSH remained. Units per mg of protein were used to express GPx activity [10].

2.3.4. Glutathione S-Transferase (GST) Activity Assay

The supernatant was then obtained by centrifugation. After the cell supernatant was collected and added to the reaction mixture including phosphate buffer, reduced glutathione, and CDNB, the absorbance rise was measured at 340 nm. Micromoles of CDNB-GSH conjugation produced per minute per mg of protein were determined and expressed the GST activity [11].

2.4. Statistical analysis

All the results were presented as means ± standard error mean (SEM) from triplicate experiments performed in a parallel manner. This current study employed statistical analysis with unpaired testing for intergroup comparisons.

3. Result

The amount of ROS (reactive oxygen species) produced by the cells is directly connected to the fluorescence level. The measurement of (ROS) reactive oxygen species in liver cancer cells exposed to monacolin X at a dose of 72.4 μM is shown in Fig. 1.

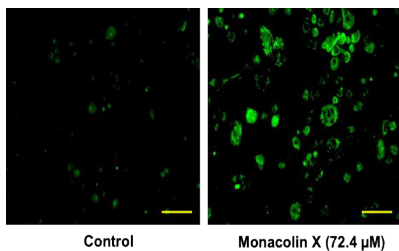


Fig.1. DCFH-DA staining of HepG2 cells treated with Monacolin X for 24 hr. (Scale bar = 50μm)

SOD, CAT, GPx and GST activity was evaluated in HepG2 liver cells following the treatment with Monacolin X at its IC₅₀ concentration (72.4 μM) for 24 h and compared with untreated control cells. As shown in the fig. 2, control HepG2 liver cells exhibited a significantly level of SOD activity, reflecting an intact cellular antioxidant defense

system. In contrast, Monacolin X-treated cells showed a marked slightly reduction in SOD activity. The other antioxidant activities of CAT (fig.3), GPx (fig.4) and GST (fig.5) are significantly decreased while compared to control liver cancer cells. As evidenced by the decreased rate of H₂O₂ decomposition. Quantitative analysis revealed that CAT, GPx and GST activity was significantly diminished in the treated group compared to the control (**p < 0.01). This reduction indicates an impaired antioxidant capacity in response to Monacolin X exposure. The observed suppression of CAT, GPx and GST activity suggests that Monacolin X induces oxidative stress in HepG2 cells, potentially by disrupting redox homeostasis and weakening enzymatic antioxidant defenses, thereby contributing to its anticancer effect [5-7].

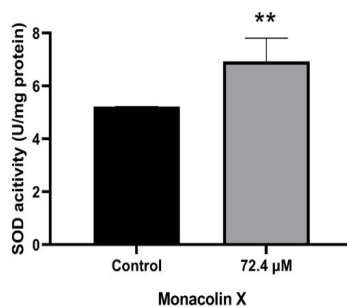


Fig. 2. Activities of SOD in HepG2 cells treated with Monacolin X for 24 hr. Statistical significance of experiment was determined using one-way ANOVA test (p < 0.01).

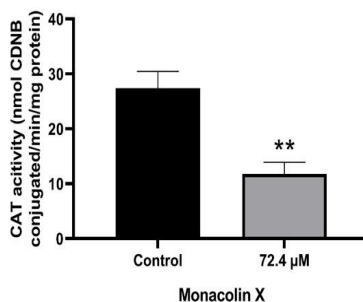


Fig. 3. Activities of CAT in HepG2 cells treated with Monacolin X for 24 hr. Statistical significance was determined using one-way ANOVA test (p < 0.01)

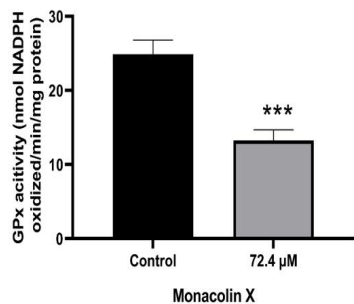


Fig.4. Activities of GPx in HepG2 liver cells treated with Monacolin X for 24 hr. Statistical significance of experiment was determined using one-way ANOVA test ($p < 0.01$).

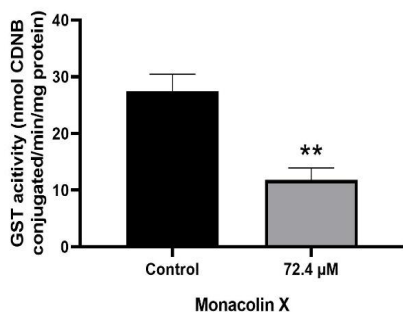


Fig.5. Activities of GST in HepG2 cells administrated with IC_{50} concentrations of Monacolin X for 24 hr. Statistical significance was determined using one-way ANOVA test ($p < 0.01$).

4. Discussion

Elevated basal levels of the free radical, reactive oxygen species (ROS) and a corresponding activation of antioxidant defense systems that promote tumor survival and proliferation are characteristics of various cancer cells, including human hepatocellular carcinoma [12]. One successful anticancer tactic is to target this redox adaptation. The beneficial properties of Monacolin X on important antioxidant enzymes including SOD, CAT, GPx, and GST which were assessed in HepG2 liver cancer cells in the present study, explaining into its potential for redox modulation [11]. In comparison to untreated control cells, our obtained results showed that Monacolin X treatment at the dose (72.4 μ M) for 24 hours caused a discernible decrease in SOD activity [7]. By accelerating the conversion of superoxide radicals into hydrogen peroxide (H_2O_2), SOD acts as the major defense mechanism against oxidative stress. Even while the decrease in SOD activity was slight, it might be enough to encourage the buildup of superoxide and start an oxidative imbalance in HepG2 cells. Downstream antioxidant enzymes showed more noticeable

effects [13]. After receiving Monacolin X, there was a significant decrease in CAT, GPx, and GST activity (** $p < 0.01$). Because catalase is important for detoxifying hydrogen peroxide, its decreased activity, as reported by a slower rate of H_2O_2 breakdown, indicates intracellular hydrogen peroxide buildup [14]. The cell's potential to neutralize peroxides and electrophilic intermediates is restricted when GPx and GST are suppressed together, further impairing glutathione-dependent detoxification pathways. Redox equilibrium is disrupted by the coordinated downregulation of CAT, GPx, and GST, which suggests a significant weakening of the cellular antioxidant defense network [15]. Growth inhibition and cell death are likely to result from such impairment, which will increase oxidative damage to cellular macromolecules. Monacolin X's capacity to inhibit these enzymes highlights its promise as a redox-based anticancer treatment because cancer cells mainly depend on antioxidant systems to combat ROS produced by increased metabolic activity.

Conclusion

Overall, conclusion from this study, it is observed that the reduction in antioxidant enzyme activity implies that Monacolin X attenuates enzymatic antioxidant defenses to cause oxidative stress in HepG2 cells, which may be a key component of its anticancer action.

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