INVESTIGATION CARDIOTOXICITY AND CARDIOPROTECTION OF (-)-EPICATECHIN IN GREEN TEA VIA THE ROLE OF ERK1/2.

*Huỳnh Thị Hồng Đào**

Title: *Nghiên cứu tính chất bảo vệ tim mạch và khả năng gây độc của (-)-Epicatechin trên tế bào cơ tim chuột H9c2 thông qua vai trò của ERK1/2.*

Từ khóa: *flavonoids, catechin, (-)-Epicatechin, bảo vệ tim mạch, độc tính trên tim tế bào H9c2, extracellular signal-regulated kinases, con đường tín hiệu protein kinase ERK1/2.*

Keywords: *Flavonoids, catechin, (-)-Epicatechin, cardioprotection, cardiotoxicity, H9c2 cell lines, differentiated H9c2 cells, extracellular signal-regulated kinases, ERK1/2.*

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TÓM TẮT

Bệnh tim mạch được Tổ chức Y tế Thế giới nhận định là một trong những nguyên nhân gây tử vong hàng đầu hiện nay nếu không được phát hiện và điều trị kịp thời. Flavoids chứa trong các thực phẩm hằng ngày có liên quan chặt chẽ đến sự điều hoà huyết áp của cơ thể và giảm các nguy cơ về tim mạch. Trong nghiên cứu này, (-)-Epicatechin – một dạng flavonoid trong trà xanh được khảo sát tác dụng gây độc cho tim thông qua mô hình stress do H2O2 trong khi các đặc tính bảo vệ tim được chứng minh bằng thử nghiệm MTT và lactate dehydrogenase trên tế bào cơ tim đã biệt hóa H9c2 của chuột. Kết quả cho thấy, sau 48 giờ (-)-Epicatechin có thể gây tổn thương tế bào từ 10 µM. Tuy nhiên, khi điều trị trước với 10, 30 hoặc 100 µM (-)-Epicatechin trong 24 giờ có thể bảo vệ các tế bào H9c2 đã biệt hóa chống lại sự chết tế bào do H2O2.

ABSTRACT

Cardiovascular disease is considered by the World Health Organization as a silent killer, which is the leading cause of death globally if it is not detected and treated promptly. Flavonoids contained in daily foods are closely related to the regulation of body blood pressure and the reduction of cardiovascular risks. In this paper, flavonoid (-)-epicatechin extracted from green tea was investigated for the cardiotoxic effects, through H2O2-induced oxidative stress modeling, while cardioprotective properties were demonstrated by MTT and lactate dehydrogenase assays on rat differentiated H9c2 cardio myoblasts with various time prolonged. Results show that after 48 hours (-)-Epicatechin can cause cell damage from 10 µM. However, pre-treatment with 10, 30, or 100 µM (-)-Epicatechin in 24 hours could protect differentiated H9c2 cells from H2O2-induced cell death.

1. INTRODUCTION:

Different protein kinases such as Akt/protein kinase B (Akt/PKB), Fyn Janus kinase 1 (JAK1), phosphoinositide 3-kinase (PI3K), mitogen-activated protein kinase (MAPK) and mitogen-activated protein kinase 1 (MEK1) have been shown that they are bind directly and activated by flavonoids and promote flavonoids properties in the inhibition of them to target

chemoprevention and therapy (Hou and Kumamoto, 2010; Spencer, 2007). Besides, MAPK, the best-characterized transduction protein kinases pathway that consist of RAS, RAF, MEK and ERK is believed to contribute in cardiopritection (Liu et al., 2018).

Extracellular signal-regulated kinase (ERK), also known as an Anchille's heel of the MAPK signaling modules (Liu et al., 2018), plays an intermediary role to stimulate the transduction between extracellular signals and the intracellular environment (Bueno, 2000). Currently, there are plenty of rodent myocardial modules are being generated to extend comprehension in the causal task of ERK1/2 in the heart, most of original studies have been investigating the role of ERK1/2 in cardiac protection. When the small G-protein Ras is initiated at the cell membrane of cardiomyocytes, the MAP3K and c-RAF that act as MAPKKKs signalling in the ERK pathway are recruited and activated. As a result, MEK1/2 the activation of ERK1/2 is set in motion via the Thr-Glu-Tyr dual phosphorylation (Goldsmiths and Bell-Pedersen, 2013; Shaul and Seger, 2007). Hence, the phosphorylation targets numerously at cytoplasmic and leads the translocation of ERK into the nucleus and bright about the initiation of transcription factors named Elk-1, c-FOS, p53, GATA4, and Ets1/2, that are crucial regulators of growth and proliferation for cardiomyocytes (Mutlak and Kehat, 2015).

As a central of MAPK pathway, the ERK signalling is contributed to variety of cellular functions from proliferative to apoptotic stages, remarkably the regulation of cell survival after oxidative stress, ischemia-reperfusion injury, and anthracycline exposure. Recent research has indicated that the active ERK1/2 signal is required for cardioprotective activities from IRI myocardium through anti-apoptotic action, extraordinarily H_2O_2-induced apoptosis (Xu et al., 2014, Daubney, 2015). According to Chinese researchers, ERK signalling is involved in a dependent pathway against oxidative stress caused by H_2O_2 declination, which benefits for anti-apoptotic mechanism of isorhamnetin (a flanovol drived from Hippophae rhamnoides L.) in the H9c2 cell lines protection (Sun et al., 2012). Although several previous research suggested that ERK cascade could partially irritate apoptosis and protect the cardiac myoblasts, there is no conclusive in vivo data demonstarting the crucial operation for this cascade when (-)-Epicatechin is presented.

The role of the ERK cascade has been confirmed in recent research by an inhibitor PD-98059, which would reverse the cardiac protection (Chen et al., 2017). PD-98059, a flavone derivative known as 2′-amino-3′-methoxyflavone, can block the phos-phorylation activation of MEK – which is downstream of ERK1/2 – specifically (Kim, 2006). Furthermore, PD-98059 is thought to be a powerful tool for clarifying the involvement of the MAPK signaling pathway in various biological processes (Dudley et al., 1995).

Catechins, such as (-)-Epicatechin (from green tea), were found to increase the occurrence of nonneoplastic lesions of the rat's heart in a research published by the United States National Toxicology Program

in 2016. Animal toxicological studies have also found signs of cardiotoxicity from Green Tea Catechins (Hu et al., 2018). While flavonoids such as Quercetin have been implicated in cardiotoxicity in differentiated H9c2 cell lines (Daubney, 2015), the cardiotoxic effects of (-)-Epicatechin have yet to be investigated.

Conclusion, the primary aims of this study are the assessment of cardiotoxicity induced by (-)-Epicatechin on H9c2 cells and illustration of the association of ERK in (-)-Epicatechin cardiac cytoprotection effects in diferentiated H9c2 model via MEK1 inhibitor factor - PD-98059. As a result, (-)-Epicatechin's cardiac cytoprotective efficacy against oxidative cell death from hydrogen peroxide exposure in the oxidative stress paradigm was highlighted.

2. LITERATURE REVIEW

2.1. (-)-Epicatechin

(-)-Epicatechin is the least plentiful catechin purified from green tea (6%) (Reygaert, 2018). However, many studies revealed that (-)-Epicatechin is abundant in variety of fruits such as grapes, apples, berries, cherries, broad beans, pears, and cocoa. According to numerous research, (-)-Epicatechin have been demonstrated its anti-inflammatory functions by iproducing less effective activation of the NF-κB signalling pathway in different tissues in both vivo and vitro. Green tea's antioxidant properties are aided by the presence of phenolic hydroxyl groups on the B-ring in (-)-Epicatechin (Mak, 2012).

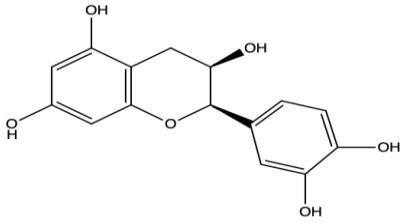


Figure 1: Chemical structure of (-)-Epicatechin (3,3', 4',5,7-pentahydroxyflavan.

2.2. Cardioprotective actions of (-)-Epicatechin

Previous studies revealed that (-)-Epicatechin, and its derivatives have the possibilities to induce cardioprotective effect on different investigation models. Scientist illustrated that (-)-Epicatechin contributes a significant reduction in infarct size of I/R injury due to its accompaniment in activation of the reperfusion injury salvage kinase (RISK) pathway such as Akt and ERK proteins. Although it is still unclear and unrelated evidence between (-)-Epicatechin Akt or ERK activation mechanism, research demonstrated that in permanent coronary occlusion models (-)-Epicatechin can confer its cardioprotective activities. Since 2010, researchers have been looking into the activation of the delta-opioid receptor and the related kinase signaling cascade as potential cardioprotective targets for (-)-Epicatechin (Testai, 2015). In recent studies, it has been discovered that the phosphatase and tensing homolog (PTEN)/phosphoinositide 3kinase (PI3K)/protein kinase B (AKT) signaling pathway is involved in the inhibition of myocardial ischemia-induced mouse cardiac injury and that (-)-Epicatechin acts

as a protective shield for cells against myocardial ischemia (Li, 2018).

(-)-Epicatechin has been studied for its possible cardioprotective qualities to protect the heart from ischemia damage as a pre-treatment. In 2014, several novel results on the cardioprotective effects of (-)-Epicatechin emerged that broaden our understanding in two ways. The first method is to give (-)-Epicatechin in a way that is appropriate for therapeutic usage after myocardial ischemia. Meanwhile, the second method is a novel mechanistic inquiry of the capabilities of (-)-Epicatechin to protect the heart by stimulating mitochondrial pyruvate transportation or oxidation via the NOS/sGC cascade. Pre-treating 1 mg/kg/day (-)-Epicatechin minimized MI size and had sustained 3-week results in a rat model of permanent coronary occlusion and IR. Additionally, a 15-minute pre-treatment with a 10 mg/kg IV dose of (-)-Epicatechin prior to reperfusion reduces the magnitude of the MI (Yamazaki et al., 2014). Dark chocolate consumption of (-)-Epicatechin has been linked to a lower risk of ischemic heart disease in epidemiological studies (Janszky et al., 2009).

In combinate treatment, (-)-Epicatechin indicated a potential pathway for efficient treatment of ischemic injury through lowering infarct tumor volume in a model of ischemia hypoperfusion injury by blocking mitochondrial swelling when it is co-administrated with Doxycycline (Ortiz-Vilchis, 2014). The advantageous cardioprotective effects of co-treatment between (-)-Epicatechin and procyanidin B2 may be due to the uncoupling of oxidation from phosphorylation, activation of phosphorylation at lower concentrations and inhibition of the respiratory chain at higher concentrations (Kopustinskiene, 2015).

2.3. ERK1/2 signalling pathway associated with cardioprotective mechanism:

Extracellular signal-regulated kinases (ERK), which has known as the first characterised MAP kinase cascade, involved significantly in cell growth, proliferation, differentiation in response to growth factor - mitogens, and survival of cells. Whilst, there are at least five different types of ERK in mammalian myoblasts which are marked from ERK1 to 5. ERK1 and ERK2 are typifing because of their higher particular studies and more abudant expressions compared to other ERK kinases (Bueno, 2002). The potential of ERK activation in cell death contribution has been demonstrated in numerous in vitro studies in neuronal cells, human medullary thyroid cancer cells, and keratinocytes. Moreover, several researchers have revealed that ERK1/2 plays an essential element for protection of ischaemic myocardium as well as the involvement of cardiac hypertrophy (Germack and Dickenson, 2004). Various flavonoids have been investigated for their potential in cardioprotection via the ERK signaling pathway. Rutin, a flavonoid, has been shown to reduce coronary heart disease in a porcine model by activating the ERK1/2 pathway (Ly et al 2018). Additionally, there are many convincing evidence about cardio protection mediated by flavonoids through ERK1/2 signalling pathways. For example, the total flavonoid found in Dracocephalum moldavica L had been illustrated its beneficial effect by

reducing the risk of myocardial ischemia reperfusion injury via ERK1/2 signalling pathways (Zeng at al., 2018).

3. METHODS:

The embryonic cardiomyoblast cell line H9c2 will be differentiated in cultivation to be the consist of elongated and multinucleated final forms and used throughout mostly majority of (-)-Epicatechin investigation associated with following experiments:

3.1. Cell culture:

To acquire and preserve H9c2 cells, the growth medium consisting of Dulbecco's Modified Eagle's Medium (DMEM) at 2mM , Foetal Bovine Serum 10% (v/v) (FBS), L-glutamine and antibiotics GIBCO including penicillin and streptomycin at same concentration 100 µg/ml would be applied. Next, at 37°C with 95% air/5% CO2 in humidified incubator, cells were conserved and subcultured (1:5 seperated ratio). After 7 days cultured in DMEM with 1% FBS (v/v) and 10 nM all-trans RA, the cells would be differentiated into a more likely cardiomyoblast phenotype.

3.2. Cardiotoxicity of (-)-Epicatechin:

To observe (-)-Epicatechin-induced cardiotoxic activity, different concentrations of (-)-Epicatechin at 3, 10, 30 and 100 µM were prepared to treat the differentiated H9c2 cells for 24, 48 and 72 hours.

3.3. H_2O_2-induced Oxidative Stress:

The cells were given 24 hours of completely supplemented DMEM. To get MTT and LDH curve responses, appropriate doses of H_2O_2 ranging from 200 µM to 1 mM were applied to the relevant wells. Differentiative H9c2 will be treated with (-)-Epicatechin at 3, 10, 30, and 100 µM for 24 hours before being exposed to 600 µM H_2O_2 in DMEM and the cardiovascular protection will be assessed using cell viability assay.

3.4. Investigating the role of ERK in (-)-Epicatechin-induced cardioprotection:

H9c2 cells were pre-treated 30 minutes with 50 M PD98059 before being treated with 30 M (-)-Epicatechin and 600 µM H_2O_2, and the investigation of cardioprotection would then be processed by measuring the features of apotosis using MTT and LDH assays to see how ERK1/2 plays a basal role in protecting cardiac myoblasts with (-)-Epicatechin effects. With the form of lyophilized powder in Dimethyl sulfoxide (DMSO) supplement, PD 98059 was being used as a scientific resource. The media from all wells would be removed after 24 hours of treatment with PD 98059, and 300 l of fresh water will be transfed to the control, PD 98059, and (-)-Epicatechin wells. After that, 300 µM of H_2O_2 solution would be introduced to wells that had been exposed to 600 µM H_2O_2 (Figure 2).

3.4.1. MTT reduction assay:

MTT assay predicated on MTT metabolism (3- (4,5-dimethylthiazol-2-yl)-2,5 diphenyl tetrazolium bromide) by respiratory enzyme in mitochondria into dark blue MTT-formazan product. The concentration of formazan, measured as the absorbance of solution at 570 nm (16 Aslantürk, 2018), is inversely proportional to the quantity of viable cells. This cell viability experiment was performed on 24-well flat-bottomed plates. Prior to 7-day differentiation, approximately 15,000 undifferentiated H9c2 cells were plated and grown in each plate with completely DMEM supplement

for 24 hours. After that, each well would be filled with 5 mg/ml MTT and incubated for 1 hour before being replaced with DMSO media, which dissolves formazan crystals.

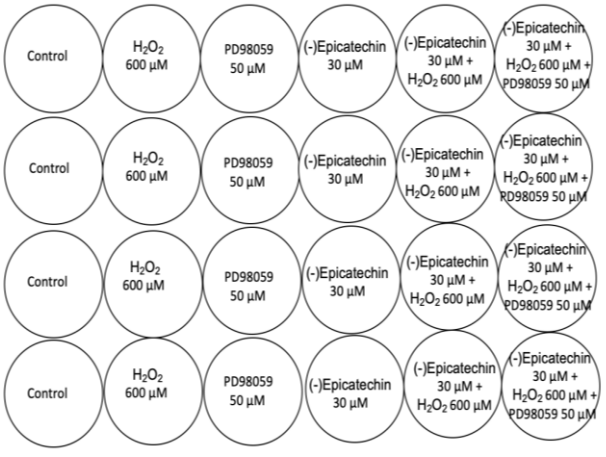


Figure 2. The way each well of 24-template were treated in MTT assay for (-)-Epicatechin-induced cardiac protection with the involvement of ERK1/2 signaling cascade inhibitor.

3.4.2. Lactate dehydrogenase assay:

Quantification of lactate dehydrogenase release, which is described as one of the indirect ways to detect cardiomyoblast necrosis, was used to assess the quantitative evaluation of cell viability and late-stage apoptosis. In this experiment, 96-well flat-bottomed plates were utilized. 5000 cells were grown and differentiated in each well in almost the same way that MTT one was. The Cyto Tox 96 non-radioactive cytotoxicity test was used to colorimetrically measure the amount of LDH released in cultured media, which was monitored at 490 nm.

3.5. Statistical analysis:

Unless otherwise stated, all data was expressed as mean S.E.M. Graph Pad Prism 8.4.3 program may use one-way analysis of variance (ANOVA) to assess the data if the p0.05 is regarded significant.

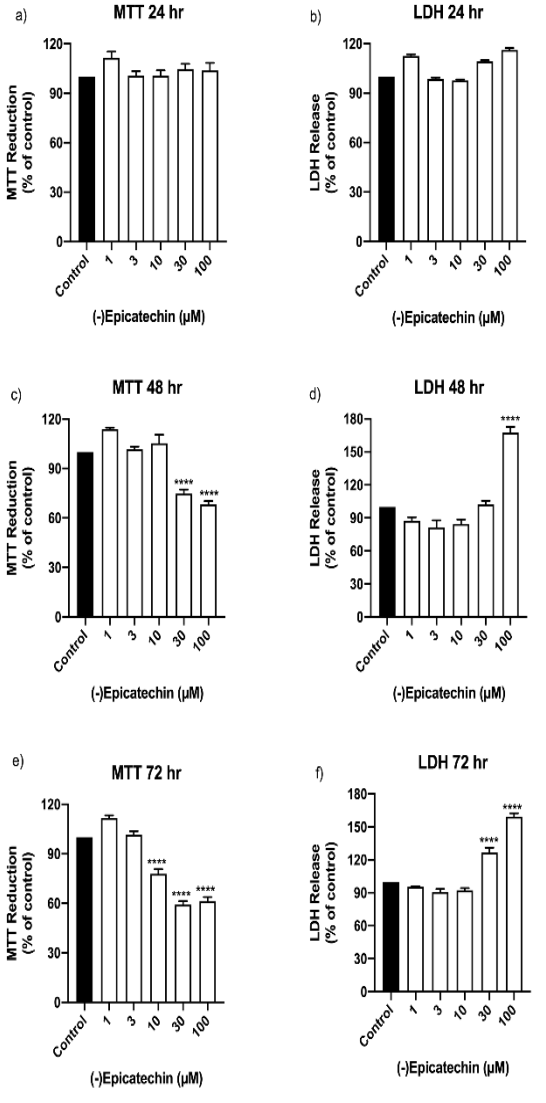
Briefly, all experiments were applicable to assess the cytotoxic and cytoprotective mechanisms of (-)-Epicatechin via the ERK1/2 pathway on differentiated cells.

4. RESULTS:

4.1. (-)-Epicatechin-induced cardiotoxicity:

The data analysis of MTT reduction and LDH release of differentiated H9c2 cell exposed to (-)-Epicatechin for 24, 48 and 72 hours were shown in Figure 3. There is no significant influence on cell viability after 24 h of treatment to (-)-Epicatechin (1-100 M) in figs. 3a and 3b. Nevertheless, 48 hours of exposure to (-)-Epicatechin resulted in a significant decrease in MTT reduction at 30 and 100 µM concentrations (fig. 3c), as well as a dramatically increase in LDH release at 100 µM. (fig. 3d). Furthermore, 10 M (-)-Epicatechin showed considerable cardiotoxic activity in the suppression of MTT decrease when the exposure time was raised to 72 hours (fig. 3e). Simultaneously, at 30 µM (-)-Epicatechin, LDH release was remarkable higher than at lower exposure times (fig. 3f). Overall, the downward trend in MTT reduction and the increasing trend in LDH release in this experiment indicate that low levels of (-)-Epicatechin from 10 µM may cause cardiovascular risk when exposed for extended periods of time.

Figure 3. The effect of varying doses of (-)-Epicatechin on cell viability in differentiated H9c2 cells (-)-Epicatechin therapy was studied using MTT and LDH



assays over a period of 24 to 72 hours. The metabolic decrease of MTT is shown in panels a), c), and e), while the release of LDH is shown in panels b), d), and f) during the requisite incubation time. The results are expressed as a percentage of control cells (100%) and represent the mean ± S.E.M. of quadruplicate MTT and sextuplicate LDH dependent tests. **** $p < 0.0001$ as compared to the control response.

4.2. (-)-Epicatechin-induced cardioprotection through recovery against H_2O_2:

The effect of (-)-Epicatechin on cardiomyoblast protection was investigated using an H_2O_2-induced cell death paradigm, and cell viability was assessed using MTT reduction and LDH release. A 2-hour exposure from 200 to 1000 μM H_2O_2 was used in this work to examine the ability of H_2O_2 oxidative stress-provoked apoptosis in H9c2 cells. The decline in MTT reduction from 400 μM H_2O_2 (fig. 4a) and the increase in LDH release from 600 μM H_2O_2 (fig. 4b) indicates that hydrogen peroxide triggers cardiac cell damage starting at 400 μM. In contrast, the MTT decrease and LDH release results from the H_2O_2-induced cell damage experiment with 24-hour (-)-Epicatechin pre-treatment were very inconsistent. As indicated in fig. 4c, the presence of (-)-Epicatechin at concentrations of 10, 30, and 100 μM could irritate MTT reduction inhibition and reduce the harmful effects of H_2O_2. Likewise, the statistically significant decrease in LDH release (fig. 4d) after pre-treatment with (-)-Epicatechin from 10 to 100 μM demonstrates that (-)-Epicatechin inhibits H_2O_2. Conclusion, this could explain (-)-Epicatechin's cardioprotective activityagainst H_2O_2-induced oxidative stress.

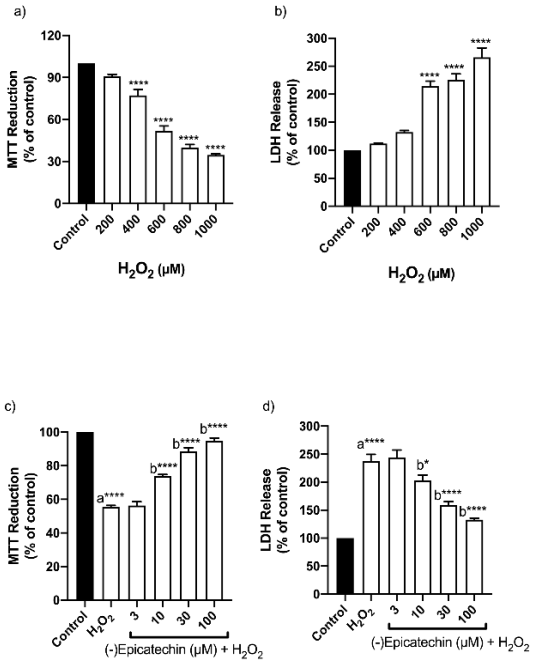


Figure 4. The comparison of cell viability measured by MTT and LDH assay on differentiated H9c2 cells with and without the present of (-)-Epicatechin in H_2O_2-induced apoptosis investigation. MTT decrease and LDH release results of differentiated H9c2 cardiomyoblasts were treated with various concentrations (200-1000 μM) of c for 2 hours in panels a) and b), respectively, with **** $p < 0.0001$ against control response. Meanwhile, the MTT reduction and LDH release data of cells that were exposed to 3, 10, 30, and 100 μM (-)-Epicatechin prior to being treated with 600 μM H_2O_2 for 24 hours are shown in panels c) and d), with * $p < 0.05$, **** $p < 0.0001$ and a and b, respectively, the comparison versus control and H_2O_2 response. All data are expressed as percentage of control cells (100%) and represent the mean ± S.E.M of quadruplicate MTT and sextuplicate LDH tests performed in quadruplicate.

4.3. Effect of (-)-Epicatechin on oxidative stress-induced via ERK1/2 activation:

In both the MTT and LDH assays, there were multiple significant results, as shown in Fig. 5. In the H_2O_2 group, treatment demonstrates that cells exposed to the H_2O_2 solution have a 50% MTT reduction and a 250% LDH release when compared to the model control group. As a result, H_2O_2 has a high proclivity for cell death. In the meantime, there was no discernible difference between the PD98059 (50 μM) and (-)-Epicatechin groups and the control group. In comparison to the H_2O_2 group, the results of the (-)-Epicatechin plus H_2O_2 group were significant, with a little decrease in MTT reduction and an increase in LDH release, revealing (-)-Epicatechin's protective ability for cardiac H9c2 cell lines. When comparing the combination group of (-)-Epicatechin, H_2O_2 with and without PD 98059, the group with PD 98059 showed a significant MTT reduction and LDH release trend, demonstrating that the presence of PD98059 causes a decline in (-)-Epicatechin cardioprotective effect against H_2O_2.

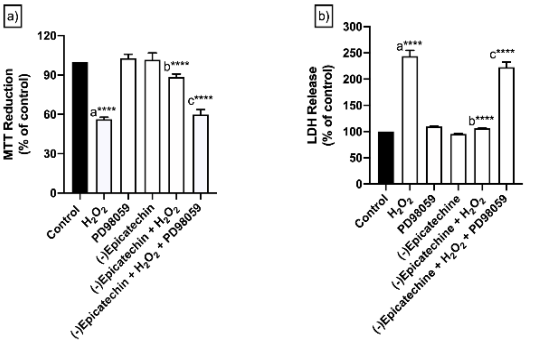


Figure 5. PD98059 inhibits (-)-Epicatechin-induced activation of ERK1/2 kinase. Differentiated cells were pre-treated

for 30 minutes with 50 μM PD 98059 (MEK1/2 inhibitor) before being treated for 24 hours with 30 μM (-)-Epicatechin in the presence of PD 98059. The MTT metabolic decrease (Fig. 5a) and LDH release (Fig. 5b) measurements were used to determine cell viability. The data are expressed as a percentage of control cells (100%) and represent the mean ± S.E.M. of quadruplicate MTT and sextuplicate LDH dependent studies.

a **** p <0.0001 compared to control cells,

b **** p < 0.0001 compared to the H_2O_2 response column, c **** p <0.0001 compared to the response of cells treated with (-)-Epicatechin and H_2O_2.

5. DISCUSSION:

5.1. (-)-Epicatechin prolonged exposure and cardiotoxic effect:

Although flavonoids have become beneficial cardiovascular preventive dietary supplements as science and medicine have progressed, their cardiotoxicity effect is still unknown, and the evaluation of (-)-Epicatechin-induce cardiac toxic is an unique concern. In this study, we looked at the possibility of (-)-Epicatechin having a negative effect on the heart in H9c2 cell lines. It is obvious that 24-hour exposure to (-)-Epicatechin, even at the highest concentration of 100 μM, had no effect on differentiated H9c2 cells. However, when H9c2 cells were exposed to (-)-Epicatechin for 48 hours, the substantial inhibition of MTT decrease at 30 and 100 μM, as well as the rise in LDH release at 100 μM, were evidence results demonstrating the cytotoxic action of (-)-Epicatechin. After 72 hours, (-)-Epicatechin-induced cardiotoxicity was more noticeable, with MTT decrease from 10 μM and increased LDH release at 30 μM. Furthermore, as compared to the LDH assay, the MTT assay has a higher effective sensitivity in cardiotoxic detection. Our findings provide the first evidence of (-)-Epicatechin-induced cytotoxicity in differentiated H9c2 cells when (-)-Epicatechin-enriched dietary supplements were given to the cells for 48 hours and, notably, at a concentration of 10 μM in the plasma. Even though this cardiotoxicity is consistent with a similar model study on another flavonoid – Quercetin, which found that prolonged quercetin treatment resulted in the generation of ROS and pro-oxidative damage as part of the mechanism of quercetin-induced cytotoxicity (Daubney, 2015), more future experiments on other experimental models are needed to draw a firm conclusion about (-)-Epicatechin-induced cardiotoxicity. To obtain additional evidence about cardiotoxic concentrations, the safety of (-)-Epicatechin long-term administration has to be explored in extensive areas and large clinical trial designs. Finally, this research tends to boost scientists' consciousness of the cardiotoxicity of not only (-)-Epicatechin, but also other catechins and flavonoids.

5.2. (-)-Epicatechin-induced cardiac protective effect:

In order to investigate the anti-apoptosis capacity of (-)-Epicatechin, we used hydrogen peroxide as a cell death

template.. When H9c2 cells were exposured to H_2O_2, the apoptosis measurements are observed by the inhibition of MTT reduction decreased significantly from the concentration of 400 μM and the LDH release increased dramatically from 600 μM H_2O_2. These findings are in line with prior research that found that inducing H_2O_2 causes oxidative stress, which leads to cardiomyocyte apoptosis via the intrinsic apoptotic pathway. Pretreatment with (-)-Epicatechin for 24 hours, on the other hand, significantly reduced H_2O_2-induced oxidative damage. (-)-Epicatechin reduced H_2O_2-induced apoptosis by dramatically inhibiting MTT decrease and LDH release starting at a dose of 10 μM. With a higher confidence P-value, the data show that the MTT assay is more sensitive than the LDH in cytotoxic detection. Previous research has shown that (-)-Epicatechin reduces the risk of cardiovascular disease and cardiomyocyte hypertrophy not only by increasing flow-mediated endothelium dilation and decreasing platelet aggregation, but also by lowering oxidative stress and improving mitochondrial structure and function. At 5 μM for 1 hour, (-)-Epicatechin has been shown to prevent myocardial ischemia-induced cardiac damage in mice (Drouin et al., 2011; Li et al., 2018). Recently, (-)-Epicatechin derived from cacao was shown to protect the heart from I/R damage, which is triggered by numerous kinase signaling pathways, including Akt and ERK (Hausenloy and Yellon, 2007). In different species, it has also been found that the most abundant Catechin, Epigallocatechin-3-gallate (EGCG), helps to the protection of cardiac injury via involving particular protein kinase activation. Noticeably, EGCG is stimulated to prevent and protect cardiac cells when Angiotensin II-induced activation of ERK1/2 is inhibited by pretreatment (Sun-mi et al, 2006). This is the first study to show that (-)-Epicatechin has cardioprotective properties by reducing H_2O_2-induced cell death in differentiated H9c2 cardiomyocytes. Overall, the findings revealed that (-)-Epicatechin caused significant cardioprotection and could be a viable anti-apoptosis reagent in the future.

5.3. The role of ERK1/2 in (-)-Epicatechin-induced cardioprotection:

In cardioprotective research, (-)-Epicatechin was suggested as a possible molecular mechanism. The ERK signaling system, which could be a therapy target for anti-apoptosis effects contributes the ability in cell apoptosis. As a result, a particular ERK kinase inhibitor – PD98059 – was used in this experiment to determine the role of ERK signaling in H_2O_2-induced cell viability reduction. PD98059 inhibited (-)-Epicatechin's cardiac protective effect against H_2O_2-induced cell death (600 μM, 2 hours). The comparable results, as shown in Fig. 5, not only confirmed that (-)-Epicatechin effectively protects differentiated H9c2 cells against H_2O_2-induced apoptosis, but also revealed that the presence of PD98059 in the combined assessment with (-)-Epicatechin and H_2O_2 significantly decreased MTT reduction and increased LDH release. Interestingly, other

flavonoids such as Isorhamnetin and Quercetin have been shown to have the ability to reduce H_2O_2-induced mitochondrial-dependent apoptosis in H9c2 cardiomyoblasts via ERK offset (Daubney, 2015). These findings are consistent with the findings of the current investigation, which show that ERK1/2 signaling inhibits (-)-Epicatechin's cardiac action against H_2O_2-induced cell death. Noteworthy, because it reduces protein kinase activation produced by H_2O_2 mediator, (-)-Epicatechin may be an inhibitor of ERK1/2. Furthermore, the inhibition of ERK1/2 by (-)-Epicatechin has been shown to protect various body organs such as auditory cells, HepG2 cells, and... (Granado-Serrano et al., 2010; Lee et al., 2010). This is the first study to indicate that cardioprotective mechanism of (-)-Epicatechin is derived from its anti-apoptotic activity mediated by the ERK1/2 signaling pathway. Furthermore, Schroeter et al. demonstrated in 2007 that green tea containing (-)-Epicatechin at a concentration of 300 nmol/L can rapidly promote not only ERK signaling but also Akt phosphorylation and CREB phosphorylation (Schroeter et al., 2007).

Another green tea catechin, (-)-epicatechin-3-gallate (ECG), has been shown to protect keratinocytes from H_2O_2-induced oxidative stress by inhibiting ERK1/2, while EGCG has been suggested to protect against oxidative stress by inducing expression of representative antioxidant enzymes like ERK1/2 signaling (Huang et al., 2007). Through the activation of ERK3, 4, and 5, (-)-Epicatechin may elicit cardio protection and cardiotoxicity. Therefore, more studies into the other ERK cascades are required in the future to find (-)-Epicatechin cardiac protective benefits.

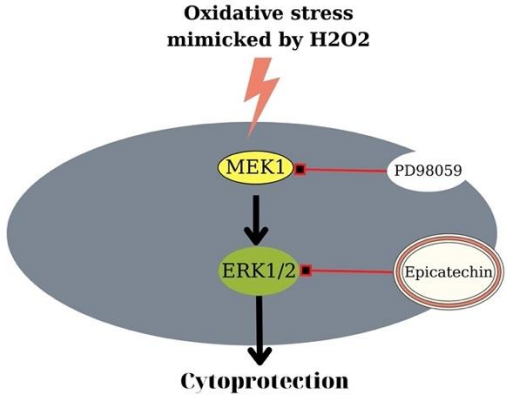


Figure 6. Mechanism of Epicatechin-induced-cardioprotection.

6. CONCLUSIONS

In conclusion, our findings support various considerations of (-)-Epicatechin as a cardioprotective agent in dietary supplement therapy. Although further research is needed to determine the cardiotoxicity and cardioprotective potential of (-)-Epicatechin via multiple kinase pathways inhibition, it is suggested in this study that differentiated H9c2 cardiomyocytes may lose cell viability after 72 hours of (-)-Epicatechin exposure, and that differentiated cell death triggered by H_2O_2 may be prevented by (-)-Epicatechin via the ERK signaling pathway.

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