REVIEW ARTICLE

Apoptosis: A Potential target site for natural bioactive agents during myocardial infarction

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ABSTRACT

Myocardial infarction (MI) is an insidious disease, gently spreading in developed and developing countries. MI is the consequence of hypoxia to myocardial tissue which may leads to apoptosis, narcosis and followed by cardiac cell death. Activation of apoptotic pathways during myocardial infarction (MI) is frequently reported in clinical, preclinical and post-mortem studies. Several apoptosis inducing factors and signalling cascades leading to death of cardiomyocytes culminate into MI. Such involvement of ischemia induced apoptosis in MI is widely accepted. Apoptosis is a natural process for regulating the homeostasis in cellular organelles. Unlike the necrosis, it is a synchronize energy dependent process which is carried out by shrinkage of the cell. This contraction of cell leads to squeezing of nucleus and nuclear chromatin into brusquely demarcated masses. However, such programmed cell death in several tissues including myocardium becomes pathogenic under certain conditions. In-addition, reactive oxygen species (ROS) and generated oxidative stress is also play a key role in production of apoptosis and several associated signalling alteration which ultimately leads to MI. Recently, certain natural products especially from the plant kingdom have been evaluated for their anti-apoptotic potential. There is an up rise in the investigations delineating the exact mechanisms through which natural phytochemicals target MI associated apoptosis. This review explores novel signalling pathways and target sites for anti-apoptotic phytochemicals having potential to check the cellular apoptosis consequent to MI. A new vista may explore in the prospective treatment of MI by using apoptosis-modulating natural products.

Keywords: Reactive oxidative stress, Caspase, Apoptosis, Myocardial infarction, Phytochemicals, Herbal plants

INTRODUCTION

Myocardial infarction (MI) is a major end result of morbidity and mortality in developed and developing countries. Myocardial ischemia is a leading cause of MI. Although, reperfusion of blood after prolonged ischemia to heart cells is necessary for cardio protection, but it additionally causes the myocardial cell damage [1]. In-addition, myocardial ischemia-reperfusion injury significantly generates ROS due to anaerobic oxidation followed by oxidative stress. ROS generated oxidative stress doubles the risk of ischemia and reperfusion injury [2]. A number of imperative studies demonstrate both ischemia/reperfusion and oxidative stress are the primary factor for generation of apoptosis which consequently leads to MI. Clinical and preclinical studies revealed the high level of ROS significantly induced MI through apoptosis [2-3]. Furthermore, I/R injury is an individual platform for apoptosis with or with generation of ROS, followed by MI [3-4]. Apoptosis is a genetically operated process of self-destruction of cells through the crumbling of DNA. It significantly differs from cellular necrosis which is premature cell death resulting from an exposure to external factors such as infection, toxins or trauma. Necrosis always leads to detrimental effects on surrounding cells. Whereas, apoptosis is a normal physiological process that eliminates DNA-damaged, or unwanted cells. Both necrosis and apoptosis cause cell death; they differ from each other in various morphological and cellular dictatorial characters. Necrosis is a process of rapid cellular swelling due to accumulation of fluid and electrolytes, premature burst of plasma membrane and distraction of cellular organelles resulting in to brisk loss of cellular homeostasis. Necrosis associated rupture and consequent leakage of broad array of cellular material through membrane culminates into the inflammation [5-6].

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In contrast to necrosis, programmed cell death or apoptosis is an energy dependent and abundantly synchronized process. Apoptosis is carried out through the breaks up of cell after shrinkage of and formation of brusquely demarcated masses of the nucleus and nuclear chromatin squeezed together. Following this, cells become isolated from the surrounding tissues. The extensions buds come out from membrane and that ultimately close off to form membrane enclosed vesicles, known apoptotic bodies. These enclosed vesicles are moreover phagocytosed by adjacent cells or endure deprivation, which seems to be the process of necrosis, entitled as secondary necrosis. Usually apoptosis does not prompt an inflammatory response as compared to necrosis [7]. Apoptosis may be activated by a stimulus or deletion of a suppressing stimulus.

Impaired supply of oxygen and blood flow to the heart initiates a pathologic condition called 'ischemic state' which initiates cascades of destructive events. Earlier, it was postulated that myocardial cells in the region of obstructed coronary artery undergo necrosis and can't regenerate. However, recent findings indicate apoptosis as another form of myocardial cell death playing an important role in the tissue destruction consequent to myocardial infarction (MI) [8]. Evidence suggests that a compartment of myocytes undergoes apoptosis during ischemia-reperfusion injury in addition to overt necrosis which was considered as a root cause of cell death in MI [4]. Thus, apoptosis may permanently damage the myocardial cells and lead to potential life-threatening arrhythmias consequently creating ventricular aneurysm. In addition to such damage, apoptosis in MI may trigger inflammation induced atherosclerotic plaque formation [9-10]. Hence, an increase in the plasma levels of C-reactive protein (CRP), a specific biomarker for inflammation, predicts the risk of MI [10-11].

Calcium deposition is another root cause of atherosclerotic plaque generation. Calcium depositions can be certainly detected by modern devices, therefore, may be an alternative predictive source in preference to classical risk factors [12-14]. Another presentation allied with untimely atherosclerosis is high level of homocysteine (non-protein amino acid) in blood and resulting homocysteinuria [15]. However, predictive significance of homocysteinemia/ homecysteinuria is debated [16]. Data suggest that oxidative stress and disproportionate nitric oxide generation play extensive roles in ischemia/reperfusion injury that derange the cardiac functions [17]. It has been well described that medicinal plants have potent anti-apoptotic property against experimentally induced MI in animal models both in vivo and ex-vivo. Numerous studies on natural products and herbal compounds delineate the molecular pathways involved in apoptosis associated MI [18]. This review lays emphasis on some potential signalling pathways and target sites for anti-apoptotic herbal compounds showing efficacy against experimentally induced MI in preclinical studies. Moreover, review also encapsulates the catalogue of herbal compounds having prospective anti-apoptotic action.

Apoptosis *vs.* Myocardial Infarction

Apoptosis, or programmed cell death, is a normal consequence in which an organized series of actions leads to the cell death. Cell death by apoptosis is an orderly and neat process instigated by the inclusive shrinkage of the cell and its nucleus and that also cause instantaneous loss of adhesion to neighbouring cells. The separation of these shrunken cells forms blebs on the cell surface and this dissects the chromatin into small fractions which is ultimately engulfed by phagocytosis [19]. Apoptosis plays important biological roles in the growth and homeostasis of cell masses, and in progression of the disease processes. Extensive as well inadequate apoptosis leads to pathogenesis of wide array of diseases including ischemia, autoimmunity, neurodegeneration, and viral infections [20]. It is experimentally demonstrated that apoptosis has been

stimulated by both external and internal initiating the 'extrinsic' and 'intrinsic' pathways respectively. A number of accumulated evidences suggest that apoptosis contributes to the pathogenic alterations in cardiomyocytes [21]. Numerous clinical, subclinical and post-mortem studies have reported activation of apoptotic pathway in myocardial infarction [22-24]. Molecular magnetic resonance imaging technique demonstrates a huge apoptotic zone in the myocardium and viable myocardium in the central-myocardium early after an ischemic injury [25]. Therefore, myocardial rescue strategies after ischemic damage are the core interest in clinical approach. Several recent studies reported that I/R induce myocardial apoptosis in the ischemic myocardium in vivo [10-11, 21]. However, whether apoptosis is triggered during ischemia or during reperfusion is still controversial. Gottlieb et al. (1994) found that the hallmark of apoptosis, nucleosomal ladders of DNA fragments, was detected in ischemic myocardium after 30 min of ischemia and 4 h of reperfusion in the rabbit, but not in ischemic-only myocardium, suggesting that apoptosis may be expressed only during reperfusion [22].

Cardiac specific overexpression of α-1 adrenoceptors in mice induces cardiac hypertrophy, apoptosis, fibrosis, and heart failure, further emphasizing the role of β-adrenergic signaling in the pathogenesis of heart failure [17]. It has showed that apoptosis in adult cardiomyocytes caused by specific β-1 stimulation is mediated by a PKA-dependent pathway, and that β-1 and β-2 adrenoceptors deliver opposing signals with respect to cell-survival. Neurohormonal factors such as angiotensin II acting via the AT-1 R have been shown to produce cardiomyocyte apoptosis. Elevated circulating levels of antiotensin II and likely in situ cardiac antiotensin II production, may play an important role in this respect. It remains however, to be shown that drugs like ACE inhibitors or ARBs reduce cardiac apoptosis while improving heart failure condition in human [10-11, 17, 21-24].

Since I/R results in the reproducible induction of cardiomyocyte apoptosis, this is the model most often used to test the effect of antiapoptotic agents on cardiomyocyte survival. The following approaches were used to test the importance of different players in the pathways mediating apopotosis: (1) if reactive oxygen species (ROS) played a major role in causing apoptosis, radical scavengers or enzymes degrading ROS should reduce apoptosis. (2) Substrate deprivation as encountered in the course of ischemia and substrate withdrawal causes endoplasmic reticulam stress; if this was important in I/R or hypoxia, overexpression of molecular chaperones should diminish apoptosis. (3) Apoptosis occurs if the balance between pro-survival and pro-apoptotic pathway is tilted toward pro apoptotic pathways. If this concept applied to cardiac disease, activation of pro-survival pathways should reduce the extent of apoptosis. (4) If apoptosis contributed importantly to the cell damage inflicted during I/R and myocardial infarction, specific inhibitors of caspases should be expected to reduce apoptosis and infarct size [22-27].

Apoptosis associated cellular pathways involve the activation of Fas-associated death domain (FADD) receptor pathway on the cell surface or alteration of mitochondria into a pro-apoptotic state, respectively called extrinsic and intrinsic pathways of apoptosis. Tumor necrosis factor receptors (TNFR) are belonging to death receptor family which induce apoptosis signalling cascade. For instance TNFR1, TRAIL, or Fas can activate aspartate-specific proteases (caspases) and ultimately accomplish proteolysis followed by cell death [21, 28]. Apoptosis is also instated by a mitochondrion-dependent pathway which leads to release of cytochrome-C under the control of Bcl-2 family. Bax and Bak are other pro-apoptotic proteins similar with that of Bcl-2 which further direct to apoptosis by inducing the discharge of cytochrome-C into the cytosolic partition. Pro-apoptotic Bcl-2 family proteins interact with both pathways to potentiate

anti-apoptotic effect in ischemia-reperfusion of cardiomyocytes [28].

Role of mitochondria in apoptosis

Mitochondria are the primary site for generation of ROS which may lead to the production of apoptosis. Mitochondria have four complexes of enzymes and at least eight distinct ROS- generating-complexes. One of these eight complexes releases ROS into an inter-membrane space from where it enters into cytosol and participates in ROS associated signalling cascade. Apart from this, complex I and complex II also possess a little potential to release ROS in inter-membrane space. The reactive species generated from ROS-generating-complexes contribute to apoptosis mediated MI [29]. Therefore, ROS maintain the homeostasis of oxidative stress associated pathophysiological events. Interestingly, ROS play a versatile role in apoptosis induction. On one hand mitochondrial ROS activate cytochrome-c that triggers caspase activation and leads to apoptosis and MI [30]. However, on the other hand ROS also possess anti-apoptotic effects. Major path for the induction of apoptosis and MI depend on Fas/Fas-ligand system and mitochondrial redox state. Mitochondrial superoxide is enhanced in apoptotic cells which were generated from reduction of oxygen, specifically during cytochrome-c release from the inter-membranous space to cytosol. This electron transfer starts activation of caspases [30-31].

Another important endogenous metabolite is nitric oxide (NO), which may increase the generation of superoxide related metabolites and oxidative stress [32]. This also enhances the intracellular free calcium ion [Ca^{+2}] and activate Ca^{+2} dependent phospholipase A2 pathway, thus increasing the gateway of membrane permeability transition (MPT) pores [33]. Indeed, Ca^{+2} induces ROS production thereby increasing oxidation of mitochondrial proteins and lipids [33-34]. However, NO depolarizes the membrane potential, promotes the cytosolic Ca^{+2}, and liberates cytochrome-c, which may activate the arrangement of cellular incidents resulting in to apoptosis and MI [35-36]. Moreover, NO gets converted into peroxynitrite by reacting with ROS. Brown and Brown [37] revealed about NO reaction with mitochondrial ROS that may cause reversible inhibition of respiration, generatng super oxide and peroxynitrite, and irreversible inhibition which further leads to mitochondrial membrane permeability transition. This mitochondrial respiration inhibition may activate necrosis or apoptosis through mitochondrial membrane permeability transition activation and ultimately leads to MI [38].

Ischemia-reperfusion (I/R) induced MI *via* apoptosis

MI is a major consequence of morbidity and mortality in modern era. Myocardial ischemia is a recognized chief root cause of MI. Even, reperfusion of blood after prolonged ischemia is necessary for cardio protection but it further leads to myocardial damage. Thus, myocardial ischemia-reperfusion injury plays a significant role in MI. ROS induced oxidative stress is a primary factor for generation of both ischemia and reperfusion. Clinical and preclinical studies revealed the high level of ROS significantly induced myocardial IR [1-2, 39]. ROS cause cellular damage through several pathways including direct or indirect activation of pro-apoptotic cascades, decrease anti-oxidant enzyme and anti-apoptotic factors [2]. The deficient supply of oxygen halts oxidative phosphorylation consequently reduced mitochondrial membrane depolarization, ATP diminution, and obstruction of myocardial contractility. Subsequently, cellular metabolism triggers the anaerobic glycolysis which ultimately increase the deposition of lactate and decrease intracellular pH (to <7.0). The intracellular deposition of proton can activate Na+/H+ ionic conversion and that squeeze out protons from cell in exchange of Na+ entry. But due to deficient ATP level during ischemia, 3Na+/2K+ ATPase working become prohibited, thus intracellular Na+ has

increased. Accordingly, reverse activation of 2Na+/Ca2+ ion exchange is increased which leads to intracellular Ca2+ overloading [40-42]. Intracellular Ca2+ overloading plays a consequent role in generation of apoptosis which is further a leading cause of MI [43]. Moreover, ROS itself results apoptosis by inducing numerous factors [2, 44]. Several evidences provide supportive data which indicates ROS play an important pathological role in cardiac injury through the apoptosis in cardiomyocytes cell death [44-45]. In-addition, I/R injury in myocardial cells significantly activate c-Jun N-terminal kinase (JNK) pathway which ultimately causes apoptosis followed by MI. JNK, or stress-activated protein kinase, is an imperative member of the mitogen-activated protein kinase (MAPK) superfamily [3]. JNKs play a key role in the activation of death receptor through both extrinsic and intrinsic apoptotic pathways. JNKs can upregulate pro-apoptotic genes through transactivation of specific transcription factors which significantly leads to activation of apoptotic signalling [46]. Prevalent evidences suggest the activation of JNK pathway during I/R injury in myocardial I/R resulting in apoptosis [47-48]. Abundance scientific literature revealed that oxidative stress can also influences the MAPK signaling pathways and associated ERK, JNK pathways [49]. Recent study also revealed apoptosis associated myocardial infarction. Additionally, author suggested tissue damage activity by apoptosis also seen after myocardial infarction [50].

Intrinsic (mitochondrial associated) pathway induced MI *via* apoptosis

Other than extrinsic pathways, several intracellular stimuli also participate in mitochondrial induced apoptosis and associated MI. Internal stimuli, such as irreversible genetic injury, hypoxia, extreme concentrations of cytosolic Ca^{+2}, viral infection, or severe oxidative stress or destructive free radicals stimulate apoptosis by the intrinsic pathway. For instance physical stress, oxidative stress and DNA damage are the commonly known intrinsic factors for generating apoptosis and MI [51]. Apoptosis induced MI is the result of imbalance between pro-apoptotic i.e. Bid, Bax and, Bak and apoptotic Bcl-2and, Bcl-xL proteins of Bcl-2 family which critically regulate this signalling cascade [52]. After activation, Bax migrates to the outer mitochondrial membrane and forms a new complex by attaching with Bak. Formation of this complex makes outer mitochondrial membrane more permeable which insist cytochrome-c with other pro-apoptotic proteins [53]. If cytochrome-c intermingles with apoptotic protease activating factor-1 (APAF-1) in cytosol, this mixture stimulates caspase cascade. Cascade starts from caspase-9 activation leading to caspases-3 and caspases-7 which ultimately cause apoptotic cell death [54]. In spite of that some other apoptogens are also released by outer mitochondrial membrane on permeable involving second mitochondria-derived activator of caspase and apoptosis-inducing factor (AIF) [55], which can also initiate cleavage of BH3-interacting domain death agonist followed by mitochondrial pathway and leads to translocation of Bax/Bak proteins and apoptogens release [56]. As apoptosis plays a significant role in MI, all intrinsic and extrinsic cofactors, apoptotic and pro-apoptotic proteins may prove to be targetable for in the quest of new approaches to prevent MI and its consequences. Figure 1.

Extrinsic (TNF associated) pathway induced MI *via* apoptosis

In the case of extrinsic pathway of apoptosis in MI, the stimulus for apoptosis is an extracellular messenger protein commonly known as tumor necrosis factor (TNF), so named due to its ability to kill tumor cells [27, 57]. TNF is generated by several cells of the immune system [58] in adverse conditions like exposure to ionizing radiation [59], high temperature [60], viral infection [61], or toxic chemical agents including cancer chemotherapeutic agents [62]. TNF binds to transmembrane receptor TNFR1 to exert its response. TNFR1, is a member of “death receptors”

family which leads to the apoptotic process [63]. The existing evidence intimates that the TNF receptor is present in the plasma membrane with accessible trimer. The cytoplasmic domain of all TNF receptor subunits include a subdivision of about 70 amino acids known as "death domain" which brings about protein–protein interactions [64]. TNF binding to trimeric receptor generates a conformation change in death receptor domain and that initiates the conscription of numerous proteins. The last proteins, procaspase-8 molecules, assemble at the inner surface of plasma membrane. Thus, the synthesis of caspases as proenzymes defend cells against unintended proteolytic injures. In-contrast, several proenzymes and procaspases reveal a low level of proteolytic movement. Therefore, consistent with one model, when two or more procaspases are aligned in close connection with each other, they are competent of splitting one another's polypeptide chain and turn other molecule into the fully active caspases. At last matured enzyme caspase-8 contains four polypeptide chains, derived from two procaspase precursors. Caspase-8 activation is comparable in tenet to the initiation of effectors by hormone or growth factor [65]. Therefore, it may be concluded that all the signalling pathways involve the binding of extracellular ligand which leads to alteration in receptor conformation ultimately causing the downstream binding and activation pathway of proteins. Caspase-8 is considered as an originator caspase since it kicks off the apoptosis [66]. Mitochondria are able to regulate functional integrity of the cardiomyocytes. ATP production and calcium ion homeostasis are the necessary parameter to normalize mitochondria associated myocardium event. TNF-α and Fas-ligand participate in inducing death of myocardial cells by formation of Death Inducing Signaling Complex (DISC). DISC, once activated, leads to activation of procaspases-8 and -10 which further downstream other cascades of different caspases like as procaspases-3 and -7 and apoptosis followed by MI [36, 67-69]. Figure 1.

Targetable protein substrates

Potential caspases targeting during the apoptosis can be carried out on either regulatory proteins or structural and "housekeeping" intensive protein substrate groups. Regulatory proteins breakage strengthens apoptotic activation, whereas cleavage of structural proteins leads to cellular breakdown. First group involves a number of kinases, like as MEK kinase 1, P21-activated kinase 2, and Mst-1. Cleavage of caspases triggers these kinases which further leads to activation of c-Jun kinase and stress-activated protein kinase in response to manage apoptotic pathways [70-71]. In-addition, caspase –associated hydrolysis of proteins may also initiate the termination of negative regulators of apoptosis. For instance, focal adhesion kinase (FAK), phosphatidylinositol-3 kinase (PI-3 kinase), and the associated protein akt, raf-1, endogenous caspase inhibitors (or IAPs), anti-apoptotic members of Bcl-2 family, and inhibitors of caspase-activated DNAse (ICAD) are such kind of proteins. On the other hand, second class of targets, concerned in apoptotic structural changes, contain nuclear lamins, actin regulatory proteins like as fodrin, spectrin, and gelsolin, transcription factors, and DNA producer and renovator proteins for instance, PARP, MCM-3 [72-73].

Protective role of anti-apoptotic herbal compounds in MI

Numerous studies provide convincing evidence that besides of necrosis; apoptosis also plays a role in tissue damage associated with myocardial infarction [74**].** Therefore, several plant extracts and their active constituent have been evaluated in animal models of ischemia–reperfusion heart injury with some success. However, till date none of the precise anti-apoptotic agents have reached the stage of clinical research.

Table 1 summarizes the list of plants and Table 2 summarizes the active constituent studied for their antiapoptotic potential in MI.

Table 1: Anti-apoptotic plant investigated for cardioprotective effects in experimental models

Sr. No	Plant	Extract/Active constituent	Animal models	Molecular mechanism	References
1.	*Ocimum sanctum*, and *Curcuma longa*	Hydro-alcoholic lyophilized extract of Ocimum sanctum and aqueous extract of Curcuma longa	Ischemia reperfusion (IR) model of myocardial Injury	Significantly reduce the Bax protein, and up-regulate Bcl-2	[75]
2.	*Ocimum sanctum, Withania somnifera, and Curcuma longa Ocimum sanctum*	Combination of herbal extracts of Ocimum sanctum, Withania somnifera, and Curcuma longa (HCB)	Left anterior descending coronary artery (LAD) ischemia and reperfusion (IR) experimental model	Attenuate the Bax,and up-regulation of Bcl-2 protien	[76]
3.	*Withania somnifera*	Purified extract (1.5% withanolides)	Doxorubicin-induced apoptosis in rats	Inhibition of bcl-2 protein	[77]
4.	*Ginkgo biloba*	*Ginkgo biloba* extract	Doxorubicin-induced Cardiac toxicity *in vitro* and *in vivo*	Suppressed Bcl-2 family protein in cardiomyocytes	[78]
			Myocardium ischemia reperfusion (IR) rat model	Down-regulate Bax, cyt-c and caspase-3. Bcl-2.	[79]
5.	*Epimedium*,	Ethanol extract of *Epimedium* (EPI-ext)	Isoproterenol induced congestive heart failure (CHF) in rats	Blocked the expression and activities of regulated Bcl-2/Bax.	[80]
6.	*Elsholtzia blanda (Benth.)*	Total flavones	Left anterior descending coronary artery (LAD) ischemia and reperfusion (IR) experimental model	Attenuate the Bcl-2/Bax expression	[81]
7.	*Corydalis yanhusuo*	*Corydalis yanhusuo* rhizome extract	Myocardium ischemia reperfusion (IR) rat model	Inhibition of myocardial apoptosis through modulation of the Bcl-2 family.	[82]
8.	*Cassia mimosoides*	Methanol Extract of *Cassia mimosoides*	Myocardium ischemia reperfusion (IR) rat model	Reduction in the cellular injury was mediated by attenuation of Bax/Bcl-2 and inhibition of caspase-3	[83]
9.	*Ocimum gratissimum*	*Ocimum gratissimum* Aqueous Extract	H_2O_2-induced apoptosis in H9c2 cardiac muscle cells	Inhibit the mitochondrial pathway and upregulated Bcl-2 expression	[84]
10.	*Dioscorea bulbifera*	Hydroalcoholic extract of *Dioscorea bulbifera*	Langendorff apparatus Induce myocardial ischemic/reperfusion in rat	Inhibition of caspase and attenuate the anti-apoptotic proteins Bax and Bcl2	[85]
11.	Cardiotonic pills(Salvia miltiorrhiza (SM),Panax notoginseng (PN), and Borneol)		Ischemia-reperfusion-induced microcirculatory disturbance and myocardial damage in rats	Inhibit expression of Bax, caspase-3 and increase expression of Bcl-2 in myocardial tissues.	[86]
12.	Lycium barbarum		Ischemia/reperfusion of rat heart.	decreased myocardium Bax	[87]

Tulsi (*Osscimum sanctum),* is a herb cultivated throughout India for religious as well as medicinal purposes. *Ocimum sanctum* L. is suggested to possess hypolipidemic [88], hepatoprotective [89],and neuroprotective [90] properties. Its anticoagulant [91], antioxidant, anti-inflammatory [92,93] and immunomodulatory [94] effects are well reported in animal models. This plant possesses cardioprotective action against experimentally induced cardiac damage in experimental animals [95]. Turmeric (*Curcuma*

longa) is a rhizomatous perennial plant belonging to *zingiberaceae* family. Curcumin is an active curcuminoid in turmeric which exhibits precise medical potential. The turmeric oil, which mostly contains curcumin is widely used for its antioxidant, antimutagenic [96], anti-carcinogenic [97], anti-bacterial [98], and anti-fungal [99,100] activities. In addition, the extracts of *osscimum sanctum* and *Curcuma longa* show synergistic action in ischemia and reperfusion (I-R) model of MI. Interestingly, this combination attenuates the apoptotic proteins [75]**.** Moreover, in-addition *Withania somnifera* with above combination has been projected to attenuate Bax and up regulate the Bcl-2 protein in cardiovascular injury [76].

Withania somnifera (WS) (Ashwagandha), is an annual herb enriched with alkaloids and steroidal lactone. The Ashwagandha roots contain diverse withanolides, essential as well as non-essential fatty acids, amino acids, sterols, catecholamines, aromatic alcohols, gamma amino butyric acid, and glycerol [101,102]. The bioactive constituents of *Withania somnifera* possess anti-inflammatory [103], anti-oxidant [104], antitumor [105], antistress [106], immunomodulatory [107] and hematopoietic properties [108]. Furthermore, it possesses anti-apoptoic potential in myocardial infarction. WS attenuates the progression of Bcl-2 protein in doxorubicin-induced cardiac toxicity in rats [77].

Ginkgo biloba (GB) (Maidenhair tree), is an exceptional member which is unique of its species with no other similar members. The leaves of *Ginko biloba* contain the ginkgolides A, B and C which possess various pharmacological activities. In addition, *GB* has an antiplatelet action, along with neuroprotective [109] and cardioprotective [110] potentials. An investigation including western blot and immunohistochemical analysis has revealed that GB effectively down-regulates the Bax, cyt-c, Bcl-2 and caspase-3 in myocardium ischemia- reperfusion (IR) model in rat [78, 79].

Epimedium is a genus of flowering plants in the family *Berberidaceae*. In addition, the major active substances of *epimedium* include icariin, epimedoside, icariside, breviflavone and ikarisoside, most of which possess anti-hepatotoxic, immunomodulatory, anti-tumor, antidepressant and anti-oxidant activities [111-112]. It has been documented that the use of *epimedium* extract attenuates myocardial apoptosis through remarkably attenuating Bcl-2/Bax protein [80]. Likewise, *Elsholtzia blanda* is a Chinese medicinal herb is reported to significantly inhibit the Bcl-2 and attenuat Bax expression in coronary occlusion induced myocardial infarction in rat [81].

Corydalis yanhusuo is a Chinese herbal medicine which contain the active constituent is protopine.Likewise, Protopine has posses the protective effects on cardiac myocytes electrophysiological [113], cerebral ischemic injury [114] ,inhibit platelet aggregation [115], anti-depression [116], antithrombotic, anti-inflammatory [117], antioxidant, and neuroprotection effects [118] action. In addition study have revealed that the *Corydalis yanhusuo* attenuate the myocardial injury is closely associated with the inhibition of myocardial apoptosis through inhibition of the Bcl-2 family [82]. However, *Cassia mimosoides* is a species of legume which belongs to fabaceae family. Studies have revealed the cardioprotection action of *Cassia mimosoides* by inhibiting apoptosis in myocardial injury. In detail, *Cassia mimosoides* reduce the cellular injury by attenuation of Bax/Bcl-2, inhibition of caspase-3 activation from procas-pase-3 and subsequent reduction in the number of apoptotic cells [83].

Ocimum gratissimum , traditionally used as folk medicine in many countries. This medicinal plant has shown potential anthelmintic [119], antibacterial[120], antifungal[121], and antiviral activities [122].In addition, it posses immunomodulation [123] activity.In one study, Ocimum gratissimum extract effectively inhibited the mitochondrial pathway and upregulated Bcl-2 expression, which may be significant in protecting

H9c2 cells from H_2O_2-induced cell death [84]. *Dioscorea bulbifera*, (air potato) belongs to family *Dioscoreaceae*. It is mainly found in Africa and Asia. Abundantly, it content steroidal saponins i.e. dioscoreanosides A to K which show various pharmacological action [124]. In one study they revealed that the *Dioscorea bulbifera* inhibit anti-apoptotic proteins i.e. Bax and Bcl2 by Western blot analysis followed by TUNEL assay [85].

Cardiotonic pills (Salvia miltiorrhiza (SM),Panax notoginseng (PN), and Borneol) is a chinease traditional medicine. Therefore, the combination of three plant produce a synergistic action. Likewise, one study demonstrated that this cardiotonic pill prevent MI by inhibition of apoptosis.Interestingly,Cardiotonic pill attenuate the Bax,caspase-3 and increase the expression of bcl-2 in myocardial cell [86]. The Lycium barbarum is a chienese herb which belong to Solanaceae family. Likewise, Lycium barbarum has a large variety of pharmaceutical activites, such as anti-aging, antitumor and immunomodulation [125-127].In addition one study Lycium barbarum possesses the antiapoptotic potential by effectively attenuate the myocardium Bax in Ischemia/reperfusion of rat heart [87].

The cardioprotective potential of medicinal plants through antiapototic pathway of myocardial preconditioning has been well evidenced in pre-clinical conditions. However, more studies are therefore needed to demonstrate a molecular mechanism of apoptosis which could certainly be a core procedure pertained to cardiovascular defensive potentials of herb. Therefore, the anti-apoptotic role of herbal plant is an imperative issue and offers new perspectives for the better exploitation of apoptosis induce cardiovascular disorders and improving cardiovascular outcomes.

Table 2: Anti-apoptotic active constituent of plants investigated for cardioprotective effects in experimental models

Sr. No	Active constituent	Animal model	Mechanism	References
1.	Betulinic Acid	Myocardial ischemia reperfusion/injury	Inhibition of expression of Bax and increase the Bcl-2	[128]
2.	Oleanolic Acid	Ex vivo perfused heart tissues	Decreased caspase-3 activity	[129]
3.	Salidroside	Acute myocardial infarction (AMI) in rat	Dramatically augment Bcl-2/Bax ratio and antagonize caspase-3 activity	[130]
4.	Lycopene	Isoproterenol induced Myocardial infarction	Inhibit the caspase-3 activity	[131]
5.	Tyrosol	H9c2 cells simulated ischemia/reperfusion (IR)	Inhibition of Apoptotic nuclei condensation, caspase-3 activity and cytochrome c release	[132]
6.	Leonurine	Mitochondria dysfunction in H9c2 cells	Inhibit the release of cytochrome c and Bax	[133]
7.	3,5-Dimethoxy-4-(3-(2-carbonyl-ethyldisulfanyl)-propionyl)-benzoic acid 4-guanidino-butyl ester	Hypoxia-induced apoptosis in cardiac myocyte.	Inhibite apoptosis by reducing the ratio of Bcl-2/Bax and caspase-3	[134]
8.	4-Guanidino-n-butyl syringate (Leonurine, SCM 198)	H9c2 rat ventricular cells from hypoxia-induced apoptosis	Reduced expression of Bax and increased BCl-2	[133]
9.	N-acetylcysteine	H9c2 cardiac muscle cells	Inhibit cytochrome c release, increase Bcl-2 and decrease Bax expression and procaspase-9 activation	[136]
10.	Rosmarinic acid (RA)	Adriyamycin-induced apoptosis in H9c2 cardiac muscle cells	Down-regulation of Bcl-2	[137]
11.	Naringenin-7- O-glucoside,	Doxorubicin-induced apoptosis in H9c2 cells	Inhibition of Bcl-2 in cardiomyocytes	[138]
12.	Tanshinone IIA (TSN), a major lipid-soluble pharmacologic constituent	Adriamycin (ADR)- induced apoptosis in neonatal rat cardiomyocytes	Reduced ratio of Bcl-2/Bax,	[139]

Betulinic Acid is a triterpenoid obtained from various plant sources. It has posses antitumor [140], anti-HIV [141], antimalarial [142], anti-inflammatory [143] activity. In one study they demonstrate that betulinic acid attenuate the BAX protein and increase the expression of bcl-2 in the myocardial ischemia/reperfusion injury with help of western blot and TUNEL assay [128].

Oleanolic Acid is a naturally occurring triterpenoid, mostly distributed in food and medicinal plants. It has a variety of biological effects, such as anti-oxidants [144], antifungal, anti-inflammatory, anti-hyperlipdemia, hepatoprotective, tumor prevention, immunomodulatory, [145], anti-HIV [147], anti-arrhythmic and cardiotonic [148]. In addition, Oleanolic acid posses antiapoptotic potential by significantly increased cardiac p-BAD/BAD and decreased caspase-3 peptide levels under high glucose perfusion condition [129].

Salidroside [2-(4-hydroxyphenyl) ethyl beta-D-glucopyranoside], active constituent of Rhodiola rosea. Salidroside possesses anti-aging, anti-cancer, anti-viral [149], anti-inflammatory [150], anti-hypoxia [151-152] and anti-oxidative [153-154] properties. The Salidroside, has been shown to exhibit protective effects in IR- and hypoxia-induced cardiomyocyte cell death [155].In one study the salidroside possesses the antiapoptotic potential by dramatically augment Bcl-2/Bax ratio and antagonize caspase-3 activity in acute myocardial infarction (AMI) in rat [130].

Lycopene is a red caratenoid found in various vegetables. Lycopene has the greatest ability of all the common carotenoids to quench singlet oxygen. It possesess the anti-cancer [156], antioxidant , low-density lipoprotein cholesterol reduction [157,158], reduces proinflammatory cytokine and chemokines [159,160].In addition,lycopene attenuate the caspase-3 protein in isoproterenol induced Myocardial infarction [131].

Tyrosol (2-(4-hydroxyphenyl) ethanol) is a natural phenolic antioxidant present in a various plants. Its mainly present in olive oil and white wine. Studies suggested that Tyrosol possesses the free radical scavenging activity [161], anti-inflammatory [162] and neuroprotective [163] effects. Addition studies support that tyrosol attenuate the Apoptotic nuclei condensation, caspase-3 activity and cytochrome c release in H9c2 cells simulated ischemia/reperfusion (IR) [132]

Leonurine (LEO), an alkaloid isolated from Leonurus cardiaca. It possesses the beneficial effect against cardiovascular diseases [164-166], ischemic stroke [167], atherosclerosis [168, 169],vasodilating [170], antioxidative [164,171], antiapoptotic [172,173], and anti-inflammatory [168] activities. Interestingly,leonurine used to treat the myocardial problem in various way. In addition, LEO inhibit the release of cytochrome c and Bax in H9c2 cells simulated ischemia/reperfusion (IR) in rat [133]. Additional studies demonstrate that the formation of new synthetic compound i.e. 3,5-Dimethoxy-4-(3-(2-carbonyl-ethyldisulfanyl)-propionyl)-benzoic acid 4-guanidino-butyl ester which possesses the antiapototic active with inhibition of Bcl-2/Bax and caspase-3 in Hypoxia-induced apoptosis in cardiac myocytes [134].Likewise, another compound synthesized form Leonurine that is 4-Guanidino-n-butyl syringate (Leonurine, SCM 198) which possesses the antiapoptotic activity by inhibiting Bax and increased expression of BCl-2 in H9c2 rat ventricular cells [135].

N-acetylcysteine(NAC) is a well known pharmaceutical agent for paracetamol poisoning. Acetylcysteine is a derivative of cysteine in which an acetyl group is attached to the nitrogen atom.NAC which contain a thiol group that responsible for scavenging the oxygen free radical [174].Additional studies revealed that NAC Inhibit the cytochrome c release and increase expression of Bcl-2 and decrease Bax expression and procaspase-9 activation in H9c2 cardiac muscle cells [136].

Rosmarinic acid (RA) is an ester of caffeic acid present in various plants. It is commonly found in species of the *Boraginaceae* and the subfamily *Nepetoideae* of the

Lamiaceae. Likewise, Rosmarinic acid has a number of interesting biological activities such as antiviral, antibacterial, antioxidant and anti-inflammatory activity [175]**.** A study reveals RA markedly inhibits the apoptotic characteristics by reducing intracellular ROS generation and by recovering the mitochondrial membrane potential. In addition, RA reverses the down regulations of GSH, SOD and Bcl-2 in adriyamycin induced apoptosis in H9c2 cardiac muscle cells [176].

Dracocephalum rupestre (*Lamiaceae*), is a rhizomatous herb mainly found in China. It contains abundant amount of flavanoids. The Eriodictyol-7-O-glucoside and Naringenin-7-O-glucoside is a major flavanoid obtained from *Dracocephalum rupestre* [177]. Experimental investigations suggest that Naringenin-7-O-glucoside reduces mRNA expression of caspase-3 and caspase-9 [178]. *Salvia miltiorrhiza* (*danshen*), also belongs to Chinese traditional herbs and is officially noted in the Chinese Pharmacopeia [179]. Active constituents of *Salvia miltiorrhiza* possess various pharmacological activities including hepatoprotective, anti-fibrotic, anti-oxidative, anti-inflammatory and anti-apoptotic activities [180-186]. Interestingly, one of these studies reveals that *Salvia miltiorrhizia* markedly reduces the ratio of Bcl-2/Bax protein in adriamycin-induced apoptosis in cardiomyocyte [139]. Taken together, it may be suggested that the multiple herbal compounds and extracts have been systematically proved to prevent myocardial infarction through apoptotic pathways. Further investigations are warranted to elucidate molecular mechanisms involved in such specific apoptosis modulating effects of these potential substances.

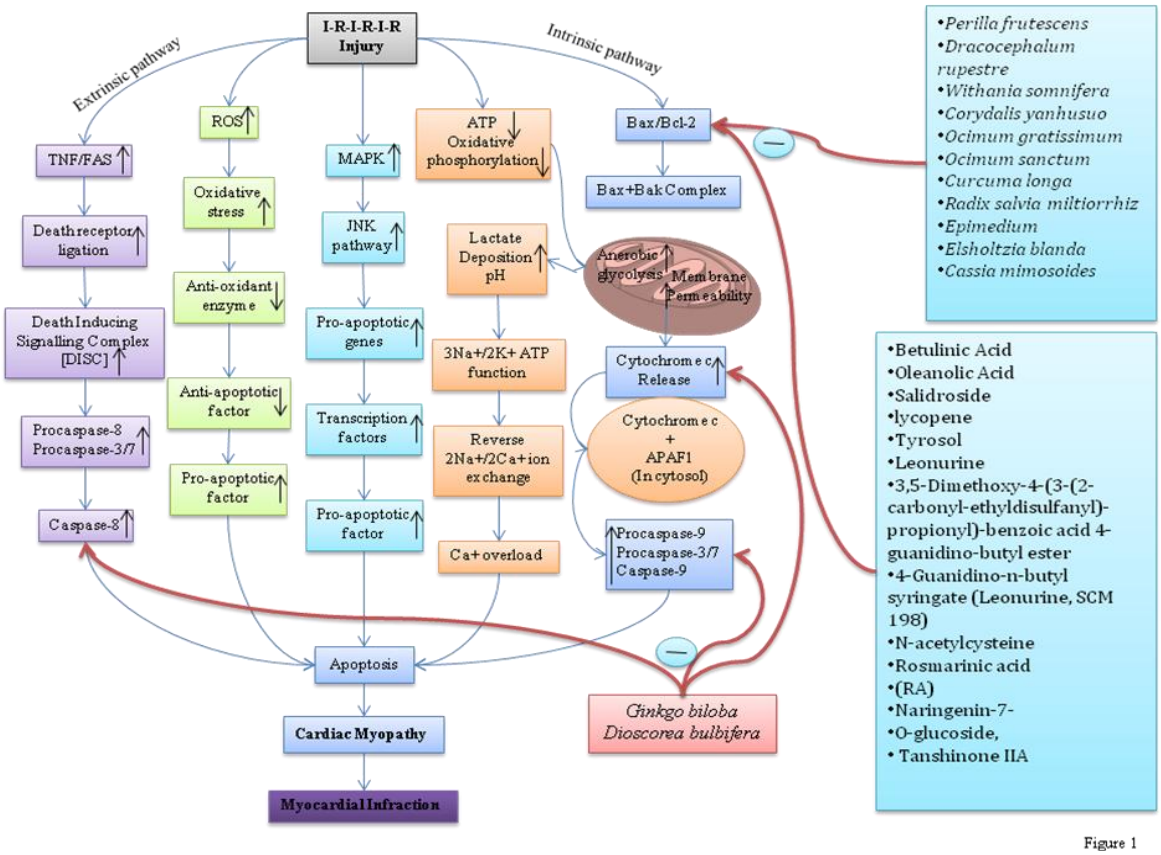

Fig. 1: The figure interprets the Ischemia/Reperfusion-associated signalling alterations and their possible contribution to apoptosis and consequently to Myocardial infarction. I/R indicates ischemia/reperfusion; TNF indicates tumor necrosis factors; FAS indicates TNF receptor superfamily, member 6; DISC indicates death inducing signalling complex; Bax indicates pro-apoptotic member of Bcl-2; APAF indicates apoptotic peptidase activating factor; ROS indicates reactive oxygen species; ATP indicates adenosine triphosphate; MAPK indicates mitogen-activated protein kinase; JNK indicates c-Jun N-terminal kinases.

CONCLUSION

Apoptosis or programmed cell death is a series of genetically controlled events that result in killing the cells. Growing body of evidence suggests that herbal medicine is increasingly gaining greater acceptance from the public and medical profession due to understanding the mechanisms by which herbs positively influence health and quality of life. Numerous pharmacological intervention apparent that experimental evaluation of herbal drugs for the treatment of cardiovascular diseases is rather impressive, but very few have reached clinical trials and still fewer have been marketed. In fact, elaborate studies with such compounds with respect to their abilities to inhibit apoptosis and understanding their mechanism of action may provide valuable information for their possible application in myocardial infarction treatment and prevention. Hence, pharmacologists need to take more active interest in the evaluation of herbal drugs for potential antiapoptotic activity and their standardization to allow them to be clinically effective and globally competitive.

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CONFLICT OF INTEREST

No conflict of interest.

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