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REVIEW: ISCHEMIC HEART DISEASE AND THE POTENTIAL ROLE OF FENUGREEK (TRIGONELLA FOENUM GRAECUM LINN.) IN CARDIOPROTECTION

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Graphical abstract

Abstract

Ischemic heart disease, often known as coronary heart disease (CHD), is a term for heart disorders caused by narrowing heart arteries (coronary arteries) that provide blood to the heart's muscles. The body is prone to heart attacks when there is less oxygen supply (hypoxia) to the heart resulting a major cell death in the heart tissues. Cardiovascular disease (CVD) is the leading cause of death worldwide. Over the last few decades, the potential therapeutic effects of herbs and other types of complementary medicines for managing risk factors for CVD have gotten a lot of attention. Fenugreek (*Trigonella foenum graecum* Linn.) is a very important spice in most Asian dishes. In Malaysia, fenugreek, locally known as 'halba', is used in the preparation of popular dishes; 'nasi dagang' for breakfast, and 'putu halba' as snacks for hi-tea and sometimes applied in drinks. Although, to date, the effect of fenugreek against diabetes and heart diseases is well investigated, most studies do not focus on its the effects at molecular levels. This review gives an insight on the ischemic heart disease and the nutritional values of fenugreek as functional food in protecting the heart.

Keywords: Ischemic heart disease, fenugreek, cardiomyocytes, hypoxia, ischemia, apoptosis

Abstrak

Penyakit jantung iskemik, sering dikenali sebagai penyakit jantung koronari (CHD), adalah istilah untuk gangguan jantung yang disebabkan oleh penyempitan arteri jantung (arteri koronari) yang membekalkan darah ke otot jantung. Badan terdedah

kepada serangan jantung apabila kurang bekalan oksigen (hipoksia) ke jantung mengakibatkan kematian sel utama dalam tisu jantung. Penyakit kardiovaskular (CVD) adalah punca utama kematian di seluruh dunia. Sejak beberapa dekad yang lalu, potensi kesan terapeutik herba dan jenis ubat pelengkap lain untuk menguruskan faktor risiko CVD telah mendapat banyak perhatian. Fenugreek (*Trigonella foenum graecum* Linn.) adalah rempah yang sangat penting dalam kebanyakan hidangan Asia. Di Malaysia, fenugreek yang dikenali sebagai 'halba', digunakan dalam penyediaan hidangan popular; 'nasi dagang' untuk sarapan, dan 'putu halba' sebagai makanan ringan untuk minum petang dan kadang-kala digunakan dalam minuman. Walaupun, setakat ini, kesan fenugreek terhadap diabetes dan penyakit jantung disiasat dengan baik, kebanyakan kajian tidak menumpukan pada kesannya di tahap molekul. Ulasan ini memberi gambaran mengenai penyakit jantung iskemik dan nilai pemakanan fenugreek sebagai makanan berfungsi dalam melindungi jantung.

Kata kunci: penyakit jantung iskemik, halba, kardiomiosit, hipoksia, iskemia, apoptosis

1.0 INTRODUCTION

Ischemic condition occurs due to organ (e.g., heart) not getting enough oxygenated blood relatively known as ischemia heart disease (IHD) also coronary heart disease (CHD) or coronary artery disease. IHD or CHD may occur due to high cholesterol in the blood that accumulate as plaques blocked the heart arteries or under strict exercise regime. Less oxygen supply (hypoxia) to the heart resulting a major cell death in the heart tissues. After a severe attempt of cells loss due to hypoxia, the body is prone to heart attacks. Heart attack causes some symptoms such as chest pain, pressure, fullness, discomfort, difficulty in breathing, dizziness, nausea and cold sweats leads to cardiac arrest, a sudden stops of heart due to malfunctions [1]. An immediate medical attention required when symptoms of heart attack occurred.

A symptom of ischemic heart disease occurs due to non-functional cardiac cell contractility. Myocardial infarction (MI) is an ischemic condition that leads to hypoxia and tissue death. Contractility of cardiac cell involve the intracellular calcium dynamic exchange [2]. Alteration in cardiac workload interfere with the gene expression pattern of physiological mechanisms. Symptoms and signs of ischemic heart disease can be monitored; however, prevention of cardiac cell death due to apoptosis will be a better outcome. Cardiac cell death due to the hypoxia is related through either apoptosis or adaptation. Drugs has been used for the treatment of ischemic heart disease, however the side effects due to the toxicity led to the practice of using an alternative therapeutic medicine from daily food product. To identify the potential therapeutic targets, a full understanding of apoptotic pathways on cardiomyocytes function is essential [3].

During hypoxia, cells are either adapting to the hypoxic condition or undergoing apoptosis. Those effects are depending on the level and duration of hypoxia exposure and concentration of oxygen [4]. Hypoxia has been associated with recruitment of apoptosis signaling such as caspase-3 [5], and calcium dynamic changes [6]. Hypoxia inducible factor (HIF) is responsible in oxygen homeostasis of cells and activated cells oxygen concentration decreased. During hypoxia, mitochondrial oxygen consumption decreases and leads to energy depletion through loss of Na^+/K^+ pump function and cells swelling. Cells swollen are due to water permeability throughout membrane towards the Na^+ gradient. Hypoxic environment also interrupts the calcium pump's function, increase of intracellular calcium; $[Ca^{2+}]_i$ leads to activation of proteases, endonucleases, phospholipase, and hydrolytic enzymes. Calcium is permeable inside the mitochondrial membrane pore and this leads to opening of mitochondria permeability pores that cause cytochrome c release into the cytosol and subsequently also activates the apoptosis signal [7]. Therefore, in mild, moderate or intermittent hypoxia cells could adapt to the environment and restore cell mechanism [8] after reperfusion but in severe hypoxia, cells may not survive throughout the environment and commits cell suicide or apoptosis [9]. Hypoxia mimicking microenvironment established *in vitro* using chemically induced-cobalt chloride ($CoCl_2$) resulted in mitochondrial dysfunction H9c2 cardiomyocytes [10].

Apoptosis is essential for cells growth, however the activities and condition of which trigger activation of apoptotic pathway should be avoided. Apoptosis process involves both morphological and biochemical changes [11], causing minimal effects of inflammation, thus making it as an important target for drug development. Two main routes of apoptotic pathways in mammalian cells are intrinsic and extrinsic pathways [12]. Intrinsic pathway involves the internal cell damage due to mitochondrial damage. However, the extrinsic pathway requires the death-receptor pathway of tumor necrosis factor alpha (TNF) receptor activating procaspase-8 molecule recruiting caspase-8, thus

triggering caspase-3 activation towards apoptosis. The apoptotic pathway may vary in different types of cells, as example in cancer cells. The ability of cells to commit suicide initiated by a network of stimuli of both extrinsic and intrinsic apoptotic pathways. Activation of executioner caspase triggered the endonucleases towards nuclear DNA defragmentation and breakdown of cytoskeleton. Cells then create a form of cytoplasmic bleb on cell surface known as apoptotic body. The apoptotic body was tagged with the ligands for phagocytic cell receptors by phagocyte. Lysis of apoptotic bodies was successfully done by the phagocyte with less stress occurs inside cells.

Lifestyle changes is important to avoid heart attack, stop smoking, balanced diet, control the blood cholesterol and blood pressure, healthy weight and physically active [13]. World Health Organization (WHO) suggested that the food lifestyle play an important role as functional foods are scientifically reported to prevent CVD [14]–[17]. Milk or total dairy product is not associated with CHD [18] but in some countries there is a risk of milk consumer that may developed IHD [19].

Since ancient times, spices are utilised as traditional remedies to treat a wide range of chronic diseases. In fact, two-thirds of the world population depends on traditional medicine for primary medical needs. Among the popular spices with medicinal value is fenugreek, a short-living annual medicinal plant belonging to *Fabaceae* family. Fenugreek is deemed as one of the oldest medicinal plants, where its health-promoting effects have been cited in Ayurveda and traditional Chinese medicine [20].

2.0 CARDIOVASCULAR DISEASES RISK FACTORS LEADING TO HEART DISEASE

Risk factors for CVD are generally divided into, controllable and uncontrollable factors. Controllable factors indicate that the risk factors is preventable or treatable such as smoking [21], [22], high and low cholesterol [23], [24], hypertension [25]–[27], physical inactivity [28]–[30], obesity [31]–[33], diabetes [34][35] and stress or anger [36]–[38]. Uncontrollable conditions include family history with the heart disease and ethnicity that may be difficult to be modified. Generally, lifestyle including type, quality, and amount of food as well as social activities are deemed as "controllable". Interestingly, in the third world countries, food has a big influence in the development of the CVD and is therefore focused in this review.

There are many foods categorized as "bad food". Bad food is processed food not meeting the food standard [39], [40] and this includes (but are not limited) foods containing high fat, high sugar, high flavouring and colouring enhancers. Some countries are very cautious of the nutritional value contained in processed food. For example, in Australia and New Zealand, sodium salt of glutamic acid is not allowed as food additive since its safety is not approved by Food Standards Australia New Zealand (FSANZ); of the steps taken by the government to control the misuse of food enhancer that may lead to the development of CVD.

Cholesterol is a fat-like, essential alcohol that can be synthesized by the body or obtained from the diet. Virtually every nucleated cell can synthesize cholesterol from acetyl-CoA. The rate-limiting step in cholesterol synthesis is the conversion of 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) to mevalonic acid which is catalysed by HMG-CoA reductase. Cholesterol is an essential lipid constituent of cell membrane and plays very important roles in the body in producing hormones, vitamin D, post translational modification of membrane proteins besides helping in food digestion. Other than, the body producing its own cholesterol as needed, cholesterol can also come from the liver or the daily diet [41]. The liver produces cholesterols for export to other cells and also remove the cholesterol by converting it to bile salts where it can be eliminated in the faeces [42]. The cells take cholesterol via receptor-mediated endocytosis. Interestingly, the liver, other organs and the cells produce 75 % of the cholesterol in the blood while remaining 25 % is from food [43]. Cholesterols can be found in foods including egg yolks, meat, and cheese.

The formation of cholesterol in the blood may cause a serious disease including atherosclerosis (building up in the blood vessel wall) [23]. Atherosclerosis leads to heart attack, stroke and peripheral vascular disease since the hardening of the plaques in the artery narrow the opening of the arteries and restrict the blood flow [44]. Plaque build-up reduces the blood flow as well as oxygen distribution to major organs like the heart. Oxygen deprivation causes hypoxic conditions leading to severe cell death [45], [46] while accumulated cells death causes a major failure of the organs.

Cholesterol biosynthesis pathway (Figure 1) starts with acetyl coenzyme A (acetyl-CoA) as an important constituent to synthesize hydroxymethylglutaryl-CoA (HMG-CoA). Acetyl-CoA is catalysed by thiolase and HMG-CoA synthase to synthesize HMG-CoA which is found in the cytosol. Cholesterol biosynthesis depend on the export of acetyl-CoA from the mitochondria and requires the helps of nicotinamide adenine dinucleotide phosphate (NADPH). However, the subsequent HMG-CoA downstream steps conversion into cholesterol occur in the endoplasmic reticulum in which HMG-CoA reductase reduces HMG-CoA to mevalonate. Mevalonate is then converted into various isoprene intermediates which requires several rounds of polymerization to produce a linear hydrocarbon molecule which is cyclized into lanosterol [47]. Lanosterol then undergoes various steps of demethylation, desaturation, and saturation for conversion into cholesterol.

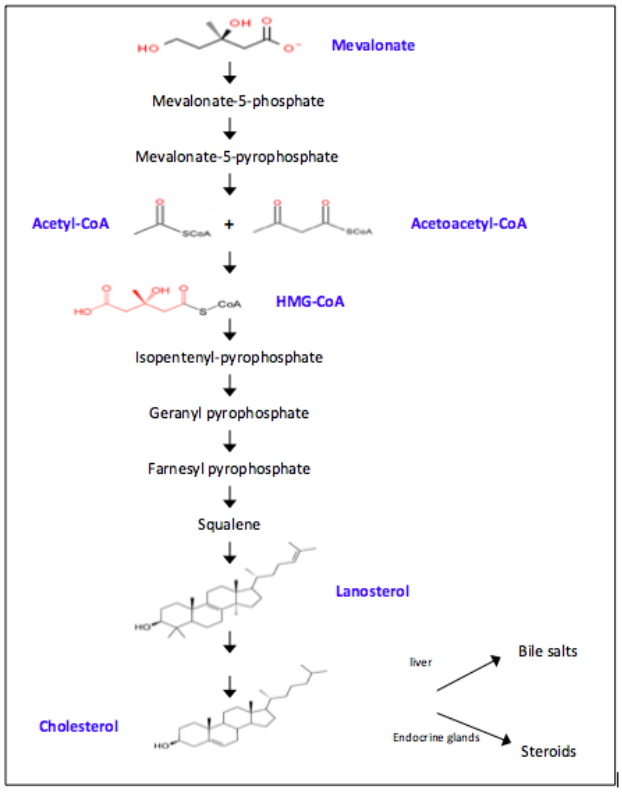

Figure 1 Cholesterol biosynthesis pathway (Adapted from Cardelle Cobas *et al.,* 2010)

There are two main types of cholesterol in the body high density lipoprotein (HDL) and low-density lipoprotein (LDL). Lipoproteins are made of fat and proteins. Excess intake of cholesterol can cause a coronary heart disease for example hypercholesterolemia HDL or the "good cholesterol", is less formed in the arteries as it helps to transport the cholesterol into the liver and expelled it out from the body. LDL also known as "bad cholesterol" since it carries cholesterol to the arteries and may build up in artery walls is the main cause of atherosclerosis. HDL level varies between males and females; where females need to have at least 55 mg/dL whereas 45 mg/dL in males [48]. As for LDL, overall, the amount should not be more than 130 mg/dL (with no health problems) or 100 mg/dL (with health problems). High LDL cholesterol levels increase the risk of atherosclerosis since it contributes to the formation of atherosclerosis plaques. In heart disease, correlation to cholesterol level is dependent on functioning LDL receptors which transport cholesterol into the cells. LDL oxidation is the main cause of atherosclerosis; however, intake of antioxidants inhibits the oxidative damage leading to myocardial infarction. Cholesterol biosynthesis pathway adapted from Cardelle-Cobas *et al*. (2010) [49].

Prevention of CVD is important to promote cardiovascular fitness. CVD risk factors and a list of the prevention methods should be listed and verified through laboratory and scientific evidence. Apart from the chemically produce drugs to treat CVD diseases, foods can potentially be an approach for the treatment. Even if the active compound in foods is isolated and generic product of the active compounds is stabilized into capsule, the effects of further implications of ingesting the said products is not fully understood.

3.0 HEART DISEASE PROMINENT TO HEART FAILURE

CVDs affect the heart muscle, valves or rhythm including blood vessels leading to arrhythmias, coronary heart diseases, hypertension, atrial fibrillation and peripheral arterial diseases. Various factors can contribute to heart failure with the most well-known condition is common being ischemic heart disease. Interestingly, ischemic heart disease is a silent killer that leads to heart attack affecting cardiac cell into programmed cell death (apoptosis) of the heart [50].

Ischemic heart disease may be to blocked arteries of heart (atherosclerosis) which impede blood supply to other parts of the body or organs, leading to unconditional apoptosis of the heart cell caused by lack of oxygen supply. Atherosclerosis begins without signs and symptom due to cholesterol accumulation or hardening of the artery wall in the heart.High blood cholesterol develops into fatty deposits, accumulate, and harden into plaque to block the artery, triggering blood clot and consequently leading to a heart attack.

Ischemic heart disease patient often experiences chest pain or discomfort like pressure, squeezing and burning in the chest. Subsequently, the individual may experience some nausea, fatigue, shortness of breath, sweating and dizziness. Typically, most individuals mistaken the chest pain with gastrointestinal problems including ulcers, muscle spasms in the oesophagus and pancreatitis. Although the signs and symptoms of ischemic heart disease can be monitored, prevention of cardiac cell death due to apoptosis will offer a better outcome. To date, modern drugs have been used for the treatment of ischemic heart disease, however due to the side effects, the practice of using alternative therapies including those based on daily food product are becoming more popular. To identify the potential therapeutic targets, a full understanding of the apoptotic pathways of the cardiac cell (cardiomyocytes) is essential [51].

To date, two main apoptotic pathways in mammalian cells (Figure 2) have been reported [52]. Intrinsic pathway involves mitochondrial death via disruption of the mitochondria physiology involves the inner cellular stimuli such as hypoxia, increased permeability of inner and outer mitochondrial membranes. Permeability permits the release of cytochrome c into cytosol thus recruiting apoptotic protease activating factor 1 (APAF1) to form an

'apoptosome' complex. As a result, caspase-9 activation triggers activation of caspase-3 hence initiating DNA fragmentation. However, the extrinsic pathway requires the death-receptor pathway of tumor necrosis factor alpha receptor (TNFR) activating procaspase-8 molecule recruiting caspase-8 thus, triggering caspase-3 activation towards apoptosis.

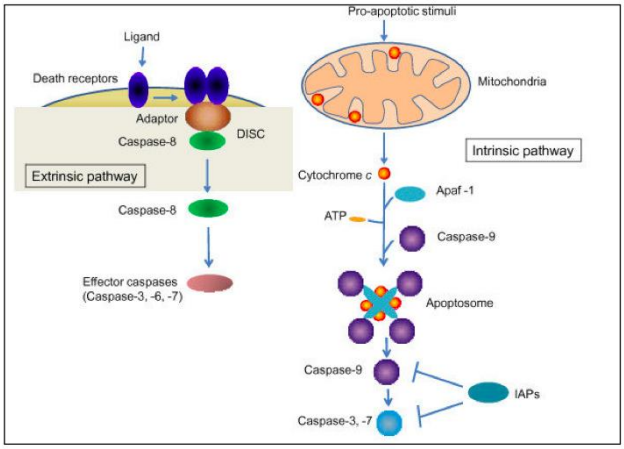

Figure 2 Apoptotic signalling pathway consists of both intrinsic and extrinsic pathways leading to activation of caspases (Li & Sheng, 2012)

Cardiac cell or the cardiomyocytes plays a major role in the heart function since the beating rhythm of healthy cardiac cell is primarily important for contractile function of the heart that enables blood circulating around the body. Cardiomyocytes are packed largely at the left ventricle of the heart, pumping blood to the systemic circulation. Cardiomyocytes cell death causes a severe injury of the heart with limited heart contraction that lead to less or zero supply of oxygenated blood to all part of the body leads to myocardial infarction [53]. Myocardial infarction is a form of heart attack that cause sudden cardiac arrest when the heart suddenly stops beating. Cardiac arrest involves the blockage of heart electrical activity due to an abnormal heart rhythm such as cardiomyopathy, tachycardia, bradycardia or ventricular fibrillation [54]. Cardiomyocytes receives some signals from the pacemaker cells causing them to contract which occur via depolarization of membrane potential produce action potential current.

The membrane action potential produces calcium-induced calcium release mechanism [55] which involve four phases, depolarization, rapid depolarization, repolarization, resting phase no depolarization allowing contraction of cardiac muscle. It started with the pacemaker potential of the pacemaker occurring via the opening of hyperpolarization-activated cyclic nucleotide-gated channel (HCN). HCN allows positively charged ion, Na^+ to enter the cells since membrane cell more permeable to sodium. In fact, membrane potential starts to slowly depolarize (-65mV to -50 mV) [56]. The cells will enter a phase 0 (-50 mV) where the voltage-gated Ca^{2+} channels open, allowing calcium to flow into the cells and causing an increase in membrane potential, reaching a peak (+10 mV) when the calcium-gated channel closes (phase 3, rapid depolarization). At +10 mV membrane potential, closing calcium channel occurs, allowing opening of the potassium (K^+) channel thus causing, potassium ions to leave the cells, causing a net outward positive current. The positively charged ion leaves the cells faster that the entry of ion causing cells to be more negatively charged on the inside or a repolarization phase.

The electrical stimulus (action potential) will then convert the signal into a mechanical response (muscle contraction). Contraction in the muscle occurs via cardiac muscle fibres, myosin and actin [57]. The binding of myosin head to adenosine triphosphate (ATP) pulls the actin filaments to the centre of sarcomere resulting in a mechanical force of contraction [58]. Contraction and membrane potential occurs very fast. A complete depolarization phase to repolarization is one cycle of heartbeat. The contraction requires healthy cells since apoptotic cells inhibit contraction, which causes a slowing of the heartbeat. Consequently, the amount of oxygenated blood being pumped in and out of the heart is affected leading to hypoxia which in turn affect the function of the cardiomyocytes resulting in heart failure or ischemic stroke.

Depolarization occurs when calcium ion move from the cardiomyocytes to the neighbouring cells through the gap junction [59]. The cardiomyocytes have a unique passageway known as T-tubules surface area of cardiomyocytes. Another important organelle involved in contractility is the sarcoplasmic reticulum which stores the intracellular calcium. When the membrane potential is reached, sodium channels open up and allow both extracellular calcium and sodium ions to move across cells [60]. During the excitation-contraction coupling in both cardiac and skeletal muscles, the t-tubule which consists of ryanodine receptors binds to the sarcoplasmic reticulum allowing the release of intracellular calcium to cause more calcium movement into the cells (calcium-induced calcium release mechanism). Subsequently, the two contractile proteins (myofilaments of actin and myosin) [61] and activated. The myosin attaches and pulls actin with the help of ATP forming a cross bridge that results in shortening of the muscle fibre.

Cardiomyocytes contractility is dependent on intracellular calcium concentration [62] 1) can be modified by sympathetic activation, 2) increases contractility or parasympathetic activation, and 3) decreases contractility. Contractility can increase independently by increased in heart rate [63]. Although cardiomyocytes can proliferate during fetal and neonatal developments, the heart will mature during adulthood to enable efficiently replacement of damaged tissue [64]. Following cardiac injury, an

adult heart normally does not regenerate significant amount of tissue damage thus leading to scarring and hypertrophy which eventually contributes to fatal arrhythmias and heart failure [65]. Therefore, investigation that allows prevention of cardiomyocytes death in future is crucial since ischemic heart disease reported to be the leading death across the world regardless of the income groups [66] or age [67].

4.0 TREATMENT AGAINST ISCHEMIC HEART DISEASE

CVD treatments may entail healthy lifestyle such as healthy diet, exercise and stop smoking. CVD treatments involve surgery such as the coronary artery bypass grafting (CABG) followed by cardiac rehabilitation, including exercise and counselling [68]. CVD is majorly treated by drugs such as inotropic, diuretics, antiarrhythmic, beta-adenoreceptor blocking, antihypertensive, anti-angina, anticoagulants, protamine, antiplatelet, anti-fibrinolytic, homeostatic and lipid-lowering agents [69]. The treatments are important to relieve the symptoms, risk, and complications. As for the treatment of ischemic heart disease, targeting the main cause of the disease which is amelioration of acute myocardial infarction and coronary blood flow by using drugs such as streptokinase, tissue plasminogen activator (tPA), antiplatelet agent, statins and beta-blockers [70], [71] are required. tPA also known as thrombolytic drugs is a type of protein involved in the breakdown of blood clots and improving blood flow. Antiplatelet agents can help reduce the risk of a heart attack by preventing blood clot. For individuals with high cholesterol level, statins can block the formation of cholesterol thus inhibiting the restriction of coronary blood flow occurring due to accumulation of cholesterol plaque. Furthermore, beta-blockers are commonly used in angina prevention and lowering of blood pressure resulting in slowing down of heartbeat to improve blood flow. Glycoside is used to treat individual with congestive heart failure to stimulate contractility [72].

Studies also show that high consumption of fried fast food as well as processed foods containing vegetable shortening leads to CVD [73]. Therefore, selection of foods containing natural fat such as extra virgin olive oil [74], canola [75], peanut [76], [77], avocado [78], soy [79], fish [80], [81], or switching to low-fat or non-fat dairy products [82] protects the heart. Consumption of large amounts of vegetables [83], nuts; walnut and almond [84], [85], wholegrain cereals [86], [87] and avoidance processed meat [87] retains healthy heart. Most importantly is reduction of uptake and ensuring selection of foods with minimal sodium amount [88]–[90]. Moreover, alternative medicines such as spices and other traditional medicine approaches have become other good alternatives in the treatment of patients with heart disease [91].

5.0 FUNCTIONAL FOODS PROMOTES HEALTH BENEFITS AGAINST ISCHEMIC HEART DISEASE

Recent studies point to the fact that functional foods like fish, milk and nuts are beneficial against CVD [16]. Health-promoting functional foods which are diet high in fruits, vegetables, cereals, beans, nuts, seeds and olive oil can help prevent the risks of chronic inflammation in the heart, consequently minimizing CVD [92]. Plant products contain large number of antioxidants that effectively inhibits oxidation and protects cells from free radicals. Nutrient rich antioxidants and polyphenols in foods reduce reactive oxygen species (ROS) concentration in tissues thus inhibiting cell damage. A review on fish and fish oils containing good omega-3-fatty acids indicated reduction in the mortality rate of patients suffering from CVD [93]. Fish and fish oils containing omega-3-fatty acid known as eicosapentaenoic acid (EPA) plus docosahexaenoic acid (DHA) as intervention has the potential to reduce mortality in post-myocardial infarction patients by reducing the risk of sudden cardiac death in controlled trials [17].

Moreover, cocoa suggested as mediator to control blood pressure, insulin resistance, and vascular ad platelet function thus have recommended as plant-derived foods to reduce risk of CVD [14]. Cocoa known as its good taste also have more beneficial on cardiovascular health as it contains polyphenols, bioavailability of nitric oxide thus induces relaxation of vascular smooth muscle.

Milk also introduced as one of the important functional foods against CVD likewise ischemic heart disease [19]. Bioactive peptide in milk proteins has potential functional and physiological roles in relation to CVD. Milk-derived tripeptides (isoleucine-proline-proline or IPP, and valine-proline-proline or VPP) significantly reduce the blood pressure thus reduce the risk of hypertension to the development of CVD [94]. A meta-analysis review by Alexander *et al.*, (2016) examined the association of dairy consumption with 12 % - 13 % reduction of CVD and cheese with 13 % - 18 % reduction of coronary heart disease.

Steroidal saponin diosgenin of yams (*Dioscorea bulbifera*) protects cardiac cell from hypoxia-reoxygenation injury through activation responsible proteins involve for cell death (Bax) and cell survival (Bcl-2, hemeoxygenase-1 and Akt) [95]. Furthermore, fenugreek shows to increase milk production of rodent models of lactation challenge resulting in pup's growth increases [96].

CVD is a disease that is associated with excessive oxidative stress responsible for various CVDs. ROS stimulates cellular apoptosis signaling, increases cardiac fibroblast proliferation and activates matrix remodelling (myocardial growth) as well as cellular dysfunction leading to heart failure [97]. Redox balance of ROS controlled by antioxidants that help to keep ROS concentrations down at picomolar range [98]. The cellular physiology requires low

amount of ROS whilst excess ROS led to oxidative stress resulting in cellular damage and eventually cell death. Antioxidant including coenzymes Q10 [99][100], beta carotene [101], lycopene [102], [103], quercetin [104], [105], resveratrol [106], vitamin C [107] and vitamin E [108] are agents that can help combat CVD associated with oxidative stress. Vitamin C and D suggest inhibition of the oxidation of low density lipoprotein cholesterol [107].

6.0 FENUGREEK, THE NOTORIOUS ANCIENT MEDICINE AS HERB AND SPICE

Fenugreek or scientifically known as *Trigonella foenum-graecum* is listed is one of the oldest medicinal plants that play a role in promoting healthy body [109]. Fenugreek is an annual herb of southern Europe and eastern Asia with white flowers, aromatic seeds and used widely in foods preparing. In traditional Chinese medicine, fenugreek (Hú Lú Bā) was used long ago for the treatment of kidney [110] and liver [111]. In India, fenugreek (methi) was used in Ayurveda and it is beneficial against hair fall and dandruff as the seeds help in moisturizing the hair [46], [112]–[114]. Fenugreek act as antibacterial against *Staphylococcus aureus*, *Pseudomonas aeruginosa* and anticancer agent for MCF-7 breast cancer cells, liver cancer HCAM cells and the non-cancerous Vero cell lines, with no cytotoxic activity [115]. Fenugreek shows properties of antidiabetic agent either through oral ingestion or injection to improved blood glucose, renal and liver functions evens at higher doses or longer duration of treatment [116]. Fenugreek antioxidants properties suggested as a potential cardioprotective against the myocardial infarction by suppression of oxidative reaction [117].

Fenugreek is used in traditional medicine since ages. The present study shows that fenugreek as growth promoter and has many therapeutic properties in animal's metabolism and physiology [118], [119]. It reduces plasma cholesterol levels [120], enhances food consumption, indicates a stimulatory influence on immune functions [121], decreases kidney/body weight ratio [122], blood glucose [116], blood lipid levels and improves hemorheological parameters. Since the systemic studies on the effect of fenugreek on heart are very limited as only on the *in vivo*, the study on *in vitro* aimed to investigate the mechanistic action of fenugreek as therapeutic cardioprotectant agents suitable as a daily food supplement against ischemic heart injury.

7.0 THERAPEUTIC USES OF FENUGREEK

Medicinal plants like fenugreek is listed in the WHO monographs as herb that gives many health benefits [109]. Favourite dishes from Malaysia such as, "Nasi Dagang" and "putu halba", as well as curry, utilise fenugreek in generous amounts as spice. In India, fenugreek was occasionally used as drinks in daily life, and they also use part of the tree likewise the fenugreek leaves in their dishes, 'Aloo Methi'. Most of country use the fenugreek in cooking to enhance and mitigate the flavour of other ingredients. Fenugreek is also used in some bakery products, frozen dairy products, condiments, spices, pickles, and beverages in addition to its ability to treat different types of diseases [123]. Despite being ubiquitous in different types of dishes across different countries, information on its nutritional value specifically in the heart at the molecular mechanism level is still lacking. Information sharing on the importance of functional food can help maintain healthy lifestyles across urbanization, globalization, and development. The needs for a continuous supply of functional foods are essential as the population grows, the demands are high.

Fenugreek is a decent candidate for functional foods as it has active phytochemical compounds that play a major role in protecting the heart from diseases. A large amount of amino acids and galactomannan is found in fenugreek seed as compare to maize, soybean [124], white lupine and Durum wheat [125]. Fenugreek is reported to lower the lipoproteins, carriers of the cholesterol in the blood for oxidation. The fiber present in fenugreek seeds binds to bile acids to reduce cholesterol level and fat absorption. Additionally, mucilage in fenugreek seeds can relieve gastrointestinal inflammation, thus markedly diminishing severity of heartburn severity [126]. Furthermore, traditionally fenugreek used as medicine worldwide (Table 1) helps combat notorious CVD diseases that contribute to the deliberately high mortality rate globally.

Phytochemical analysis reveals the presence of steroids, alkaloids, saponins, polyphenols, flavonoids, lipids, carbohydrates, amino acids, and hydrocarbons in fenugreek play an important role in medical (Table 2) [127]. Fenugreek seed extract has been reported to treat gastric [128]. Additionally, fenugreek has beneficial effects in nutraceutical products, for a smoother and softer skin since it contains natural oil, fats, and mucus that help hydrate and moisturize. Additionally, fenugreek purifies the blood, cleansing the lymphatic system, and detoxifies the body [123]. Fenugreek is investigated in preclinical and clinical research as antidiabetic, antinociceptive, antihyperlipidemic, antiobesity, anticancer, anti-inflammatory, antioxidant, antifungal, antibacterial, galactogogue and for miscellaneous pharmacological effects, including improving women's health [129].

Table 1 Fenugreek, a traditional medicine used across the countries in the world

Country /Region	Fenugreek Plant Parts	Treatment	References
China	Seed	Cervical cancer and kidneys problem	[130]
Middle East Balkans	Aerial	Abdominal cramp due to diarrhoea	[131]
India	Roasted Seed, Stem, Leaves	Dysentery; cooling agents for smallpox patients; hair loss, flavouring agent	[132]
Sudan	Seed oil	Dysentery and stomach disturbances and antimicrobial	[133]
Egypt	Seeds	Winter drink, a remedy for diabetes, ease sleep at night, ease childbirth and increase milk flow	[116]
Iran	Leaves	Cold cough, splenomegaly, hepatitis, backache, and bladder cooling reflex	[134]
USA	Absolute compounds	Post-menopausal vaginal dryness and dysmenorrhea, flavouring	[135][136]
Morocco	Seeds	Stimulate appetite and preventive medicine for stomachache	[137]
Ethiopia	Seeds, Aerial	Flavouring, treat diabetes	[138]
Swiss	Seeds	Flavouring cheese	[123]
Pakistan	Seeds	Antioxidant	[139]

Similarly, fenugreek has many therapeutic properties in animals [140] and have been investigated as a growth promoter [141]. Fenugreek 1) reduces plasma cholesterol via selective reduction of LDL-cholesterol in a rabbit [142], 2) enhances food consumption of male Wistar rats due to increased food intake which occur within 24 hours [143], 3) decreases kidney or body weight ratio via inhibition of kidney stone formation [144] as well as blood glucose level (due to the fiber fraction) [145], 4) reduces blood lipid levels [146], 5) improves hemorheological parameters [147] and 6) is also effective against gastric ulcer acting as a gastro-protectant in wistar rats [148].

A combination of Korean black ginseng with fenugreek protects TM3 leydig cells of mouse from oxidative stress, increases testosterone levels, and regulates cell survival mechanism via activation of the Erk kinase enzyme [149]. Fenugreek seed powder has been demonstrated to protect rats from aluminium-chloride (AlCl3)-induced Alzheimer's disease (AD) by 1) inhibition of acetylcholinesterase, and activating Akt/GSK3β pathway [150] as well as 2) attenuation of the AlCl3-induced memory deficits, amyloid and tau pathology, oxidative stress, and inflammation in AD rats [151]. Apart from afore-mentioned diseases, there is limited effect of fenugreek on the heart. Investigating the activity at the molecular level rather than focusing on the physiological effect may uncover the mechanisms involved.

Overall, fenugreek's application in ameliorating various diseases across the world is varied. In China, the seeds are used to treat cervical cancer and kidney problems. On the other hand, the aerial parts of the plant are used to treat abdominal cramps during diarrhoea in the Middle East and the Balkans. In southern India, the seeds are roasted and are used to treat dysentery or as a cooling agent for patients with smallpox. Additionally, fenugreek can reduce blood sugar and cholesterol levels in diabetic patients. A low dose of fenugreek (less than 5%) improves the rats' liver function [120]. Fenugreek contains a healthy polysaccharide that triggers the breakdown of fat and sugar metabolism in the body. Various other uses of fenugreek include antispasmodic [136], appetite stimulant [152], wounds [121], blood cleanser [153] and as expectorant [154].

An active agent, protodioscin identified in fenugreek induces apoptosis in the leukemic cell line, HL-60 [155]. In addition, crude methanol extract of fenugreek seeds potentially induces apoptosis in hepatocellular carcinoma cell line, HepG2 through p53, Bax and proliferating cell nuclear antigen that upregulate caspase-3 activation [156]. The anti-tumor properties of fenugreek were further investigated on growth-inhibitory effect on breast cancer, MCF-7 human breast cancer cells [157], [158]. Alshatwi *et al*. (2013) reported that fenugreek methanol extract inhibits MCF-7 cell line growth at certain time and dose-dependent upregulation of Fas receptor expression induced apoptosis in non-dependent manner on caspases, FADD or p53 activation [158]. A recent finding on fenugreek against breast cancer AMN-3 (Ahmed-Mohamed-Nahi-3) and normal cell lines of REF (rat embryo fibroblast), found that total alkaloids extract of ethanolic fenugreek extraction reduces AMN-3 cell viability [159].

Table 2 Bioactive compounds isolated from fenugreek and its major functions in promoting good health

Bioactive Compounds in Fenugreek	Medical Properties
Saponin (fenugreekine, diosgenin)	Reduces anxiety [160], inhibit cholesterol absorption [161], alleviate diabetes associated liver damage [162], ameliorates dyslipidemia [163], acts as phytoestrogen in skeletal system of post-menopausal condition [164]
Alkaloids (trigonelline, gentianine, carpaine)	Hypoglycemic agent [165], antioxidant effects [166]
Amino acids	Improved properties of food products [167], and livestock feed preparation [124].
Flavonoids (Quercetin, kempferol, apigenin, catechin, vitexin, isovitexin)	Antioxidant and anticancer [168][169], antinociceptive [170], anti-inflammatory [171] and antimicrobial activities [172]
Galactomannan	Emulsifier, stabilizer and thickening agent [173], as prebiotic [174],
Polyphenol	Maintains hemoglobin [175]; is gastro-protective [148].
Lipids (Neutral lipids, glycolipids, phospholipids)	Endogenous-sleep inducing factor [176].

Fenugreek seeds extract inhibit apoptosis of neoplastic cell as seen on the human acute myeloblastic leukaemia cell line, KG-1 thus suggesting a significant chemotherapeutic effect [177]. On the other hand, fenugreek seed oil has similar effect in to promoting cytotoxicity and mitochondrial-mediated apoptosis in human HepG2 via ROS generation [178].

Upregulation of pro-apoptotic gene expression (p53, Bax, caspase-3, caspase-9) and downregulation of anti-apoptotic gene (Bcl-2) has been reported in HepG2 cells treated with fenugreek seed oil at two doses (50 and 100 µg/mL. Furthermore, apoptotic effect of fenugreek seed extract on ethanol-induced toxicity in Chang liver cells was also seen [179] where the extract protects the ethanol-induced toxicity and apoptotic of cells coupled with reduction in the production of ROS and lipid peroxidation. Depending on the concentration and cell types, variable cytotoxicity of fenugreek alkaloid activity against cancer and normal cell lines were observed [180]. Additionally, fenugreek promotes cardioprotection via inhibition of apoptotic expression although its mechanism remains unclear.

8.0 FENUGREEK PROMOTES CARDIOPROTECTION

To date, there are limited studies available on cardiovascular drugs which can either cure or prevent heart diseases since many studies focus only on a few number of heart diseases such as heart attack involving clear-up the clogged arteries [181], blood pressure lowering [120] and cholesterol reduction for clearing up plaque formation [182].

Moreover, the study on the mechanisms of fenugreek as therapeutic cardioprotection drugs at the molecular level confirms reduction in lipid peroxidation and enhanced antioxidant activities in a male wistar albino rats [183]. It also showed that fenugreek prevented a high-fat diet induced plasma lipid elevation and fat accumulation in mice [184]. Clinical studies on 60 patients with type 2 diabetes showed lower blood glucose levels after 24 weeks of administration of 25 g of fenugreek seed powder [185], [186] and had a significant effect on improving lipid metabolism without side effects [187]. Diabetes increases the risk of coronary artery disease, myocardial infarction, high blood pressure, and dyslipidemia. Other than fenugreek, some other foods recommended to prevent cardiomyocytes apoptosis include broccoli [188], flaxseed [189], blueberry [190] and ginseng [191]. Aqueous formulation of fenugreek seeds can reduce the hepatic lipid accumulation associated with heart attack and stroke [192].

The main active constituents of fenugreek are diosgenin, trigonelline, 4-hydroxyisoleucine and galactomannan. The alkaloid trigonelline is believed to decrease diabetic glycosuria [184]. Galactomannan and potassium present in fenugreek are beneficial to the heart [193]. The high potassium level prompts sodium excretion from the kidneys through the urine. Since sodium is hygroscopic in nature, it pulls water together thus resulting in the elimination of higher number of fluids from the body which can help lower the blood pressure. It also helps to control arrhythmias by regulating the heart rhythm. Fenugreek seeds can enhance the heart function by regulating both the blood pressure and heart rate. Flavonoids, natural phenolic compounds found in vegetables and fruits stimulate the survival pathways by preserving the survival of pancreatic beta cell to prevent both extrinsic and intrinsic signaling pathways involved in apoptosis [194]. Fenugreek or a combination of fenugreek with garlic ameliorates the high cholesterol level of isoproterenol-induced myocardial infarction in wistar rats by alleviating of oxidative stress through alteration of myocardial Ca2+-ATPase and antioxidant enzymes activities [117].

9.0 CONCLUSION

In summary, this article examines ischemic heart disease and fenugreek as a potential functional food in the treatment of heart disease. The acceptance of functional foods as treatment agents is encouraging compared to the use of pharmaceuticals. Thus, further research may highlight the importance of phytochemical compounds present in fenugreek in the treatment of heart disease.

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