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Article

Clinacanthus nutans: Its potential against diabetic vascular diseases

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Diabetes is an independent risk factor for the development of cardiovascular disease, with approximately 80% of cardiovascular mortality and morbidity linked to vascular complications such as atherosclerosis. It has been estimated that up to one-third of patients with diabetes mellitus use some form of complementary and/or alternative medicine. One plant that has received attention from diabetic patients for its perceived antidiabetic properties is *Clinacanthus nutans*, a member of the Acanthaceae family that is known as snake grass. Ethnomedical applications of this herb have been identified for the treatment of certain conditions, including fever, diabetes, skin rashes, and insect bites. This review aims to assess the potential of *C. nutans* to be used in the prevention and/or treatment of diabetic vasculopathy. Evidence for antidiabetic, anti-inflammatory, and dyslipidemic properties of *C. nutans*, as shown from experimental studies, is presented and discussed. Diabetes, inflammation, and hyperlipidemia are known to play significant roles in the initiation and severity of diabetic cardiovascular disease; thus, targeting these factors might be beneficial for preventing and/or treating diabetic vasculopathy.

Keywords: *C. nutans*. Diabetes. Inflammation. Cardiovascular disease

INTRODUCTION

Diabetes mellitus is a metabolic disorder characterized by altered glucose and lipid metabolism, which leads to persistent hyperglycemia. Individuals with diabetes have a greater risk of developing vascular complications despite intensive glycemic control, stressing the need for novel approaches to lessen the burden of diabetic-mediated macrovascular injury. The vascular endothelium plays a significant role in diabetes-associated atherosclerosis through the regulation of vessel permeability, inflammation, coordination of leukocyte trafficking, and thrombosis (Funk *et al*., 2012; Kolka *et al*., 2013; Sharma *et al*., 2012). Endothelial cells regulate vascular function and structure because of their strategic anatomic position between the vessel wall and circulating blood. In normal endothelial cells, biologically active substances are synthesized and released to maintain nutrient delivery and vascular homeostasis and to ensure adequate blood flow, while also preventing leukocyte extravasation and thrombosis.

Nitric oxide (NO) is produced by endothelial NO synthase (eNOS) in the endothelial cells through five-electron oxidation of the guanidine–nitrogen terminal L-arginine. NO causes vasodilation by activating guanylyl cyclase on subjacent vascular smooth muscle cells (Creager *et al*., 2003). NO also protects blood vessels from endogenous injury by mediating molecular signals that inhibit leukocyte and platelet interaction with the vascular wall and prevent vascular smooth muscle proliferation and migration (Kubes *et al*., 1991;

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Radomski *et al*., 1987; Sarkar *et al*., 1996). A reduction in endothelium-derived NO increases the activity of the pro-inflammatory transcription factor nuclear factor-kappa B (NF-κB), resulting in over-expression of leukocyte adhesion molecules and an increase in the production of cytokines and chemokines. These activities promote vascular smooth muscle cell and monocyte migration into intima and the formation of foam cells, characterizing the initial morphological changes of atherosclerosis (Creager *et al*., 2003). Endothelial dysfunction, as characterized by impaired endothelium-dependent NO-mediated relaxation, has been reported in both cellular and experimental models of diabetes (Mokhtar *et al*., 2016). In addition, endothelial dysfunction, diabetes, and atherosclerosis are linked to a heightened state of oxidative stress and its main mediator, superoxide anion. Superoxide scavenges free NO to form peroxynitrite, another potent reactive oxygen species (ROS), which significantly contributes to lower NO bioavailability (Sharma *et al*., 2012). A majority of the metabolic derangement known to occur in diabetes, including hyperglycemia, insulin resistance, and excess free fatty acid liberation, mediate abnormalities in endothelial cell function by affecting the synthesis or degradation of NO (Creager *et al*., 2003; King, 1996) (Figure 1).

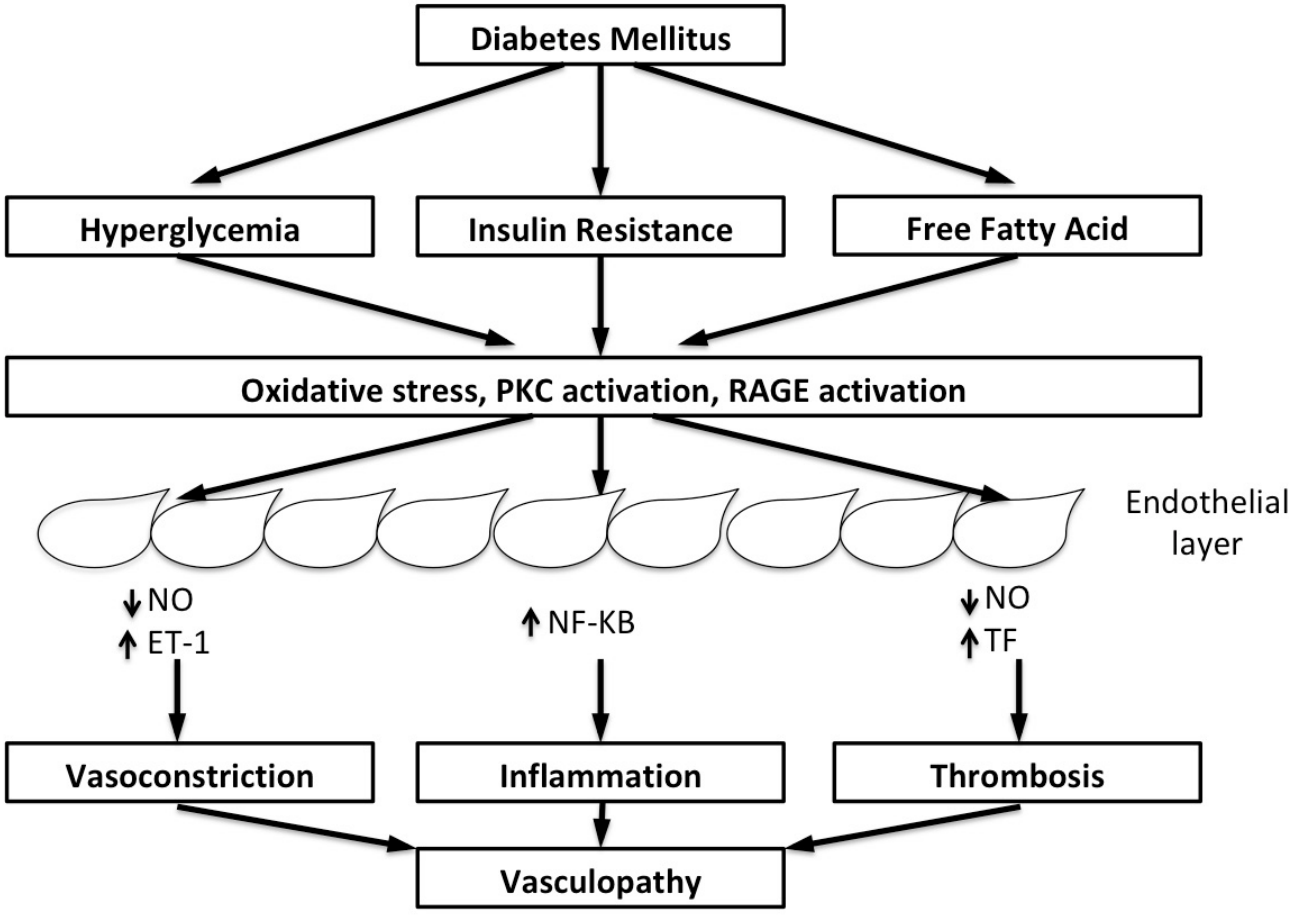

FIGURE 1 - The metabolic abnormalities that characterize diabetes, particularly hyperglycemia, insulin resistance, and free fatty acids, act to stimulate molecular mechanisms that change the function and structure of blood vessels. These include increased oxidative stress, disturbances of intracellular signal transduction (activation of protein kinase C [PKC]), and activation of receptors for advanced glycation end product (RAGE). Consequently, there is decreased availability of NO, increased production of endothelin (ET-1), activation of transcription factors such as NF-κB, and production of prothrombotic factors such as tissue factor (TF).

Insulin resistance characterizes type 2 diabetes mellitus. Insulin increases NO production in endothelial cells by increasing the activity of NO synthase (NOS) via activation of Akt kinase and phosphatidylinositol-3. Thus, insulin increases endothelium-dependent vasodilation. In diabetic subjects, insulin resistance impairs the phosphatidylinositol-3 kinase pathway and causes a reduction in insulin's ability to activate NOS and produce NO (Creager *et al*., 2003). In addition, insulin resistance is associated with elevations of free fatty acid levels. In the diabetic state, levels of free fatty acids are elevated because of their excess liberation from adipose tissue and diminished uptake by skeletal muscle (Boden, 1999; Creager *et al*., 2003; Kelley, Simoneau, 1994). High free fatty acid levels cause endothelial dysfunction through several mechanisms, including activation of PKC, increased production of oxygen-derived free radicals, and exacerbation of dyslipidemia (Creager *et al*., 2003; Dresner *et al*., 1999; Inoguchi *et al*., 2000). It has been proven that free fatty acids reduce endothelium-dependent vasodilation in human and animal models *in vivo* (Creager *et al*., 2003; Steinberg *et al*., 1997), and elevation of free fatty acid concentrations activate PKC and decrease insulin receptor substrate-1 associated phosphatidylinositol-3 kinase activity (Creager *et al*., 2003; Dresner *et al*., 1999; Griffin *et al*., 1999). These effects reduce NOS activity, as discussed above.

Ethnopharmacology of *C. nutans*

Clinacanthus nutans (Burm. f.) Lindau, commonly called Sabah Snake Grass, or "Belalai Gajah" in layman's terms, is widely used in Malaysia, Thailand, and Indonesia as a traditional medicine. The name Sabah Snake Grass is derived from its application by traditional practitioners as an antivenin for poisonous snakes (Kamarudin *et al*., 2017). Traditionally, it has been used to treat diabetes, cancer, herpes infections, inflammation, and various skin problems (Alam *et al*., 2017; Sakdarat *et al*., 2006; Sangkitporn *et al*., 1995; Shim *et al*., 2013). In Malaysia, the fresh leaves of *C. nutans* are commonly consumed as an herbal tea for the treatment of diabetes, skin rashes, and fever, as well as to induce diuresis (Kamarudin *et al*., 2017; Shim *et al*., 2013). In addition, the fresh leaves of *C. nutans* are consumed in the treatment of insect bites (ant, bee, hornet, mosquito, and wasp), stings by Chilopoda (centipede) and Arachnida (scorpion), sea creature bites (catfish and jellyfish), snake bites, eye diseases, and allergic responses (Daduang *et al*., 2008; Kamarudin *et al*., 2017; Uawonggul *et al*., 1986).

Bioactivities of *C. nutans*

Natural products continue to be one of the major sources of drug discoveries owing to their ability to evoke complex cellular pathways in preventing and alleviating various diseases (Kamarudin *et al*., 2017). Therefore, research of natural products for chronic diseases is of major significance as it may lead to the development of newer therapeutic agents with potentially fewer side effects. Previous studies and scientific investigations have supported the traditional use of *C. nutans* in various diseases. Scientifically, extracts of *C. nutans* are reported to have a number of pharmacological properties, with effects that include being antihyperglycemic (Alam *et al*., 2017), antibacterial (Huang *et al*., 2015), anti-inflammatory (Ho *et al*., 2013; Wanikiat *et al*., 2008), anti-herpes (Kunsorn *et al*., 2013; Wanikiat *et al*., 2008), antioxidant (Sangkitporn *et al*., 1995; Sarega *et al*., 2016), and antihyperlipidemic (Sarega *et al*., 2016).

Pharmacologic Properties of *C. nutans* That Are Potentially Useful for Preventing or Ameliorating Diabetic Vasculopathy

Antihyperglycemic effect

Diabetes is an endocrine disease characterized by hyperglycemia arising from insulin insufficiency and/or insulin resistance. In type 2 diabetes, macrovascular and microvascular complications are associated more with postprandial hyperglycemia than with fasting glucose levels. Postprandial hyperglycemia is a marker of vascular risk for microvascular and macrovascular complications in not only type 2 diabetes but also for those with impaired glucose tolerance (DiNicolantonio *et al*., 2015). Postprandial hyperglycemia causes vascular endothelial dysfunction by inducing oxidative stress responses that decrease NO bioavailability. Major sources of oxidative stress during postprandial hyperglycemia include the production of mitochondria-derived ROS, induction of NF-κB-dependent pro-inflammatory enzymes and cytokines, and uncoupling of eNOS (Mah *et al*., 2012).

Alpha-glucosidase is an enzyme that regulates postprandial glucose levels by hydrolyzing carbohydrates into glucose in the small intestine (Tundis *et al*., 2010).

Inhibition of the α-glucosidase enzyme in the small intestine is an effective approach for the management of carbohydrate metabolic disorders, including type 2 diabetes mellitus (Olubomehin *et al*., 2013). Therefore, a natural product that has activity as an α-glucosidase inhibitor becomes one of the main targets in searching for new compounds for the therapeutic management of diabetes (Alam *et al*., 2017).

Available studies on the antihyperglycemic effects of *C. nutans* were all performed *in vitro.* Lee *et al.* (2014) demonstrated that the methanol extracts of the leaves and stem of *C. nutans* exhibited α-glucosidase inhibitory activity (13.57% and 17.67%, respectively) at the dose of 5 mg/mL. Further, Wong *et al.* (2014) reported that treatment with a higher dose (50 mg/mL) of aqueous extract of *C. nutans* showed high levels of inhibition of α-glucosidase activity (88.2%, IC_{50}: 30 mg/mL). In another recent report, Khoo *et al.* (2013) showed that extract from oven-dried leaves of *C. nutans* (70% ethanol with sonication) inhibited α-glucosidase activity up to 41% at a concentration of 5000 ppm, or approximately 5 mg/mL.

In addition, a recent study by Alam *et al.* (2017) reported on the α-glucosidase inhibitory activity of *C. nutans* methanol extract and its fraction at six different dilutions (6.25, 12.5, 25, 50, 100, and 200 μg/mL). Butanol, ethyl acetate, and hexane fractions revealed similar α-glucosidase inhibitory activities of 72.16% (IC_{50}: 37.47 μg/mL), 70.76% (IC_{50}: 53.69 μg/mL), and 69.94% (IC_{50}: 44.57 μg/mL), respectively, whereas the methanol extract showed moderate inhibitory activity at 58.21% (IC_{50}: 61.39 μg/mL). The butanol fraction had significantly higher α-glucosidase inhibitory activity, with an IC_{50} of 37.47 μg/mL, which is close to the standard quercetin (positive control) compound (IC_{50}: 38.54 μg/mL).

Previous studies showed that α-glucosidase inhibitory activity in extracts mostly involved flavonoid compounds (Alam *et al*., 2017; Fornari *et al*., 2012). Khoo *et al.* (2013) showed that application of the oven-dried method with sonication during 70% ethanol extraction resulted in greater levels of the phenolics, terpenoids, and sulfur-containing glucosides that contributed to the α-glucosidase inhibitory activity. Based on data reported by Alam *et al.* (2017), the phytoconstituents that were identified from the methanol extract and four fractions of *C. nutans* included neophytadiene, squalene, lupeol, tocopherols, vanillic acid, syringic acid, myo-inositol, glycolic acid, butanedioic acid, 4-coumaric acid, and stigmasterol. Extensive literature searches document that the identified compounds were responsible for various biological activities, including acting as antioxidant, antidiabetic, anti-inflammatory, chemotherapeutic, and antihyperglycemic agents (Alam *et al*., 2017; Ow, Ieva, 2003). The structural orientation of the polyphenolic compound, owing to the lactones/quinones or 4-oxo-pyran moiety, is responsible for the digestive enzyme inhibitory activity (Alam *et al*., 2017). Previous studies showed that phenolic compounds from *C. nutans* helped to reduce intestinal digestive enzyme activity and were able to oxidize body fat, owing to their thermogenic properties (Alam *et al*., 2017; Alterio *et al*., 2007), whereas the terpenoids and terpenes class of compounds can act as antihyperglycemic agents (Alam *et al*., 2017; Tundis *et al*., 2010).

In summary, based on previous studies, it appears that the methanol extract of *C. nutans* leaves is the best extract type for exhibiting α-glucosidase inhibition activity, although the crude extract showed moderate activity. In addition, the butanol fraction from the methanol extract of *C. nutans* appears to show a greater inhibitory effect on α-glucosidase (Alam *et al*., 2017).

Antioxidant properties and effects on insulin resistance

Many studies have provided evidence for the significant role of oxidative stress in insulin-resistant states such as type 2 diabetes, obesity, and metabolic syndrome (Pitocco *et al*., 2013). Oxidative stress occurs due to an imbalance between endogenous antioxidant systems and the generation of ROS. ROS overproduction has been reported to be an important trigger of insulin resistance and a contributing factor in the development of type 2 diabetes (Houstis *et al*., 2006), and previous studies have suggested that ROS and endothelial dysfunction play significant roles in the pathogenesis of diabetic vasculopathy (Schaffer *et al*., 2012). It has been shown that, in diabetes, the endothelium fails to produce adequate amounts of NO, and blood vessels fail to relax in response to endothelium-dependent vasorelaxants such as acetylcholine, shear stress, and bradykinin (Ceriello *et al*., 2001). High levels of plasma glucose lead to increased mitochondrial formation of superoxide, a ROS that produces peroxynitrite when reacting with NO. It has been reported that peroxynitrite production is increased in the platelets of diabetic individuals (Ceriello *et al*., 2008). The levels of nitrotyrosine (a marker of ROS) in endothelial cells, myocytes, and fibroblasts significantly relate to the

degree of cell death (Tannous *et al*., 1999) in the hearts of both diabetic patients and streptozotocin-induced diabetic rats (Frustaci *et al*., 2000). Peroxynitrite causes cellular damage through various mechanisms, including degradation of different biomolecules in the vascular endothelium, myocardium, and vascular smooth muscle and the depletion of tetrahydrobiopterin, the cofactor of eNOS for NO biosynthesis (Pitocco *et al*., 2013).

Free radical species can be neutralized through dismutation or reduction by endogenous antioxidants like superoxide dismutase (SOD) and catalase, as well as through direct scavenging or electron transfer by exogenous antioxidants such as vitamins C and E (Kohen, Niska, 2002). Pannangpetch *et al*. (2007) first reported the antioxidant activity of *C. nutans* where the ethanol extract (50%) of the dried leaves exhibited a dose-dependent ability to scavenge 2,2-diphenyl-1-picrylhydrazyl (DPPH), with an IC_{50} value of 110.4 ± 6.59 µg/mL. Although a 50% scavenging effect was achieved at a dose of 110 µg/mL, the highest dose (1000 µg/mL) did not show complete DPPH inhibition but, rather, did show a moderate radical scavenging activity of 67.65%. While the positive control, ascorbic acid (IC_{50}: 9.56 ± 0.56 µg/mL), exhibited an almost complete radical scavenging effect, the DPPH scavenging efficacy of *C. nutans* was only 0.08 times that of ascorbic acid.

Yong *et al*. (2013) showed that the semipolar chloroform extract of *C. nutans* leaves demonstrated the most effective DPPH radical scavenging effect when compared to methanol and aqueous extracts, with high levels of antioxidant capacity of 7852.63 ± 449.90 µg Teq/g extract at different concentrations (12.5, 25, 50, and 100 µg/mL). The aqueous extract showed the lowest activity, with an antioxidant activity value of 864.11 ± 73.49 µg Teq/g extract. The antioxidant effect of the *C. nutans* extracts decreased in the order of chloroform > methanol > aqueous. In addition, the chloroform extract showed a greater galvinoxyl radical scavenging effect, with a high value of 12,248.82 ± 173.50 µg Teq/g extract. However, that study also demonstrated that NO and hydrogen peroxide radical scavenging activities were lower than those of the positive controls ascorbic acid and quercetin (both at 100 µg/mL), which exhibited more than 50% activities. The IC_{50} value and the antioxidant activity obtained in the study (aqueous extract) were similar to the first study on the antioxidant activity of *C. nutans* extract against DPPH (Pannangpetch *et al*., 2007).

In a different study, Arullappan *et al*. (2014) demonstrated that methanol, ethyl acetate, and petroleum ether extracts of *C. nutans* leaves exhibited greater DPPH radical scavenging activity, between 70% and 82%, at higher concentration ranges of 4–10 mg/mL. Similarly, Wong *et al*. (2014) showed that the aqueous extract of *C. nutans* at 10 mg/mL resulted in 60% DPPH radical scavenging activity. Furthermore, the aqueous extract of the whole plant demonstrated metal chelating activity of up to 90% in a dose-dependent manner (1–10 mg/mL). Sulaiman *et al*. (2015) reported that chloroform and ethyl acetate extracts of *C. nutans* showed high levels of DPPH radical scavenging activity when compared to ethanol and hexane extracts. The hexane extract of *C. nutans* exhibited the greatest antioxidant activity (720 mg Teq/100 g extract) in the β-carotene bleaching assay, while the ethanol extract showed the lowest activity in this study. In contrast, when tested using an oxygen radical absorbance capacity (ORAC) assay, the ethanol extract demonstrated the most effective antioxidant activity (229.5 mMol TE/100 g extract), followed by the ethyl acetate, chloroform, and hexane extracts. However, the study lacked positive controls in all their assays, and the authors did not properly indicate the concentration range used; therefore, it is difficult to determine the dose-dependent relationship.

Wanikiat *et al*. (2008) reported that the methanol extract of *C. nutans* showed a weak scavenging activity, with an EC_{50} of 240 ± 15.3 mg/mL, as compared to the positive control Trolox (EC_{50}: 7.5 ± 2.3 µg/mL). Although the majority of DPPH radical scavenging of methanol extract showed a weak antioxidant activity, *in vitro* administration of *C. nutans* methanol extract (0.1–100 µg/mL) dose-dependently suppressed *N*-formyl-methionyl-leucyl-phenylalanine (fMLP)-induced superoxide anions in human neutrophils, with an IC_{50} of 24.3 ± 3.1 µg/mL. Although the positive control indomethacin (0.01–100 µg/mL) inhibits superoxide anions more effectively (IC_{50}: 0.82 ± 0.2 µg/mL), the *C. nutans* inhibitory activity against fMLP-induced superoxide anions was significant and effective compared to untreated cells (Wanikiat *et al*., 2008).

Excessive production of reactive nitrogen species (RNS), which includes NO and peroxynitrite (ONOO-), has induced aberrant inflammation by causing oxidative and nitrosative or nitrative stress, lipid peroxidation, and disruption of cell membrane integrity. These processes can lead to the disruption of the mitochondrial electron transport chain (ETC) complexes that alter mitochondrial function, inducing mitochondrial leakage, with enhanced ROS production (Osto *et al*., 2008; Ott *et al*.,

2007; Preiser, 2012). Additionally, the aqueous extract of *C. nutans* showed the ability to scavenge NO in a dose-dependent manner, with a moderate scavenging activity of 32.33 ± 0.97% at 100 µg/mL (Yong *et al*., 2013). This moderate NO radical scavenging activity of *C. nutans* was supported by a recent finding that demonstrated that the whole aqueous extract of *C. nutans* scavenged NO in a concentration-dependent manner at a higher dose range of 1, 5, and 10 mg/mL. The aqueous extract of *C. nutans* at a dose of 10 mg/mL showed more than 90% NO scavenging activity (Wong *et al*., 2014).

Furthermore, a recent report demonstrated that the antioxidant activity of *C. nutans* is associated with its ability to modulate the expression of various antioxidant genes (Sarega *et al*., 2016). Administration of aqueous and aqueous methanol leaf extracts of *C. nutans* upregulated the expression of SOD1, SOD2, catalase, glutathione reductase, and glutathione peroxidase. These then significantly increased hydroxyl radical scavenging activities and suppressed lipid peroxidation in rats fed a high-fat and high-cholesterol diet (Sarega *et al*., 2016). Additionally, both hexane and methanol leaf extracts showed the greatest DPPH, 2,2-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), and ferric reducing ability (FRAP) radical scavenging activities because of the high amounts of phenolic compounds present in the extracts (Sarega *et al*., 2016). Generally, all studies indicated the antioxidant activities of *C. nutans* to be moderate, with efficacy of approximately 60–80% radical scavenging activity at higher concentration ranges (in mg/mL).

All except one study (Sarega *et al*., 2016) used the DPPH method to assess the antioxidant properties of *C. nutans*. The DPPH method has been reported to be an easy, fast, and reliable method that does not require a special device and reactions, as compared to other antioxidant assays (Aksoy *et al*., 2013). Based on the above data, it appears that aqueous and methanol leaf extracts of *C. nutans* showed significant antioxidant activity in different assays, including DPPH, NO scavenging, SOD, ABTS, and FRAP, when compared to other crude extracts, and both are suggested to be the best extracts of *C. nutans* for exhibiting antioxidant activity.

Anti-inflammatory effects

Inflammation is the body's protective response to infection or injury, but excessive inflammation can have detrimental effects and contributes to the progression of chronic and/or prolonged diseases such as atherosclerosis, rheumatoid arthritis, and systemic lupus erythematosus (Keane, Strieter, 2000).

Inflammation may promote vasculopathy by causing endothelial dysfunction. Endothelial dysfunction is defined as the failure of the vascular endothelium to subserve its normal role in vasodilatation and/or vascular homeostasis, and it commonly results from an imbalance between endothelium-derived contracting and relaxing factors (Rahman *et al*., 2007). Inflammation can modify the synthesis and degradation of vasodilators and vasoconstrictors, including NO, and it impairs NO bioactivity. A previous study showed that rats treated with the NOS inhibitor N-nitro-L-arginine methyl ester (L-NAME) had higher blood pressure than control rats (Baylis *et al*., 1992). The study also demonstrated that inflammation downregulated NOS activity. In addition, c-reactive protein (CRP) and tumor necrosis factor (TNF) have been shown to reduce NO production by impairing the expression of eNOS mRNA (Verma *et al*., 2002; Yan *et al*., 2008). Further, a previous study showed that inhibition of TNF restored endothelial-dependent vasodilation in humans (Maki-Petaja *et al*., 2006). Another cytokine, IL-17, has been demonstrated to cause endothelial dysfunction by activating Rho-kinase, which leads to phosphorylation of the inhibitory eNOS residue, threonine 495 (Nguyen *et al*., 2013). Inhibition of eNOS increases vascular tone (Baylis *et al*., 1992) and, consistent with this condition, Rho-kinase has been demonstrated to contribute to increased cerebral vascular tone *in vivo*.

Carrageenan-induced paw edema is an acute model of inflammation that is widely used for investigation of the anti-inflammatory effects of different compounds. Paw edema formation is a result of synergism between inflammatory mediators that increase blood flow and microvascular permeability (Wanikiat *et al*., 2008). This acute model involves two phases: The early phase observed at 1 hour is associated with the release of serotonin, histamine, and bradykinin and, to a minor extent, prostaglandins. The delayed phase (after 1 hour) is associated with polymorphonuclear (PMN) leucocyte infiltration and the continuation of prostaglandin generation (Di Rosa *et al*., 1971; Gilligan *et al*., 1994). The release of PMN leucocyte derived free radicals, NO, ROS, and pro-inflammatory cytokines such as IL-1 and TNF-α are associated with the delayed phase of carrageenan-induced inflammation (Halici *et al*., 2007; Nacife *et al*., 2004).

The anti-inflammatory activity of the butanol fraction of *C. nutans* leaves has been reported to reduce carrageenan-induced edema (Kamarudin *et al*., 2017; Kittisiripornkul, Witthayasat, 1984). Studies have also shown that topical application of the whole-plant methanol extract of *C. nutans* (3, 6, 9 mg/20 µL acetone/ear) suppressed ethyl phenylpropiolate (EPP)-induced ear edema in rats in a significant concentration-dependent manner at all time points (15 min, 30 min, 60 min, and 120 min), and the highest dose of *C. nutans* (9 mg/20 µL acetone/ear) exhibited more edema inhibition than the positive control (indomethacin, 2 mg/20 µL acetone/ear) at all time points (Kittisiripornkul, Witthayasat, 1984). Wanikiat *et al.* (2008) also reported that oral administration of the whole-plant methanol extract (50, 100, and 200 mg/kg) decreased carrageenan-induced edema in rat hind paws in a dose-dependent manner, resulting in 30%, 49%, and 59% inhibition, compared to the positive control (indomethacin, 20 mg/kg), with 76% inhibition. Pongphasuk *et al.* (2003) reported that treatment with the ethanol extract of *C. nutans* leaves (95%) at the dose of 5 g/kg showed 17% and 36% inhibition against carrageenan-induced paw edema at 3 and 6 hours, respectively.

Wanikiat *et al.* (2008) also reported that pretreatment with the methanol extract of *C. nutans* (IC_{50}: 2.7 ± 0.6 µg/mL) suppressed fMLP-induced chemotaxis and chemokinesis in human neutrophils in a dose-dependent manner. The inhibition was significant when compared to the non-treated group, although there was less activity than in the positive control indomethacin (IC_{50}: 56.3 ± 3.5 ng/mL).

Toll-like receptor (TLR)-4 is the first line of host defense against acute and chronic inflammation and is one of the key pro-inflammatory signaling receptors (Mai *et al*., 2016). Activation of TLR-4 by lipopolysaccharides (LPS) enhances the production of NO and inflammatory cytokines through activation of NF-κB and IRF3, and the inhibition of TLR-4 activation may produce anti-inflammatory effects because TLR-4 is the upstream receptor that activates both NF-κB and IRF3 signaling, the hallmark of inflammation (Mai *et al*., 2016). It has been reported that pretreatment with *C. nutans* polar leaves extract (LP), non-polar leaves extract (LN), polar stem extract (SP), and non-polar stem extract (SN) at 1.5625–100 µg/mL significantly reduced the production of NO in RAW 264.7 macrophage and TLR-4 activation in TLR-4-transfected human embryonic kidney cells. In addition, the *C. nutans* polar leaves extract (1.5625–100 µg/mL) demonstrated anti-inflammatory activity against LPS by suppressing TNF-α, IFN-γ, IL-1β, IL-6, IL12p40, and IL-17 production and inhibiting the activation of p38 mitogen-activated protein kinases (MAPK), extracellular signal regulated kinase (ERK), c-Jun-N-terminal kinase (JNK), NF-κB p65, and interferon regulatory factor 3 (IRF3) (Mai *et al*., 2016). LPS produces a powerful inflammatory response through activation of TLR-4, resulting in activation of NF-κB and the production of NO and inflammatory cytokines, including TNF-α, IFN-γ, IL-1β, IL-6, IL12p40, and IL-1.

Generally, studies on anti-inflammatory activities of *C. nutans* extracts in carrageenan-induced paw edema and EPP-induced ear edema have shown significant inhibitory effects on edema formation. It has been suggested that the *C. nutans* extract strongly inhibited the release and/or effects of histamine and serotonin that are released by inflammatory mediators during inflammation in an EPP model. *C. nutans* extract also affected the synthesis and release of mediators during both phases of the responses in carrageenan-induced rat paw edema. In addition, *C. nutans* extract was found to attenuate neutrophil chemokinesis in fMLP-induced chemotaxis and chemokinesis. Additionally, activation of neutrophils resulted in both intracellular and extracellular production of the radical superoxide (O2-), which may be attributed to distinct pools of nicotinamide adenine dinucleotide phosphate (NADPH) oxidase (Wanikiat *et al*., 2008). It was shown that fMLP triggered neutrophil O2- generation in a concentration-dependent manner, which was significantly inhibited by *C. nutans* extract. The suppression of the production of NO and cytokines (TNF-α, IFN-γ, IL-1β, IL-6, IL12p40 and IL-1) suggests that the anti-inflammatory activity of the *C. nutans* extract is associated with the inhibition of TLR-4 activation (Mai *et al*., 2016). *C. nutans* is suggested to exert its anti-inflammatory activity by preventing the activation of TLR-4 receptors, thus reducing the production of inflammatory cytokines and TLR-4 related inflammatory proteins. The methanolic extract of *C. nutans* leaves is suggested to show the best anti-inflammatory activity because of its significant effect in different assays in reducing carrageenan-induced paw edema and EPP-induced ear edema and in inhibiting fMLP-induced chemotaxis and chemokinesis.

Antihyperlipidemic effects

Dyslipidemia is one of the major risk factors for cardiovascular disease in diabetes mellitus. The

characteristic features of diabetic dyslipidemia are low levels of high-density lipoprotein cholesterol (HDL-C), high plasma triglycerides (TG), and increased levels of small dense low-density lipoprotein cholesterol (LDL-C) (Mooradian, 2009). The HDL-C particles induce the removal of cholesterol from cells, including those in atherosclerotic plaques, and carry them to the liver. However, the mechanism by which HDL-C confers protection from atherosclerosis involves more than just reverse cholesterol transport (Bitzur *et al*., 2009). HDL-C particles seem to have anti-inflammatory and antioxidant properties, inhibiting the oxidation of LDL-C and the expression of cellular adhesion molecules as well as monocyte recruitment (Bitzur *et al*., 2009).

High TG levels are the markers for several types of atherogenic lipoproteins. Hypertriglyceridemic states are associated with increased very low-density lipoprotein cholesterol (VLDL-C) production and delayed VLDL-C clearance from circulation (Talayero, Sacks, 2011). Insulin resistance is believed to contribute to this atherogenic dyslipidemia by increasing the hepatic secretion of VLDL-C and other apolipoprotein (apo) B-containing lipoprotein particles as a result of increased free fatty acid flux to the liver (Bitzur *et al*., 2009). This may also be the result of a diminished suppressive effect of insulin on apoB secretion, either at the level of the regulation of apoB degradation or through inhibition of microsomal TG transfer protein activity (Bitzur *et al*., 2009; Malmstrom *et al*., 1997).

Through the action of the cholesterol ester transfer protein, TG are transferred from VLDL-C to HDL-C, creating TG-rich HDL-C particles, which are hydrolyzed by hepatic lipase and rapidly cleared from the plasma (Bitzur *et al*., 2009). A similar cholesterol ester protein-mediated transfer of TG from VLDL-C to LDL-C contributes to the formation of small dense LDL-C particles. Similar to oxidized LDL (Ox-LDL), these cholesterol-enriched, TG-poor species are subject to endothelial accumulation and uptake by macrophages to form foam cells (Talayero, Sacks, 2011).

Hypercholesterolemia is a lipoprotein metabolic disorder characterized by altered metabolism of cholesterol, which promotes the production of ROS through modulation of the activities of enzymes such as NADPH oxidase and xanthine oxidase. The altered activities of these enzymes have been demonstrated to increase endothelial superoxide anion production, resulting in JNK-mediated inactivation of endothelium-derived NO and subsequent increases in oxygen radical production and inflammation (Osto *et al*., 2008; Sarega *et al*., 2016).

Hypercholesterolemia leads to accumulation and oxidation of LDL-C within the intima of the vessel wall and causes endothelial dysfunction (Osto *et al*., 2008; Sarega *et al*., 2016). Cholesterol, in particular LDL-C, is generally considered a major contributor to atherosclerosis susceptibility. LDL-C and other lipoproteins cross the endothelial cell via vascular transport, may be modified by oxidation, aggregation, or glycation, and are associated with proteoglycans or are incorporated into immuno-complexes (Rahman *et al*., 2007). LDL-C molecules are oxidatively modified at the subendothelial space into ROS that are generated by macrophages, endothelial cells, and smooth muscle cells (Rahman *et al*., 2007). Ox-LDL facilitates monocyte recruitment, activation, and differentiation to become larger macrophages, which bind and internalize Ox-LDL particles with cholesteryl ester accumulation and then become foam cells. Foam cells stimulate inflammatory mediators and alter both the structure and function of endothelial cells (Rahman *et al*., 2007). Ox-LDL increases the adhesion of circulating monocytes to damaged endothelium, increasing their migration into the vascular intima (Baumgartner-Parzer *et al*., 1995; Tesfamariam *et al*., 1991). Ox-LDL also decreases NO production by reducing NO synthase (Wautier, Guillausseau, 2001), thus contributing to defective vasodilatation.

Sarega *et al.* (2016) reported that in rats fed a high-fat and high-cholesterol diet, the methanol extract of *C. nutans* leaves and phenolic-rich aqueous at doses of 125, 250, and 500 mg/kg/day/rat exhibited good antihyperlipidemic activity that was comparable to the lipid-lowering drug simvastatin (10 mg/kg/day). After 7 weeks of treatment, the aqueous and methanol extract treated groups showed improvement in their lipid profiles, including reduced total cholesterol (TC), TG, LDL-C, and VLDL-C levels and increased HDL-C levels. The data showed that the methanol and aqueous extracts dose-dependently reduced TC levels (28% and 18% reduction, respectively) at the dose of 500 mg/kg. The methanol extract dose-dependently decreased both VLDL-C and TG levels and increased levels of HDL-C but not LDL-C, whereas the aqueous extract showed dose-dependent effects on TG and LDL-C but had no effect on HDL-C and VLDL-C levels. Further, at the highest dose of 500 mg/kg, improvements were seen in TG, HDL-C, and VLDL-C levels with comparable efficacy to simvastatin treatment.

Recently, Abdulwahid *et al*. (2017) reported the effects of methanol extract of *C. nutans* at different doses (500, 1000, and 1500 mg/kg) on lipid profiles in diet-induced obese mice. Their results showed that plasma TC levels were reduced in *C. nutans* treatment groups when compared to high-fat diet control mice.

It has been suggested that plant polyphenols may reduce plasma cholesterol by decreasing cholesterol absorption, forming insoluble precipitates of cholesterol, and decreasing bile acid-induced micellar solubility (Abdulwahid *et al*., 2017; Anandh Babu *et al*., 2006). Sarega *et al.* (2016) also reported that weight loss and antihyperlipidemic activities of *C. nutans* were associated with multiple phenolic compounds present in the extract, with protocatechuic acid being the most abundant. It can be hypothesized that the synergy between phenolic compounds and their ability to upregulate various hepatic antioxidant genes that suppress oxidative stress mainly contribute to the antihyperlipidemic properties of *C. nutans.* Thus, the hypocholesterolemic activity of the polyphenols in *C. nutans* could be due to decreased cholesterol absorption, enhanced cholesterol excretion, and inhibition of cholesterol biosynthesis (Abdulwahid *et al*., 2017). Based on previous studies, it appears that the methanol extract of *C. nutans* has better antihyperlipidemic effects than the aqueous extract.

CONCLUSION

Scientific findings, predominantly experimental studies, have shown that *C. nutans* extracts possess antihyperglycemic, antioxidant, anti-inflammatory, and antihyperlipidemic effects. *C. nutans* extracts possess significant antihyperglycemic properties, as demonstrated by its α-glucosidase inhibitory activity. In terms of antioxidant properties, it has shown greater DPPH radical scavenging activity in a dose-dependent manner. It also upregulated the expression of SOD1, SOD2, glutathione reductase, and glutathione peroxidase and showed the greatest effects on ABTS and FRAP radical scavenging. *C. nutans* have significant anti-inflammatory properties, as shown by its effect in both the rat carrageenan-induced paw edema model and the EPP-induced rat ear edema model. For antihyperlipidemic studies, *C. nutans* extracts improved the lipid profile in rats. Phenolic compounds also reduced hyperlipidemia-induced oxidative stress. With these pharmacological properties, *C. nutans* has the potential to reduce the severity of or prevent diabetic cardiovascular complications, as these properties can act synergistically to reduce diabetic vasculopathy. Therefore, future studies on *C. nutans* in diabetes should assess its potential effects as an anti-atherosclerosis agent to reduce cardiovascular complications in diabetes. The present review provides the information, groundwork, and important directions for researchers in conducting further *in vivo*, *in vitro*, and clinical investigations of *C. nutans* in diabetic cardiovascular related studies.

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DECLARATION OF INTEREST STATEMENT

The authors report no conflicts of interest.

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