Mechanisms of Action of *Andrographis Paniculata* As Anti-Atherosclerotic Agent

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ABSTRACT
Andrographis paniculata isaherbaceous plant in the family of Acanthaceae. Andrographolide, an active component isolated from *Andrographis paniculata*, has been reported to prevent oxygen radical production and thus prevent inflammatory diseases. Atherosclerosisis characterized by the presence of intimal lesions called atheromas or atheromatous plaque. There are two dominant theories regarding atherogenesis. One is emphasizing intimal cellular proliferation in response to endothelial injury and the other focusing on repeated formation and organization of thrombi. *Andrographis peniculata* plays important roles in preventing atherosclerosis from becoming worse. Its role includes the promotion of endothelial cell survival by blocking the cytochrome c release from mitochondria, preventing the activation of caspase – 3 and - 9, that result inthe suppression of endothelial cell apoptosis. The anti-atherogenesis activity of *Andrographis peniculata*would be attributed to its anti-oxidative action and the ability to inhibit Macrophage-1 expression. It was also shown that it has vaso-relaxant effects by activating the nitric oxide synthase and guanylylcyclase to release nitric oxide from endothelial cells. Andrographolide has enhanced the dephosporylation of NF-kB subunit p65 Ser536 through activation of protein phosphatase 2A in vascular smooth muscle cells thus preventing endothelial inflammation and formation of atherosclerosis. It was demonstrated that *Andrographis paniculata* can reduce amount ofiNOS protein through synergistic action by reducing de novo protein synthesis and increasing degradation of iNOS protein. In this review we are focusing on the available literature which had described the mechanisms of action of*Andrographis paniculata* as anti-atherosclerotic agent.

Keywords: *Andrographis paniculata*, Andrographolide, Atherosclerosis.

1. INTRODUCTION

Figure 1: *Andrographis Paniculata*

Andrographis paniculata is aherbaceous plant in the family Acanthaceae.It is widely cultivated in Southern and Southeastern Asia, where it is used to treat infections and some diseases, often being used before antibiotics were created. Mostly the leaves and roots are used for medicinal purposes (Jarukamjorn&Nemoto, 2008).

Andrographis paniculata is an erect annual herb extremely bitter in taste in all parts of the plant body. The plant is known in northeastern India as Maha-tita, literally "king of bitters". In Malaysia, it is known as HempeduBumi, which literally means 'bile of earth' since it is one of the bitterest plants that are used in traditional medicine.

Andrographolide, an active component isolated from *Andrographis paniculata*, has been reported to prevent oxygen radical production and thus prevent inflammatory diseases and may represent a candidate of therapeutic agent for atherosclerosis (Chen, et al. 2004).

Rao, et al. (2004) reported that the major bioactive components in *Andrographis paniculata* are flavonoids, diterpenoids, and polyphenols. The four main components of flavonoids which are 7-Omethylwogonin, apigenin, onysilin and 3, 4-dicaffeoylquinic acid are anti-atherosclerotic (Choa& Lin, 2010).

While, the major component of diterpenoid, which is Andrographolide, is abundant in leaves and can be easily isolated from the crude plant extracts as crystalline solid (Choa, et al., 2009). Generally, the pharmacological properties of Andrographolide are anti-inflammatory, anti-cancer, immune-modulatory, anti-infective, anti-hepatotoxic, anti-hyperglycemic effect, anti-oxidation and anti-atherosclerotic.

Atherosclerosis is one of the types of arteriosclerosis, characterized by the presence of raised lesions called atheromas (Hansson, 2005). The name of atherosclerosis comes from the Greek words *athero* (meaning gruel or paste) and *sclerosis* (hardness). Atheromas also known as atheromatousplaques, are intimal lesions composed of soft lumps lipid cores (cholesterol with necrotic debris) covered by fibrous caps. Atheromas can cause blockage in vascular lumina and easily rupture, resulting in thrombosis. Plaques also weaken the underlying media, at times leading to formation of aneurysm, the localized blood-filled bulge.

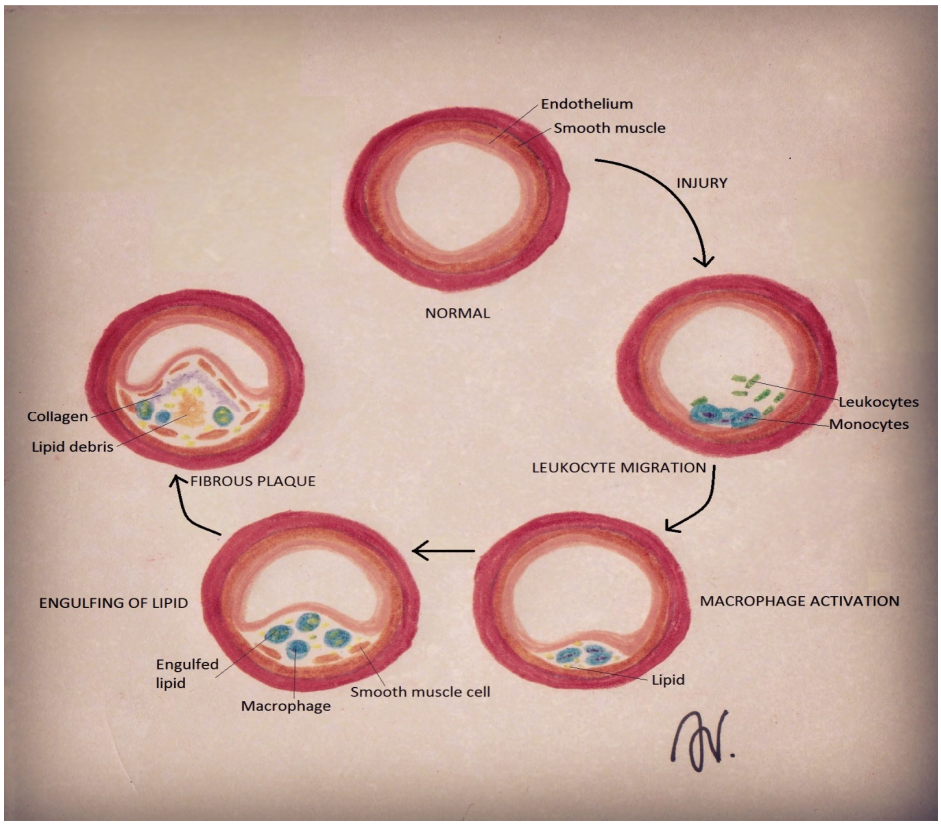

Figure 2: Response to injury in atherogenesis:**1,**Normal.**2,**Endothelial injury with monocyte and platelet adhesion.**3,**Monocyte and smooth muscle cell migration into the intima, with macrophage activation.**4,**Macrophage and smooth muscle cell uptake of modified lipids and further activation.**5,** Intimal smooth muscle cell proliferation with ECM elaboration, forming a well-developed plaque.

2. MECHANISMS OF ACTION

Chen et al. 2004 has reported that the maintenance of vascular homeostasis, as well as regulation of the permeability of plasma lipoproteins, adhesion of leukocytes, and release of growth factors and vascular regulators are key roles played by endothelial cells. The pathogenesis of atherosclerosis is induced once endothelial cells are impaired (Libby, 2000). Andrographis peniculata promotes endothelial cell survival as well as protects against apoptosis. Apoptotic endothelial cells could donate to the weakening of atherosclerotic plaques and thrombosis (Tricot et al., 2007). Suppression of endothelial cells apoptosis may represent a crucial step to alleviate atherosclerosis.It was demonstrated that *Andrographis paniculata* restricted cytochrome c release as well as the subsequent activation of the caspase-9/-3 cascades which resulted in repressing the mitochondrial pathway of apoptosis (Kennedy, et al., 1999; Hermann, et al., 2000). Protein kinase Akt, a survival factor in a several experimental systems has been denoted as the mediator upstream of the mitochondrium. Similar to growth factors such as vascular endothelial growth factor (Fujio& Walsh, 1999) and oestrogens (Spyridopoulos, et al., 1997), *Andrographis paniculata*was found to be activating Akt via the PI3K-dependent pathway that resulted in suppression of cell apoptosis in HUVECs. By activating the PI3/Akt pathway, apoptosis of human umbilical vein endothelial cells (HUVECs) that was induced by growth factor deprivation can be suppressed by *Andrographis paniculata* (Chen, et al., 2004). *Andrographis paniculata*also significantly inhibited thrombin-induced platelet aggregation through the inhibition of ERK1/2 pathway (Thisoda, et al., 2006). In a conclusion, *Andrographis paniculata* served as an anti-atherosclerosis by suppressing HUVECs apoptosis via enhancement of PI3K-Akt activity as well as inhibiting thrombin-induced platelet aggregation via ERK1/2 pathway.

Davi and Falco (2005) suggested that systemic inflammation was associated with increased risk of chronic diseases such as cardiovascular disease, cancer and insulin resistance. Inflammation involves macrophage and T lymphocyte activation as well as the release of pro-inflammatory mediators, such as tumor necrosis factor (TNF)-α, interleukin (IL)-1, IL-6, interferon (IFN)-γ, nitric oxide (NO) and cell adhesion molecules, which in turn amplify the inflammation (O'Shea and Murray, 2008). O'Keffe, et al. (2008) reported that effective modulation of the aberrant production of these molecules may reduce inflammation.

A previous study by Chiou, et al. (1998) demonstrated that Andrographolide inhibited nitric oxide (NO) production and the expression and stability of inducible synthase (iNOS) protein in lipopolysaccharide (LPS)-stimulated RAW264.7 (RAW) cells. It also inhibited oxygen radical production in neutrophils (Shen, et al., 2002). Next, it inhibited macrophage migration (Tsai, et al., 2004), NF-κB activity (Xia, et al., 2004) as well as TNF-α and IL-12 production(Qin, et al., 2006).Andrographolide acts more like protein synthesis inhibitor. Since iNOS mRNA expression is not altered, this means that Andrographolide acts at post-transcriptional level to inhibit nitric oxide formation. Andrographolide is able to inhibit iNOS expression by impairing protein de novo synthesis. Hence, Andrographolide can reduce amount of iNOS protein through synergistic action which are reduction of de novo protein synthesis and increasing degradation of iNOS protein. These anti-inflammatory activities of Andrographolide may be a result of its interference with protein kinase C dependent pathway, extracellular signal-regulated kinase1/2 (ERK1/2) or PI3K/Akt signaling pathway (Wu, et al., 2008).Anti-oxidative activity and inhibition of Mac-1 expression has also been attributed to the anti-atherogenesis activity of *Andrographis paniculata* (Shen, et al., 2002).

Bao et al. (2009) and Hsieh et al.(2010) reported that Andrographolide possessed anti-inflammatory activities which inhibited the expression of inflammatory mediators in macrophages and lung epithelial cells. Andrographolide was shown to inhibit LPS/IFN-γ-induced inducible nitric oxide synthase (iNOS) and matrix metalloproteinase-9 (MMP-9) expressions in vascular smooth muscle cells (VSMCs). Dephosphorylation of the p65 subunit of nuclear factor-kB (NF-kB) by protein phosphatase 2A (PP2A) may contribute to Andrographolide's protective actions in VSMCs. It was also reported that andrographolide can suppress the phosphorylation of p65 Ser536 through IKK inhibition in lung epithelial cells but not affect IKK activation in VSMCs (Sakurai, et al. 1999; Li et al. 2006).

NF-kB is asignificant transcription factor in trans-activating inflammatory genes, and it's activation can be regulated by PP2A, it appeared to be a promising molecular target in treating inflammatory vascular diseases. Andrographolidealso can diminish p65 kB oligonucleotide binding in LPS/IFN-γ-stimulated VSMCs. At the same time, PP2A may contribute to these actions of andrographolide. In a conclusion Andrographolide was found to suppress the phosphorylation of NF-kB subunit p65 Ser536 through activation of PP2A in VSMCs and thus preventinginflammation and formation of atheroma (Sakurai, et al. 1999).

Zhanget al. (1996, 1997) reported that *Andrographis paniculata* can lower systolic blood pressure (SBP) and produce a significant decrease in mean arterial blood pressure (MAP) without significant fall in heart rate. *Andrographis paniculata* also showed vaso-relaxant effects by activating the NOS and guanylylcyclase to release NO from endothelial cells (Zhang and Tan, 1998,1999).

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