BIOACTIVE CONSTITUENTS FROM *Andrographis paniculata* NESS WITH HEPATOPROTECTIVE POTENTIALS THROUGH *in silico* METHOD

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Abstract

Sambiloto (Andrographis paniculata Ness) has wide range of medicinal and pharmacological application. It is used in different traditional system of medicine and exhibit anti-inflammatory, antiviral, antioxidant, hepatoprotective and various other activities. Most liver disease are accompanied by inflammatory processes. Hence in the present study an attemp has been made to study the hepatoprotective potency of the active constituents in A. paniculata through in silico methods. Compounds were prepared for docking study by their energy minimization in Marvin Sketch. Tumor necrosis factor-á (TNF-á), represents a key mediator in pathological situation induce liver damage, served as target (receptor). Docking simulation was done using Autodock vina programm. The analysis results showed that andrographolactone in Andrographis paniculata has potency as hepatoprotective agent with possible mechanis by inhibition of TNF-á.

Keywords: *Andrographis paniculata, hepatoprotective, TNF-á, docking simulation*

ISBN : 978-979-18458-5-4

INTRODUCTION

Liver is one of the important organs concerned with methabolism of endogenous substances as well as xenobiotic and is the first target for various toxic insults. The liver regulates several important metabolic functions and the hepatic injury is associated with distortion of these metabolic functions. Thus, liver desease remain one of the serious health problems.

Most liver deseases are accompnied by inflammatory processes. Therefore, pharmacological strategies focus on attenuating this inflammatory response exerted by immune cells, such as Kupffer cells (KC). KC as part of the mononuclear phagocyte play an important role in host defence. Upon inflammatory stimuli, KC as well as macrophages trigger signals for the production of diverse inflammatory mediator such as tumor necrosis factor-á (TNF-á). TNF-á represents a key mediator in pathophysiological situations, such as endotoxin-, alcohol-, ischemia/reperfusion-, or virus-induced liver damage. An early rise of TNF-á level induced proinflammatory genes, including inducuble nitric oxide synthase (iNOS) and cyclooxygenase-2 (COX-2). Toxic exposure could induce oxidative stress in the liver due to its metabolisation into highly reactive free radicals. However, excessive production of TNF-á, significantly contributes to inflammatory processes (Kiemer et.el., 2002). Oxidative stress triggers the TNF-á release from Kupffer cells and injured hepatocyte, which further activates NF-êB, allowing its nuclear translocation. Consequently, NF-êB stimulates the expression of iNOS and COX-2 at the level of transcription, translation and the enzyme level. The final product of iNOs and COX-2, NO and prostaglandins, respectively, contributes to nitrosative stress and initiate the cascade of inflammatory respons in injured liver. Persistent hepatic injury and inflammation may lead to the progressive liver damage, fibrosis, and finally cirrhosis. The inhibition of proinflammatory cytokines and enzymes may offer a new theurapeutic strategy against inflammatory liver deseases (Akarasereenont et.al., 1995; Ramadory and Ambrust, 1999; Domitrovic et.el., 2011).

In spite of tremendous strides in the modern medicine, there are not much drugs available for the treatment of liver deseases. There are number of medicinal preparations recommended in the traditional medicine for the treatment, which there are scientific claims to offer significant relief as hepatoprotective. In recent years, investigation have been carried out to provide experimental evidence, confirming that many of traditional plant remedies do indeed have hepatoprotective properties.

Andrographis paniculata Ness (family Acanthaceae), a medicinal herb with an extremely bitter taste, has wide range of medicinal and pharmacological applications. It is used in different traditional system of medicine and exhibits anti-inflammatory, anti-HIV, antibacterial, antioxidant, antiparasitic, antispasmodic, antidiabetic, anticarcinogenic, antipyretic, hepatoprotective, nematocidal and various other activity(Niranjan et.al, 2010). The herb contains diterpenoids, flavonoids and polyphenols as the major bioactive components. Active compounds extracted with ethanol or methanol from the whole plant, leaf and stem of Andrographis paniculata. Andrographolide ($C_{20}H_{30}O_5$) is the major diterpenoid, making up about 4%, 0.8~1.2% and 0.5~6% in dried whole plant, stem and leaf extract respectively and can be isolated from the crude plant extract as crystalline solid.

Andrographis paniculata was concluded as plant was useful remedy for treatment of infective hepatitis. Andrographolide exhibits protective effects in carbon tetrachloride induced hepatopathy in rats. Andrographolide was found to be more potent (0.75-12 mg/kg) than silymarin, standard hepatoprotective agent. The hepatoprotective action of andrographolide is related to activity of certain metabolic enzymes. The inhibitory effect of plant extract and andrographolide on hepatic cytochrome P450s (CYPs) activities using rat and human liver microsomes has also been reported.

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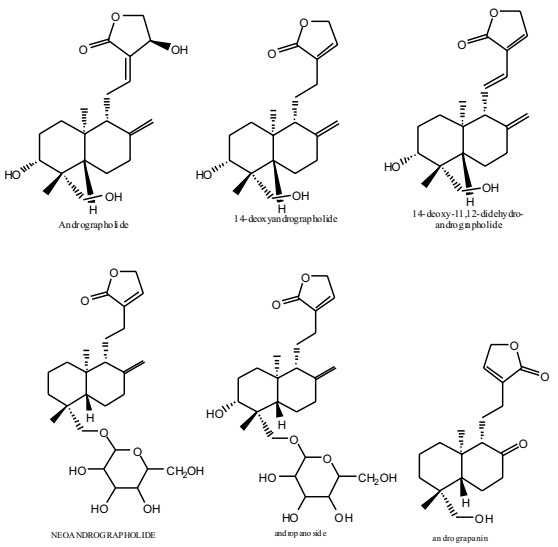
Hepatoprotective effect of andrographolide against hexachlorocyclohexane-induced oxidative injury in mice model has been established.

Hence in the present study an attemp has been made to study the hepatoprotective potency of the active constituents in *Andrographis paniculata* through in silico methods.The binding interactions between these compounds and TNF-á were studied by docking methods using AutoDock vina software. The aim of this study was to know which a better ligand that could inhibit production of TNF-á, and to better understand the interactions between the inhibitor and the protein's binding sites via computational docking methods. We hope, this compound will get success to clear out all the phases ofclinical trial and it will be effective drug as hepatoprotector.

METHODS

In this study, 16 structures of bioactive compounds in *Andrographis paniculata* were obtained from Chao and Lin (2010)



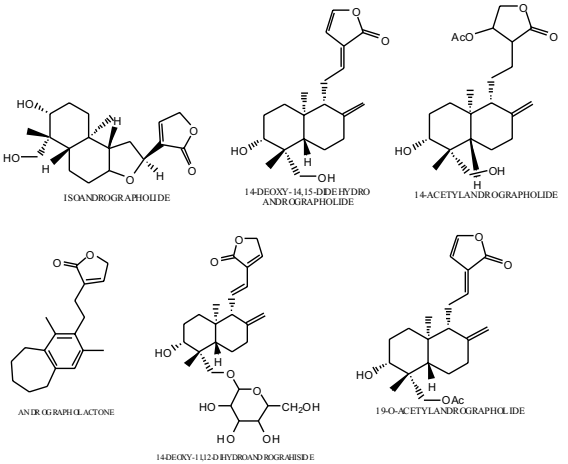


Figure 1. Structures and bioactive with diterpene skeleton isolated from *A. paniculata*

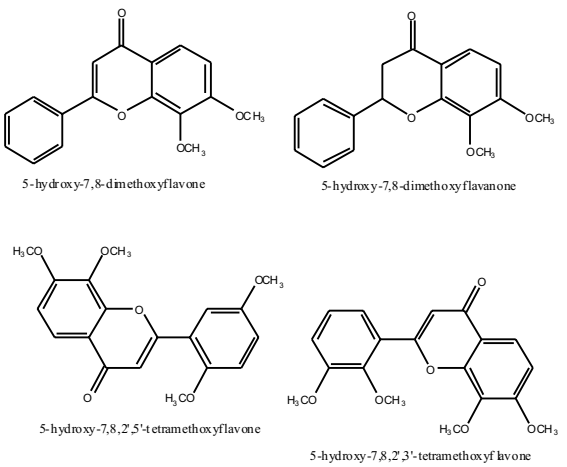


Figure 2. Structures and bioactive of flavonoids isolated from *A. paniculata*

Three dimensional (3D) structure of TNF-áas protein receptor was retrieved from Protein Data Bank of RCSB (Research Collaboratory for Structural Bioinformatics) code 2AZ5.

The docking of ligands to the catalytic triad of TNF-á protein was performed using AutoDock Vina software. The docking protocol was validated by predicting the binding mode of known inhibitor previously.The interactions of

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complex TNF-á protein-ligand conformations, including hydrogen bonds were analyzed using Pymol.

RESULTS AND DISCUSSION

Docking simulation was performed to obtain a population of possible conformations and orientations for the ligand at the binding site. Using the software, polar hydrogen atoms were added to the TNF-á protein and its nonpolar hydrogen atoms were merged. All bonds of ligands were set to be rotatable. The grid box with a dimension of 40 x 40 x 40 points was used around the catalytic triad to cover the entire target receptor binding site and accommodate ligands to move freely. The best conformation was chosen with the lowest docked energy, afterthe docking search was completed.

Predicted binding energies for bioactive compounds of *Andrographis paniculata* with tumor necrosis factor-á (TNF-á) are summarized in Table I. The result show that andrographolactone had the lowest docked energy. Andrographolactone, possessing an unprecedented diterpene skeleton, was isolated from the EtOAcextract of the aerial parts of Andrographis paniculata. Its structure was established by NMR, IR, UV, andHRESIMS data and subsequently confirmed by X-ray diffraction analysis by Wang et.al (2009).

Tabel I. Predicted binding energies for bioactive compounds of *Andrographis paniculata* with tumor necrosis factor-α (TNF-α)

No	Compound	ΔG (kkal/mol)
1.	14-deoxy-11,12-dihydroandrographiside	-8,6
2.	Andropanoside	-7,9
3.	5-hydroxy-7,8,2',3'-tetramethoxyflavone	-7,5
4.	5-hydroxy-7,8-dimethoxyflavanone	-7,5
5.	5-hydroxy-7,8-dimethoxyflavone	-7,7
6.	Neoandrographolide	-8,2
7.	14-deoxy-11,12-didehydroandrographolide	-7,5
8.	5-hydroxy-7,8,2',5'-tetramethoxyflavone	-7,5
9.	14-deoxy-14,15-didehydroandrographolide	-8,1
10.	Andrographolide	-8,1
11.	Andrographolactone	-8,7
12.	14-deoxyandrographolide	-7,6
13.	Andrograpanin	-7,5
14.	19-o-acetylanhydroandrographolide	-7,7
15.	14-acetylandrographolide	-7,3
16.	Isoandrografolid	-7,9

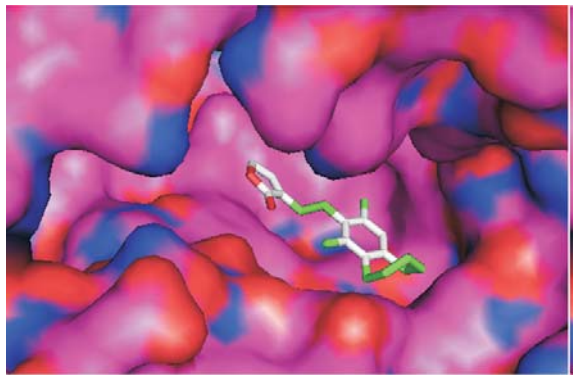

Figure 1. Docking representations of andrographolactone inside the pocket of TNF-α

Based on results of docking simulation, there are not demonstrated outstanding scores which mean interaction between substituents in *Andrographis paniculata* with TNF alpha. In other hand, some literature has shown that *A.paniculata* can act as hepatoprotector. Thus there is the possibility of synergistic effects between the compounds in *Andrographi spaniculata* when it protects the liver or hepatoprotective mechanism of this plant is not in inhibiting TNF-á.

CONCLUSION

It can be concluded that andrographolactone hold a stronger potencial than other substituens in *Andrographis paniculata* for acting as hepatoprotector to inhibit TNF-á as hepatotoxic mediator which initiation and propagation of inflammatory respons tissue damage.

ACKNOWLEDGEMENTS

This study is financial supported by insentive SINas programm of Ministry for Research and Technology, Republic of Indonesia.

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