ANTIDIABETIC PROPERTIES OF *ANDROGRAPHIS PANICULATA* NEES AND *EUGENIA POLYANTHA* WIGHT LEAVES IN WISTAR RATS BY ORAL GLUCOSE TOLERANCE TEST

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Abstract

Ethanol and water extract of *Andrographis paniculata* Nees, and ethanol extract of *Eugenia polyantha* Wight leaves were evaluated for their hypoglycemic effects in healthy albino Wistar rats by oral glucose tolerance test. Both ethanol extract of *Euginia polyantha* (200 mg/kg bw) and water extract of *Andrographis paniculata* (200 mg/kg bw) showed significant increased of blood glucose level at T\(_{30}\) and T\(_{60}\) (T\(_{30}\) = 30 minutes after glucose loading or 60 minutes after drugs administration, T\(_{60}\) = 60 minutes after glucose loading or 90 minutes after drugs administration). Meanwhile, ethanol extract of *Andrographis paniculata* (200 mg/kg bw) showed significant increased in blood glucose level up to T\(_{90}\). Therefore, water extract of *Andrographis paniculata* (200 mg/kg bb) was considered faster to lower blood glucose level than the ethanol extract at the same dose of administration. Furthermore, water extract of *Andrographis paniculata* and *Eugenia polyantha* leaves could be selected for further research as they reduce the blood glucose level steadily and does not cause hypoglycemia such as in glibenclamide.

INTRODUCTION

*Andrographis paniculata* Nees (known as sambiloto in Indonesia) is also well known as king bitter. Ethanol extract of *A. paniculata* contains andrographolide, a diterpene, that can lower plasma glucose (Dai, 2006). Andrographolide without glucosidase inhibitory activity may also exert antidiabetic effect. They concluded that α-glucosidase inhibitory activity was the reason or at least one of the reasons that the constituents of *A. paniculata* had antidiabetic effects. The promotion of the glucose metabolism was found when treating diabetic rat with the plant extract or andrographolide. So, it can be deduced that extracts of *A. paniculata* and andrographolide lower plasma glucose by inhibiting the disaccharide metabolism and/or promoting the glucose metabolism (Dai, 2006).

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*Eugenia polyantha* Wight leaves (known as daun salam in Indonesia) is also known as bay leaves. It is used in Indonesian culinary food additive, and as medicine in ulcer, diabetes, inflammation and diarrhea (Lelono, 2009). The antioxidant properties of the water extract *Eugenia polyantha* Wight infuse is bactericide against *V. cholera* and *E. coli* enteropathogen (Hendradjatin, 2009). *E. polyantha* Wight infusa is also can be used to reduce the uric acid in potassium oxonate-induced male white mice (Ariyanti, 2007). The dose of 1.25 g/kg bw; 2.5 g/kg bw and 5.0 g/kg bw were able to reduce the uric acid level by 79.98% (P = 0.000), 112.27% (P = 0.004) and 112.75% (P = 0.006) in the male white mice induced with 300mg/kg bw potassium oxonate. Moreover, extract of bay leaf (*Eugenia polyantha*) was proven to lower blood glucose levels of hyperglycemic white rats (*Rattus norvegicus*). Bay leaf extract (*Eugenia polyantha*) at doses of 252 mg / kg bw and 314.87 mg/kg bw, giving a decrease of blood glucose levels, which are similar when compared with glibenclamide as standard (Nugroho, 2010).

Although there are many studies on the hypoglycemic effect of *Andrographis paniculata* and *Eugenia polyantha*, they were not carried out together at the same time and interestingly, the Thin Layer Chromatography profiles of the ethanol and water extract of *Andrographis paniculata* were different. Therefore, the present study was conducted to differentiate the effect of the ethanol and water extract of *Andrographis paniculata*, also the hypoglycemic effect of *Eugenia polyantha* extract.

**MATERIALS AND METHOD**

This study was carried out in the Faculty of Pharmacy Catholic University Widya Mandala, Surabaya during August-October 2010 to evaluate the antidiabetes effect of the *Eugenia polyantha* extract and the water and ethanol extract of *Andrographis paniculata* as well as the Thin Layer Chromatography profiles from both of the extracts.

**MATERIALS**

*Ethanol and water extracts of Andrographis paniculata* Nees and *Eugenia polyantha* Wight leaves extracts were obtained from PT. Natura Laboratoria Prima; metformin and glibenclamide from PT. Bernofarm; Accu Check Advantage glucometer and Accu Check Advantage sticks from Roche. Gavage, syringe, water, and glucose were used for the experiment.

Wistar rats were obtained from UD Wistar, Jogjakarta and were certified by drh. Slamet Raharjo, MP from Gadjah Mada university. The animals were maintained on rat pellet feed and treated water given ad-libitum in a normal uncontrolled condition (Exposed to natural day and night order). The rats were kept for one week for acclimatization before the experimental sessions. The experiment was conducted after the Animal Ethical clearance no 084-KE was obtained.

**METHOD**

Thin Layer Chromatography

The TLC profile was made from *Andrographis*
paniculata ethanol and water extract, where andrographolide was employed as standard. The stationary phase was silica gel GF254, and the eluents used in this experiment were chloroform-methanol (8:2).

Oral glucose tolerance test
Wistar rats, weighing 100-150 grams, were fasted for 10 hours prior the experiment, but water was provided *ad libitum*. The blood glucose concentrations of the fasted rats were taken from the tail veins and the blood glucose levels were measured by Accu Check Advantage glucometer and then rats which have blood glucose between 65-100 mg/dl were divided into 6 groups. The first two groups were the positive controls, and were given glibenclamide 0.45 mg/kg bw or metformin 63 mg/kg bw per oral. The third group was the negative control, which was given water, while the rest of the group were given 200 mg/kg bw of *Andrographis paniculata* Nees (water or ethanol extract) and *Eugenia polyantha* Wight leaves extracts from PT. Natura Laboratoria Prima. After 30 minutes, the rats were given 2 g/kg bw of glucose. Then every 30 minutes up to 150 minutes after glucose administration, the blood glucose was taken.

RESULT AND DISCUSSION
The Thin Layer Chromatography profile (UV 254 nm) of the *Andrographis paniculata* extract (extracted by PT Natura Laboratoria Prima) can be seen in Figure 1. The TLC profile coded by number 1 was derived from *Andrographis paniculata* ethanol extract, the second profile was taken from *Andrographis paniculata* water extract and the third one was the andrographolide standard compound. It could be seen andrographolide was not detectable in the water extract.

Moreover, the oral glucose tolerance test result can be seen in Table 1, Figure 1 and Figure 2.
All the blood glucose at T₃₀ (30 minutes after glucose loading or 60 minutes after the control or drugs administration) illustrated in Figure 3 differed significantly (increasing blood glucose level) towards T₀ (fasting blood glucose), which showed that the method was valid. Glibenclamide 0.45 mg/kg bb started to lower the blood glucose
at T_{60}, although significant difference was obtained at T_{120} and T_{150}. On the other hand, the other positive control, metformin 63 mg/kg bw showed blood glucose reduction at T_{120}, although it rose again at T_{150}. The raise at T_{150} was considered as normal as the second raise of blood glucose after glucose loading happens normally in glucose metabolism.

Water or negative control gave the highest raise in blood glucose level at T_{30} which is as expected. The blood glucose level differed significantly throughout the experiment, except at T_{150}, when the blood glucose level returned to normal.

Both *Eugenia polyantha* leaves of 200 mg/kg bw and water extract of *Andrographis paniculata* (200 mg/kg bw) showed significant increase of blood glucose level at T_{30} and T_{60} (T_{30} = 30 minutes after glucose loading or 60 minutes after
drugs administration, $T_{60} = 60$ minutes after glucose loading or 90 minutes after drugs administration). Meanwhile, ethanol extract of *Andrographis paniculata* obtained from PT. Laboratoria Natura Prima (200 mg/kg bw) showed significant increased in blood glucose level up to $T_{90}$. Therefore, water extract of *Andrographis paniculata* (200 mg/kg bb) was considered faster to lower blood glucose level than the ethanol extract at the same dose of administration. *Andrographis paniculata* water extract and *Euginia polyantha* leaves extract reduced the blood glucose level steadily and did not cause hypoglycemia such as in glibenclamide.

Although *A. paniculata* has antidiabetic properties, we should not neglect the toxicity. The toxic effects of its aqueous crude extracts were studied in 20 male white rats over a period of 28 days by Adedapo, and co-workers (2007). The rats were administered with 400mg/kg, 800mg/kg and 1,600mg/kg, doses of the extract respectively. They observed hepatic degeneration and necrosis with mononuclear cellular infiltration. The kidney showed glomerular degeneration with protein casts in the tubules. However, there were no pathological changes were observed in the testes. As a result of the histopathological changes in the kidney and the liver caution should be exercised in its use for medicinal purpose (Adedapo, 2007).

**CONCLUSION**

Water extract of *Andrographis paniculata* (200 mg/kg bb) had a promising antidiabetic properties compared to ethanol extract at the same dose of administration. Both *Andrographis paniculata* water extract and *Euginia polyantha* leaves extract did not demonstrated hypoglycemia effect such as in glibenclamide. This fact suggests a beneficial effect of the both extract in lowering glucose level.
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