

Antihyperglycaemic Effects of Ethanol Extracts of *Andrographis paniculata* on Streptozotocin-Induced Diabetic Rats.

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Abstract Single oral dose administration of 20% ethanol extract of *Andrographis paniculata* (Acanthaceae) was found not to lower the blood glucose level of streptozotocin-induced diabetic rats but inhibited the rise in blood glucose level of glucose loaded normal rats in glucose tolerance test[1]. The present study was conducted to determine the effect of chronic administration of different ratios of aqueous ethanolic extracts of this plant on the blood glucose levels of diabetic rats as well as their effects on insulin levels. Six groups of streptozotocin-induced diabetic rats (n=6) were treated orally either with metformin (0.5 g/kg body weight), tap water, 20%, 50%, 95% ethanolic or water extracts of *A. paniculata* (0.5 g/kg body weight) twice daily for fourteen days respectively. The results showed that only the blood glucose levels in 50% and 95% ethanol extracts treated rats were significantly reduced ($p < 0.05$). It suggests that the antidiabetic compound(s) in this plant is/are relatively non polar and different from the compound(s) that inhibit the increase of blood glucose levels in glucose loaded normal rats. Moreover, different extracts were subjected to thin layer chromatography (TLC). The results showed that the presence of andrographolide in all the different ratios of aqueous ethanolic extracts examined. However, it is still to be determined how andrographolide[2], found in these plant extracts contributed to the differences in mechanisms of actions.

Keywords: *Andrographis paniculata*; Diabetes mellitus; Streptozotocin; hyperglycaemia; rats.

Abstrak Pemberian dos tunggal ekstrak beretanol 20% *Andrographis paniculata* (AP) gagal memberi kesan penurunan paras glukosa darah pada tikus teraruh diabetik oleh streptozotocin tetapi berjaya memberi kesan penurunan ke atas tikus normal yang diaruh hiperglisemia dengan pemberian glukosa di dalam ujian toleransi glukosa [1]. Oleh itu kajian selanjutnya dijalankan untuk menentukan pemberian ekstrak akues beralkohol AP secara kronik dapat memberi kesan terhadap paras glukosa dan insulin. Enam kumpulan yang mengandungi enam ekor tikus teraruh diabetik oleh Streptozotocin dirawat mengikut kumpulan samada dengan metformin (500 mg/kg), air paip, ekstrak AP berair, 20%, 50% atau 95% beretanol dengan dos 500mg/kg dua kali sehari selama 14 hari. Didapati ekstrak AP beretanol 50% dan 95% dapat menurunkan paras glukosa secara signifikan ($p < 0.05$). Ini menunjukkan bahawa kandungan aktif didalam tumbuhan tersebut adalah berlainan dari kandungan aktif di dalam ekstrak beretanol 20% yang berkesan menurunkan paras glukosa darah di dalam ujian toleransi glukosa memandangi ia adalah kurang polar. Analisa kromatografi lapisan nipis menunjukkan bahawa kesemua jenis ekstrak mengandungi andrographolide. Walaubagaimanapun adalah perlu kepastian bagaimana andrographolide di dalam tumbuhan tersebut memberi kesan perbezaan dari segi mekanisme tindakannya.

INTRODUCTION

Andrographis paniculata Nees (AP) (Acanthaceae) vernacularly known as King of Bitter, The Creat, Kariyat, Hemptu Bumi and Pokok Cerita is an annual herb. It is an erect and branched plant with lanceolate green leaves growing to a height of 60 – 70 cm. It grows

abundantly in southeastern Asia; from India to Indo-China including northern Malay Peninsula and Java [3]. The plant has been quoted in the Indian pharmacopoeia and used in the traditional Chinese medicine for treating upper respiratory and gastrointestinal tract infections, fever, sore throat and a variety of other chronic and infectious diseases [4]. In the Peninsular of

Malaysia, the plant was also reported to be used in folk medicine for treating diabetes mellitus [5,6].

Single oral dose administration of the aqueous extract of AP has been reported to lower the blood glucose levels of diabetic rats [7,8]. Other studies showed that multiple doses of ethanol extract of the plant were required to lower the blood glucose levels [9]. However, in a recent study we found that acute administration of the 20% ethanol extract of the plant has antihyperglycaemic effects on normal rats in intraperitoneal glucose tolerance test (IPGTT) but did not lower the blood glucose level of diabetic rats [1].

Therefore, in present study the antihyperglycaemic effect of chronic administration of different ratios of aqueous ethanol extracts of this plant was studied in diabetic rats. Besides the blood glucose levels, the insulin concentration was also determined to find the correlation between the two parameters and possible explanation for the different antihyperglycaemic effects of this plant extracts [1,2].

MATERIALS AND METHODS

Plant Materials

The plant material used in this study was dried aerial parts and leaves of the plant, *Andrographis paniculata* was authenticated, standardized and supplied by Malaysian Research on Agriculture Development Institute (MARDI) Telong, Kota Bharu, Kelantan.

Preparation of Extracts

The dried plant material was ground to powder form using a milling machine. The dried powder was then divided equally into four portions and macerated in water, ethanol 20%, 50% and 95% (v/v) respectively at 60°C for three consecutive days. They were then filtered and the extracts were obtained evaporated under vacuum to dryness. The extracts were dried further in the oven at 60°C until the weight is constant.

The percentage of water, ethanol 20%, 50% and 95% extracts obtained were 10.3 %, 13.29, 11.46 and 8.7% (w/w), respectively.

Thin layer chromatography analysis

The solvent system of the upper layer of butanol: acetic acid: water, at ratio of 4:1:5, was used. Pre-coated silica gel 60 F₂₅₄ aluminum plate (Merck) was used as solid phase. Andrographolide was used as a reference.

Experimental Animal

Normoglycaemic Sprague-Dawley rats, weighing 200 – 250 g of either sex, were used in this study and were obtained from the animal house of the School of Pharmaceutical Sciences, Universiti Sains Malaysia, Penang. The animals were kept in plastic cages and provided with standard animal diet and tap water. The animal study has been approved by ethical committee in animal research of Universiti Sains Malaysia, Penang.

Sample collection

Fasting Blood Glucose level

All the rats were fasted overnight before blood glucose level determination. Blood samples were obtained by making a small nick at the tail of the rat and the blood glucose levels were determined using clinical glucometer (Accutrend Advantage II Clinical Glucose meter by Roche®).

Insulin assay

Blood obtained from the tail nick was collected by using capillary tube and centrifuged. The plasma was then collected and stored at -20°C until assayed. A commercial ELISA kit of rat insulin (Crystal Chem Inc, IL, USA) was used to determine the level of insulin in the plasma.

Drugs used

Streptozotocin (Sigma®) 65 mg/kg was administered intraperitoneally to induce diabetes in the rats. The antidiabetic activity of the plant extracts were compared with oral metformin 500 mg/kg of body weight (500 mg tablet, Glucophage®) as positive control.

Experimental Protocol

Sprague-Dawley rats (200 – 250g) of either sex were treated with Streptozotocin 65 mg/kg intraperitoneally. Three days after the diabetic induction, the fasting blood glucose and insulin levels of each rat were determined. Rats with blood glucose levels above 15mMol/L were considered diabetic and used for the study. The diabetic rats were randomly divided into six groups, with each group comprising of six rats (n=6). The groups were respectively treated with

tap water as negative control, metformin (500 mg/kg of body weight) as positive control and the test groups of water, ethanol 20%, 50% and 95% of extract of *A. paniculata* (500 mg/kg, orally, twice daily) for 14 days. The fasting blood glucose and insulin levels after three days of the diabetic induction and 14 days after chronic oral treatment were recorded.

Statistical analysis

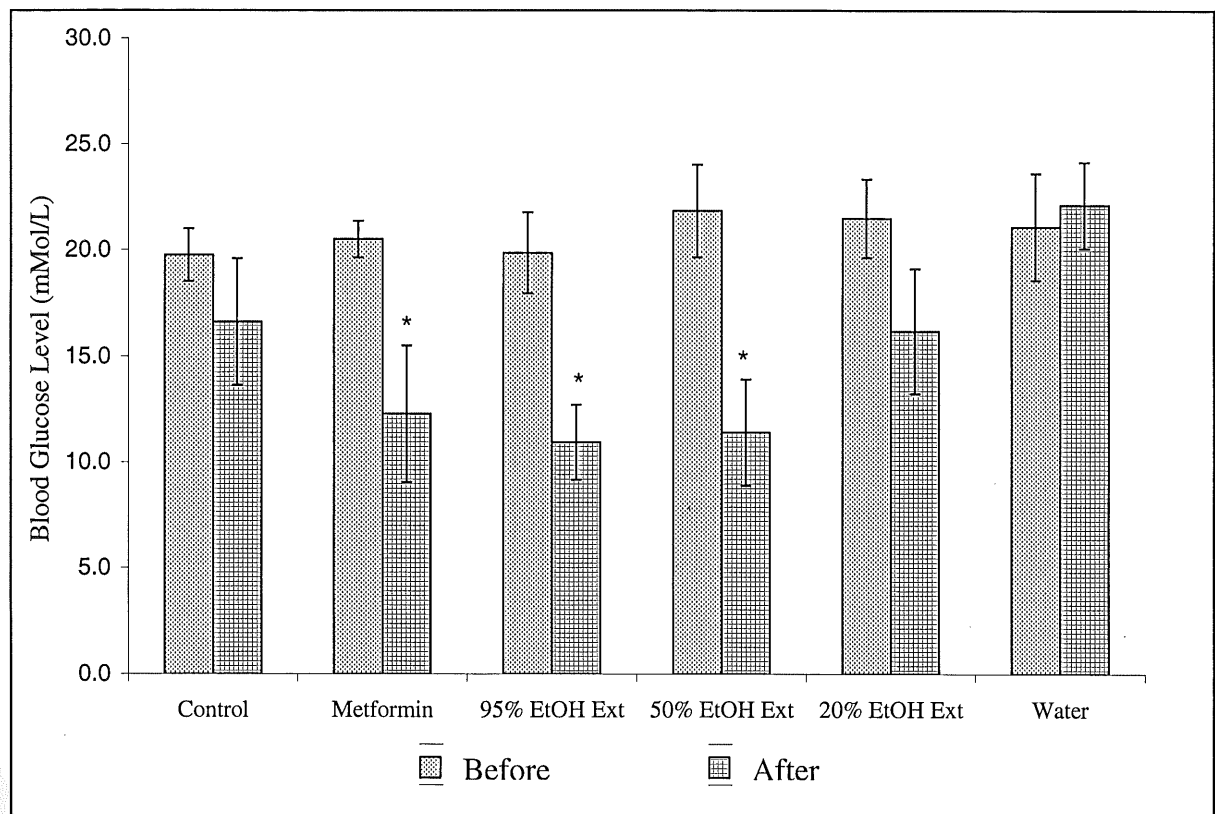
All data were expressed as mean \pm SEM. The means differences between pre- and post-

treatment were analyzed by paired *t*-test and considered significant at $p < 0.05$.

RESULTS

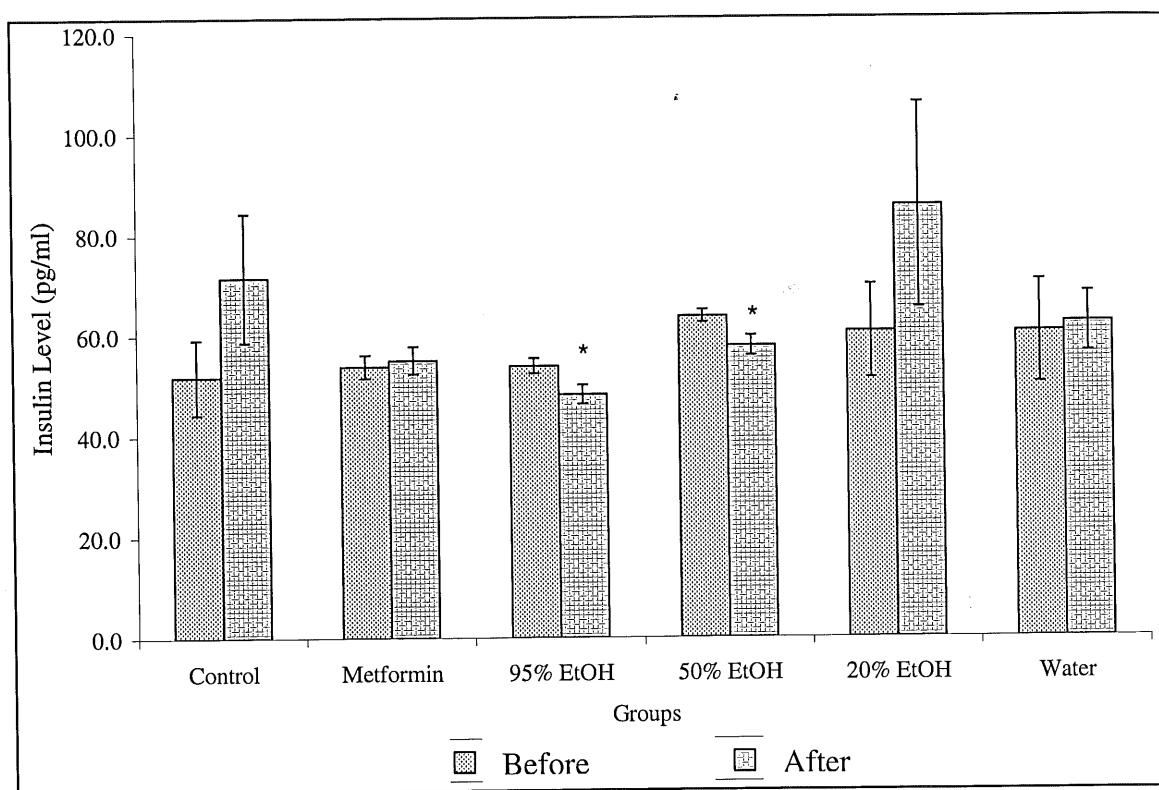
Figure 1 shows that the fasting blood glucose levels of metformin, 50% and 95% ethanol extracts of *A. paniculata* treated rats were significantly reduced compared to the pre-treatment level ($p < 0.05$). However, only treatment with 50% and 95% ethanol extracts of the plant significantly lower the insulin levels (Fig 2).

The thin layer chromatography revealed that all extracts have dark brown bands when visualize at 254 nm with Rf value 0.87. This was equivalent with andrographolide's band.



$n=6; *p < 0.05$

Figure 1. The effect of oral treatment with water, ethanol 20%, 50% and 95% extracts of *A. paniculata* 500 mg/kg and Metformin 500 mg/kg twice daily for 14 days on fasting blood glucose levels of streptozotocin-induced diabetic rats. Symbols are given as \pm SEM of six experiments.



$n=6; *p < 0.05$

Figure 2. The effect of oral treatment with water, ethanol 20%, 50% and 95% extracts of *A. paniculata* 500 mg/kg and Metformin 500 mg/kg twice daily for 14 days on insulin levels of streptozotocin-induced diabetic rats. Symbols are given as \pm SEM of six experiments.

DISCUSSION AND CONCLUSION

Daily treatment with metformin, 50% and 95% ethanol extracts of *A. paniculata* for two weeks significantly reduced the fasting blood glucose levels of streptozotocin-induced diabetic rats (figure 1). The lowering of blood glucose levels after 14 days of treatment with 95% ethanol extract of *A. paniculata* has been reported previously by Zhang & Tan, 2000 [9]. This suggested that similar to metformin the 50% and 95% ethanol extracts of *A. paniculata* possessed antidiabetic activity. It also suggested that the antidiabetic compound(s) in the *A. paniculata* is/are relatively non-polar as similar treatment with the more polar extracts of 20% ethanol and water extract of the plant did not lower the blood glucose level.

Chronic treatment of the diabetic rats with 50% and 95% ethanol extract significantly reduced the insulin levels, unlike those treated with

metformin, water and 20% ethanol extracts (figure 2). The result on the metformin-treated group is expected as antidiabetic drug metformin, a biguanide, is known not to stimulate the release of insulin from the β -cells of pancreas. It indicates that the antidiabetic activity of 50% and 95% ethanol extracts are not through the mechanism of the stimulation of insulin released. The lowering of the insulin levels by these extracts was presumably as a result of physiological response to the lowering of the blood glucose levels.

The TLC analysis showed that andrographolide was present in all the *A. paniculata* extracts. However, the results showed that only 50% and 95% ethanol extracts produced the antidiabetic activity. It suggested that either andrographolide was not involved in the antidiabetic activity or the concentration of andrographolide in the water and 20% ethanol extracts of *A. paniculata* was not

sufficient to cause significant lowering of blood glucose level.

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