39th Meeting of National Working Group on Indonesian Medicinal Plant

PROCEEDING

INTERNATIONAL CONFERENCE AND TALK SHOW
ON MEDICINAL PLANT

Effective, Safe and Qualified Herbal Medicine for Diabetes Mellitus Treatment

Jakarta, 19th – 21st of October 2010

Organized by:
Agency for the Assessment and Application of Technology (BPPT)
National Working Group on Indonesian Medicinal Plant (POKJANAS TOI)
Association of Indonesian Herbal Medical Doctors (PDHMI)

Supported by:
Deutscher Akademischer Austauch Dienst (DAAD)
PROCEEDING INTERNATIONAL CONFERENCE AND TALK SHOW ON MEDICINAL PLANT “Effective, Safe and Qualified Herbal Medicine for Diabetes Mellitus Treatment”


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INDONESIA
Praise and gratitude towards Allah who has given His Grace so that the International Conference and Talk Show on Medicinal Plant could be conducted in BPPT Jakarta on the 19th – 21st October 2010.

This International Conference and Talk Show on Medicinal Plant which is also the 39th Meeting of National Working Group on Indonesian Medicinal Plant is organized by BPPT in collaboration with the National Working Group on Indonesian Medicinal Plants (POKJANAS TOI), Deutscher Akademischer Austauch Dienst (DAAD) and the Association of Indonesian Herbal Medical Doctors (PDHMI). This Conference is an effort to develop the national herbal medicine industry and as a venue for researchers, policy makers, clinicians, industrialists and observers of the herbal medicine to gather and discuss the issues regarding herbal medicine.

The theme of this Conference is “Effective, Safe and Qualified Herbal Medicine for Diabetes Mellitus Treatment”. This theme was chosen because diabetes mellitus is one of diseases that could become a serious threat to public health in the future and currently herbal medicine has not been optimally utilized for the treatment of various diseases, especially diabetes mellitus. This Conference is expected to provide an overview of recent advances in herbal medicine technology, herbal medicine role in the treatment of diabetes, the problems faced by the herbal medicine industry and alternative solutions.

This conference has brought together 88 research papers from researchers of various institutions with a variety of topics, ranging from the cultivation aspects to clinical trials which provide an overview of the research currently conducted by research institution in Indonesia. Through this Conference, it is expected that research institutions with similar interest could collaborate to accelerate research in Indonesian and also as a venue for facilitating collaboration between research institutes, universities and industries.

The committee realizes that there are still many shortcomings that may be less than satisfactory to many parties. Criticisms and suggestions are expected to improve this organization of seminars in the future.
On this occasion the committee expressed the highest appreciation to the
guest speakers, papers contributors, participants and sponsors for their
cooperation in organizing the International Conference and Talk Show on
Medicinal Plant

Jakarta, 19th of October 2010

Organizing Committee

International Conference and Talk Show on Medicinal Plant
Assalamu Alaykum wa rahmatullahi wa barakatuh.

Good morning, Selamat Pagi, Salam Sejahtera untuk kita semua.

May God Almighty blesses us in conducting this event

Let us start by expressing our gratefulness to God almighty. His bless and mercy has given us opportunity to be here in the great and special event of International Conference and Talkshow on Medicinal Plants which focus on the theme of “Effective, Safe and Qualified Herbal Medicine for Diabetes Mellitus Treatment”.

I am delighted to welcome all distinguished guests, invited speakers and participants. To speakers coming from overseas, welcome to Jakarta and welcome to Indonesia. Jakarta is very wet at the moment, due to heavy rain in the last couple of months. I hope this will not discourage you to enjoy your visit to Jakarta.
In this very important moment, allow me to thanks to the committee who has been working very hard to bring this event into reality. I also would like to express a special thank to the Assosiation of Indonesian Herbal Medical Doctors (PDHMI) who has been actively involved and support to this event. And to Deutscher Akademischer Austauch Dienst (DAAD) or German Agency for Academic Exchanges as a main sponsor, gratitude thanks and most appreciation shall be addressed.

Ladies and Gentlemen,

I would like to draw your attention that BPPT as a research agency under coordination of the Ministry of Research and Technology, highly supports the development of herbal medicines as one of the BPPT program on health and pharmaceutical sector especially in fulfilling the national self-sufficiency on medicines. Beside Health and medicine, BPPT is conducting other focused program, namely food, energy, ICT, transportation, defence, environment, and manufacturing.

I would like to highlight here that herbal medicine development is strategic choice for developing country like Indonesia where mega biodiversity is one of its comparative advantages. Currently, chemical and semi-chemical drug industries has been dominated by global pharmaceutical industries in developed countries. For this reason, competition in this area is not beneficial for Indonesian pharmaceutical industries where its industrial structure in general, is still very weak and need to be further developed. The choice to promote program on herbal medicine development is the fact that this program has strong national capacity building from upstream to downstream processes by using locally available resources and involving various stakeholders.

Ladies and gentlemen,

This is our main mission to relate the most suitable technology to significantly increase the added value of our bioresources in order to support our national herbal medicine industries. Knowledge and technology would be a fundamental tools in the development of herbal medicine industries which enable to support national economic growth (known as knowledge based economy).

I do realise that to achieve the noble goal is not trouble-free. We need an active participation of all players not only from government institutions as facilitatory and regulatory bodies, but also from private sectors, academicians as well as researchers to work hand-in-hand toward the best
in the utilization of knowledge and local resources in natural product development.

*Ladies and gentlemen,*

Since the enthusiasm of back to nature have been gaining popularity and acceptance in recent decade, national herbal medicine industries should become one of the pillars of national economic development. This is also supported by the fact that Indonesia has a second largest biodiversity in the word, with more than 30,000 species of plants. The number of herbal medicine industries keep increasing, numbering to 1,500 companies, big and small corporation. According to Herbal Medicine Entrepreneur Association *(GP Jamu)* data in 2007, not less than 3 million people works on herbal medicine sector from upstream to downstream industries. The market share of herbal medicine has growing significantly, up to 15-20% yearly.

We all hope that herbal medicine will not only fulfil local and national market, but more than that will expand to other countries, as export commodity.

*Ladies and gentlemen,*

As I have just mentioned before, BPPT, through the Centre of Pharmaceutical and Medical Technology - fully supports the herbal medicine development. I hope throughout this great forum, all stakeholders involved in the utilization of medicinal plants will stumble on the best and most suitable ways to develop our biological resources as health products. We would like to utilize our biodiversity as source of natural products to prevent and cure diseases, especially degenerative diseases such as diabetes mellitus, and also to promote our well being. To PDHMI, the Associsiation of Indonesian Herbal Medical Doctors we would certainly relay on your share in using herbal medicine as part of formal health services.

At last, we should work closely with all experts and institutions who concern in this area not only from our national side but also from abroad. Therefore, BPPT is continually welcome to all collaborators from national as well as international institutions based on our mutual benefit for each parties involved.

*Ladies and gentlemen,*

Once again, I would like to thanks to the committee, sponshors, speakers and participants and wishing all a successful symposium.
Finally, by saying “Bismillahirrahmanirrahim”, in the name of GOD Almighty, the Most Gracious and the Most Merciful, I have the great pleasure to declare the **International Conference and Talkshow on Medicinal Plants** is officially opened.

Thank you very much for your passion and kind attention,

*Wassalamu’alaykum warahmatullahi wabarakatuhu*

BPPT Chairman,

Dr. Ir. Marzan Azis Iskandar
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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 *Eugenia polyantha* (200 mg/kg bw) and water extract of *Andrographis paniculata* (200 mg/kg bw) showed significant increase of blood glucose level at T₃₀ and T₆₀ (T₃₀ = 30 minutes after glucose loading or 60 minutes after drugs administration, T₆₀ = 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 T₉₀. 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.

**Keyword**: *Andrographis paniculata* Nees extract, *Eugenia polyantha* Wight leaves extract, oral glucose tolerance test

**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).

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 glibenclamid 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, 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, Jogiakarta 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.

Figure 1. The TLC profile: (1) Andrographis paniculata ethanol extract, (2) Andrographis paniculata water extract, (3) andrographolide as a reference compound

Moreover, the oral glucose tolerance test result can be seen in Table 1, Figure 1 and Figure 2.

Table 1. Blood glucose in treated Wistar rats (n=3, Average ± SEM)

<table>
<thead>
<tr>
<th>Groups</th>
<th>T0</th>
<th>T30</th>
<th>T60</th>
<th>T90</th>
<th>T120</th>
<th>T150</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glibenclamid 0.45mg/kg bw</td>
<td>73.00 ± 4.04</td>
<td>97.33 ± 1.33*</td>
<td>82.33 ± 11.23</td>
<td>65.33 ± 9.57</td>
<td>55.33 ± 6.9*</td>
<td>45.67 ± 3.76*</td>
</tr>
<tr>
<td>Metformin 63 mg/kg bw</td>
<td>81.33 ± 2.03</td>
<td>118.33 ± 2.34*</td>
<td>86.67 ± 2.34</td>
<td>90.33 ± 3.18*</td>
<td>77.67 ± 3.29*</td>
<td>88.00 ± 1.53</td>
</tr>
<tr>
<td>Negative control (Water)</td>
<td>71.00 ± 3.06</td>
<td>129 ± 3.52*</td>
<td>110.33 ± 9.7*</td>
<td>99.33 ± 0.67*</td>
<td>91.00 ± 1.16*</td>
<td>83.00 ± 4.59</td>
</tr>
<tr>
<td>Euginia polyantha leaves 200 mg/kg bw</td>
<td>75.67 ± 4.18</td>
<td>92.33 ± 3.29*</td>
<td>95.67 ± 11.48*</td>
<td>86.33 ± 6.99</td>
<td>80.00 ± 2.00</td>
<td>73.33 ± 6.37</td>
</tr>
<tr>
<td>Water extract of A. paniculata 200mg/kg bw</td>
<td>75.67 ± 3.53</td>
<td>106.33 ± 5.82*</td>
<td>96.67 ± 7.70*</td>
<td>85.33 ± 5.93</td>
<td>78.67 ± 2.85</td>
<td>68.67 ± 3.18</td>
</tr>
<tr>
<td>EtOH extract of A. paniculata 200 mg/kg bw</td>
<td>72.67 ± 3.76</td>
<td>115.67 ± 2.41*</td>
<td>118.33 ± 1.20*</td>
<td>93.67 ± 1.67*</td>
<td>81.67 ± 3.18</td>
<td>81.67 ± 1.45</td>
</tr>
</tbody>
</table>

*Significantly different (P<0.05) toward fasting blood glucose (T0)
Figure 2. Blood glucose levels at T\textsubscript{0}-T\textsubscript{150} (mg/dl)

All the blood glucose at T\textsubscript{30} (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 T\textsubscript{0} (fasting blood glucose), which showed that the method was valid. Glibenclamide 0.45 mg/kg bb started to lower the blood glucose at T\textsubscript{60} although significant difference was obtained at T\textsubscript{120} and T\textsubscript{150}. On the other hand, the other positive control, metformin 63 mg/kgbb showed blood glucose reduction at T\textsubscript{120}, although it rose again at T\textsubscript{150}. The raise at T\textsubscript{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\textsubscript{30}, which is as expected. The blood glucose level differed significantly throughout the experiment, except at T\textsubscript{150}, when the blood glucose level returned to normal.

Both *Euginia 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\textsubscript{30} and T\textsubscript{60} (T\textsubscript{30} = 30 minutes after glucose loading or 60 minutes after drugs administration, T\textsubscript{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\textsubscript{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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