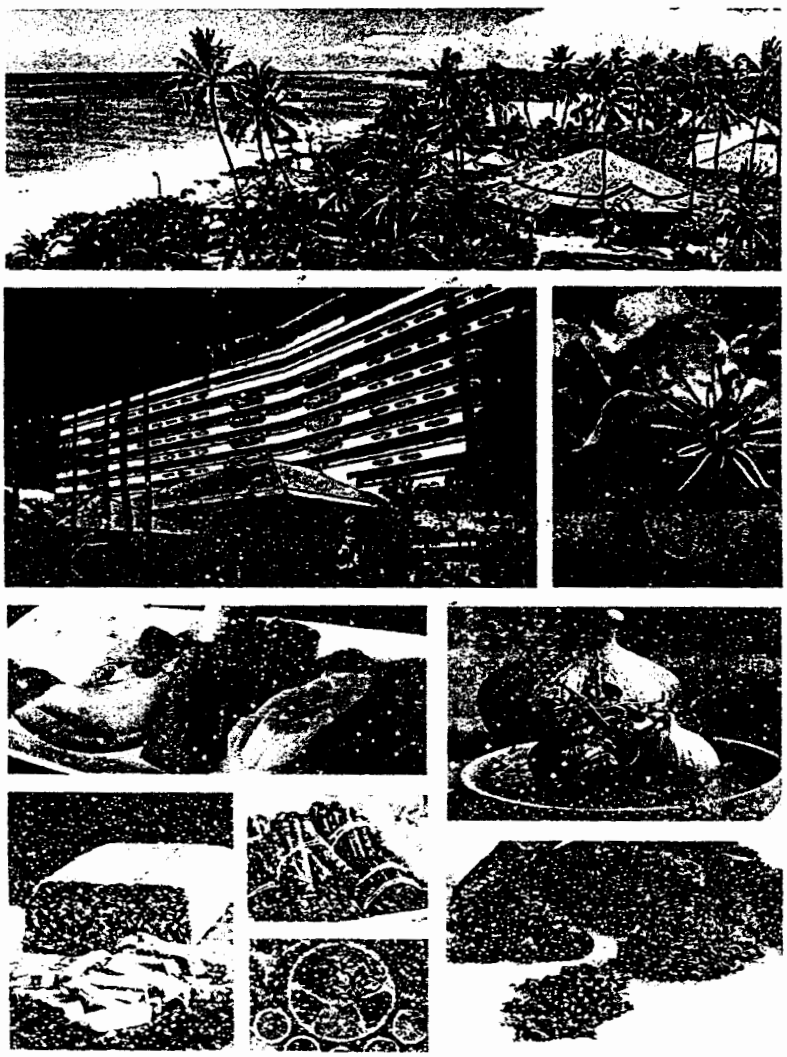
INTERNATIONAL CONFERENCE, EXHIBITION &SHORT COURSE ON

NUTRACEUTICALS & FUNCTIONAL FOODS

Inna Grand Bali Beach
Bali Indonesia
October 11-15, 2010



SHORT COURSE:
MON-TUE, OCTOBER 11th - 12th, 2010

CONFERENCE:
TUE-FRI, 12th - 15th, 2010

Indonesian Agency for Agricultural Research and Development, Ministry of Agriculture

International Society for Nutraceutical and Functional Foods

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O73. AN OVERVIEW OF BOTANICAL SUPPLEMENTS IN AMELIORATING THE SYMPTOMS OF METABOLIC SYNDROME

Akhtar A. Ali – National Center for Toxicological Research US FDA (USA)

ABSTRACT NOT AVAILABLE AT TIME OF PRINTING

O74. ANTIHYPERGLYCAEMIC EFFECT OF *AZADIRACHTA INDICA* J ETHYL ACETATE EXTRACT ON ALLOXSAN –INDUCED DIABETIC RATS

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The aim of this study is to assess the potential of ethyl acetate extract of neem leaves (*Azadirachta indica* J) which has the effect antihyperglycemic using white rat experimental animals. Phytochemical screening of neem leaves was obtained which contained the active ingredients are flavonoids, saponins, and tannins. 18 male white rats aged 4-5 months was divided into six treatment groups: first as a normal control group who were given aquadest, second as a negative control group who were given aquadest, a third group as a positive control were given glibenclamide; fourth group as the treatment group one (P1), i.e. groups with a ethyl acetate extract of neem leaves dose of 30 mg/kg BW; fifth as the treatment group two (P2), i.e. groups with a ethyl acetate extract of neem leaves dose of 60 mg/kg BW; sixth as the treatment group three (P3), i.e. groups of rats fed ethyl acetate extract of neem leaves dose of 90 mg/kg BW. All groups except normal controls were induced by alloxan dose 150 mg/kg BW. Glucose levels in rats examined before the induction of alloxan (H0), after induction of alloxan (h6) and after a given treatment (H10). The results obtained showed that the ethyl acetate extract of neem leaves significantly affected the decline in blood glucose of rats. Ethyl acetate extract of neem leaves 90 mg/kg BW showed a decrease of blood glucose the most optimum. The research concluded that the ethyl acetate extract of neem leaves has antihyperglycemic effect.

The Activity of Aethyl Acetate Extract of Neem Leaves on Anti Hyperglicemia Rats Induced by Alloxan

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ABSTRACT

The aimed of this study is to assess the potency of aethyl acetate extract of neem leaves which has anti-hyperglycemia effect in white male rats Spraguey Dawley. Eighteen rats age 4-5 months was divided into six groups: first as a normal control group and negative control group (K1 and K2) which have been given aquadest, a third group as a positive control (K3) which has been given glibenclamide; the treatment group (KP1, KP2, KP3) have been given aethyl acetate extract of neem leaves dose of 30, 60, 90 mg/kg bw. All groups except K1 were induced by alloxan dose 150 mg/kg bw. Phytochemical screening of neem leaves contained the active ingredients such as flavonoids, saponins, and tannins. Blood glucose levels in rats was examined before and after induction of alloxan and after treatments. The results showed that the aethyl acetate extract of neem leaves significantly affected the decreased of blood glucose levels. The effective dose to decreased blood glucose levels are 60 and 90 mg/kg bw. Neem leaves as a herbal remedy is seen as less expensive. It can at this stage be recommended as a phytotherapeutic agent.

Keywords: extract, aethyl acetate, neem leaves, alloxan, anti-hyperglycemia.

INTRODUCTION

Background

Indonesia is a country that has a high biodiversity in the world. Thousands of plant species have been used as traditional medicine (Bermawie et al. 1996). Many of the traditional ingredients of a good product that has been processed with modern technology and simply circulate in the community. The need for testing to prove the efficacy of a material nature is needed, because there are still based on empirical evidence. Scientific research will reveal problems associated with natural materials such as: potency, chemical contents and possible development for use in modern medicine. One of the plants which can be developed into drugs is neem (*Azadiracta indica* J).

In Java the name of neem is imbo, imbau or umbo. Neem usually used by many communities as a vegetable pesticide materials, as well as a reforestation crop (Kardinan & Ruhnayat 2003). The seed is used to cure itching, the leaves to repellant flies on cattle and the stem can be used for domestic purposes (Soewita 1995). Some people use as a traditional medicine such as allergies, heart disease, arthritis (rheumatoid arthritis), cancer, cough, fever, rheumatism, kidney, high blood pressure, cholesterol, and diabetes mellitus (Kardinan & Ruhnayat 2003). Neem is also known to have a property as anti-hyperglycemia (lowering blood glucose levels).

Hyperglycemia is a condition of high blood glucose levels due to the entry of glucose into the blood can not be transferred into muscle cells, kidney, adiposit, and can not be converted into glycogen and fat. Hyperglycemia may occur due to lack of insulin, insulin receptor, and glucose carrier so buried in the blood glucose. Hyperglycemia is one of the symptoms of diabetes mellitus. Research on effects of neem leaves as a lowering of blood glucose has been widely applied. According to El-Hawary & Kholief (1990), neem leaves extract produce hypoglycemic agents in normal rats and can lower blood sugar in rats suffering from hyperglycemia. The availability of abundant medicinal plant, neem plants in particular makes this plant accessible and less expensive. This study is expected to provide information for the medical community and society, about the role of neem leaves as an alternative for traditional medicine, particularly as anti-hyperglicemic drug.

MATERIALS AND METHODS

Neem leaf is obtained from BALITRO Research Institute and were identified by Herbarium Bogoriences in LIPI Cibinong. Powdered neem leaves was maceration extracted. Results maceration is filtered and piaced in the tube.

Content of organic compounds commonly identified are alkaloids, tannins, flavonoids, saponins, steroids, triterpenoid, and hydroquinone. Eightteen male white rats Spargue Dawley aged 4-5 months were used. The animals were divided into six groups, each group consisted of three rats. Carried out during 10 days of treatment. Grouping of experimental animals are:

1. Normal controls (K1) was injected with NaCl 0.9% 1ml + 1ml distillated water
2. Negative control (K2) was injected alloxan 150 mg/kgbw + 1ml distillated water
3. Positive control (K3) was injected alloxan 150 mg/kgbw + glibenclamide dose of 3.5×10^{-6} mg/kgbw
4. Treatment 1 (KP1) was injected alloxan 150 mg/kgbw + aethyl acetate extract doses 30 mg/ kgbw
5. Treatment 2 (KP2) injection of alloxan 150 mg / kg bw + ethyl acetate extract dose of 60 mg/kgbw
6. Treatment 3 (KP3) injection of alloxan 150 mg / kg bw + aethyl acetate extract dose of 90 mg/kgbw

Blood glucose levels were measured after the animals were kept for ± 16 hours, and before alloxan induced. Subsequent measurement of blood glucose levels on days 6^{th} were intended to determine an increase in blood glucose levels. On days 5^{th} were measured to determine blood glucose levels after given treatment. Rats blood glucose was measured using a Blood Glucose Meter Finetest TM brand. The trick with a drop of blood from rat tail vein glucose dropped on the strip that has been included in glucometer. Value is indicated on glucometer blood glucose levels value with units mg/dL.

Body weight was measured four times, before giving alloxan, after induced with alloxan, and after treatment. Measurement of body weight using grams coarse scales.

Statistically analyzed using analysis of variance ANOVA test followed by Duncan test area to see whether there is any difference.

RESULTS AND DISCUSSION

Changes of Body Weights

Observation of the rat's body weight during the three weeks of adaptation, showed the trend increased in rats body weight. This marks the rats had begun to adapt to there new environment. K1 have continued to increased body weight and growing normally. Group K2, K3, KP1, KP2 and KP3 which were injected by alloxan, showed the decreased body weight (Table 1) but the statistical analysis showed no different response ($p > 0.05$).

Table 1 Average body weight of rats

Treatment	Initial weight (grams)	Weight before induction (g)	Weight after induction (g)	Weight after treatment (g)
K1	187.67±12.85	203.67±14.01	213.67±17.21	223.00±17.34
K2	173.67±4.50	189.00±8.54	172.67±7.02	166.33±3.51
K3	163.00±30.80	176.67±29.95	159.33±12.34	147.00±10.39
KP1	155.00±7.00	179.00±5.19	168.33±9.50	144.33±7.37
KP2	197.00±10.53	212.33±12.66	174.00±13.74	176.00±33.45
KP3	197.33±8.32	210.67±7.09	180.67±6.42	180.67±10.06

Decreased in body weight of rats caused by rats which has been suffering from hyperglycemia (Table 1). The pathophysiology of the disease involves impaired entry of glucose into the cells and accumulation of glucose in the blood. This process results in increased plasma osmolarity and urinary loss of glucose, accompanied by excess loss of water and sodium (polyuria). The resulting dehydration triggers compensatory mechanisms such as thirst (polydipsia). The inability of the cells to utilize glucose resembles a state of cellular starvation, stimulating hunger (polyphagia) and triggering the activation of compensatory responses to increase the release and availability of fuel substrates though activation of lipolysis and proteolysis. Gluconeogenesis can be derived from lipolysis and proteolysis that reduced muscle mass as indicated by decreased body weight (Scobie 2007).

Blood Glucose Levels

Alloxan is a chemical that can cause damage to the pancreatic β cells and used as a material for inducing the occurrence of hyperglycemia in experimental animals (Ellenberg & Rifkin 1970). Measurement of blood glucose performed immediately before and six days after

induction of alloxan, and five days after dosing ethyl acetate extract of neem leaves. Measurement of glucose levels on 6 days with the assumption that all rats which have been suffering from hyperglycemia. Hyperglycemia rats diagnosed when blood glucose levels over 250 mg/dL (Gutirerrez & Vargas 2006).

Table 2 showed that mean blood glucose level in each group. There was significant ($P< 0.05$) increased of blood glucose levels in group K2 compare to treatment groups, implying an increased plasma glucose in K2 and a significant decreased in the blood glucose level of test rats administered aethyl acetate extract of neem leaves (KP1, KP2, KP3) and glibenclamide (K3). In the normal group (K1) increased blood glucose was detected.

Table 2. The effect of ethyl acetate extract of neem leaves on blood glucose level (mg/dl) in rats

Group	Days	
	0	5
K1	$62,33\pm9,81^{a}$	$85,67\pm24,37^{a}$
K2	$454,33\pm40,20^{b}$	$454,00\pm36,37^{b}$
K3	$451,67\pm101,08^{bc}$	$182,00\pm145,34^{c}$
KP1	$370,00\pm16,64^{cd}$	$290,67\pm39,80^{d}$
KP2	$414,00\pm18,52^{bcd}$	$260,33\pm29,4^{bc}$
KP3	$363,00\pm32,78^{d}$	$213,33\pm14,57^{bc}$

*Superscript in the same column and row mean difference significantly ($P <0.05$)

There were no significant differences decreased of blood glucose level in group KP2 and KP3 compare to K3. Ethyl acetate extract 60 mg/kgbw and 90 mg/kgbw lowered the blood glucose levels is equivalent to glibenclamide. According Sukrasno & Tim Lantern (2003), the hypoglycemic activity of leaf extracts and neem seed same with glibenclamide.

The statistical analysis showed that administration of ethyl acetate extract of neem leaves in groups of KP1, KP2 and KP3 significant ($p <0.05$) to decreased blood glucose levels. Ethyl acetate extract with different dose can lower blood glucose levels. These results are consistent with Biswas et al. (2002) which states that the leaves, stems, bark and neem seed oil has the effect of hypoglycemic (lowering blood glucose levels).

The result of phytochemical screening test showed positive for flavonoids, saponins, and tannins. According to Gahukar (2010) that the results of phytochemical neem leaves contain more than 40 biologically active compounds, including lomonid, flavonoids, polysaccharides and

compounds of sulfur. Substances suspected to affect the activity of ethyl acetate extract anti-hyperglycemia neem leaves are tannins and flavonoids. According to Zabri *et al.* (2008), flavonoid function can lowering blood sugar. Tadera *et al.* (2005) also states that the compound has the potential of tannins and flavonoids anti-hyperglycemia with reversible noncompetitive inhibition mechanism glucosidase enzyme. Glucosidase is an enzyme that is needed in the process of carbohydrate metabolism which is located at the edge of the surface of intestinal cells. Glucosidase enzymes break down carbohydrates into glucose in the intestines. Compounds that can inhibit the enzyme activity showed an indication that the compound has the potential to reduce blood sugar levels.

Conclusion and recommendation

Conclusion

Ethyl acetate extract of neem leaves contain flavonoids, tannins and saponins. The effective dose to lower blood glucose levels are 60 and 90 mg/kgbw (KP2 and KP3).

Suggestion

Further study is necessary to know about the mechanism of neem leaves in lowering blood glucose levels, histopathological observations and histopathologic studies.

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