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Comparison On Anti-Hyperglycemic Activity Of Sambilotoleaves (Andrographis Paniculata (Burm. F.) Nees) And Turmeric Rhizome (Curcuma Domestica Val) Ethanol Extracts In White Male Mice

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Abstract

Sambiloto (King of Bitters) and turmeric are empirically used by Indonesiansfor antidiabetic Mellitus. Therefore,thisstudyaims to examine the anti-hyperglycemic activity fromvarious doses of Sambilotoleaves and turmeric rhizome on white male mice. The studyusedan antihyperglycemic activity test with alloxan induction administered intraperitoneally (IP). Furthermore, 35 mice were used and divided into 7 groups (n=5), namely, the comparison with glibenclamide (0.013 mg/20grBW), the control with Na-CMC, Dose 1 Sambiloto 100 (1.12 mg/20grBW), Dose II Sambiloto - Turmeric 75:25, Dose III Sambiloto - Turmeric 50:50, Dose IV Sambiloto - Turmeric 25:75,and Dosage V Turmeric 100 (1.4 mg / 20 g BW). The data wereanalyzed statistically using the Annova method with a 95% confidence level. Moreover,the results showed that all test groups in 1 to 5 doses (P values 0.001, 0.0061, 0.0004, 0.0029, 0.0348,and 0.0011) hadasignificant effect on reducing blood glucose levels in mice compared to the control (p-value < 0.005). Based ontheseresults, the Sambilotoethanol extract, turmeric, and the combination had activity in reducing blood glucose levels.

Keywords: Diabetes mellitus, turmeric, Sambiloto, a combination of turmeric - Sambiloto, and alloxan

Introduction

Diabetes mellitus is a syndrome withimpaired carbohydrate, fat, and protein metabolism caused by decreased insulin secretion or tissue sensitivity,whichleadsto chronic complicationssuch as microvascular, macrovascular, and neuropathic disorders.[1,2,3,4]

In most cases, diabetes mellitus develops slowly after the age of 30 which is oftenbetween the ages of 50 and 60,therefore, it is called adult-onset diabetes. However, there has been an increasing numberof younger individual cases with type 2 diabetes mellitus recently. This is mainly related to the increased obesity prevalence which is the most important risk factor for type 2 diabetes in everyindividual.[1]

DM patients have impaired basal and prandial insulin secretion tomaintain blood glucose levels within normal limits in the fasting state and after eating. Understandingthis mechanism showsthatthe essence of DM treatment is to decrease blood glucose levels at the fasting state and after eating. Moreover,blood glucosechecks include Random Blood Glucose (GDS),Fasting Blood Glucose (GDP), and Oral Glucose Tolerance Test (OGTT) for diagnosis.[5.6]When the blood glucose level remains high after the checks, an additional HbA1c examination is required.This HbA1c reflects the average plasma glucose for the past 2 to 3 months andis performed withoutspecial preparations such as fasting. Furthermore,itis the standard for assessing glycemic control in diabetic patients and also as an option todetermine the glucose tolerance in undiagnosed diabetic patients.[7,8,9]

Managing DM begins with a non-pharmacological approach such aspublic enlightenment, diet planning, physical activity, and weight loss. Moreover, Indonesia has abundant natural resources and some aremedicinal, therefore, they are used as traditional medicineingredients from animals, minerals, and plants. Furthermore, the country has 30,000 plant species and 3,698 have medicinal properties which are used bythecommunity since ancient times asherbal medicines to prevent, maintain,and improve health. One of these plants includes anti-hyperglycemic while Turmeric is contained in the category of spices and drugs [10.11]. The properties of Turmeric are antidiabetic, typhoid, dysentery, vaginal discharge, pain during menstruation, facilitate breastfeeding, tonsillitis, and diarrhea. Furthermore, itschemical constituents include curcuminoids which consist of curcumin, desmetoxicumin, bisdemethoxycurcumin, essential oils, fats, carbohydrates, proteins, and vitamin C. Meanwhile, Sambiloto (Andrographis paniculata (Burm. F.) Nees) usually grows in lowlands ofapproximately 700 m above sea level anditsefficacyincludes antidiabetic, dysentery, typhoid, pneumonia, malaria, toothache, fever, acid, cancer, and others Thechemical constituents in the leaves are deoxy andrographolide, neo-andrographolide, 14-deoxy-11-12 didehydrographolide, and homo andrographolide. [12]

Therefore,thisstudy aims to examine the anti-hyperglycemic activity from various doses of Sambilotoleaves and turmeric rhizome on white male mice.

Materials and Methods

The study design is experimental with the alloxan induction test method and comparative. Meanwhile,theexperimental animals required were 50 mice weighing 20 to 40 grams and aged 6 to 8 weeks. These animalswere divided into various groups with 5 mice for each group, namely control, comparison, test doses 1, 2, 3, 4, and 5 on Sambilotoleaves, and turmeric extracts. The mice fasted for 18 hours before treatmentandwere induced with alloxan intraperitoneally and given a 10 % glucose drink. After 3 days, the blood glucose of all the experimental animals was measured with anda value of 200 mg/dL was obtained using Gluco-DR®.Thetreatment conditions are as follows:

1. The comparison group was given glibenclamide (0.013 mg/20grBB)
2. The control group was given 1% Na-CMC
3. Test group dose 1 (Sambiloto 100): Sambiloto ethanol extract of 1.12 mg/20 gr BW)
4. Combination test group dose 2 (Sambiloto - Turmeric 75:25) : Combination of Sambiloto 75% : Turmeric 25% ethanol extract
5. Combination test group dose 3 (Sambiloto - Turmeric 50:50): Combination of Sambiloto 50%: 50% turmeric ethanol extract
6. Combination test group dose 4 (Sambiloto - Turmeric 25:75): Combination of Sambiloto 25% and Turmeric 75% ethanol extract
7. Test group dose 5 (Turmeric 100): Turmeric ethanol extract 1.4 mg/20 g BW)

On the 1st, 7th, 14th, and 21st days after alloxan administration, blood glucose levels of all groups were measured. The measurement data were processed using statistical methods of Annova and Dunnett multiple comparisons.

Results and Discussion

Sambiloto leaves and turmeric rhizome were from Karawang Regency and the determination was carried out at the Biology Laboratory of Halu Oleo University, Southeast Sulawesi. Furthermore, wet and dry sorting was carried out for simplification and the Simplicia obtained was extracted using a maceration technique. This was carried out by soaking each Simplicia powder with 96% ethanol using a sample and solvent ratio of 1:10. The soaking process was observed for 3 x 24 hours and the extracted solvent was replaced daily with a new solvent. Furthermore, the filtrate was collected and concentrated until a thick extract was obtained and used for the testing process. From 1 kg of Simplicia leaves extract, a thick extract of 46.323 grams was obtained (4.63% yield) while 1.5 kg of turmeric rhizome powder was from a thick extract of 140.233 grams (9.35%).

Experimental Animal

Experimental animals used were male Swiss Webster strain mice weighing > 20 Gr, in good health, and acclimatized for 1 week at a temperature of 25^0C. The ethical clearance was from the Faculty of Medicine, Tanjung Pura University, West Kalimantan with No: 2191/UN22.9/TA/2021.

Plant Determination

Sambiloto and turmeric plants were obtained in the Kawarang area, West Java Province. Meanwhile, the determination was carried out at the Biology Laboratory of Halu Oleo University in Southeast Sulawesi which showed Sambiloto plants with species of Andrographis paniculata (Burm. F.) Nees and turmeric of Curcuma domestica Val.

Identification of Chemical Ingredients

Identification of chemical content was carried out to determine the content of secondary metabolites in a plant being tested.

Table 1. Chemical Content of Sambiloto and Turmeric Ethanol Extract

Sample	Observation Result			
	Flavonoid	**Saponins**	**Quinone**	**Steroid/Triterpenoid**
Turmeric Rhizome	+	-	+	+
SambilotoLeaves	+	+	-	+

The identification results of the chemical constituents showedthatthe turmeric rhizome contained secondary metabolites of flavonoids, quinolones, and steroids/triterpenoids while the Sambiloto leaves contained flavonoids, saponins, and steroids/triterpenoids.

Antidiabetic Activity Testing

The data obtained were analyzed using One Way Annova. Based on this test, all groups showed a significant effect on reducing blood glucose levels in mice compared to the control group as shown in Figure 1.

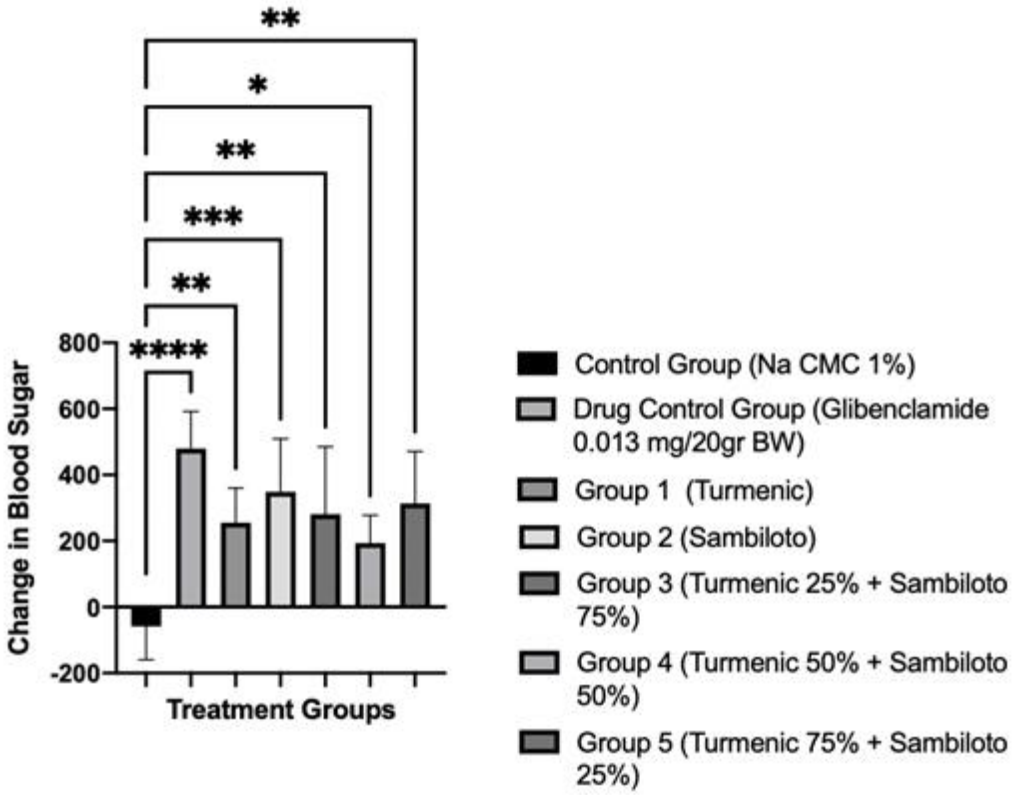

Figure 1: Antidiabetic Ethanol Extract of Sambiloto Leaves and Turmeric Rhizome

The figure above showed that the highest decrease in glucose levels occurred in the comparison group (glibenclamide 0.013mg/20 g BW) with an average of 479.4 mg/dL from the baseline blood glucose level. This was followed by the sambiloto test group (Sambiloto 100) with an average value of 348.8 mg/dl, the combination test group (Turmeric-Sambiloto 75:25) with 313.4 mg/dl, the combination test group (Turmeric-Sambiloto 25:75) of 281.4 mg/dl, the turmeric test group (Turmeric 100) with an average of 255.8 mg/dl, and the combination test group (Turmeric-Sambiloto 50;50) with 193.4 mg/dl. Meanwhile, the activity of decreasing blood glucose levels was statistically significant (P-value < 0.05) as shown in Table 1.

Table 1 Activities for decreasing blood glucose levels in the control group

Group	Average Decrease in Blood Glucose Levels (mg/dL)	P Value
Turmeric 100 (Turmeric 100)	255,8	0,0061*
Sambiloto 100 (Sambiloto 100)	348,8	0,0004*

Turmeric-Sambiloto Combination (KS 25:75)	281,4	0,0029*
Turmeric-Sambiloto Combination (KS 50:50)	193,4	0,0348*
Turmeric-Sambiloto Combination (KS 75:25)	313,4	0,0011*
Comparison of Glibenclamide (0.013 mg/20 g BW)	479,4	0,0001*

***One Way Annova (significant if p<0,05)**

Based on the One Way Annova test, to find the test group that has a similar effect with the drug group, the Dunnett multiple comparison tests were performed without the control group. Among all test groups, only the Turmeric - Sambiloto 50:50 combination test group different effect in decreasing blood glucose levels while the other groups had the same effect as the comparison group. This is shown in Figure 2.

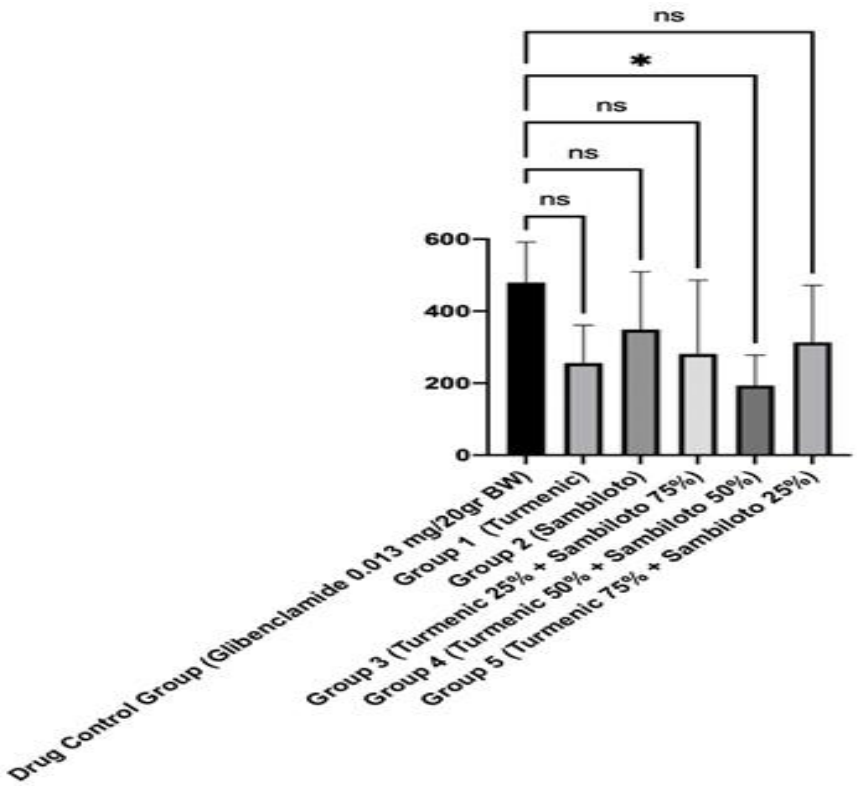

Figure 2: Comparison of the activity of decreasing blood glucose levels between the test and the comparison groups

The results above showed that there was a decrease in blood glucose levels on all test groups while both single and combination were statistically significant. These results are strengthened by a study by Simkumar et al which stated that single-use of sambiloto showed a decrease in blood glucose levels that prevent diabetes and cause a protective effect on kidney tissue in experimental rats. [13] Other studies related to sambiloto have been conducted by combining with neem leaves and the results have an impact on decreasing glucose levels, therefore, have the potential as antidiabetic.[14] This is also supported by Anggarini et al. study which combined sambiloto and soursop leaves for antidiabetic. Based a on previous study, there is an antidiabetic activity in the combination of Sambiloto - Soursop (50:50).[15]

Study on turmeric as an antidiabetic, conducted by Mohamad Andrie et al, showed that turmeric tamarind herb has antidiabetic activity as characterized by a decrease in blood glucose levels and an improvement in the pancreatic islets of Langerhans in streptozotocin-induced diabetic rats with an effective dose of 1.90mL/200gBW.[16]Other research showed that the combination of turmeric and garlic has anti-hyperglycemic activity and not effective as glibenclamide. Furthermore, the combination has been clinically proven not to affect liver and kidney function as well as hematological profiles.[17]

The main compounds in sembung (Blumea balsamifera)leaves with biological activity are diterpene lactone groups whichconsist of andrographolide, neoandrographolide, 14-deoxyandrographolide, andrographin, 14-acetylandrographolide, 14-deoxydehydroandrographolide, and homoandrographolide. Meanwhile, Andrographolide is the most dominant diterpene lactone compound which consistsapproximately 4% of all plants and it is activeas an antidiabetic. However, its mechanism as an antihyperglycemic agent is not well known. Several parameters used to determine the activity of Andrographis paniculata as an antidiabetic include preprandial and postprandial blood glucose levels, GLUT-4 protein expression in muscle tissue, hypoglycemic activity of glibenclamide, and the HOMA-IR (homeostatic model assessment-insulin resistance) index. [18]Moreover, the curcumin found in turmeric (Curcuma longa Linn) is widely consumed and generally believed to be beneficial for human health. Curcumin extract from turmeric rhizome has been shown to contain anti-inflammatory and anti-diabetic properties. Therefore, it delaysthedevelopment of type 2 diabetes, improves cell function, prevents cell death, and reduces insulin resistance in experimental animals.[19]

Conclusion

Based on the results, the ethanolic extracts of Sambiloto (Andrographis paniculata (Burm. F.) Nees) and Turmeric rhizome (Curcuma Domestica val) have antidiabetic activity in single-use or combination. Meanwhile, the combination of turmeric-sambiloto in ratio 50:50 showed insignificant ow statistics when compared to the comparison group (Glibenclamide 0.013 mg/20 gr BW)

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Conflict of Interest

All authors state that there is no potential conflict of interest with the research, authorship, and/or publication of this study.

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