WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 8.084
Volume 12, Issue 20, 1088-1103. Research Article ISSN 2277– 7105

ANTIDIABETIC AND ANTIDYSLIPIDEMIC ACTIVITIES OF COMBINED BANDOTAN (*AGERATUM CONYZOIDES*) AND SAMBUNG NYAWA (*GYNURA PROCUMBENS*) ETHANOL EXTRACTS IN INSULIN-RESISTANT RAT MODELS

Yani Mulyani*, Patonah Hasimun and Ella Fazila and Khairani Asfa

Pharmacy Study Program, Faculty of Pharmacy, Bhakti Kencana University, Bandung, Indonesia.

Article Received on
01 Oct. 2023,

Revised on 22 Oct. 2023,
Accepted on 12 Nov. 2023

DOI: 10. 20959/wjpr202320-30231

***Corresponding Author**
Yani Mulyani
Pharmacy Study Program, Faculty of Pharmacy, Bhakti Kencana University, Bandung, Indonesia.

ABSTRACT

Unhealthy lifestyles are known to increase the risk of degenerative diseases, such as cardiovascular diseases, which greatly contribute to mortality worldwide. This condition often arises due to factors such as diabetes mellitus and dyslipidemi. Meanwhile, bandotan (*Ageratum conyzoides*) and sambung nyawa (*Gynura procumbens*) have been reported to possess antidiabetic and antidyslipidemic activities. Therefore, this study aimed to determine the antidiabetic and antidyslipidemic activities of bandotan and sambung nyawa ethanol extracts in insulin-resistant rat models. A pre and post-test control group design was used with 36 rats divided into six treatment groups. These included normal control (0.5% CMC), positive control (high-fat and fructose emulsion), metformin, simvastatin, as well as doses 1 (125:125) and 2 (250:250) mg/kgBW. In addition, the rats were induced into insulin resistance by administering a high-fat and fructose emulsion for 42 days, followed by 14 days of treatment. Blood glucose levels and lipid profiles were monitored at a specific time. The results showed that the combination dose of bandotan: sambung nyawa at 125 mg/kgBW: 125 mg/kgBW demonstrated significant potential for antidiabetic and antidyslipidemic activities.

KEYWORDS: Antidiabetic, antidyslipidemic, bandotan, sambung nyawa, insulin resistance.

INTRODUCTION

Unhealthy lifestyles are known to increase the risk of degenerative diseases, such as cardiovascular diseases, which greatly contribute to mortality worldwide. This condition often arises due to factors such as diabetes mellitus and dyslipidemia. Diabetes mellitus is characterized by hyperglycemic conditions as well as disruptions in fat, carbohydrate, and protein metabolism due to insulin resistance. This can lead to dyslipidemia through increased lipolysis and sugar production in the liver as well as reduced glucose uptake in muscle or fat cells.[1,2]

Across the globe, herbal medicines are recognized for their efficacy, with 80% of the population utilizing various materials for treatment. Furthermore, the World Health Organization (WHO) recommends the use of traditional herbal medicines for the maintenance, prevention, and treatment of chronic and degenerative diseases. Generally, traditional medicines are considered safer than modern ones due to their relatively fewer side effects.[3,4] Some plants utilized for herbal treatment include bandotan and sambung nyawa.

Previous studies reported that bandotan and sambung nyawa extracts lowered blood glucose levels by enhancing insulin sensitivity as well as reducing total cholesterol, triglycerides, and LDL levels.[5,6,7] Therefore, this study aimed to examine the antidiabetic and antidyslipidemic activities of combined bandotan and sambung nyawa ethanol extracts in male rats with insulin resistance. Histopathology examination of the liver and adipocyte cells was also conducted to observe improvements after treatment.

MATERIALS AND METHODS

Preparation of Tools and Materials

The tools used in this study included oral gavage for rats, rat cages, an analytical balance, water bath, rat scale (Mettler Toledo®), beaker glass (Pyrex®), a funnel, parchment paper, measuring glass (Pyrex®), volumetric flask (Pyrex®), mortar, pestle, spatula, stirring rod, 100 mL bottles, Glucometer and test strips (Accu Check®), micropipette, centrifugation, Microlab 300, blue tips, yellow tips, white tips, microtubes, ointment pot, and hematocrit capillary tubes.

The materials used comprised male white rats, bandotan and sambung nyawa herbs, 96% ethanol, metformin®, CMC-Na, lipid emulsion (lipomed®), distilled water, HCl, fructose, Neutral Buffered Fixative (NBF) 10%, CMC-Na, Total Cholesterol Reagent, Triglyceride

Reagent, LDL Reagent 1 and 2, HDL Reagent 1 and 2, Trucal U, Trulab N, Cholestest N Calibrator (CNC), and Cholestest Control (CC).

Preparation of Bandotan and Sambung Nyawa Simplicia

The bandotan and sambung nyawa were obtained from the Research Institute for Spice and Medicinal Crops (BALITRO) Bogor. The plants were then identified in the Biology Department Laboratory, Faculty of Mathematics and Natural Sciences, Padjajaran University, Jatinangor. Additionally, freshly harvested herbs with a total of 8 kg were subjected to simplicia preparation, including wet sorting, washing, slicing, drying, and dry sorting.

Simplicia Characterization

Characterization was conducted to obtain safe simplicia with good quality, standardization, and stability. Several parameters used in this stage included drying shrinkage, as well as moisture, ash, water-soluble, and ethanol-soluble content.

Preparation of Combined Bandotan and Sambung Nyawa Ethanol Extracts

The pulverized bandotan and sambung nyawa simplicia were placed in separate containers. The 96% ethanol was added at a ratio of (1:6) until the simplicia was completely submerged. The sample was left submerged and stirred every few hours, while the 96% ethanol was replaced daily for 3 consecutive days. The resulting macerate was filtered using filter paper and then concentrated with a rotary evaporator.

Phytochemical Screening of Bandotan and Sambung Nyawa Ethanol Extracts

Alkaloid Test

The sample was added with Mayer, Wagner, and Dragendorff reagents. The formation of a white, brown-red, and orange-yellow precipitate after the addition of the reagent respectively, indicated the presence of alkaloids.[8]

Flavonoid Test

A 2 mL sample of concentrated HCl and a piece of Mg metal were placed in a test tube, then color changes were observed. The formation of a reddish-orange color change implied the presence of flavonoids.[8]

Polyphenol and Tannin Test

The sample was mixed with hot distilled water and left to cool, then 3-4 drops of 10% NaCl were added. The mixture was stirred, filtered, and subsequently divided into 2 parts. Part 1

was combined with 5 mL of gelatin and 5 mL of NaCl solution. The formation of a white precipitate indicated the presence of tannin. Meanwhile, part 2 was mixed with FeCl3 and a color change to blackish green indicated the presence of polyphenols.[8,9]

Saponin Test

The sample was added with 10 mL of hot distilled water, allowed to cool, and then shaken for 10 seconds in a test tube. Any observed changes were noted and a drop of 2 N HCl was added. Saponins were confirmed when foam with a height of approximately 1 to 10 cm was formed.[8]

Glycoside Test

The sample was added with chloroform and evaporated over a water bath. The solution was dissolved in 5 mL of acetic anhydride P and then added with 10 drops of sulfuric acid. A color change to green or blue indicated the presence of glycosides.[10]

Steroid and Terpenoid Test

The sample was mixed with 15 mL of ethanol and then added with 3 drops of acetic anhydride, along with concentrated sulfuric acid through the tube wall. A positive steroid result was indicated by a color change to bluish-green, while a positive terpenoid result was demonstrated by the formation of a brownish or violet ring after the addition of concentrated sulfuric acid.[9]

Preparation of Test Animals

Rats acclimatized for 1 week were divided into 6 groups, each receiving oral treatments at specified dose levels as follows:

Group I: Negative control (0.5% CMC-Na)

Group II: Positive control (Induction of lipid emulsion (Lipomed®) + 70% fructose + 0.5% CMC-Na)

Group III: Induction of lipid emulsion (Lipomed®) + 70% fructose + Metformin suspension

Group IV: Induction of lipid emulsion (Lipomed®) + 70% fructose + Simvastatin suspension

Group V: Induction of lipid emulsion (Lipomed®) + 70% fructose + Combined bandotan and sambung nyawa ethanol extracts as dose 1

Group VI: Induction of lipid emulsion (Lipomed®) + 70% fructose + Combined bandotan and sambung nyawa ethanol extracts as dose 2

Antidiabetic Activity Test

The rats were induced into insulin resistance by administering a high-fat and fructose emulsion for 42 days. Afterward, the test compounds were administered according to the treatment groups for 14 days until day 56. The glucose levels were monitored on days 0, 14, 28, 42, and 56 using a glucometer through the rat tail vein. Blood was collected from the lateral tail vein by gently piercing the tip. Subsequently, the blood absorbed by the glucometer showed the glucose levels.[11,12,13]

Antidyslipidemic Activity Test

The rats were induced into insulin resistance by administering a high-fat and fructose emulsion for 42 days. Afterward, the test compounds were administered according to the treatment groups for 14 days until day 56. Measurement of lipid profile levels began with the collection of blood serum samples. The test animals were anesthetized by inhaling CO2, and blood was collected through the orbital sinus. A microhematocrit was gently inserted into the corner of the eyeball while rotating gently towards the back until blood flowed and was then collected in a 1.5 ml microtube. The collected blood was centrifuged for 15 minutes at 5000 rpm to separate serum and pellets. The separated clear serum was collected and placed in 4 separate microtubes, each containing at least 20 μl. Measurements were conducted using a Microlab 300 by reacting the serum with total cholesterol, triglyceride, LDL, and HDL reagents enzymatically.

Insulin Tolerance Test

The insulin tolerance test was conducted on day 42 (post-induction) and 56 (post-treatment) by administering Novorapid® insulin at a dose of 10 U/kgBW intraperitoneally. Subsequently, blood glucose levels were rechecked through the tail vein every 15 minutes for 60 minutes using a glucometer. The regression coefficient (r) or slope was determined with linear regression where the axis represented time and the ordinate indicated fasting glucose levels. The Insulin Tolerance Test Constant (KTTI) value was obtained from the calculation using the formula: KTTI = r x 100.[13,14]

Histopathology Examination of the Liver and Adipocyte Cells

On day 56, the rats were anesthetized using carbon dioxide (CO2) and then dissected to take the liver and fat tissues. Fat washing was performed using 0.9% saline (NaCl) and fixed with 10% Neutral Buffered Formalin (NBF) for further histopathology preparation. The

preparation was stained with Hematoxylin Eosin (HE) and observed using a light microscope at 400x magnification.[15,16]

Data Analysis

Data was stored in Ms. Excel and statistically analyzed using the SPSS (Statistical Package for the Social Sciences) program. Normality and homogeneity tests were conducted as the initial stage of statistical analysis. Since both tests resulted in data having normal and homogeneous distributions with a significance value (p) of (>0.05), the analysis proceeded using One-way ANOVA. When the test results showed differences between groups, a post hoc test was performed for further analysis.

RESULTS AND DISCUSSION

Characterization of Bandotan and Sambung Nyawa Simplicia

Characterization was conducted to obtain safe simplicia with good quality, standardization, and stability.[17] Several parameters used in this stage included drying shrinkage, as well as moisture, ash, water-soluble, and ethanol-soluble extract content. The characterization results of bandotan and sambung nyawa simplicia are shown in Table 1.

Table 1: Characterization results of bandotan and sambung nyawa simplicia.

Characteristics	Bandotan Simplicia	Sambung Nyawa Simplicia
Drying shrinkage	7.120%	9.734%
Moisture content	5.5%	9%
Ash content	13%	11.5%
Water-soluble extract content	27%	23%
Ethanol-soluble extract content	10%	15%

The drying shrinkage and moisture content tests fulfilled the quality requirements set by the Indonesian Herbal Pharmacopoeia, namely ≤ 10%. However, the ash content test did not meet the requirements due to discrepancies attributed to the indirect sun drying process which introduced external impurities such as smoke, dust, and sand to the simplicia. The water-soluble and ethanol-soluble extract content tests indicated that the bandotan simplicia tended to have polar compounds, while the sambung nyawa simplicia had non-polar compounds.[18,19]

Phytochemical Screening of Bandotan and Sambung Nyawa Ethanol Extracts

Phytochemical screening of the bandotan and sambung nyawa ethanol extracts was conducted using various methods to determine the contained compounds. The results of both extracts are depicted in Table 2.

Table 2: Phytochemical screening results of bandotan and sambung nyawa extracts.

Compound class	Test Results	
	Bandotan Extract	Sambung Nyawa Extract
Alkaloids	+	+
Saponins	+	+
Tannins	+	+
Phenols	+	+
Flavonoids	+	+
Glycosides	+	+
Triterpenoids	+	+
Steroids	+	+

Description: +: contains secondary metabolite compounds

The phytochemical screening indicated the presence of several secondary metabolite compounds, including alkaloids, phenols, saponins, tannins, flavonoids, glycosides, triterpenoids, and steroids in both bandotan and sambung nyawa extracts. These results were consistent with previous studies on the phytochemical screening of bandotan and sambung nyawa extracts.[20,21]

Antidiabetic Activity Test

Table 3: Average blood glucose levels.

Group	Average Blood Glucose Levels (mg/dL) (Mean ± SD)				
	0	14	28	42	56
Normal	79.7 ± 2.52	94.7 ± 6.03	95.7 ± 4.73	83.0 ± 7.57^{b}	92.7 ± 2.52^{bc}
Positive	89.3 ± 6.03	111.3 ± 2.52	112.0 ± 7.00	101.7 ± 10.69^{ac}	121.7 ± 13.32^{a}
Metformin	88.7 ± 3.21	105.0 ± 6.24	109.7 ± 5.51	123.7 ± 10.97^{a}	106.3 ± 4.16^{b}
Dose 1	82.7 ± 4.04	105.7 ± 4.93	98.3 ± 3.51	110.7 ± 11.59^{ac}	97.3 ± 5.51^{bc}
Dose 2	92.7 ± 2.52	105.3 ± 10.97	108.7 ± 12.10	108.3 ± 3.79^{ac}	97.3 ± 9.71^{bc}

Description: a: sig <0.05 significantly different from the normal group; b: sig <0.05 significantly different from the positive group; c: Not significantly different from the metformin group (p>0.05); T0: Initial blood glucose level before induction; T14: Blood glucose level after 14 days of induction; T28: Blood glucose level after 28 days of induction; T42: Blood glucose level after 42 days of induction; T56: Blood glucose level after 14 days of test compound administration.

Table 3 presents the monitoring results of fasting blood glucose levels on day 42, indicating a significant difference ($p<0.05$) between the normal control and all other groups. This suggested that the administration of fat emulsion and fructose for 42 days increased fasting blood glucose levels in all treatment groups except the normal control. Moreover, the results on day 56 showed a significant difference between the positive control and all other groups. This indicated a decrease in fasting blood glucose levels in the normal and test groups due to the administration of medicines and test compounds over 14 days. Both dose 1 and 2 test groups yielded statistically non-significant different results compared to the metformin group. This suggested that the combined bandotan and sambung nyawa ethanol extracts reduced fasting blood glucose levels almost equivalent to the metformin group. Based on the calculation results, the dose 1 group reduced the percentage of blood glucose levels closest to the metformin group. Therefore, it was considered to have the greatest potential as an antidiabetic agent.

Antidyslipidemic Activity Test

Total cholesterol (KT) level test

Table 4: Average total cholesterol levels.

Average Total Cholesterol Levels (mg/dL)					
Group	**T0**	**T42**	**%Change after induction**	**T56**	**%Change after treatment**
Normal	55.8±1.4	60.4±2.7$^{bcd\#}$	+7.7%	58.8±3.4$^{bd\#}$	-2.7%
Positive	54.5±0.4	72.3±0.7^{a}	+24.6%	71.6±0.6$^{acd\#}$	-1%
Simvastatin	54.7±1.9	70.6±0.4^{a}	+22.5%	60.4±1.3^{ab}	-14.5%
Dose 1	52±1.4	71.7±0.7^{a}	+27.5%	67.2±1.5^{abc}	-6.3%
Dose 2	54.3±2.7	73.6±0.9^{a}	+26.2%	65.5±0.8^{abc}	-11.1%

Description: a: sig <0.05 significantly different from the normal group; b: sig <0.05 significantly different from the positive group; c: sig <0.05 significantly different from the simvastatin group; d: sig <0.05 significantly different from dose 1 group; #: sig <0.05 significantly different from dose 2 group; T0: day 0 before induction; T42: day 42 after induction and before treatment; T56: day 56 after treatment; (+): percentage increase; (-): percentage decrease.

The results of change after induction in Table 5 demonstrated an increase in triglyceride levels across all groups except the normal. Meanwhile, Table 4 showed a decrease in total cholesterol levels across all groups except the normal and positive control, which were not given treatment. The normal control did not experience significant change, indicating the Na-CMC carrier had no cholesterol-lowering activity, although there was a normal decrease.

Statistical significant differences were observed between all groups and the normal (P<0.05). The positive control did not experience significant change after induction cessation and demonstrated a significant difference with all groups (P<0.05). Furthermore, the simvastatin group differed significantly (P<0.05) from the normal and positive control. Dose 1 and 2 showed a significant difference (P<0.05) compared to the simvastatin and normal groups. However, dose 1 and 2 did not significantly differ from each other (P>0.05), indicating that the increase in total cholesterol levels in these groups was not significantly different. Dose 1 produced the most potent effect with a 6.28% reduction percentage for total cholesterol levels.

Triglyceride Level Test

Table 5: Average triglyceride (TG) levels.

Average Triglyceride Levels (mg/dL)					
Group	**T0**	**T42**	**% Change after induction**	**T56**	**% Change after treatment**
Normal	75.7±3.8	93.07±21.0$^{bc\#}$	+18.7%	93.4±5.4^{bd}	-0.4%
Positive	70.4±3.8	134±5.1^{a}	+47.5%	130.3±2.8$^{acd\#}$	-2.8%
Simvastatin	81.7±4.3	128.9±9.6^{a}	+36.6%	96.13±3.7^{bd}	-25.4%
Dose 1	67.3±3.7	99.5±7.4	+32.4%	80.1±2.3$^{abc\#}$	-19.5%
Dosis 2	66.7±1.0	123.1±4.6^{a}	+45.8%	95.3±6.6^{bd}	-22.6%

Description: a: sig <0.05 significantly different from the normal group; b: sig <0.05 significantly different from the positive group; c: sig <0.05 significantly different from the simvastatin group; d: sig <0.05 significantly different from dose 1 group; #: sig <0.05 significantly different from dose 2 group; T0: day 0 before induction; T42: day 42 after induction and before treatment; T56: day 56 after treatment; (+): percentage increase; (-): percentage decrease.

Low-Density Lipoprotein (LDL) Level Test

Table 6: Average Low-Density Lipoprotein (LDL) levels.

Average LDL Levels (mg/dL)					
Group	**T0**	**T42**	**%Change after induction**	**T56**	**% Change after treatment**
Normal	19.6±0.61	19.9±0.4$^{bc\#}$	+1.5%	19.8±0.3$^{bcd\#}$	-0.5%
Positive	19.6±1.18	25.1±2.3^{a}	+21.9%	24.4±1.7^{acd}	-2.8%
Simvastatin	14.8±2.56	23.6±1.1^{a}	+37.3%	16.5±1.4$^{ab\#}$	-30.1%
Dose 1	15.4±0.9	21±1.3	+26.7%	17.5±1.2$^{ab\#}$	-16.7%
Dose 2	19.5±0.8	29.6±2.2^{a}	+34.2%	23.2±0.8^{acd}	-21.6%

Description: a: sig <0.05 significantly different from the normal group; b: sig <0.05 significantly different from the positive group; c: sig <0.05 significantly different from the

simvastatin group; d: sig <0.05 significantly different from dose 1 group; #: sig <0.05 significantly different from dose 2 group; T0: day 0 before induction; T42: day 42 after induction and before treatment; T56: day 56 after treatment; (+): percentage increase; (-): percentage decrease.

The results of change after induction showed an increase in LDL levels across all groups except the normal. Conversely, Table 6 demonstrated a decrease in LDL levels in all groups except the normal and positive control, which were not given treatment. The normal control did not experience significant change, indicating the Na-CMC carrier had no LDL-lowering activity, although there was a slight decrease. Statistical significant differences were observed between all groups and the normal (P<0.05). The positive control did not experience significant changes after induction cessation and demonstrated a significant difference with all groups (P<0.05) except dose 2. The simvastatin group differed significantly (P<0.05) from the normal, positive, and dose 2 groups. The dose 1 and 2 groups showed significant differences from each other and differed significantly from the normal (P>0.05). However, dose 1 did not differ significantly (P>0.05) from the simvastatin group, resulting in its consideration as the best dose for LDL levels.

Table 7: Average High-Density Lipoprotein (HDL) Levels.

Average HDL Levels (mg/dL)					
Group	**T0**	**T42**	**%Change after induction**	**T56**	**% Change after treatment**
Normal	12.6±1.2	11.9±1.2$^{b\#}$	-6.0%	11.6±0.7$^{bcd\#}$	-2.6%
Positive	21.1±2.5	15.7±1.4^{ad}	-25.7%	16±0.9$^{acd\#}$	+1.7%
Simvastatin	15.9±1.7	13.7±2.1	-13.84%	35.4±2.9$^{abd\#}$	+61.3%
Dose 1	15.4±0.8	12.5±0.6^{b}	-19%	23.1±2.7$^{abc\#}$	+45.9%
Dose 2	18.0±2.2	14.3±0.6^{a}	-10.7%	29.5±1.6^{abcd}	+51.5%

Description: a: sig <0.05 significantly different from the normal group; b: sig <0.05 significantly different from the positive group; c: sig <0.05 significantly different from the simvastatin group; d: sig <0.05 significantly different from dose 1 group; #: sig <0.05 significantly different from dose 2 group; T0: day 0 before induction; T42: day 42 after induction and before treatment; T56: day 56 after treatment; (+): percentage increase; (-): percentage decrease.

The results of change after induction demonstrated an increase in HDL levels across all groups except the normal control. Meanwhile, Table 7 showed an increase in HDL levels

across all groups except for the normal and positive control, which were not given treatment. The normal control did not experience significant change, indicating the Na-CMC carrier had no HDL-improving activity, although there was a slight decrease within the normal range, possibly due to food factors. The positive control showed a significant statistical difference from all groups. In terms of %change, this group experienced change within the normal range. This implied that the group continued to experience distress after induction cessation for 2 weeks. Dose 1 and 2 differed significantly ($P<0.05$) from other groups. However, the POST-HOC LSD HDL statistical analysis in Appendix XIII of the Lipid Profile Test Results showed that dose 1 significantly differed from the simvastatin comparator compared to dose 2. Based on the results, dose 2 most effectively increased HDL levels with a percentage of 51.5%. Flavonoid compounds are known to promote the maturation and development of HDL cells.

Insulin Tolerance Test

Table 8: Insulin tolerance test (KTTI) constant values.

Group	KTTI Values	
	After Induction (T42)	**After Treatment (T56)**
Normal	2.12 ± 0.81^{b}	3.13 ± 0.72^{bc}
Positive	0.54 ± 0.40^{ac}	0.42 ± 0.45^{a}
Metformin	0.68 ± 0.29^{a}	2.58 ± 1.10^{b}
Simvastatin	0.90 ± 0.80^{a}	3.60 ± 3.30^{b}
Dose 1	0.54 ± 0.73^{ac}	2.41 ± 0.88^{bc}
Dose 2	0.15 ± 0.11^{ac}	1.93 ± 0.74^{bc}

Description: a: Significantly different from the normal control ($p<0.05$); b: Significantly different from the positive control group ($p<0.05$); c: Not significantly different from the metformin group ($p>0.05$); T42: KTTI value on day 42 after induction; T56: KTTI value on day 56 after treatment.

Based on the results on days 42 and 56 in Table 8, the normal control provided average KTTI values of 2.12 and 3.13. These values indicated good body sensitivity to insulin. In contrast, the positive control experienced a decrease from 0.54 to 0.42 due to the administration of fat emulsion and fructose without treatment. The metformin group demonstrated an increase in KTTI values from 0.68 to 2.58, indicating that metformin administration restored insulin sensitivity. Furthermore, the dose 1 group showed an increase from 0.54 to 2.4, similar to the dose 2 which also experienced an elevation from 0.15 to 1.93. Statistically, the test groups did not significantly differ from the metformin group, indicating both groups have

antidiabetic activity by enhancing insulin sensitivity. Direct comparison between the test groups showed that dose 1 had KTTI values close to the metformin group.

The obtained KTTI values demonstrate the success of administering combined bandotan and sambung nyawa ethanol extracts as an antidiabetic treatment in insulin-resistant rats. In normal conditions, glucose in the blood enters the muscles and is converted into energy. When the blood contains excessive free fatty acids, the muscles carry out oxidation and this causes an increase in acetyl-CoA levels in the macrolide, potentially inhibiting glucose uptake leading to hyperglycemia. At normal free fatty acids levels, pancreatic β cells compensate for hyperglycemia by producing insulin to lower blood glucose levels. However, when there is an excess of free fatty acids in the body, the insulin produced by the pancreatic β cells becomes excessive (hyperinsulinemia), potentially disrupting the insulin signal transduction pathway in β cells. This condition causes resistance which refers to reduced sensitivity of body tissues to insulin.[22]

The dose 1 group had a more significant effect on the percentage reduction of fasting blood glucose levels and increased insulin sensitivity compared to dose 2. Additionally, it had close fasting blood glucose levels and KTTI values to the metformin group. The increase in KTTI values resulted in a decrease in blood glucose levels due to a rise in the sensitivity of tissue receptors to insulin. The decrease in blood glucose levels and the increase in KTTI values were attributed to the main compounds present in bandotan and sambung nyawa extracts, namely flavonoids, alkaloids, and tannins.

Histopathology Examination of Liver and Adipocyte Cells

Table 9: Liver histopathology.

Treatment Group	Description
Normal	No inflammation
Positive	Inflammation
Simvastatin	Inflammation
Dose 1	Inflammation
Dose 2	Inflammation

The liver histopathology results showed that the normal group did not undergo any changes in hepatocyte cells, suggesting the Na-CMC carrier had no significant effect on histopathology changes in the liver. In the positive control, hepatocyte cells experienced inflammation, indicating the induction for 42 days caused inflammation and required a longer time to induce liver necrosis. In the simvastatin, dose 1, and dose 2 groups, hepatocyte cells

experienced inflammation comparable to the positive control. This was consistent with Setiawan and Sari (2012) stating that high-fat induction for 30 days was not sufficient to induce liver degeneration and necrosis.

Table 10: Adipocyte Cell Diameter.

Treatment Group	Adipocyte cell diameter (µm)
Normal	52.582
Positive	75.846
Simvastatin	51.439
Dose 1	62.990
Dose 2	66.482

According to Table 10, the positive control induced with lipid emulsion (Lipomed®) and 70% fructose without treatment produced the largest adipocyte cell diameter of 75.846 µm compared to the other groups. This increase in diameter was due to the accumulation of fat in obese rats. In the positive control, adipocyte cells experienced hypertrophy (enlargement) compared to the other groups. However, both dose 1 and 2 showed adipocyte cell size similar to the normal and metformin groups. This indicated that the administration of bandotan and sambung nyawa ethanol extracts improved insulin resistance conditions as evidenced by the absence of adipocyte cell enlargement.

The decrease in blood glucose levels and lipid profiles as well as the increase in KTTI values were attributed to the main compounds found in bandotan and sambung nyawa extracts, namely flavonoids, alkaloids, and tannins. Due to their antidiabetic potential, flavonoids protect β cells, as well as restore and enhance receptor sensitivity to insulin. The flavonoids present in the bandotan plants include quercetin, while those in the sambung nyawa plants comprised myricetin and kaempferol. These 3 compounds have antidiabetic potential by acting as antioxidants. Quercetin, in particular, increases insulin secretion, improves resistance, reduces lipid levels, inhibits inflammation and oxidative stress, as well as lowers liver fat accumulation. Flavonoids can restore gut microbiota and endotoxemia-related imbalances mediated by induction of the TLR-4 pathway.[23,24]

Alkaloids enhance insulin sensitivity by aiding glucose absorption as well as GLUT-4 translocation to muscles and tissue cells, resulting in decreased blood glucose levels. These compounds are potent antioxidants that can reduce lipid elevation and oxidative stress, thereby restoring insulin sensitivity and increasing adiponectin release. Antioxidants increase glucose uptake into cells, consequently reducing blood glucose levels. Additionally, alkaloids

have other mechanisms such as inhibiting dipeptidyl peptidase-4, α-glucosidase, and α-amylase enzymes, leading to delayed glucose absorption. These compounds also prevent an increase in blood glucose levels by synthesizing glycogen through increased hexokinase activity in muscle tissue cells. Furthermore, tannins enhance glucose and fat metabolism, preventing fat accumulation, and also modulate insulin signaling through the P13 pathway, resulting in increased glucose uptake.[23,24]

CONCLUSION

Based on the results, it was concluded that:

1) The combined bandotan (*Ageratum conyzoides*) and sambung nyawa (*Gynura procumbens*) ethanol extracts showed antidiabetic and antidyslipidemic activities in male white rats (Rattus norvegicus) of the Wistar strain.
2) The combined extract at a dose ratio of 125 mg/kgBW:125 mg/kgBW produced the most effective antidiabetic and antidyslipidemic activities.
3) The combined extracts also ameliorated insulin resistance as evidenced by the absence of adipocyte cell enlargement in adipose tissue but did not improve liver inflammation.

ACKNOWLEDGEMENTS

The authors are grateful to the Faculty of Pharmacy, Bhakti Kencana University, for the study grant.

REFERENCES

1. Jumayanti, Wicaksana, A. L., & Sunaryo, E. Y. A. B. Kualitas Hidup Pasien dengan Penyakit Kardiovaskular di Yogyakarta. Jurnal Kesehatan, 2020; 13(1): 1–12.
2. Jialal, I., & Singh, G. Management of Diabetic Dyslipidemia: An update. World Journal of Diabetes, 2019; 10(5): 280–290.
3. Nurmalasari, N., & Hexa Apriliana Hidayah, dan. Studi Kasus Pemanfaatan Tumbuhan sebagai Obat-Obatan Tradisional oleh Masyarakat Adat Kampung Naga di Kabupaten Tasikmalaya. Biosfera. 2012; 19(3).
4. Sumayyah, Shofiah dan Salsabila, Nada. Obat Tradisional : Antara Khasiat dan Efek Sampingnya. Majalah Farmasetika, 2017; 2(5).
5. Aprilani, D., & Mustofa, S. Utari Gita Mutiara. Efek Pemberian Daun Sambung Nyawa (Gynura procumbens) Terhadap Kadar Gula Darah. Majority, 2019; 8(2).

6. Delisma. Counseling About the Efficacy of Bandotan Leaves on Decreasing Blood Sugar Levels at Talun Kenas Health Center. In Jurnal Pengabdian Masyarakat Putri Hijau, 2020; 1(1).
7. Mulyani, yani, suhardiman, aris, & fajarwati, kania. Uji Aktivitas Antidiabetes dan Antidislipidemia Ekstrak Herba Bandotan, Sambung Nyawa, dan Kombinasinya Pada Tikus Diabetes Yang Diinduksi Aloksan, 2022.
8. Muthmainnah, B. Skrining Fitokimia Senyawa Metabolit Sekunder dari Ekstrak Etanol Buah Delima (Punica granatum L.) dengan Metode Uji Warna. *Media Farmasi*, 2017; *13*(2): 36.
9. Simareme, Eva Susanty. Skrining Fitokimia Ekstrak Etanol Daun Gatal (Laportea decumana (Roxb.) Wedd). *PHARMACY*, 2014; 11(1): 98-107.
10. Malik, A., Edward, F., & Waris, R. Flavonoid Total Ekstrak Metanolik Herba. *Jurnal Fitofarmaka Indonesia*, 2014; *1*(1): 1–5.
11. Aligita, W., Muhsinin, S., Susilawati, E., Dahlia., Pratiwi, D.S., Aprilliani., Artarini, A., Adnyana, I.K. Antidiabetic Activity Of Okra (Abelmooschus esculentus L.) Fruit Extract. *Rasayan J.Chem.*, 2014; 12(1): 157-167.
12. Aligita, Widhya., Susilawati, Elis., Sukmawati, Ika Kurnia., Holidayanti, Lusi., Riswanti, Jejen. Antidiabetic Activities of Muntingia calabura L. Leaves Water Extract in Type 2 Diabetes Melitus Animal Models. *The Indonesian Biomedical Journal*, 2018; 10(2): 16-170.
13. Susilawati, Elis., Adyana, I Ketut., Fisheri, Ketut. Kajian Aktivitas Antidiabetes dari Ekstrak Etanol dan Fraksinya dari Daun Singawalang (*Petiveria alliacea L.*). *PHARMACY*, 2016; 13(2): 182-191.
14. Fauzi, Novi Irwan., Febriani, Yessi., Musthofa, Risma Ayu. Uji Aktivitas Insulin-Sensitizer Ekstrak Etanol Buah Malaka (Phyllanthus emblica L.) Pada Tikus Jantan Galur Wistar yang Diinduksi Diet Tinggi Lemak. *Indonesian Journal of Pharmaceutical Science and Technology*, 2017; 6(2): 22-28.
15. Nuralifah, N., Muhammad Fitrawan, L. O., Parawansah, P., & Trisetya, M. Histopatologi Organ Pankreas Tikus DM tipe 2 yang diberi Ekstrak Etanol Daun Gedi Merah (Abelmoscus manihot L. Medik). *Journal Syifa Sciences and Clinical Research*, 2022; *4*(1): 141–151.
16. Magitasari, H. D., Hidayaturrahmah, Santoso, H. B., & Sari, D. K. Gambaran Histologi Pankreas Tikus Putih (Rattus norvegicus) Hiperglikemia Setelah Pemberian Biskuit Ikan

Patin (Pangasius hypothalmus). *Prosiding Seminar Nasional Lingkungan Lahan Basah*, 2019; 4(1): 211–216.

17. Febriyenti, Suharti, N., Lucida, H., Husni, E., & Sedona, O. 178-674-5-Pb. Sains Farmasi Dan Klinis, 2018; 5(1): 23–27.
18. Aziz, Y. S. Standarisasi Parameter Non Spesifik Simplisia Rimpang Kunyit (Curcumae Domestica Rizhoma) dan Temulawak (Curcuma xanthorrhiza Roxb.) di Kabupaten Ponorogo. *Jurnal Delima Harapan*, 2019; *6*(2): 89–94. https://doi.org/10.31935/delima.v6i2.84.
19. Kementrian Kesehatan Republik Indonesia. *Farmakope Herbal Indonesia Edisi II*. Jakarta: Kementrian Kesehatan Republik Indonesia, 2017.
20. Maulidya, S. A. I., Nuari, D. A., Suryana, S., & Almarifah, S. Antibacterial Activity of Bandotan (*Ageratum conyzoides* L) Leaves Extracts Against Methicillin-Resistant Staphylococcus aureus. Borneo Journal of Pharmacy, 2020; 3(4): 243–248.
21. Nasiruddin, M., & Sinha, S. N. Phytochemical screening and antioxidant, antibacterial efficacy of *Gynura procumbens* (Lour.) Merr. Asian Journal of Medical and Biological Research, 2020; 6(2): 187–195.
22. Wangsaatmadja, A. H., Ulfah, M., & Rospina, Y. Uji Aktivitas Antidiabetes Ekstrak Etanol Herba Suruhan (Peperomia pellucida (L.) Khunt) Pada Tikus Wistar Jantan yang diinduksi Pakan Tinggi Lemak dan Karbohidrat. Jurnal Sains Dan Teknologi Farmasi Indonesia, 2022; 10(1): 23.
23. Haryoto, H., & Devi, E. S. Efek Pemberian Ekstrak Etanol Daun Dan Batang Ubi Jalar Ungu (Ipomoea batatas L.) Terhadap Penurunan Kadar Glukosa Darah Pada Tikus Jantan Galur Wistar Yang Diinduksi Aloksan. Talenta Conference Series: Tropical Medicine (TM), 2018; 1(3): 139–143.
24. Ajie, Rizky Bayu. White Dragon Fruit (*Hylocereus undatus*) Potential As Diabetes Mellitus Treatment. *J MAJORITY*, 2015; 4(1): 69-72.