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Combined Antidiabetic and Antidyslipidemic Activity of *Ageratum conyzoides* and *Gynura procumbens* in Alloxan-induced Diabetic Rats

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ABSTRACT

Unhealthy lifestyle habits greatly increase the risks of various degenerative illnesses, for example, diabetes mellitus as well as dyslipidemia. Recent studies have shown that the pharmaceutical drugs used for the treatment of these conditions have undesirable side effects. This indicates that it is necessary to find more effective and safe alternative treatment options, particularly in medicinal plants, such as *Ageratum conyzoides* and *Gynura procumbens*. Therefore, this research examined the combined antidiabetic and antidyslipidemic activity of *Ageratum conyzoides* and *Gynura procumbens* extracts in alloxan-induced diabetic rats. The insulin sensitivity of the test animals was assessed at the beginning of the experiment using the oral glucose tolerance test (OGTT) through the administration of 3 g/kgBW glucose. Pancreatic destruction was induced with the intraperitoneal injection of a single dose of 150 mg/kgBW alloxan, and the rats were treated with ethanol extract for 14 days. High-density lipoprotein cholesterol (HDL-C), Triglycerides (TG), total cholesterol (TC), and low-density lipoprotein cholesterol (LDL-C) were measured using the enzymatic method. The results showed that the single extracts and their combination exhibited high antidiabetic and antidyslipidemic activity. This was indicated by a substantial reduction in fasting blood sugar, TG, TC, and LDL-C ($p<0.05$), and an insignificant increase in HDL-C. The activity of the combined extract was similar to the single extracts, but it was not better in decreasing TG levels. Based on these findings, 95% ethanol extract of *Ageratum conyzoides*, *Gynura procumbens*, and their combination exhibited high antidiabetic and antidyslipidemic activity in alloxan-induced rats.

Keywords: alloxan; *Ageratum conyzoides*; *Gynura procumbens*; antidiabetic; antidyslipidemic

INTRODUCTION

Unhealthy lifestyle habits are one of the major contributors to decreased cellular function in the body, leading to various degenerative complications, such as obesity, diabetes, and cardiovascular disease (Rahman, 2021). Diabetes, in particular, serves as a risk factor for cardiovascular diseases, and affected adults are two to three times more prone to heart attack and stroke. Furthermore, this condition caused a total of 1.5 million fatalities in 2019 (World Health Organization, 2021). The main driving factor behind the high morbidity and mortality in these patients is the long-term complications of atherosclerosis caused by diabetic dyslipidemia, affecting approximately 72%–85% of individuals with type 2 diabetes mellitus (Agbafor et al., 2015; Athyros et al., 2018; Jialal & Singh, 2019). According to previous reports, this disorder is indicated by hyperglycemic conditions, and impaired fat, carbohydrate, and protein metabolism caused by insulin resistance. This, in turn, can cause dyslipidemia through increased lipolysis and sugar production in the liver, as well as decreased glucose uptake in the muscle or fat cells. Dyslipidemia patients have been reported to be more prone to cardiovascular disease compared to those suffering from hyperglycemia, including individuals with diabetes. Therefore, diabetic patients need to control the level of lipids in their blood (Jialal & Singh, 2019).

Pharmaceutical drugs currently used for treating diabetes and dyslipidemia have been linked with undesirable consequences and contraindications, including hypoglycemia, weight increase, lactic acidosis, rashes, skin reactions, acute porphyria, bone marrow damage, gastritis, and stomach discomfort (Osadebe et al., 2014). This indicates that it is important to explore more effective and safe alternative treatment options. Medicinal plants have long served as a valuable source for addressing various health problems. Furthermore, two such plants, *Ageratum conyzoides* (bandotan) and *Gynura procumbens* (sambung nyawa) belonging to the family *Asteraceae,* have exhibited promising antidiabetic and antidyslipidemic properties. Based on previous studies, *Asteraceae* is rich in flavonoids, polyphenols, terpenoids, and other metabolites

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known for their disease-fighting ability, particularly in reducing the risk of cardiovascular diseases. Bandotan and sambung nyawa have strong antioxidant, antihyperlipidemic, antidiabetic, vasodilatory, and anti-inflammatory activities. These consequences are associated with the mitigation of cardiovascular diseases, such as atherosclerosis and coronary heart disease (Atawodi et al., 2017; Chahal et al., 2021; Guo et al., 2021; Huang et al., 2019; Michel et al., 2020).

Prior studies showed that various parts of *Ageratum conyzoides* and *Gynura procumbens* possess the ability to reduce triglycerides (TG), blood glucose levels, total cholesterol (TC), and low-density lipoprotein cholesterol (LDL-C) while increasing high-density lipoprotein cholesterol (HDL-C) in diabetic rats (Atawodi et al., 2017; Hu et al., 2018; Ojewale et al., 2020). Although several reports have explored the individual benefits of these plants as antidiabetic and antidyslipidemia agents, there are no studies on their combined activity. Therefore, this research analyzed the combined effect of 95% ethanol extract of *Ageratum conyzoides* and *Gynura procumbens* in treating diabetes and dyslipidemia in diabetic rats. A histopathological examination was then conducted on the pancreas to examine the improvement after being treated with the extract suspension.

METHODOLOGY

Plant material

The fresh parts of *Ageratum conyzoides* and *Gynura procumbens* were collected from the Research Institute of Spices and Medicinal Plants (Balittro), Bogor. The samples were designated at the Department of Biology, Faculty of Mathematics and Natural Sciences, Padjadjaran University, Jatinangor, with letter numbers 49/HB/12/2021 and 50/HB/12/2021. Furthermore, the drying process was conducted in an oven at 40°C for 4 days, and the simplicia was mashed using a blender. According to the Indonesian Herbal Medicine Pharmacopoeia, the simplicia characterization test included water content, drying shrinkage, water-soluble extract, ash content, and soluble ethanol extract (Kemenkes RI, 2017). Simplicia was irrigated with 95% ethanol until it was submerged at a ratio of 1:6 to the solvent using 3,000 mL of 95% extract. The solution was allowed to stand for 24 h, stirred occasionally, and filtered to obtain the filtrate, followed by soaking with 1,500 mL of the same solvent for 1 day. The obtained maceration was concentrated using a rotary evaporator after this procedure was carried out three times through immersion. To achieve a thick extract, the obtained extract was evaporated in a water bath, and the yield was estimated (Kemenkes RI, 2017).

Phytochemical screening

The viscous extract was subjected to phytochemical screening to provide a qualitative description of its metabolite compounds using color reagents, including flavonoids, alkaloids, polyphenols and tannins, saponins, steroids, and triterpenoids (Shaikh & Patil, 2020; Yadav et al., 2017; Yelin & Kuntadi, 2019).

Test for alkaloid. About 2 mL of the extract was dissolved in 6 mL of distilled water, continued by adding 1 mL of 2 N HCL. The solution was then heated in boiling water, cooled, and 1 mL was placed in three test tubes. Subsequently, 2 drops of Dragendorff, Mayer, and Wagner reagents were added to each test tube. Positive alkaloid results were indicated by the presence of red, white, and brownish-red precipitates using Dragendorff, Mayer, and Wagner reagents, respectively.

Test for flavonoid. As much as 2 ml of extract was dissolved in 50 ml of distilled water, continued by heating, and filtration. Furthermore, 1 ml of the solution was mixed with 1 mL of ethanol, heated for 5 minutes, and a few drops of HCl and 0.025 g of Magnesium turning were added. Positive results of flavonoids were indicated by the appearance of a dark red (magenta).

Test for tannin. Approximately 1 mL of the extract was dissolved in 3 mL distilled water, and 3 drops of the solution were placed on a drip plate. The process was then continued with the addition of 2-3 drops of 1% FeCl3 and positive results for tannins were indicated by the appearance of a dark blue or greenish-black color.

Tes for polyphenol. As much as 2 mL extract was diluted in distilled water, continued by dropping 0.5 mL of 1% ferric chloride solution. The formation of a bluish-black color indicated positive results for polyphenols.

Test for saponin. Approximately 2 mL extract was diluted in 2 mL of distilled water, followed by heating for 2-3 minutes and cooling. The saponin test was carried out by shaking the solution vigorously for 15 minutes and then adding 2 drops of HCl. A positive result for saponins was indicated by the appearance of a stable foam layer with a height of 1-10 cm.

Test for terpenoids and steroids. A total of 2 ml extract was liquefied in 6 ml of distilled water. Subsequently, 1 ml of the solution was mixed with 1 ml of methanol, and 2-3 drops of the final mixture were placed on the drip plate. The process then continued with the addition of 2 drops of anhydrous acetic acid and sulfuric acid. Positive test results for triterpenoids and steroids were

indicated by the formation of indica red/orange and greenish-blue colors, respectively (Yelin & Kuntadi, 2019).

Experimental animals

Six groups of 24 male Wistar rats, weighing 180-250 g each, were created at random, namely I as non-diabetic control (CMC-Na 0.5% only), II as diabetic control (alloxan + CMC-Na 0.5%), III as diabetic metformin/positive control (alloxan + metformin 9 mg/200 g BW), IV as diabetic with extract of *Gynura procumbens* (alloxan + single extract of *Gynura procumbens* 500 mg/kg BW), V as diabetic with extract of *Ageratum conyzoides* (alloxan + single extract of *Ageratum conyzoides* 500 mg/kg BW), and VI as diabetic with combined extract (alloxan + extract of *c* and *Gynura procumbens* each 250 mg/kg BW). The test animals were fed standard pellets and provided access to drinking water ad libitum. Furthermore, this research was approved by the Padjadjaran University Research Ethics Commission with Registration Number 2112021292.

Oral Glucose Tolerance Test (OGTT)

The test rats fasted for 12 h with access to drinking water, and their initial blood glucose level (T0) was checked. Each animal was treated orally with a predetermined dose and the treatment was continued after 30 min with the oral administration of a 3 g/kg BW glucose solution except in the negative control group. Post-prandial blood glucose levels were examined for a total of 2.5 h with a 30 min interval, namely at 30 (T 30), 60 (T 60), 90 (T 90), 120 (T 120), and 150 (T 150) min. Subsequently, the blood sample collection was made by injuring the end of the rat's tail with a lancet and measuring using an Easytouch® glucometer (Derosa et al., 2022).

Induction of diabetes in rats

Intraperitoneal injections of 150 mg/kg BW alloxan monohydrate solution were used to induce diabetes. The blood sugar levels of the test animals were measured after 72 h using a glucometer. Furthermore, rats with blood sugar levels above 200 mg/dL were declared diabetic and proceeded to the next stage. They were then treated for 14 days, receiving preparations orally based on the respective groups, one time daily. The blood sugar levels were monitored on days 7 and 14 (Ojewale et al., 2020).

Assay of lipid profile

The test animals were anesthetized after 14 days of treatment using CO_2 inhalation, and blood was drawn through the orbital sinus. The blood was collected in a microtube and allowed to clot for 10 min, continued by centrifugation for 5 min at 5000 rpm and separation of serum (Ojewale et al., 2020). The TG, TC, LDL-C, and HDL-C levels were measured enzymatically using the TC reagent CHOD-PAP (ProLiNE®, Prodia Diagnostic Line, Cikarang, Indonesia, catalog number: 11300 99 10 022), GPO-PAP (ProLiNE®, Prodia Diagnostic Line, Cikarang, Indonesia, catalog number: 1 5710 99 10 192), LDL-C reagent kit (Sekisui®, Sekisui Medical CO., LTD, Tokyo, Japan, catalog number: 30173000), and HDL-C reagent kit (Sekisui®, Sekisui Medical CO., LTD, Tokyo, Japan, catalog number: 30169000). Furthermore, the measurement processes were carried out using the Microlab 300.

Histopathological analysis

The rats were sacrificed at the end of the assay on day 15, and their pancreas was removed and fixed with 10% formalin solution in paraffin. The pancreas tissue was then cut to a size of 5 µm and stained with Gomori. Subsequently, the samples were analyzed and evaluated under an Olympus BX51 light microscope (Pashapoor et al., 2020; Ratwita et al., 2019).

Statistical analysis

The test results were analyzed and displayed as mean ± standard deviation. The statistical examination was performed with the help of the Statistical Package for Social Sciences program with a one-way analysis of variance (ANOVA) test and post hoc with the LSD method. Furthermore, no significant difference was found when the p-value is >0.05 and a considerable difference existed at p-value <0.05.

RESULT AND DISCUSSION

Characterization results of Gynura procumbens and Ageratum conyzoidessimplicia

A simplicia characterization or standardization was performed to determine the values of the various parameters that had been previously set, as shown in Table I (Kemenkes RI, 2017).

The simplicia extraction results with ethanol solvent were expressed as extract yield, which indicated the ratio between the amount of extract obtained and the material used. This value showed the content of the substance extracted during the process. Based on the results, the yield of *Gynura procumbens* and *Ageratum conyzoides* was 17.79% and 8.73%, respectively (Kemenkes RI, 2017; Mulyani et al., 2021).

Table I. Characterization results of simplicial

Characteristic	*Gynura procumbens* (%)	*Ageratum conyzoides* (%)
Drying shrinkage	9.734	7.120
Water content	9	5.5
Ash content	11.5	13
Water soluble extract content	23	27
Ethanol soluble extract content	15	10

Table II. Phytochemical screening results of *Gynura procumbens* and *Ageratum conyzoides*

Test	*Gynura procumbens* extract	*Ageratum conyzoides* extract
Alkaloids	−	+
Flavonoids	+	+
Tannins	+	+
Polyphenols	+	+
Saponins	+	+
Steroids/triterpenoids	+	+

Information: (−) does not contain compounds; (+) contains compounds.

Phytochemical screening results of Gynura procumbens and Ageratum conyzoides extracts

The viscous extract was subjected to phytochemical screening to provide a qualitative description of its metabolite compounds using color reagents (Shaikh & Patil, 2020). Table II shows the phytochemical screening results of *Gynura procumbens* and *Ageratum conyzoides*.

The test results showed that *Gynura procumbens* and *Ageratum conyzoides* ethanol extracts contained flavonoids, tannins, polyphenols, saponins, and steroids/triterpenoids. However, there was no formation of either a white or brown precipitate when analyzing the alkaloids in *Gynura procumbens* extract using Mayer's reagent or Dragendorff's reagent, respectively. This indicated that the sample used did not contain alkaloids, but *Ageratum conyzoides*are tested positive in the test. These results were also in line with Gulo and Silitonga (2021) that the ethanol extract of *Gynura procumbens* leaves was negative for alkaloids (Gulo A, 2021).

Oral Glucose Tolerance Test (OGTT) results

OGTT determined the body's capacity to accept glucose administration. The results indicated that the administration of the test material reduced blood glucose levels. Meanwhile, the intake of high doses of loading glucose monohydrates caused an increment in the levels. The diabetes condition induced in the rat was reversible (temporary) because it did not destroy the pancreas. According to Hidayaturrahmah*et al*. (2020), the normal blood sugar of white rats was in the range of 90.4–97.6 mg/dL. The measurement results of blood glucose levels in the test animals are shown in Table III.

Values were expressed as mean ± SD (n=4). [a]Significantly different ($p < 0, 05$) when compared to the non-diabetic control; [b]Significantly different ($p < 0.05$) when compared to the diabetic control. (c): Considerably different ($p < 0.05$) when compared to the positive control. T, time (min).

The oral glucose tolerance test indicated no statistical difference among the treatment groups before induction (T0). This indicated that all test animals used in this study had normal and homogeneous blood glucose levels. After 30 min of glucose administration (T30), there was an increase in the levels due to hyperglycemia, as demonstrated by a significant difference between all glucose-charged treatment groups and non-diabetic control ($p < 0.05$). Furthermore, this condition showed a sugar absorption process in the blood, which triggered hyperglycemia in rats. The diabetic control group demonstrated the highest gain in blood glucose levels in contrast to the others. Based on these findings, the carrier, CMC-Na, had no antidiabetic activity.

The positive control treated with metformin showed a significant difference with the diabetic control at 30 min (T30). This indicated that the use of metformin could prevent high blood glucose levels. Furthermore, the drug was selected in this study due to its antidiabetic activity, where it functioned by inhibiting the process of gluconeogenesis, reducing glucose absorption in

Table III. Profile of mean blood glucose levels on OGTT

Group	Profile of average blood glucose level (mg/dL)					
	T0	T30	T60	T90	T120	T150
Non-diabetic control	77 ± 5.57	72.67 ± 4.7[b]	74.33 ± 73.67	73.67 ± 3.21	77.67 ± 3.51	80.67 ± 13.05
Diabetic control	87.33 ± 4.04	182.67 ± 22.30[a]	170.67 ± 8.08[a]	154.67 ± 14.05[a]	145.00 ± 12.12[a]	132.67 ± 12.06[a]
Positive control (Metformin)	83.33 ± 7.57	131.67 ± 28.99[a]	120.67 ± 12.22[ab]	118.33 ± 6.51[ab]	107.00 ± 17.58[b]	89.00 ± 4.36[b]
Single *Gynura procumbens* extract	82.33 ± 12.01	144.00 ± 18.73[a]	138.67 ± 11.06[abc]	101.67 ± 7.64[ab]	95.00 ± 11.27[b]	76.33 ± 8.74[b]
Single *Ageratum conyzoides* extract	84.00 ± 2.65	155.00 ± 10.54[a]	134.33 ± 8.14[ab]	132.33 ± 10.12[ab]	109.00 ± 22.61[ab]	81.67 ± 15.18[b]
Combined extract	78.00 ± 2.65	150.33 ± 9.71[a]	128.67 ± 3.21[ab]	126.67 ± 15.89[ab]	84.67 ± 22.30[b]	81.33 ± 5.51[b]

the intestine, and improving the sensitivity of liver and muscle tissue to insulin. These processes then caused an increase in peripheral glucose uptake, thereby lowering blood sugar levels (Zhou et al., 2018).

The maximum decrease in average blood glucose levels took place at 150 min. The results revealed a significant difference among the administration of metformin, single *Gynura procumbents* extract, single *Ageratum conyzoides*extract, and their combination when corresponding with diabetic control. Nevertheless, no substantial variation was found between the three test extract against the positive control (Metformin). This indicated that the single extracts of *Gynura procumbens* and *Ageratum conyzoides* at 500 mg/kgBW, as well as their combination had the same antihyperglycemic activity as metformin. The results of the LSD at 150 min also stated that the single extract did not significantly differ from the other, as well as their combination in lowering blood glucose levels. According to these results, the extract of the combination of bandotan herbs and sambung nyawa at a ratio of 1: 1 had a comparable antidiabetic activity with the single extract.

Effect of single and combined extract administration in alloxan-induced diabetic rats

Antidiabetic testing using the insulin deficiency method aimed to define the capacity of the test material to stimulate insulin secretion from pancreatic cells with partially damaged conditions, thereby lowering blood glucose levels. Furthermore, Alloxan caused diabetes through a mechanism that involved the partial degradation of the pancreatic cells in the islets of Langerhans, affecting the quality and quantity of the hormone produced (Ighodaro OM, Adeosun AM, 2018). Table IV shows the measurement results of blood glucose levels with the insulin deficiency method.

Values are expressed as mean ± SD (n=4). [a]Significant different ($p < 0.05$) when compared to the non-diabetic control; [b]significantly different ($p < 0.05$) when compared to the diabetic control; [c]considerably different ($p < 0.05$) when compared to the positive control. H0: BGL (Blood Glucose Level) on the day before induction. H0′: BGL on the day after induction. H7: BGL on the day after the seventh day of treatment. H14: BGL on the 14th day after treatment.

The statistical analysis results in Table IV showed an increase in blood glucose levels in all groups of alloxan-induced rats after 3 days of induction (H0′). This was indicated by a substantial difference when corresponding to the non-diabetic control ($p < 0.05$), where the induced group of rats had very high blood glucose levels (>200 mg/dL). Furthermore, the increased glucose levels showed the success of alloxan induction as the indicator of diabetes. Several studies had shown that alloxan could change blood glucose levels from normal conditions to hyperglycemia.

Statistical test on the 7th day (H7) revealed that the single extracts of *Gynura procumbens* and *Ageratum conyzoides* and the combined extract had significant differences when compared to the diabetic control (< 0.05). However, no substantial dissimilarity was found between the positive

Table IV. Profile of the average blood glucose levels on alloxan-induced diabetic rats

Group	Profile of average fasting blood glucose level (mg/dL)				% Decrease
	H0	H0′	H7	H14	
Non-diabetic control	82.67 ± 4.16	81.33 ± 3.21	74.00 ± 11.14	69.33 ± 8.14	−14.75
Diabetic control	78.00 ± 11.27	295.67 ± 76.00[a]	294.67 ± 36.30	217.00 ± 26.46	−26.61
Positive control (Metformin)	70.00 ± 6.24	303.00 ± 89.60[a]	230.33 ± 68.70[a]	85.00 ± 19.00[b]	−71.95
Single *Gynura procumbens* extract	67.67 ± 7.37	252.00 ± 43.7[a]	129.00 ± 60.89[bc]	88.67 ± 23.46[b]	−64.81
Single *Ageratum conyzoides* extract	71.67 ± 16.65	394.33 ± 50.01[a]	105.67 ± 18.04[bc]	76.33 ± 19.50[b]	−80.64
Combined extract	66.00 ± 8.54	544.67 ± 51.98[a]	57.33 ± 28.10[bc]	65.00 ± 25.94[b]	−88.07

(Metformin) and diabetic controls ($p > 0.05$). Based on these findings, the administration of the test extracts on the 7th day reduced blood glucose levels of alloxan-induced diabetic rats, while metformin had no significant effect on reducing the condition yet. This was consistent with Ayoade et al. (2010) and Derosa et al. (2022) that metformin had not displayed a substantial reduction in blood glucose levels within 2-8 days, because the intervention had no direct activity against pancreatic cells and their subsequent impact on the liberation of nonesterified fatty acids from TG stored in adipose tissue.

The average profile of blood glucose levels on day 14 displayed a significant decrease in the test group. Furthermore, the administration of metformin, *Gynura procumbens,* and *Ageratum conyzoides* extracts, as well as the combined extracts significantly differed from the diabetic control ($p < 0.05$) but not particularly diverse compared to the non-diabetic control ($p > 0.05$). This indicated that the animals test in this group had returned to normal condition. On the 14th day, the single extracts *Gynura procumbens* and *Ageratum conyzoides*, and the combination extracts were not significantly different compared to metformin. Therefore, the administration of single extracts *Gynura procumbens* and *Ageratum conyzoides* at 500 mg/kg BW each and also the combined extracts produced antidiabetic activity compared to metformin.

Based on the percentage of decreased blood glucose levels, the combined extract gave the most effective proportion of reduction than other treatment groups, namely 88.07%. The next highest percentage was sequentially given by single *Ageratum conyzoides*, metformin, and single *Gynura procumbens*. However, the results of the LSD test revealed no considerable dissimilarity between the three extract treatment groups in lowering the blood glucose levels of alloxan-induced diabetic rats on the 14th day. Therefore, the combined extracts of *Gynura procumbens* and *Ageratum conyzoides* herbs with a ratio of ½: ½ had an antidiabetic activity that was comparable to the single extracts.

Lipid profile measurement results

Damage to the pancreas by alloxan caused insulin deficiency in the body, activating intracellular hormone-sensitive lipase, which led to the liberation of nonesterified fatty acids from TG accumulated in adipose tissue. The high circulating levels of NEFA increased the formation of triglyceride in the liver and this was correlated with a gain in the secretion of apolipoprotein B (apoB). Furthermore, TG had a unidirectional relationship with cholesterol, LDL-C, and HDL-C (25, 26). Table V shows the results of lipid profile measurements in the test animals.

Values are expressed as mean ± SD (n=4). [a]Significantly different ($p < 0.05$) when compared to the non-diabetic control; [b]Significantly different ($p < 0.05$) when compared to the diabetic control. LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; TC, total cholesterol; TG, triglycerides.

Table V. Results of lipid profile measurement after 14 days of treatment

Test group	Lipid profile (mg/dL)			
	TC	TG	LDL-C	HDL-C
Non-diabetic control	30.43 ± 3.25[b]	40.57 ± 8.13[b]	14.20 ± 3.2[b]	30.40 ± 4.36[b]
Diabetic control	53.67 ± 4.37[a]	102.17 ± 17.64[a]	26.00 ± 3.04[a]	16.67 ± 3.33[a]
Positive control (Metformin)	33.60 ± 2.71[b]	53.97 ± 6.13[b]	21.87 ± 3.46[a]	27.03 ± 5.17[b]
Single *Gynura procumbens* extract	28.17 ± 2.10[b]	43.93 ± 13.18[b]	17.03 ± 0.55[b]	18.00 ± 6.58[a]
Single *Ageratum conyzoides* extract	42.77 ± 2.86[b,a]	53.50 ± 6.12[b]	14.67 ± 3.42[b]	23.40 ± 9.45
Combined extract	43.43 ± 3.99[b,a]	95.57 ± 7.91[a]	13.57 ± 3.74[b]	18.23 ± 2.86[a]

Values are expressed as mean ± SD (n=4). [a]Significantly different ($p < 0.05$) when compared to the non-diabetic control; [b]Significantly different ($p < 0.05$) when compared to the diabetic control. LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; TC, total cholesterol; TG, triglycerides.

Table VI. Average area of the islets of Langerhans, the total of alpha cells and beta cells after 14 days of treatment

Group	Islets of Langerhans average area (µm)	Total	
		Alpha cells	Beta cells
Non-diabetic control	184558.60 ± 28289.13[b]	6.50 ± 1.03[b]	85.13 ± 10.72[b]
Diabetic control	57518.95 ± 28487.38[a]	24.66 ± 7.12[a]	18.20 ± 4.70[a]
Positive control (Metformin)	103139.40 ±14636.54	14.00 ± 1.63	41.86 ± 1.67[a]
Single *Gynura procumbens* extract	118419.4 ± 67132.55	14.67 ± 2.25	61.73 ± 14.37[b]
Single *Ageratum conyzoides* extract	64941.86 ± 19548.09[a]	12.46 ± 3.55[b]	36.47 ± 19.31[a]
Combined extract	92621.00 ± 38828.49	11.00 ± 4.58[b]	61.33 ± 12.66[b]

Value are expressed as mean ± SD (n=4). [a]Significantly different ($p < 0.05$) with the non-diabetic control; [b]Significantly different ($p < 0.05$) with the diabetic control.

Statistical tests showed that the diabetic control differed significantly ($p < 0.05$) from the normal non-diabetic control. This indicated that alloxan induction could improve TC, TG, and LDL-C levels as well as reduce HDL-C. Based on the one-way ANOVA, there was a significant difference ($p < 0.05$) between metformin, *Gynura procumbens*, *Ageratum conyzoides*, and the combined extracts compared to non-diabetic control. This showed that the administration of these treatments for 14 days had an effect in reducing TC, TG, and LDL-C in diabetic rats. Regarding the increase in HDL-C levels, only the metformin group differed significantly ($p < 0.05$) from the non-diabetic control. Meanwhile, the increase in HDL-C for the single and the combined extract groups was not statistically significant ($p > 0.05$) but not different from the metformin treatment. The combination group showed similar properties to the single extract. According to the analysis results, there was no significant difference ($p > 0.05$) in the decrease in TC, LDL-C, and an increase in HDL-C with the individual extract. The combined extract was shown to have the ability to reduce triglyceride levels but was not statistically significant.

Based on Hu *et al*. (2018), the supplementation of 500 mg/kg BW life-sustaining ethanol extract to streptozotocin-induced diabetic rats reduced TC, TG, and LDL-C, and increased HDL-C after 7 days of treatment. Ojewale*et al*. (2020) stated that the intake of *Ageratum conyzoides* root ethanol extract at a dose of 500 mg/kg BW reduced TC, TG, LDL-C, and significantly increased HDL-C compared to the diabetes group after 4 weeks of treatment. These findings were consistent with this current study, where the *Gynura procumbens* and the *Ageratum conyzoides* groups had significant differences from the diabetes control after 14 days (Hu et al., 2018).

Pancreatic histopathology results

Histopathological observations were conducted on the test rat's pancreas to asess the impact of the extract on the improvement by observing the density of cells in the islets of Langerhans using Gomori staining, as shown in Figure 1 and Table VI.

The non-diabetic control group had the most extensive islets of Langerhans compared to others. Meanwhile, the diabetic control group had the smallest area of Langerhans islets due to the

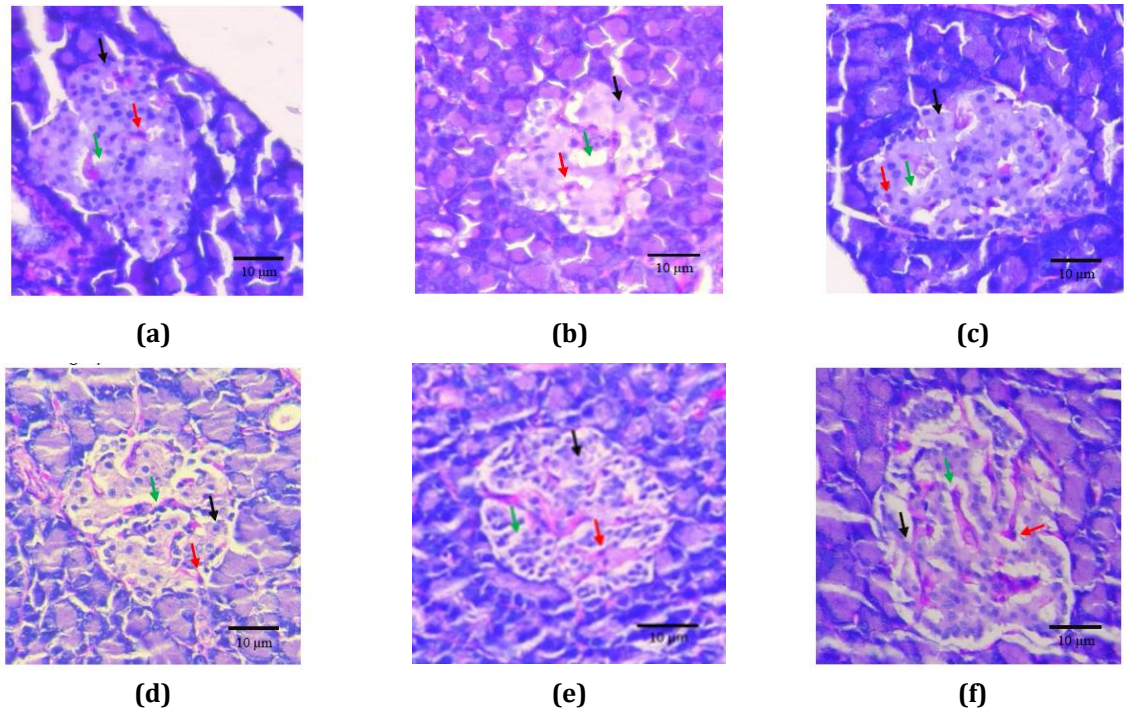

Figure 1. Pancreatic histopathology results 400× optical magnification

(a) non-diabetic control, (b) diabetic control, (c) Positive control (Metformin), (d) diabetes + *Gynura procumbens* extract 500 mg/kg BW, (e) diabetes + *Ageratum conyzoides* extract 500 mg/kg BW, and (f) diabetes + combined extract. (→):showed alpha cells, (→): beta cells, (→);empty space without anycells.

alloxan induction. The findings indicated a notable rise following the application of metformin at a dosage of 500 mg/kg BW, *Ageratum conyzoides* at a dosage of 500 mg/kg BW, as well as their combined extract. Nonetheless, the gain was not statistically significant.

The results revealed the presence of high alpha and low beta cells in the diabetic control group. This indicated that there was an increase in the amount of insulin produced by beta cells, while the production of glucagon increased. Furthermore, these changes had various effects on carbohydrates, fat, and protein metabolism. Metformin could suppress alpha cells and increase beta cells, but it was insignificant ($p > 0.05$) than the diabetic control, as shown in Table VII. Figure 1C showed that the cells' density began to increase, but there was a significant space in the condition. This was due to the indirect functional mechanism of metformin on the pancreas, which involved increasing insulin sensitivity, reducing gluconeogenesis in the liver, and improving glucose transport to tissues (Anderson et al., 2020). The administration of *Gynura procumbens* at 500 mg/kg BW could insignificantly suppress alpha cells, but the increase in beta cells was significant ($p < 0.05$) than the diabetic control. Figure 1D demonstrated that the cell density on the islets of Langerhans was still loose with much free space. The distribution of *Ageratum conyzoides* extract could suppress the number of alpha cells significantly, but the increase in beta cells was not significant. The density began to close together, but the alpha and beta ratio was relatively large compared to the number. The outcomes also revealed that the combined extract distribution could significantly suppress the alpha cells and increase the beta cells ($p < 0.05$) than the diabetic control group. Figure 1F revealed the occurrence of regeneration in the islets of Langerhans, as evidenced by the cells that had started to close and the reduction in space due to necrosis. This indicated that the combination had the best ability to repair pancreatic cells. However, based on the post hoc test, the statistical results showed no significant difference between the combined and the single extract, as well as with metformin.

The ability of *Gynura procumbens* and *Ageratum conyzoides* to lower blood sugar and lipid levels was caused by the presence of various compounds, including phenolics and flavonoids. The flavonoid compounds could reduce ROS, as

well as restore cell integrity and increase viability. Phenolics and flavonoids were known to function as antioxidants by reducing oxidative stress caused by alloxan, and they could regenerate damage from pancreatic cells. Furthermore, the ability to lower lipid levels was mediated by the presence of saponins, which prevented excessive intestinal cholesterol absorption. Flavonoids could suppress glucose levels, lower plasma cholesterol, significantly reduce TG, and increase insulin secretion from the pancreatic islets of Langerhans. This insulin secretion indirectly improved lipid metabolism in the body, as it inhibited lipolysis and increased fatty acid uptake for adipose tissue and triglyceride synthesis (Adelakun et al., 2018; Agbafor et al., 2015; Al-Ishaq et al., 2019; Algariri et al., 2014; Atawodi et al., 2017).

CONCLUSION

Based om the results, this research concluded that the administration of 95% ethanol extract of *Ageratum conyzoides* and *Gynura procumbens* herbs had antidiabetic and antidyslipidemic activity in alloxan-induced rats. The indications were shown by the substantial decrease in blood sugar, TC, TG, and LDL-C levels, as well as the insignificant increase in HDL-C. Furthermore, the combination of *Ageratum conyzoides* and *Gynura procumbens* extracts in a ratio of ½:½ lowered blood sugar activity, TC, LDL-C, and increased HDL-C, and the effects were comparable to the single extracts. Based on TG reduction, its activity was not better compared to the single extracts. The histopathological test results showed that the single extract facilitated pancreatic repair, while the combination caused a significant decrease in alpha and an increase in beta cells.

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