Antidiabetic Effect Test of Ethanol Extract of Cep Cepan Leaf (*Saurauia cauliflora* Dc.) in Male Mouse (*Mus musculus*) Streptozotocin Induced

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Abstract

Cep-cepan leaves (Saurauia cauliflora DC.) have an antidiabetic effect due to the flavonoid compounds contained in them. As an antidiabetic so that it can be an alternative treatment for diabetes that is beneficial to improve quality of life, control diabetes and efficiency of medical costs. The approach employed in addressing antidiabetes involves the exploration of novel antidiabetic chemicals derived from medicinal plants. The Karo people in Medan, North Sumatra frequently utilize the cep-cepan plant (Saurauia Cauliflora DC.), a member of the Fagaceae family, as a medicinal remedy. The objective of this study is to investigate the impact and optimal dosage of cep cepan leaves produced by Saurauia cauliflora DC. The present study employs an experimental design utilizing a one-group pretest-posttest design. The study was carried out in Langkat Regency between May and December 2023. The population in this study was cep cepan as much as 5kg. The research sample was 3kg of cep cepan leaves taken by purposive sampling which had been dried. The dried cep cepan leaves were then blended to obtain a fine powder. Rats used in this study were rats that had blood glucose levels >200 mg/dl. Data analysis with the post hoc test used was the Mann-Whitney U Test, processing research data using SPSS software version 23. The results showed that the administration of Cep Cepan (Saurauia cauliflora DC.) leaf extract to mice (Mus musculus) at a dose of 200 mg/kg Body Weight/day for 12 days can reduce blood glucose levels in Balb/C male mice (Mus musculus) which is equivalent to glibenclamide.

Keywords: Diabetes Mellittus, Mus Musculus ,Saurauia cauliflora Dc, Streptozotocin

INTRODUCTION

Indonesia has millions of varieties of medicinal plants that are far greater than in other countries, where these medicinal plants have the potential to be developed to add value to the herbal medicine industry, such as herbal medicine, standardized herbal medicines, and phytopharmaceuticals. Indonesia holds the distinction of being the foremost global supplier of herbal medical products, owing to its extensive collection of over 30.000 plant species and marine resources. Nevertheless, it is worth noting that around 9.600 species of plants and animals, which possess recognized therapeutic characteristics, are not fully harnessed as alternative medicines (BPOM RI, 2021).

Medicinal plants are less popular than other types of plants, such as fruits, tubers and so on. Medicinal plants are highly favored by nature enthusiasts, particularly those who have adopted a lifestyle shift known as "back to nature." The phenomenon of "Back to nature" has not only influenced individuals' consumption habits but has also permeated various industries, including the field of medicine (Zamroni et al., 2017).

According to Regulation of the Minister of Health of the Republic of Indonesia Number 6 of 2016, traditional medicine refers to a combination of plant, animal, mineral, medicinal, or galenic materials that have been used for generations for treatment. These materials can be applied according to the prevailing norms in the community. In the context of Indonesia, native Indonesian herbs refer to medicinal plants that are indigenous to the country and have been cultivated for generations, serving various health-related functions (Kemenkes RI, 2016).

In 2013, Indonesian undertook a comprehensive Basic Health Research study that encompassed 33 provinces. In 2013, the Riskesdas reported a prevalence rate of 2.1% for Diabetes Mellitus. The current value exceeds the previous value of 1.1% recorded in 2007. Among the 33 provinces that were examined, it was observed that 31 provinces (93.9%) exhibited a noteworthy rise in the prevalence of Diabetes Mellitus (Kemenkes RI, 2013). Data obtained from the Integrated Disease Surveillance Data report in 2019, namely the percentage of people with Diabetes Mellitus in North Sumatra was 249.519 patients and those who received health services were 144,521 patients or 57.92%. The remaining 104.998 did not access health services (Dinkes Sumut, 2020).

Diabetes mellitus is a degenerative disease that needs proper treatment to minimize the onset of complications such as kidney failure, heart disease and nervous system damage (WHO, 2022). People living around the Tangkahan forest of Gunung Leuser National Park, Langkat Regency, North Sumatra have used cep cepan leaves (*Saurauia cauliflora DC.*) as a remedy for abdominal pain, indigestion, wound medicine and sugar medicine that can be developed as an antidiabetic drug. Nevertheless, its utilization remains inherited and has not undergone scientific investigation (Salim et al., 2017).

Based on research conducted by Syahputra et al., (2022) It was found that the content of flavanoids, alkaloids, triterpenoids, anthraquinone glycosides and tannins in the leaves of Cep cepan (*Saurauia cauliflora DC.*) had antifungal activity against candida albicans and microsporum canis fungi. Research Salim et al., (2017), stated that cep cepan leaves (*Saurauia Cauliflora DC.*) have an analgesic effect, while for the antidiabetic effect of cep cepan leaves (*Saurauia cauliflora DC.*).

Based on the above problems, therefore researchers are interested in conducting research with the title "Test of the Antidiabetic Effect of Cep Cepan Leaf Ethanol Extract (*Saurauia cauliflora DC.*) on male white rats (Rattus norvegicus) Induced Streptozotocin". The purpose of this study was to determine the effect and effective dose of cep cepan leaves (*Saurauia cauliflora DC.*).

METHODS

The type of research used is the experimental method. The research design used is One group pretest-posttest design. Where in this research design, there are groups given treatment or treatment, and then the results of treatment are as independent variables, and results are as dependent variables.

This research was conducted in the Langkat district from May to December 2023. The population in this study was cep cepan as much as 5kg found in Langkat district, North Sumatra. The sample used in this study was cep cepan leaves taken by purposive sampling which had been dried to a total of 3kg.

Materials used cep cepan leaves, 95% ethanol in maceration, and Strepzotocin, flannel cloth, Na.CMC 0.5%, glibenclamide 5 mg. The tools used were a maceration vessel, scales, water bath, beaker glass, volume pipette, vaporizer cup, stirring rod, glucometer, rotavapor, 1 mL spoit, blood sugar strips, pH meter.

Cep cepan leaves used in this study are cep cepan leaves found in Langkat district, North Sumatra. A total of 5 kg of cep cepan leaves were collected sorted and cleaned of foreign objects attached, and then wash with running water to remove dirt that sticks. Then, the leaves were chopped and dried in the oven. The dried cep cepan leaves are then blended to obtain a fine powder. Extraction of cep cepan leaves using maceration method with 96% ethanol solvent. 300 grams of cep cepan leaf powder was dissolved in 1.5 liters of 96% ethanol for 24 hours. The dregs from the filtering results were re-macerated with 750 ml of ethanol. The resulting extract was concentrated on a water bath for 5 days. A thick extract of 312 grams was obtained.

Preparation of cep cepan leaf extract using maceration method with 96% ethanol solvent because ethanol is a universal solvent (Lloyd R et al. 1997). Cep cepan leaf powder of as much as 312 grams was macerated with 96% ethanol for as much as 1.5 liters for 24 hours. The extract obtained was evaporated in a water bath. The dregs of maceration were re-macerated twice, each time using 750 ml of ethanol. The results of maceration obtained a thick extract of 63.14 grams. The yield of cep cepan leaf extract obtained is 20.18%.

Examination of blood glucose levels was carried out using a blood glucose strip test kit or Auto Check. Balb / C mice aged 2-3 months as many as 5 heads were measured blood glucose levels before treatment, then induced with Stz solution 40 mg/kg BW intraperitoneally left for 3 days, on the 3rd day blood glucose levels were measured and then compared with blood glucose levels before induction. Male Mouse used in this study with blood glucose levels > 200 mg/dl. Male rats with blood glucose levels > 200 mg/dl are then given ethanol

extract 50 mg/kg BW, 100 mg/kg BW and 200 mg/kg BW for 12 days, every 3 days measuring blood glucose levels.

The test used to determine whether the data is homogeneous is Levene's variance test, while the test to determine whether the data is normally distributed is the Shapiro-wilk test. The bivariate analysis test used was the Kruskal-Wallis test because the data did not meet the requirements of the parametric test. To see if there is a difference between each treatment group, further tests or post hoc tests were conducted. The post hoc test used was the Mann-Whitney U Test. Data processing in this study used the help of SPSS version 23 software.

RESULTS

Table 1: Extract Identification

Identification	Conclusion
Alkaloids	(+)
Saponins	(-)
Tannins	(+)
Flavanoids	(+)
Steroids	(+)

Based on Table 1. The results show that (+) contains Alkaloid, Tannin, Flavanoid, and Steroid compounds, while (-) contains saponins.

Table 2: Average Percentage of Blood Glucose Level Reduction

Group	Mean Blood Glucose Level Reduction (mg/dL)
Na CMC 1%	173.4
Glibenclamide 5 mg/70 kg BW	83
EEDCC 50 mg/kg BW	117.8
EEDCC 100 mg/kg BW	129.6
EEDC 200 mg/kg BW	148.4

Based on Table 2. The results showed that the results of the research treatment were able to reduce blood sugar levels.

Table 3. Normality test results

Blood Sugar Levels	N	p-value
Induction blood sugar level	25	0.200
Blood sugar level after treatment	25	0.080

Based on Table 3. The results of the Kolmogorov test exhibited a normal distribution, as evidenced by a significance value of 0.200, suggesting that all the data conformed to a normal distribution. The homogeneity test yielded a significance value of 0.200, which is greater than the predetermined threshold of 0.05. This suggests that there is no significant variation in the variance of the percentage reduction in blood glucose levels among the five treatment groups. Consequently, it can be inferred that the research data exhibits homogeneity. The data pertaining to the percentage reduction in blood glucose levels has been determined

to exhibit a normal distribution and homogeneity, so satisfying the criteria for conducting ANOVA analysis. The ANOVA test results are as follows:

Table 4. Blood sugar levels after treatment

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	5378,160	4	1344,540	3,581	0,023
Within Groups	7508,400	20	375,420		
Total	12886,560	24			

According to the data shown in Table 4. The results of the ANOVA test indicate a statistically significant disparity in the mean percentage decrease in blood sugar levels among the various treatment groups. The probability of this difference is less than 0.05, as evidenced by the value (1344,540) of 3.581 and the corresponding value of 0.023. after the ANOVA test was carried out, continued with the Tukey HSD Post Hoc Test with the following results:

Table 5. Hasil Uji Post Hock Tukey HSD

(I) Treatment Group		Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Positive control	Negative control	-5,200	12,254	0,993	-41,87	31,47
	Extracts 50	34,200	12,254	0,075	-2,47	70,87
	Extracts 100	24,000	12,254	0,321	-12,67	60,67
	Extracts 200	15,800	12,254	0,700	-20,87	52,47
Negative control	Positive control	5,200	12,254	0,993	-31,47	41,87
	Extracts 50	39.400*	12,254	0,031	2,73	76,07
	Extracts 100	29,200	12,254	0,161	-7,47	65,87
	Extracts 200	21,000	12,254	0,448	-15,67	57,67
Extracts 50	Positive control	-34,200	12,254	0,075	-70,87	2,47
	Negative control	-39.400*	12,254	0,031	-76,07	-2,73
	Extracts 100	-10,200	12,254	0,917	-46,87	26,47
	Extracts 200	-18,400	12,254	0,573	-55,07	18,27
Extracts 100	Positive control	-24,000	12,254	0,321	-60,67	12,67
	Negative control	-29,200	12,254	0,161	-65,87	7,47
	Extracts 50	10,200	12,254	0,917	-26,47	46,87
	Extracts 200	-8,200	12,254	0,961	-44,87	28,47
Extracts 200	Positive control	-15,800	12,254	0,700	-52,47	20,87
	Negative control	-21,000	12,254	0,448	-57,67	15,67
	Extracts 50	18,400	12,254	0,573	-18,27	55,07
	Extracts 100	8,200	12,254	0,961	-28,47	44,87

. The mean difference is significant at the 0.05 level.

Based on Table 5. Tukey HSD results were used to determine differences between groups,, and the Tukey HSD test results at the 95% confidence level showed significant differences.

Table 6. Differences in blood sugar levels at induction and after treatment

			Standard ize	Point Estimate	95% Confidence Interval	
					Lower	Upper
Pair 1	Induction blood sugar level - Blood sugar level after treatment	Cohen's d	37,651	3,464	2,409	4,508
		Hedges' correction	38,252	3,410	2,371	4,437

Based on Table 6. T-test results showed a significant difference in reducing blood sugar levels induction and after treatment in all groups.

DISCUSSION

Castanopsis costata, commonly known as "Cep-cepan", is one of the plants used empirically as an antidiabetic drug by the people of North Sumatra, Indonesia. Recent studies have reported that extracts and fractions of Cep-cepan leaves have antidiabetic effects tested in vivo. (Alkandahri et al., 2021). The antidibetic activity of the Cep-cepan plant is thought to be due to the flavonoid compounds contained therein (Alkandahri et al., 2016; Alkandahri et al., 2022). Flavonoid compounds are reported to have antidiabetic activity by inhibiting GLUT-2 in the intestinal mucosa, thereby reducing the absorption of glucose and fructose from the intestine, which causes a decrease in blood glucose levels (Al-Ishaq et al., 2019).

This study demonstrated a notable disparity in the reduction of blood glucose levels following induction and therapy across all groups. Alkaloids, tannins, flavonoids, saponins, and steroids were identified as constituents of the cep cepan leaf extract using phytochemical screening. Alkaloids possess pharmacological properties that exhibit antidiabetic and antihypertensive effects within the health sector (Putri et al., 2020). Alkaloids also have activity as anti-microbial and anti-parasitic (Aniszewski, 2007; Adha et al., 2021). Saponins have pharmacological activity as antibacterials (Anggraeni Putri et al., 2023). Saponins have antidiabetic reducing activity (Elekofehinti, 2015).

Saponins possess attributes that contribute to the reduction of cholesterol levels, as well as exhibit anti-coagulant, anti-carcinogenic, hepatoprotective, immunomodulatory, antidiabetic, neuroprotective, anti-inflammatory, and anti-oxidant properties (Syahla et al., 2023). Tannins has antioxidant properties and are employed for their cardioprotective, anti-carcinogenic, anti-inflammatory, and anti-mutagenic effects. Tannins have the potential to enhance glucose absorption and impede adipogenesis, hence offering potential therapeutic

applications for the management of Diabetes Mellitus (Sudjaroen et al., 2005; Kumari et al., 2012; Munawwaroh et al., 2022).

Ethanol extract of cep cepan leaves is proven to control blood glucose levels in type 2 Diabetes Mellitus model mice induced with Streptozotocin (STZ) (Azhar et al., 2022). Various types of plants are proven to have pharmacological activity as anti-bacterial. Secondary metabolite compounds in plants that function as antibacterials include flavonoids with the mechanism of action of inhibiting nucleic acid synthesis, cell membranes and energy metabolism (Alfaridz et al., 2019). Steroids have antibacterial and anti-tumor properties, while flavonoids offer antioxidant, antibacterial, and anti-diabetes mellitus properties (Parubak, 2013).

CONCLUSIONS

Based on the results of the study, it can be concluded that the administration of cep cepan leaf extract (*Castanopsis costata* (Blume)) to mice (*Mus musculus*) at a dose of 200 mg /kg Body Weight /day for 12 days reduces blood glucose levels in Balb/C mice comparable to glibenclamide. It is recommended for further researchers to test the pharmacological effects in a longer period of time, so that it can be seen hispatology than mice.

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