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RESEARCH ARTICLE

Antidiabetic activity of Akar Kuning (*Fibraurea tinctoria* Lour) Extract in Alloxan-Induced Diabetic White Male Rats

Supomo Supomo[1]*, Eka Siswanto Syamsul[1], Hayatus Saadah[1], Kintoko Kintoko[2], Hardi Astuti Witasari[2]

[1]Departement of Pharmaceutical Biology, Departement of Pharmacology, Departement of Pharmaceutical Technology Sekolah Tinggi Ilmu Kesehatan Samarinda, East Kalimantan, Indonesia.

[2]Departement of Pharmaceutical Biology, Faculty of Pharmacy, Ahmad Dahlan University, Yogyakarta, Indonesia.

*Corresponding Author E-mail: **fahmipomo@gmail.com**

ABSTRACT:

The *Akar kuning* (*Fibraurea tinctoria* Lour) is a well-known plant for its potential for the treatment of liver damage and hyperglycemia. However, the main constituent responsible for the anti-diabetic effect is unknown. This study was conducted to investigate the effect of administration of *Fibraurea tinctoria* Lour ethanol extract on the blood sugar levels in alloxan-induced diabetic male albino rats. All animals were acclimatized with normal feed, and were subsequently divided into 5groups (3rats/ group). Group 1 (negative control group) was given 0.3% NaCMC; Groups 2, 3, and 4 (treatment groups), were given 50, 100, and 200mg/kg BW of *Fibraurea tinctoria* Lour ethanol extract, respectively once daily for 15 days, while Group 5(positive control group) received 0.65mg/kg BW glibenclamide. Before the treatments, the rats were induced intraperitoneally with 175mg/kg BW alloxan monohydrate. The blood glucose levels were observed every five days. The results showed that the ethanol extract at the doses of 50, 100, and 200mg/kg BW caused a significant decrease ($p < 0.05$) in the blood glucose levels compared to the negative control group. Also, the highest decrease in the blood glucose level was observed in the 100mg/kg BW treatment group. The findings from this study indicate that the ethanol extract of *Fibraurea tinctoria* Lour has an antidiabetic effect and the 100mg/kg BW dose was the most effective in lowering blood glucose levels.

KEYWORDS: Alloxan, *Akar kuning*, Blood glucose, Body weight, Diabetes mellitus, *Fibraurea tinctoria* Lour.

INTRODUCTION:

Recently, the disease trend has shifted from infectious diseases to metabolic syndromes like diabetes mellitus (DM). DM is a diverse and complex metabolic disorder due to the disturbances in lipids, proteins, and glucose metabolism in response to insulin deficiency or insensitivity.[1] Worldwide, DM has become highly prevalent and has been associated with severe complications. In 2019, 351.7 million people aged 20-64 years old had diabetes, which is projected to be 417.3 million by 2030.[2]

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Clinical symptoms that arise from DM include polyuria, polydipsia, and polyphagia.[3] There are two types of DM; Type 1 or insulin-dependent and Type 2 or non-insulin-dependent. Type 2 diabetes mellitus (T2DM) can be treated by regulating blood sugar levels using hypoglycemic compounds, alpha-glucosidase enzyme inhibitors, and a low-sugar diet.[4]

Management of T2DM using medicinal herbs is widespread in Indonesia, particularly in the forestry region in Kalimantan. *Fibraurea tinctoria* Lour can be abundantly found in the Samboja Special Purpose Forestry Area, Kutai Kartanegara, East Kalimantan, Indonesia. The plant is used in folklore practice to treat jaundice, malaria, dysentery, and fever by the Dayak, Banjar, and Kutai tribes.[5] Several studies revealed that secondary metabolites from the root and stem of

Fibraurea tinctoria Lour extract possess anticancer, antioxidant, antimicrobial, increased immune system, antidiabetic, and hepatoprotective properties.[6-12] These effects are mainly attributed to the constituent phenolic compounds. However, the main component which exerts the antidiabetic property is poorly understood. Therefore, this study was aimed at exploring the potential of *Fibraurea tinctoria* Lour ethanol extract as an antidiabetic agent in alloxan-induced diabetic white male rats.

MATERIALS AND METHODS:

Materials:

Source of plant material:

Fibraurea tinctoria Lour sample was purchased from the Samboja Special Purpose Forestry Area, Kutai Kartanegara, East Kalimantan, Indonesia and was characterized by Bina Swasta Sitepu, an MSc. student at the same institution. The specimen was identified and assigned a herbarium number: 129/AK/2020 using W252 specimens as a comparison and then deposited in the Herbarium Wanariset.

Source of experimental animals and ethical approval:

Fifteen white male rats aged 2-3 months (150-250 g) were obtained from the Pharmacology and Toxicology Laboratory, Samarinda College of Health Sciences. The rats were acclimatized under laboratory conditions for one week with food and water available *ad libitum*. During this period, the body weight was measured, and health condition was checked periodically. Only healthy rats with no change in body weight exceeding 10% during acclimatization and exhibited normal behavior were used in the study. All the experimental procedures were approved by the Animal Experiments Local Ethics Committee of the Faculty of Pharmacy, Mulawarman University, with the "Ethical Exemption"No.: 50/KEPK-FFUNMUL/EC/EXE/01/2021.

Preparation of *Fibraurea tinctoria* ethanol extract:

Before extraction, 800g of *F. tinctoria* was washed and dried at room temperature until a stable dry weight (200 g) was achieved.[7] Dry powder was sieved with 40-mesh and macerated with 2L of 95% ethanol. The preparation was stirred slowly during the first two hours until all of the powder was immersed in the solvent. After 24h soaking in ethanol, the solvent was squeezed and filtered out. The macerated powder was then soaked again twice as described. Finally, the extract was evaporated in a rotary evaporator apparatus with a temperature of 50°C to obtain 44.25g of concentrated ethanol extract.[13]

Induction of diabetes in experimental animals:

Diabetes was induced in the experimental animals as described,[14] with modification. Alloxan tetrahydrate (Sigma-Aldrich, USA) was prepared in a solution at a dose of 175mg/kg BW and administered intraperitoneally to 15 animals (experimental group) after 18h of fasting (water was still allowed). The sham group was injected with sterile normal saline. Following the induction of diabetes, the animals were given food and drink containing 10% glucose for two days. Afterward, 10% glucose was replaced with water, and the rats were transferred to a single housing cage. Hyperglycemic rats were determined on day three using a glucose test meter with glucose test strips (Gluko Dr. Blood) and characterized by blood glucose levels ≥ 200 mg/dl.[13] Preprandial and postprandial blood glucose levels were measured in blood samples obtained from the rat's tail.

Preparation and administration of treatment extract:

The treatment extract was made by mixing *Fibraurea tinctoria* Lour ethanol extract with 0.3% NaCMC (Sigma-Aldrich, USA) suspension and heating over a water bath 20times. The preparation was then weighed, crushed, and added to the volume aquadest until it reached the 100mL. Hyperglycemic rats were divided randomly into five experimental groups (n = 3rats/ group). Group 1 was the negative control and received 0.3% NaCMC. Groups 2, 3, and 4 were the treatment groups and received the treatment extract once daily at doses of 50, 100, and 200mg/kg BW, respectively. Finally, Group 5 was a positive control and received glibenclamide at a dose of 0.65mg/kg BW. The treatment period was started after confirmation of hyperglycemia (day 4) and lasted until day 18.

Evaluation of glycemic profile:

Blood glucose levels of the experimental rats were measured on days 5, 10, and 15 as described previously. Then, the percentage reduction in blood glucose levels was calculated according to the following formula:

$$\text{The percentage reduction in blood glucose levels (\%)} = \frac{Ko - Kd}{Ko} \times 100 \quad \ldots..(1)$$

Where Ko: Blood glucose level on day 4 when hyperglycemia was identified; Kd: Blood glucose levels on days 5, 10, and 15.

Data Analysis:

The data were statistically analyzed using GraphPad Prism version 7.04. The results are presented as mean ± SD from three independent experiments (n = 3) unless otherwise stated. An unpaired t-test was used for two-group comparison, while the Tukey test and one-way ANOVA were used for group comparison. $P < 0.05$ was considered statistically significant.

RESULT:

Alloxan-induced hyperglycemia in the experimental group animals was confirmed by measuring the preprandial and postprandial blood glucose levels as depicted in Tables 1 and 2

Table 1: Preprandial blood glucose levels of experimental rats after intraperitoneal injection of alloxan.

Group	Preprandial Blood Glucose Levels (mg/dL)			
	Observation time (day)			
	0	1	2	3
Treatment	104.67± 4.67	121.33± 3.84	153.67± 4.67	205.67± 67*
Sham	86± 7.02	105.33± 9.61	121.67± 7.26	154.33± 25.91

Data are presented as mean ± SD. The experimental group was animals induced with alloxan, while the sham group was injected with normal saline. *: Significant increase ($p<0.05$) was calculated using an independent t-test.

Table 2: Postprandial blood glucose levels of experimental rats after intraperitoneal injection of alloxan.

Group	Postprandial Blood Glucose Levels (mg/dL)			
	Observation time (day)			
	0	1	2	3
Treatment	112.67± 7.67	132± 3.78	166.667± 1.67	289± 4.16*
Sham	95± 12.53	118.67± 9.26	145±10.41	174.33± 35.89

Data are presented as mean ± SD. The experimental group was animals induced with alloxan, while the sham group was injected with normal saline.*: Significant increase ($p<0.05$) was calculated using an independent t-test.

Body weight data was used to determine the significant increase between the treated and normal rat groups. As indicated in Table 3, there was an increase in the body weights across all the groups

Table 3: The mean body weights of the experimental rats on days 0, 7, and 14.

Experimental Group		Mean body weight on day (g)		
		0	7	14
	1	191.86	214.47	237.54
	2	170.31	200.54	224.03
	3	213.3	239.94	249.16
	4	204.68	230.24	248.18
	5	203.75	243.98	286.24

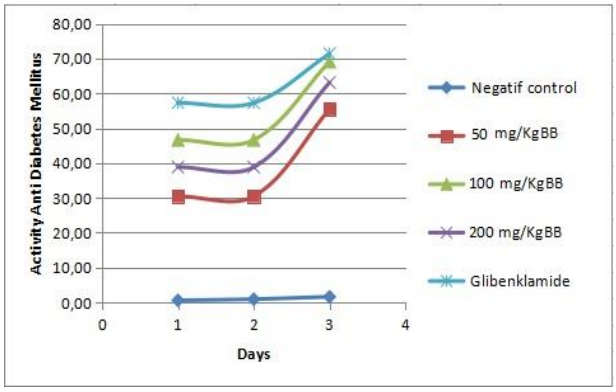
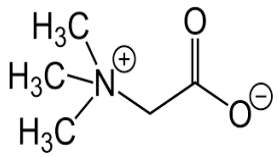


Figure 1: Mean percentage reduction in blood glucose levels of experimental animal groups.

Analysis of the chemical content of the ethanol extract of *Fibraurea tinctoria*, by using LC-HRMS analysis the chemical information has been obtained. Namely: berberin, α oleostearic acid, betaine, choline, glaucine, oleamide as shown in Figure 2.



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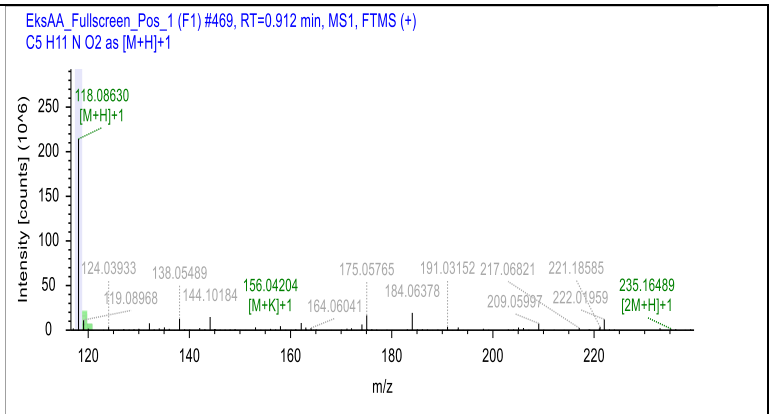
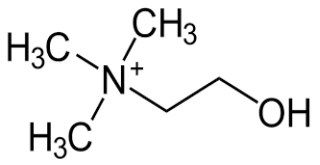
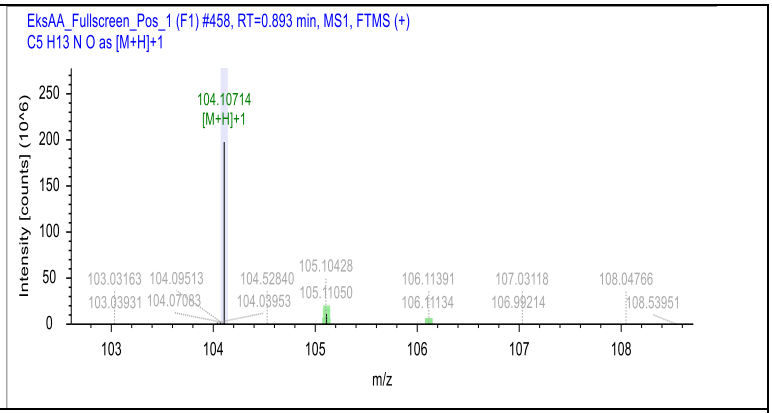


Betaine chromatogram





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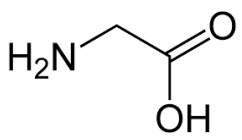
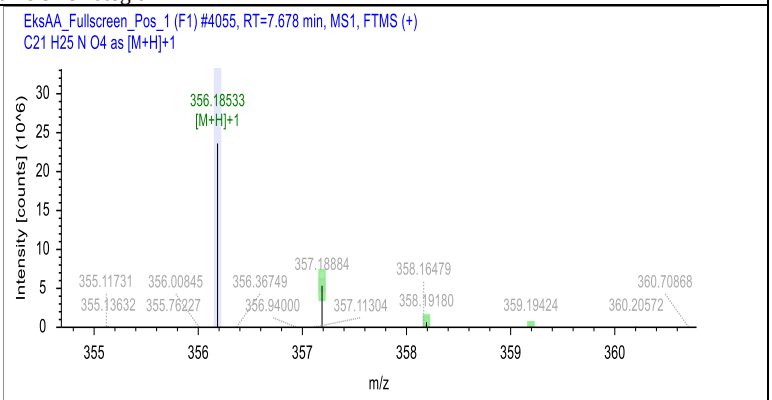
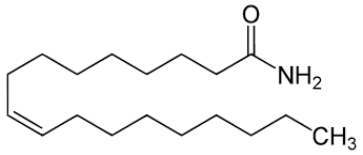
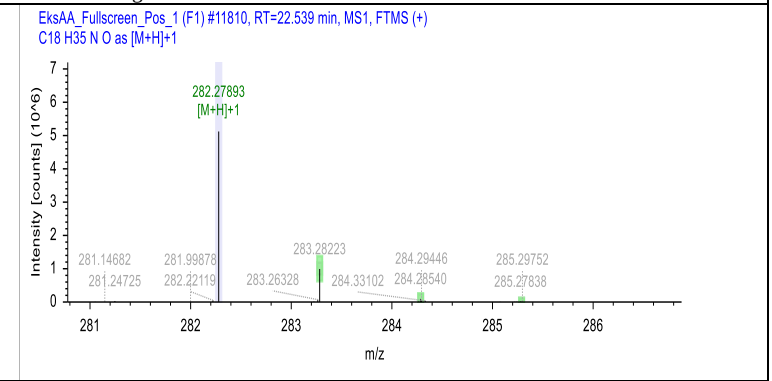


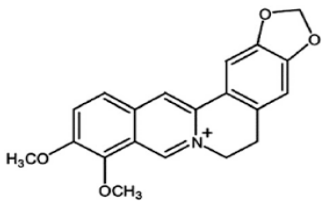


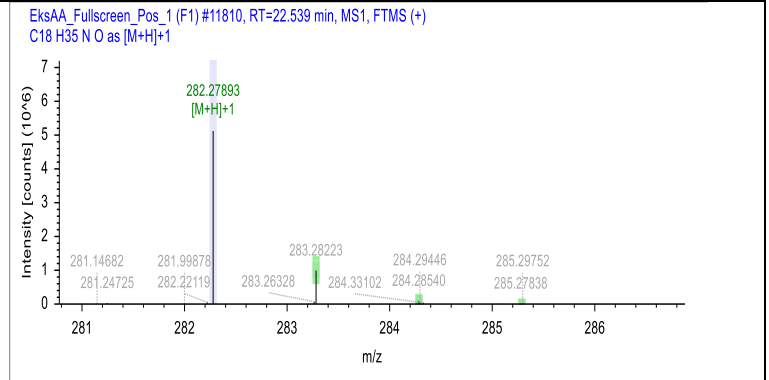
Struktur Glaucine | Glaucine chromatogram





Struktur Oleamide | Oleamide chromatogram





Struktur Berberine | Berberine chromatogram

Figure 2: Analysis of the chemical content of the ethanol extract of *Fibraurea tinctoria*, by using LC-HRMS

DISCUSSION:

Hyperglycemia evaluation:

Alloxan-induced hyperglycemia in the experimental group animals was confirmed by measuring the preprandial and postprandial blood glucose levels as depicted in Tables 1 and 2. It was observed that blood glucose levels increased significantly ($p<0.05$) in the treatment groups compared to the sham group. This observation indicated that alloxan successfully induced hyperglycemia in the treatment groups compared to the sham group. Alloxan tetrahydrate is a diabetogenic substance that selectively acts on pancreatic β cells that produce insulin. Alloxan can bind to GLUT-2 (glucose transporter), allowing it to enter the β cell cytoplasm of the pancreas. Within the cell, alloxan causes excessive mitochondria depolarization due to Ca^{2+} entry ions. Besides, excessive use of energy leads to severe deprivation in the cell. These two mechanisms cause damage to both the number and mass of pancreatic cells resulting in a decrease in insulin release and hyperglycemia.[15]

Weight and treatment evaluations:

Body weight data was used to determine the significant increase between the treated and normal rat groups. As indicated in Table 3, there was an increase in the body weights across all the groups. This parameter is one of the characteristics of insulin resistance that supports the assumption that the animals were already resistant. Figure 1 shows the antidiabetic activities of *Fibraurea tinctoria* Lour ethanol extract at doses of 50, 100, and 200mg/kg BW. There was a significantly higher ($p <0.05$) blood glucose reduction in the ethanol extract treatment groups compared to the negative control group. This indicates that the ethanol extract of *Fibraurea tinctoria* Lour has an antidiabetic effect in all the test doses. There was an increase in blood glucose levels during the treatment in the negative control group but was not significant. The negative control group was only given 0.3% NaCMC, an inert substance that does not lower blood glucose levels.

The Tukey test's statistical analysis revealed that the 50, 100, and 200mg/kg BW ethanol extract groups showed a significant difference ($p<0.05$) when compared to the negative control group. However, comparison between the 100 and 200mg groups showed no significant difference ($p>0.05$). The antidiabetic effect of *F. tinctoria* is due to its berberine content. Berberin regulates glucose metabolism possibly through multiple mechanisms and signal pathways, such as increasing insulin sensitivity, activating the adenosine monophosphate (AMP)-activated protein kinase (AMPK) pathway, modulating gut microbiota, inhibiting gluconeogenesis in the liver, stimulating glycolysis in peripheral tissue cells, promoting intestinal glucagon-like protein-1 (GLP-1) secretion, upregulating hepatic low-density lipoprotein receptor mRNA expression, and increasing glucose transporter.[16-19]

The antidiabetic effect of berberine is also facilitated by inhibition of the α-glucosidase enzyme, which leads to disruption of carbohydrates conversion into glucose, thereby reducing glucose levels in the blood. Therefore, the α-glucosidase inhibitor enzyme reduces postprandial blood sugar levels.[20,21] Berberine belongs to a class of chemical compounds called alkaloids. This substance is a polar compound that is bound to a polar solvent like ethanol. *Fibraurea tinctoria* Lour contains secondary metabolites such as alkaloids, flavonoids, and saponins.[22] Flavonoids are also chemical compounds with antidiabetic properties and are polar because they have a hydroxyl group (-OH) where hydrogen can be formed. Because alkaloids and flavonoids are polar, they dissolve and bind quickly in ethanol.[23] Saponins are also substances found in *F. tinctoria* and have antidiabetic properties because they can inhibit the function of the α glucosidase enzyme in the intestine which acts to convert carbohydrates into glucose.[10] Saponins are also polar chemical compounds that can dissolve in ethanol solvent. Analysis of the chemical content of the ethanol extract of *Fibraurea tinctoria*, by using LC-HRMS analysis the chemical information has been obtained. Namely: berberin, α oleostearic acid, betaine, choline, glaucine, oleamide as shown in Figure 2. Studies are underway to further elucidate the mechanism of blood glucose lowering effect of the extract of *Fibraurea tinctoria* Lour[24,25].

CONCLUSION:

The findings from this study reveal that the ethanol extract of *Fibraurea tinctoria* Lour showed an antidiabetic effect at doses of 50, 100, and 200 mg/kg BW. They significantly ($p <0.05$) reduced the blood glucose levels in hyperglycemic male white rats. Furthermore, the 100 mg/kg BW treatment dose was the most effective in lowering blood glucose levels.

CONFLICT OF INTEREST:

The authors have no conflicts of interest regarding this investigation.

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