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ACTIVITY OF ETHYL ACETATE FRACTIONS OF TONGKAT ALI ROOTS (*EURYCOMA LONGIFOLIA*, JACK) ON BLOOD GLUCOSE LEVELS IN STREPTOZOTOCIN INDUCED DIABETIC RATS

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ABSTRACT: This study is to evaluate two ethyl acetate fractions of "Tongkat Ali Roots" (TAR) namely fractions-1 and fraction-2 (F-1; F-2) on blood glucose levels in streptozotocin (STZ) induced diabetic rats. Each fraction used sixty rats which grouped into six groups (n=10). Group 1 is normal rats which received aqua dest; group 2 is diabetic rats which received STZ 60 mg/kg b.w., group 3 is diabetic rats with standard anti-diabetic metformin 10 mg/kg b.w. and group 4, 5, and 6 are diabetic rats which received tested fractions at doses of 25, 50 and 100 mg/kg b.w. Diabetic rats were induced by STZ intraperitoneally. The fasting blood glucose were assessed on days 0, 7th after diabetic inducing and 15th after giving tested fraction by using a digital glucometer (GCU Easy Touch) from the tip of the tail vein rat. The data were analyzed by using one-way analysis of variance (ANOVA). The fraction-2 (F-2) which was made from the extract of TAR by Vacuum Liquid Chromatography (VLC) using mobile phase ethyl acetate-ethanol-water in ratio 80: 30: 1 has potency as anti-diabetic ($P<0.05$) when compared with fraction 1 using the same mobile phase in ratio 100: 30: 1. This study demonstrated the fraction-2 has the ability to lowering blood glucose. The fifty effective dose (ED_{50}) values of F-2 are 1.71 mg/dL and 67.48 mg/dL for F-1 on days 15th after giving tested fractions.

INTRODUCTION: Many scientific studies have informed the efficacy of traditional medicines and their preparations in controlling diabetes mellitus. The plant *Eurycoma longifolia* Jack (ELJ) is belonging to the family of Simaroubaceae and locally known as "Tongkat Ali's Root" in Malaysia and "Pasak Bumi" in Indonesia. These roots extract most popular in studying drug activity, and processing of the roots extract usually taken after 4 years of cultivation. The roots of this plant commonly used as a single or a mixture of compound with other traditional medicine [1].

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The pharmacological evaluation of TAR has many activities such as antimalarial, antiulcer, antitumor [2, 3] antiparasitic, anti-inflammation [4], antipyretic [5] and anti-diabetic [6]. Their preparations also used in lowering blood pressure, reduces blood glucose and treat of rhinitis, asthmatic and stomach disorders. Tongkat Ali Roots are also most widely used for aphrodisiac [7] and as tonic agents. Several classes of compounds have been identified in the roots such as quassinoids [8, 9] cathin-6-one alkaloids, *β*-carboline alkaloids, tirucallane-type triterpenes, squalene derivatives, and biphenylneolignans [10].

The aqueous extract of TAR at dose 150 mg/kg b.w. in STZ induced diabetic rats have demonstrated to low the blood glucose by 38% ($P< 0.05$) [11]. The activity of *n*-hexane, ethyl acetate and methanolic extracts of TAR in alloxan-induced diabetic rats to have ED_{50} values 684.72, 15.01, 131.42 mg/kg b.w. orally [12].

Based on the points above, this study was examined for knowing the influence of two ethyl acetate fractions of TAR on blood glucose levels in STZ induced diabetic rats. We hope the results of this study would give way out of managing blood glucose levels in diabetics.

MATERIALS AND METHODS:

Materials: All chemicals and drugs used in this study were analytical grades, streptozotocin was purchased from (Sigma Chemical Company). A digital glucometer (GCU Easy Touch) was used for the assessment of the blood glucose levels of the experimental animals. The ethyl acetate fractions of TAR were made of TAR. The roots were washed, cleaned properly to remove foreign material by using flowed water, then cut in a small piece and dried in the shade for 15-25 days and pulverized. About 10 kg powder of TAR was macerated with ethanol then shaken every 4 h until 24 h. Every 24 h the ethanolic extracts were filtered and changed with new ethanol. After maceration (3 × 24 h), all the extracts were concentrated, evaporated and weighed. The percentage yield of extract was calculated to air-dried material. This extract was fixed on silica gel 60 and subjected to VLC then eluting with gradients polarity of mobile phase ethyl acetate-ethanol-water (100: 30: 1 and 80: 30: 1) and provided two fractions (F-1 and F-2). The yields were 1% and 1.25% respectively.

Study Design: This study used a randomized with post-test only with control group design, and was approved by The Institutional Ethical Committee, Faculty of Medicine, Syiah Kuala University with number: 46/KE/FK/2017).

Experimental Animal: One hundred and twenty albino Wistar rats, healthy, ± 3 months old and weighed 150-200 g were used for the study of F-1 and F-2 on diabetic rats. The rats were housed in plastic bottom cages at 23-25 ºC throughout the experiment and allowed free access to standard animal laboratory feed and water *ad libitum*. Each fraction group consists of sixty animals which is divided into six groups (n = 10). The cages were well ventilated with 12 h dark and 12 h light and placed in Laboratory of Pharmacology, Faculty of Medicine, University of Syiah Kuala.

Study Procedure: Before diabetic induction, all the animals fasted for 12-16 h with free access to water. The diabetic induction was carried out by a single intraperitoneal injection of fresh streptozotocin (60 mg/kg b.w.) in 0.1M citrate buffer pH 4.5. Then the diabetic animals were randomized into experimental groups and kept in the cages. Rats with glucose level above 200 mg/dL were selected for the study.

After diabetic induction, the animals were randomly divided into six groups. G-1: nondiabetic rats; G-2: diabetic rats; G-3: diabetic, rats were given 10 mg/kg b.w. of metformin; G-4-5-6: diabetic rats were fed with 25, 50, and 100 mg/kg b.w. of each fraction. Before treatment, all rats fasted for 12 h. The drugs were given orally, once daily to the rats in 0.1% dimethyl sulfoxide (DMSO) as the vehicle.

The Assessments of Blood Glucose Levels: Fasting blood glucose levels of each group was determined on days 0, 7^{th} after diabetic inducing and 15^{th} after treatment with F-1 and F-2. Blood samples were taken from the tail vein of the rats, then glucose levels determined by using blood glucose strips with a digital glucometer (GCU Easy Touch). The data of blood glucose were expressed in mg/dL. The concentration of blood glucose levels was represented as mean ± S.D for 10 animals in each group. Data were analyzed using one-way ANOVA, then followed by Post Hoc tests. The results were considered statistically significant if the P-values were ≤0.05. The activity of the tested drug is valued by the value of fifty effective doses (ED_{50}) which is calculated by using regression probit analysis.

RESULTS AND DISCUSSION: Although, various of oral anti-diabetic are currently available along with insulin for the treatment of diabetes mellitus, there is growing research in traditional medicine due to the side effects associated with the existing of diabetic therapy. Two ethyl acetate fractions of Tongkat Ali Roots (F-1; F-2) were evaluated on blood glucose levels in streptozotocin (STZ) induced diabetic rats. The normal blood glucose levels are measured before the rats inducing with STZ which performed after the seventh day of acclimatization. Besides that, the blood glucose levels of diabetic rats were assessed after the seventh day of diabetic induction. Further measurements of fasting blood glucose levels were

performed at days 15th after giving tested fractions. The mean data of normal and diabetic blood glucose levels of rats were shown in **Table 1**. The data showed a difference in blood glucose levels before and after induction with STZ at a dose of 60 mg/kg BW in all experimental groups of tested fractions (F-1 and F-2), except for the normal group (G-1).

TABLE 1: BLOOD GLUCOSE LEVELS IN NORMAL AND DIABETIC RATS

Group	Normal blood glucose (mg/dL)	P-value	Diabetic blood glucose (mg/dL)	P-value
1	122.83 ± 10		117.87 ± 15	
2	132.00 ± 21		282.77 ± 14	
3	113.67 ± 12	0.607	224.78 ± 16	0.892
4	118.83 ± 11		238.85 ± 11	
5	107.83 ± 15		278.63 ± 10	
6	113.50 ± 14		210.60 ± 22	

TABLE 2: PERCENTAGE OF DIABETIC PROTECTION BY ETHYL ACETATE FRACTIONS OF TAR ON DAY 15th

Group	Fraction-1	P-value	Fraction-2	P-value
1	0		0	
2	0		0	
3	16.37	0.039	13.37	0.023
4	40.09		48.80	
5	74.92		82.89	
6	85.04		84.21	

Percentage of diabetic protection in rats after giving tested fractions on days 15th is shown in **Table 2**. The percentage of diabetic protection of F-1 and F-2 on day 15th is to determine the ED_{50} value of each fraction through regression probit analysis **Table 3**. The presence of drug activity *in-vivo* is determined by the value of fifty effective doses (ED_{50}). The fifty effective dose value of anti-diabetic is the number of doses of the tested drugs or fractions that have anti-diabetic activity in 50% of experimental animals.

TABLE 3: THE FIFTY EFFECTIVE DOSES VALUE (ED_{50}) OF ETHYL ACETATE FRACTIONS OF TAR

The polarity of Ethylacetic Fractions of TAR	The Fifty of Effective Doses (ED_{50})
Fraction 1 (Ethyl acetate: Ethanol: Water 100: 30: 1)	67.48
Fraction 2 (Ethyl acetate : Ethanol: Water 80: 30: 1)	1.71

The result of fractionation of ethanolic extract of TAR with a mobile phase of ethyl acetate- ethanol-water in ratio 100: 30: 1 was obtained ± 10 g (1%) of fraction-1 and fraction-2 ± 12.60 g (1.25%) which used the same mobile phase in ratio 80: 30: 1. The difference in the acquisition of each fraction is estimated as a result of polarity differences of the mobile phase used during the fractionation. The measurement of normal blood glucose levels before administration of diabetic inducer (STZ) is done to prove that the animal was not diabetic, while the measurement of blood glucose levels after administration of the diabetic inducer to ensure that the experimental animal is already diabetic and ready for anti-diabetic activity testing. The metabolic disorder that characterized with increase in blood glucose levels is known as diabetes mellitus. Treatment of diabetes mellitus is to reduce the blood glucose levels to normal and avoid complications.

In the present study, diabetes was induced using STZ. Streptozotocin is a glucosamine-nitrosourea compound which damages pancreatic β cells that caused hypoinsulinemia and hyperglycemia [13, 14]. This diabetic inducer being a destroyer agent causes alkylation of DNA in the beta cells of pancreas, degranulation, and destruction of the beta cells which leads to an acute decrease in the level of insulin [13, 14]. Cellular metabolism of STZ causes the release of free radicals like nitric oxide and induces poly adenosine di-phosphate ribosylation that lowering of beta cell nicotinamide adenine dinucleotide (NAD^+), which further aggravates the DNA strand damage of the pancreatic cells [15].

The percentage of diabetic protection by ethyl acetate fractions of TAR (F-1 and F-2) illustrates the protective ability of blood glucose levels by each fraction. The ability of diabetic protection of F-1 on day 15th (74.92%) at dose 50 mg/kg b.w. The fraction-1 was made by Vacuum Liquid Chromatography using mobile phase consist of ethyl acetate: ethanol: water with ratio 100: 30: 1. This mobile phase is less polar when compared with the mobile phase of fraction-2. Although there is a statistically significant difference in the percentage of diabetic protection in tested fractions (F-1 and 2), it is not yet certain that the most potent

fraction is the fraction with the highest percentage of diabetic protection. This is probably due to the difference in chemical compounds, concentration, and chemical characters. The ED_{50} value has not shown that one of the fractions in the dose can lower blood glucose levels to normal levels. This value is only a hint and more testing needed to estimate the dose that can reduce the blood glucose to normal levels. Based on the study that has been done for 15^{th} days of observation after giving the tested fraction, it turns out the smallest ED_{50} value on day 15^{th} is 1.71 mg/kg b.w. This ED_{50} value is obtained in fraction with mobile phase consist of ethyl acetate: ethanol: water in ratio 80: 30: 1. The data can be concluded that the fraction-1 is less polar than fraction- 2. The smallest ED_{50} value of fraction 2 (1.71 mg/kg b.w.) describes its potential as anti-diabetic after 15 days administration of the fractions. This study confirms the fraction-2 has anti-diabetic activity more potent than fraction-1.

CONCLUSION: The conclusions of this study are ethyl acetate fraction of TAR with mobile phase consist of ethyl acetate-ethanol-water in the ratio of 80: 30: 10 (fraction-2) has anti-diabetic activity with an ED_{50} value of 1.71 mg/kg b.w. after 15^{th} days of treatment.

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CONFLICT OF INTEREST: The authors declare no conflicts of interest in this study.

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