ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

Blood Sugar Lowering Effectiveness of *Cassytha filiformis* Fractions on Diabetic Mice

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ABSTRACT

Blood sugar lowering effect of *Cassytha filiformis* fractions on alloxan induced diabetic mice has been conducted. A number of 45 male mice with the fasting blood glucose level of ≥126 mg/dL (weighing of 20-30 g and aged of 2-3 months) were divided such where they were treated with Butanol (BuOH), ethyl acetate (EtOAc) and water fractions of Cassytha filiformis at dose of 10 mg/kg for 1, 3 and 7 days respectively. As comparison, a goup of Glibenclamide and vehicle were used. Blood glucose level of the animal was measured before and one day after each last dose. Data was analyzed using Two Way ANOVA followed by Duncan Multiple Range T-Test and the significant was taken at 95% confidence interval. Results showed that the blood glucose level of water, BuOH and EtOAc fractions treated rats were significantly ($p<0.05$) decreased by 18.2±9.33; 78.6 ±10.1 and 25.2 ±9.33 % respectively, compared to 28.2 ±9.3 % of glibenclamide treated rat, while blood glucose of vehicle treated rat was increased by 33.7 ±9.3 %. Blood glucose lowering effect of C. filiformis was increases by duration of administration ($p<0.05$) with the average % decrease of blood glucose after 1, 3 and 7 days fractions treatment were -3.5±7.2; 23.0 ±7.2 and 51.5 ±7.6, respectively. These indicated that all fractions of *Cassytha filiformis* produced blood glucose lowering effect where BuOH fraction is the most potent followed by EtOAc and water fractions. Effect of water and EtOAc fractions was equal to that of glibenclamide while BuOH was much better.

Keywords: diabetes, Cassytha filiformis, BuOH fraction, EtOAc fraction, water fraction, blood glucose

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ISSN: 0975-8585

INTRODUCTION

Medicinal plants, one of Indonesia's natural wealth, have long been used by the society as an attempt to overcome health problems. Most of the use of herbs are still based on the beliefs, experiences and often associated with cultures. Manuscript of “Daun Lontar Husodo” (Java), “Usada” (Bali), “Lontarak Pabbura” (South Sulawesi), “Serat Primbon Jampi” and “Serat Racikan Boreh Wulang Dalem” documents, as well as the relief of Borobudur temple depicting the dispensing of medicinal herbs as raw materials are some evidences for the use of natural materials as drugs by the Indonesian ancestors centuries ago [1].

The use and development of traditional medicines all over the world are necessary to be supported continuously as stated in a WHO resolution, namely The Traditional Medicine Strategy 2014-2023. The mission is to help save lives and improve human health [2]. One of the herbs that has been recognized and is an option to be developed in Indonesia is dodder (*Cassytha filiformis* L.), which is known as a parasitic herb. This herb is reported to contain a variety of bioactive components such as vanillic acid, isoquinolon alkaloids, triterpenoids, flavonoids, sesquiterpene-lactone, sesquiterpenoids, phytosterols/steroid, fatty oils, essential oils, lignan, saponin, vitamins, minerals, sugars and organic acids [3]. Traditionally, in Indonesia, this herbaceous plant is used to eliminate intestinal worms and combined with nutmeg to treat stomach and intestinal discomforts. In Malaysia, a lotion containing *Cassytha filiformis* powder and sesame oil is used to stimulate hair growth. In Philippines, a decoction of the fresh herb is taken to induce labor and stop hemoptysis. In Taiwan, the dodder stems are used to encourage urination, treat gonorrhea and kidney disorders. Furthermore, in Vietnam this herb is used for the treatment of syphilis and lung disease [4]. In Africa *Cassytha filiformis* is used to treat cancer [5].

Several studies on the benefits of dodder as a potential raw material for medicine have been reported. Although it is slightly toxic [5,6], it is also reportedly outstanding effective as anti-trypanosomal [7]; increase bleeding time in mice [8], vasorelaxant [9]; antidiabetic effect [10]; antipyretic and analgesia activities [11], antihypertensive effect in rats induced by prednisone and salt [12,13] and as antioxidant [14].

To complete the research data of the efficacy of blood sugar lowering effect from dodder herb, the study needs to be continued on the various factions of dodder in diabetic mice. Parameters observed are fasting blood glucose levels and liver and pancreas weight ratio. This study will reveal the fraction(s) which exhibit antidiabetic effect and the effective duration of its administration. This study is expected to promote dodder herb to be developed as standardized herbal medicine.

MATERIALS AND METHODS

Plant materials

Cassytha filiformis L. was collected on July 2015 in Padang, West Sumatra, Indonesia. The extract was prepared by means of our previous study [12]. The extract continued to fractionation phase to obtain ethyl acetate, butanol and water fractions [6].

Animal preparation

A number of 45 male mice weighing 20-30 g and aged 2-3 months were prepared and acclimatized to normal experimental condition. The mice were fed with normal diet and water was available *ad libitum*. To obtain diabetic mice, all animals were induced with alloxan (150 mg/kg single dose, *ip*). Mice with fasting blood glucose level above 126 mg/dL were used for the study. The mice were divided into 5 groups receiving ethyl acetate fraction, butanolic fraction, and water fraction at the dose of 10 mg/kg. Two other groups were treated as negative control and comparator that received glibenclamide 2 mg/kg. Each groups was divided into 3 subgroups containing 3 animals each of which treatments were given daily for 1, 3 and 7 days, respectively. During the treatment, all animals continued to receive normal diet and water ad libitum. Blood glucose levels of the animal were measured before and one day after each last dose. At the end of the experiment, the animals were sacrificed, pancreas and liver were taken to determine their weight ratios.

ISSN: 0975-8585

Data analysis

Data of percentage change of blood glucose level and liver and pancreas weight ratios were presented as mean ± SD and analyzed with two-way ANOVA. The significant level was taken at $p<0.05$. The Duncan's Multiple Range Test was used as the post hoc test.

RESULTS AND DISCUSSION

From 1 kg fresh herb of *Cassytha filiformis* L, 2.87 g (0.287 %) N-hexane fraction, 706 g (0.7%) ethyl acetate fraction, 9.816 g (0.98%) butanolic fraction and 44.367 g (0.44%) water fractions were obtained.

Administration of *Cassytha filiformis* fractions to diabetic mice could lower animal blood glucose level significantly ($p <0.05$). Duration of the treatment was also affected animal blood glucose level significantly ($p<0.05$). Animals given fractions for 7 days showed the greatest percent decrease in blood glucose level ($P <0.05$). There was an interaction effect between the fraction types and duration of fraction administration to blood glucose levels. In this case, blood glucose of animal treated with ethyl acetate fraction for 1 to 3 days showed a slight increase, and when the treatment continued to 7 days, the mice blood glucose levels dropped precipitously to 60%. On the other hand, on glibenclamide treated mice, administration of such fraction for 3 days produced a decrease in blood glucose for 30%, but when the administration continued, it did not further decrease the blood glucose level. The average decrease in the blood glucose level after administration of water, butanol, ethyl acetate fractions, positive control (glibenclamide) animals were 18.2; 78.6; 25.2; 28.6 %, respectively. Meanwhile, control animals showed an increase for 33.7%. Furthermore, the average reduction in blood glucose in drug use over the last 1, 3 and 7 days were -3.54; 23 and 51.49% respectively (Table 1 and Figure 1).

Table 1. The influence of some fraction of *Cassytha filiformis* to the blood glucose level of diabetic mice

Time (Days)	Change of blood glucose level after the treatment of fractions (%)					Averages
	Water	Butanol	Et. Acetate	Glibenclamide	Vehicle	
1	-6.13 ± 16.6	-68.75 ± 16.6	-14.51 ± 16.6	-3.91 ± 16.6	111.03±16.6	3.54±7.23
3	-6.70 ± 16.6	-79.91 ± 16.6	7.60 ± 16.6	-42.25 ± 16.6	6.11 ± 16.6	-23.03±7.23
7	-41.83 ± 16.6	-91.46 ± 19.79	-68.59 ± 16.6	-39.60 ± 16.6	-15.99 ± 16.6	-51.49±7.58
Averages	-18.22±9.33	-78.61±1`0.08	-25.16±9.33	-28.59±9.33	33.72±9.33	

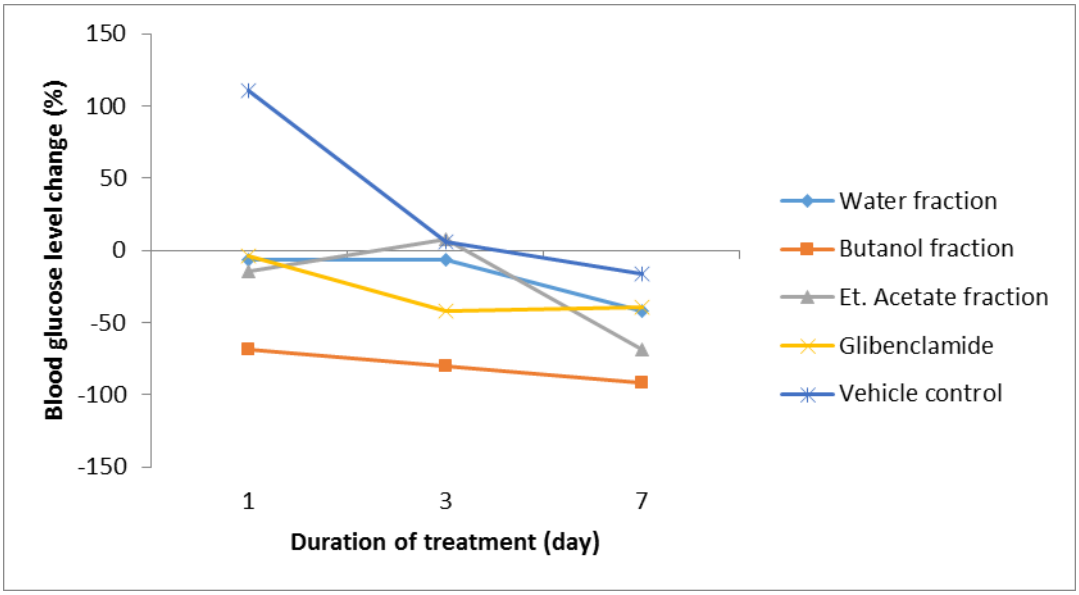

Figure 1. Time - blood glucose lowering effects of some factions of *Cassytha filiformis* after 1, 3 and 7 days treatment on diabetic mice.

ISSN: 0975-8585

There was no significant effect of fractions, duration of treatment and interaction of both factors ($p>0.1$) to the liver ratio of the diabetic mice. Liver ratio of the animal ranged from 4.777±0.509 to 5.907±0.509 % (Table 2 and Figure 2).

Table 2. The influence of some fractions of *Cassytha filiformis* to the liver weight ratio of diabetic mice

Time (Days)	Liver weight ratio after the treatment of fractions (%)					Averages
	Water	Butanol	Et. Acetate	Glibenclamide	Vehicle	
1	5.16 ±0.51	5.393±0.509	5.650±0.509	4.777±0.509	5.407±0.509	5.277 ± 0.228
3	5.47± 0.51	5.917±0.509	5.627±0.509	4.803±0.509	5.307±0.509	5.425 ± 0.228
7	5.907±0.509	6.240±0.624	4.937±0.509	4.937±0.509	5.803±0.509	5.607 ± 0.228
Averages	5.511±0.294	5.850±0.317	5.404±0.294	5.404±0.294	5.506±0.294	

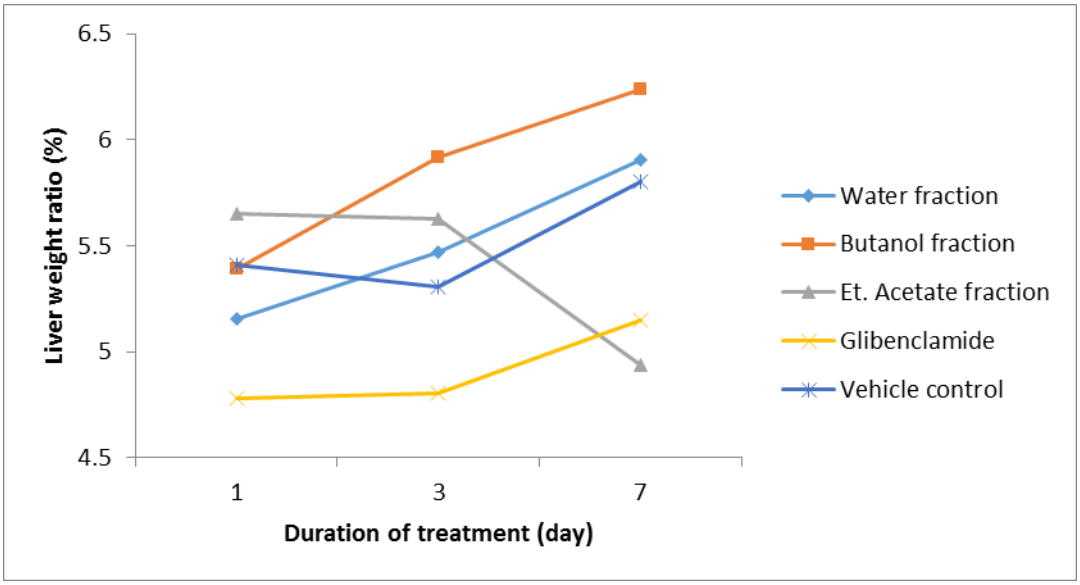

Figure 2. Time - liver weight ratio of some factions of *Cassytha filiformis* after 1, 3 and 7 days treatment on diabetic mice.

Only animal treated with glibenclamide showed different pancreatic ratio ($p<0.05$), while other groups showed non-significant different pancreatic ratio ($p>0.1$). The average pancreas weight ratio of diabetic mice was 0.383±0.04, while the average pancreas weight ratio of other animals ranged from 0.511±0.04 to 0.597±0.04 while that of negative control animal was 0.484±0.04. There was also non-significant effect of duration of treatment and interaction of fraction and the duration of the treatment to the pancreas weight ratio of the animals ($p>0.1$) (Table 3 and Figure 3).

Table 3. The influence of some fractions of *Cassytha filiformis* to the pancreas weight ratio of diabetic mice

Time (Days)	Pancreas weight ratio after the treatment of fractions					Averages
	Water	Butanol	Et. Acetate	Glibenclamide	Vehicle	
1	0.43 ± 0.067	0.50 ± 0.067	0.50 ± 0.067	0.41 ± 0.067	0.46±0.067	0.461±0.030
3	0.56 ± 0.067	0.69± 0.067	0.51 ± 0.067	0.37± 0.067	0.53 ± 0.067	0.533±0.030
7	0.54 ± 0.067	0.61 ± 0.067	0.58 ± 0.067	0.53 ± 0.067	0.46± 0.067	0.506±0.031
Averages	$0.511±0.04^{b}$	$0.597±0.04^{b}$	$0.532±0.04^{b}$	$0.383±0.04^{a}$	$0.484±0.04^{b}$	

ISSN: 0975-8585

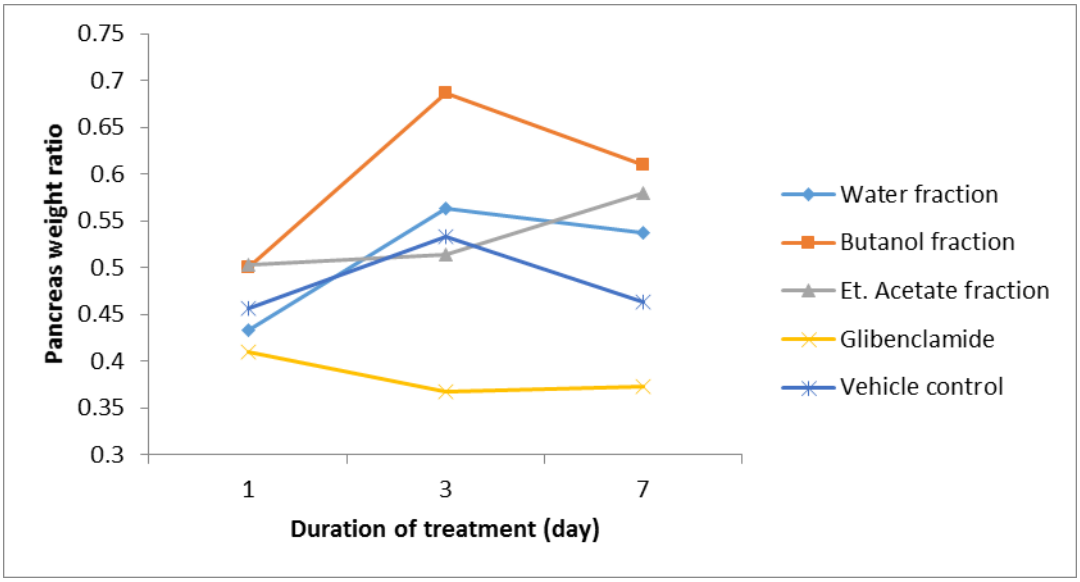

Figure 3. Time - pancreas weight ratio of some factions of *Cassytha filiformis* after 1, 3 and 7 days treatment on diabetic mice.

Our previous study showed that the crude ethanolic extract of *Cassytha filiformis* showed blood glucose lowering effect at single dose of 100 – 200 mg/kg BW [10]. The later sub-chronic toxicity study indicated that the defatted crude extract is slightly toxic [6]. To create a low toxicity material for drug, separation of this extract was performed. Three fractions have been obtained: aqueous, butanol and ethyl acetate fractions. By this effort it is expected that one or more fractions will produce a specific pharmacological activity with a lower effective dose but relatively less toxic.

It was surprising that all fractions (water, butanol and ethyl acetate fractions) exhibited different antidiabetic and anticoagulant effectiveness at low enough dose (10 mg/kg). In this situation, butanolic fraction posed a very effective antidiabetic effect. It could decrease blood glucose level up to 70 percent after single dose administration and up to 90% after 7 days of consecutive administration. Ethyl acetate fraction was the second most potential for antidiabetic effect that could decrease blood glucose level up to 70% after 7 consecutive days of administration while water fraction could only decrease up to 40% blood glucose at the same dose and duration of administration on diabetic mice. Ethyl acetate and water fraction produced a gradual effect with less effectiveness after one to three consecutive days of administration.

The plant genus Cassytha (Lauraceae) provides a rich source of the phytochemical constituents. This genus is recognized to contain flavonoid components, i.e., quercetin 3-O-robinobioside, quercetin 3-O-rutinoside, quercetin 3-O-galactoside, kaempferol 3-O-robinobioside, isorhamnetin 3-O-rutinoside and isorhamnetin 3-O-robinobioside [15]. Mythil *et al*. reported that this plant also contains aporphine alkaloids such as cathafiline, cathaformine, actinodaphnine, N-methylactinodaphnine, predicentrine and ocoteine. The total alkaloid content in the plant is estimated to be around the value of 0.11-0.43 % [14].

Cassytha filiformis extracts such as hexane, ethyl acetate and methanol were reported to have antioxidant activity where methanolic extract was found to show potent antioxidant activity on comparison with the standard Butylated hydroytoluene (BHT). This fraction is suggested to be a promising therapeutic potential and could be further applied as a potential source for the drug development by the pharmaceutical industries [14]. Our previous study indicated that the ethanolic extract and its defatted one produced anti diabetic activity [8]. Due to its toxicity, we tried to separate the compounds by fractionating the defatted extract of *C. filiformis* into 3 fractions: ethyl acetate, butanolic and water fractions. By this effort it is expected that each fraction will have more specific action and less toxic.

Butanolic fraction of *C. filiformis* is assumed to contain glycoside flavonoids. These compounds were reported to have structure function-relationships such as flavans, flavanones, flavones, flavanonols, flavonols, catechins, anthocyanidins and isoflavones. The biological properties of flavonoids include antioxidant, anti-

inflamatory, antitumoral, antiviral and antibacterial, as well as a direct cytoprotective effect on coronary and vascular systems, the pancreas and the liver [16]. Therefore it is assumed that a blood sugar lowering effect of butanolic fraction of this plant is then related to pancreatic protection of its flavonoid content as seen in this study.

Ethyl acetate fraction of the plant is assumed to contain semipolar compounds (i.e. non-glycoside flavonoids). Flavonoid compounds such as vitexin and isovitexin from *Vigna angularis* show high α-glucosidase inhibitory activity that lead to glucose absorption in the gastrointestinal tract [17]. On the other hand, these compounds are also reported to inhibit glucose movement to the cell *in vitro* which is contradictory to the blood glucose lowering effect. That's why the fraction containing this compound (ethyl acetate) had lower blood glucose lowering effect.

Furthermore, in diabetes mellitus, the pancreatic *Langerhans* structure will be irregular due to hyperplasia/hypertrophy and inlfitration of inflammatory cells [18]. In this study, the pancreatic ratio of diabetic mice treated with C. *filiformis* fractions was greater compared to those of negative control animals while pancreatic ratio of negative control animals was smaller to that of positive control animals. These may due to toxic effect of fractions to pancreatic cells, as also reported previously in our previous study for its defatted extract toxicity to liver organ [6].

These results indicate that all fractions (water, butanol and ethyl acetate) of C. *filiformis* produce blood sugar lowering effect, where butanolic fraction is the most potential fraction, followed by ethyl acetate and water fraction, respectively. Further research is still needed to identify the most potential compound(s) as antidiabetic and whether the fractions produce anticoagulant activity as well.

ACKNOWLEDGMENT

This research was conducted under the sponsor of BOPTN of University of Andalas in the scheme of Riset Guru Besar (Contract No. -523/XIV/UNAND-2016, May 9th, 2016).

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