Hypoglycemic effects of *Enhydra fluctuans* aerial extract on alloxan-induced diabetic rats

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**Abstract.** *Enhydra fluctuans* is one of the medicinal plants of the Minangkabau tribe, West Sumatra, Indonesia which is widely used by the community to treat various diseases including diabetes mellitus. However, scientific studying of its hypoglycemic activity is very limited. In this study, we evaluated the hypoglycemic activity of *E. fluctuans* aerial ethanol using an oral glucose tolerance test (OGTT) in stable hyperglycemia rats and its phytochemical screening. Twenty-five diabetic rats were divided into five groups with five replications: negative control, positive control (glibenclamide), ethanol extract dosage of 250, 500, and 1000 mg/kg. Blood glucose is measured at 0, 30, 60, 120, and 180 minutes. Blood glucose level, the area under the curve (AUC) value, and the percentage of blood glucose reduction is calculated. It was found that a dosage of 500 mg/kg was the best hypoglycemic activity in stable hyperglycemia rats and significantly different (p<0.05) compared with the negative control. Hypoglycemic activity of this dosage was longer if compared with glibenclamide. *E. fluctuans* aerial ethanol extract contained saponins, phenolic, terpenoids, steroids, and coumarin compounds. Our findings demonstrate that *E. fluctuans* have potent hypoglycemic effects in diabetes.

1. Introduction

Diabetes mellitus (DM) is serious human health identified by chronic hyperglycemia ensuing from defects in secretion of insulin, the action of insulin, or both \([1]\). Hyperglycemia contributes to the development of the microvascular and macrovascular complications of diabetes, that account for the morbidity and mortality of diabetes \([2]\). Search for the compound that normalizes hyperglycemia is a very important objective in preventing diabetes-associate complications. None of the presently used medications reverse the current failure of beta-cell function \([3]\). The explore of newer medicine from natural sources, that are efficient and safe could open new avenues for the intervention of diabetes disease and diabetes-associated complications.

In recent past, natural sources, especially herbs were shown to reduce blood glucose spikes \([4, 5]\), improve the condition of hyperglycemia and prevent complications \([6]\), and regenerate pancreatic \(\beta\) cells \([7, 8, 9]\). One of the herbs used to treat various diseases including diabetes by traditional medicine practitioners is *Enhydra fluctuans*.

*E. fluctuans* (Asteraceae) is one of the Minangkabau tribal medicinal plants known as *cikarau*, which is widely used as a cure for various diseases \([10]\). This herb contains flavonoids \([11]\), saponins,
phenols, tannins, terpenes, steroids compound \cite{11, 12}, and known to be hepatoprotective \cite{14} and antioxidants \cite{15}. In India, \textit{E. fluctuans} aerial cooking water is used to treat diabetes \cite{16, 17}. Scientifically, this herb has been reported to have antidiabetic activity \cite{18}. Although, their study has several drawbacks including testing the hypoglycemic activity of this herb using unstable hyperglycemia rats (7.80 ± 0.44 mmol/L = 140.54 mg/dL), did not use controls, and only one test dosage. Unstable hyperglycemia is not appropriate for the assessment of antidiabetic drugs. It also leads to illusory assumptions regarding the antidiabetic importance of the test compounds \cite{19}. However, the hypoglycemic activity of this herb using stable hyperglycemia rats has not been reported. The limited scientific information about the hypoglycemic activity of this herb is very unfortunate because this information will confirm the truth of this herb as a diabetes drug.

Here, we evaluate the hypoglycemic effects of \textit{E. fluctuans} aerial ethanol extract in stable hyperglycemia rats and its screening of phytochemicals. The hypoglycemic effects were determined from the blood glucose level, AUC value, and the percentage of blood glucose reduction.

2. Material and Methods

2.1. Plant Materials
\textit{E. fluctuans} aerial was taken Tanah Datar District, West Sumatra Province, Indonesia in mid-September, and the first week of October 2018. This herb was later authenticated by plant taxonomists in the Herbarium of the Department of Biology, Andalas University, Padang, Indonesia. After collection, the aerial herbs were washed with running water and then dried at room temperature that shelters from direct sunlight for 15 days. The dried plant material is cut into small pieces and ground with a blender, and then sieved with a sifter.

2.2. Preparation of extract
532 grams of \textit{E. fluctuans} aerial powder was extracted with 96% distillate ethanol solvent, using the maceration method. After macerating, the maserat is separated from the pulp using a flannel cloth. The extract is then evaporated and concentrated at 40°C, using a rotary evaporator. The concentrated extract obtained 123, 257 grams.

2.3. Animals
Male Wistar albino obtained from the animal house, the department of biology education, FTK IAIN Batusangkar. Rats aged 3-4 months were being cared for and given standard pellet feed (BR2) and water ad libitum. The rats cared at a temperature of 27 ± 30°C, humidity 65-95%, and under conditions 12 hours light-dark. The handling of rats in the study was under the guidelines for the maintenance and bring of laboratory test animals established by the Research Ethics Committee of the Medicine Faculty, University of Andalas, Padang. License passes ethical review No: 038/KEP/FK/2019.

2.4. Hyperglycemia Induction
Healthy male Wistar rats weighing 180-250 grams were fasted overnight (15 hours) but were still given drinking water ad libitum. The alloxan dosage of 125 mg/kg dissolved in 0.9% NaCl is then administered intraperitoneally. Blood glucose was checked with a glucometer (GlucoDr. AutoTM, Model AGM-4000, Korea) after 96 hours. Rats that had blood glucose above 200 mg/dL were applied for the study.

2.5. Hypoglycemic Effect
The method of Mandlik, Desai, and Naik, 2008 was modified in this research \cite{20}. Male diabetic rats, 5 per group fasted overnight (15 hours). Na-CMC 0.5% solution, glibenclamide 0.45 mg/kg, ethanol extract doses 250, 500 and 1000 mg/kg were administered to diabetic rats. Blood glucose was evaluated after fasting. All of the rats were administered extract according to treatment and waited for
60 minutes. Shortly before glucose 1.35 mg/kg was administered, the blood glucose of the rats was measured (for 0 minutes). The blood glucose is then measured at 30, 60, 120, and 180 minutes after glucose administration. Blood is drawn through the tail after the first cleaning with 70% ethanol. The rat’s tail was cut with sharp surgical scissors of approximately 2 mm. The blood is then dropped on a glucometer strip.

2.6. Phytochemical Screening

*E. fluctuans* aerial ethanol extract was subjected to phytochemical tests. Alkaloids, flavonoids, saponins, terpenoids, steroids, phenolics, and coumarin were tested. Phytochemical testing is carried out based on qualitative standard methods qualitatively [22, 12].

2.7. Data Analysis

The blood glucose profile data were analyzed descriptively from blood glucose data obtained during OGTT. The blood glucose levels for each measurement, the percentage of plasma glucose reduction, and AUC\(_{0-180}\) values are expressed in the form of mean ± SD. The percentage of blood glucose reduction is determined by the formula:

\[
\text{Percentage reduction in blood glucose levels} = \left(1 - \frac{W_e}{W_c}\right) \times 100
\]

Where \(W_e\) are the blood glucose in the glibenclamide or the extract treatment, and \(W_c\) is the control of blood glucose [13, 14].

AUC\(_{0-180}\) values are calculated using the trapezoid formula:

\[
\text{AUC}_{0-180} = \frac{V_0 + V_1}{2} (t_1 - t_0) + \frac{V_2 + V_3}{2} (t_2 - t_1) + \frac{V_3 + V_4}{2} (t_3 - t_2) + \frac{V_4 + V_5}{2} (t_4 - t_3)
\]

Where \(V_0, V_1, V_2, V_3, V_4\) are blood glucose values at each time (minutes) of measurement, “\(t_0 = 0, t_1 = 30, t_2 = 60, t_3 = 120, t_4 = 180\) minutes” [25].

Blood glucose level, percentage of reduction in blood glucose, and AUC\(_{0-180}\) values were tested for normality and homogeneity. If the distribution is normal and homogeneous, proceeded with the one-way ANOVA test. If there are significant differences followed by the Duncan test at the 0.05 level. \(P < 0.05\) was said to be significantly different. Data analysis using SPSS 21 software. Phytochemical screening data were analyzed descriptively.

3. Results

3.1. Hypoglycemic Effect

Evaluation of the hypoglycemic effect of *E. fluctuans* aerial ethanol extract using OGTT in stable hyperglycemia rats. The mean fasting blood glucose of rats before treatment was 434.8 mg/dL (data not shown). Blood glucose levels above 350 mg/dL have been proved to have stable blood glucose in diabetic rats. The glucose loading dosage of 1.35 mg/kg was carried out after 60 minutes of giving the extract. Immediately before loading glucose, blood glucose was measured and assigned as 0 minutes. Blood glucose was then evaluated at 30, 60, 120, and 180 minutes. The hypoglycemic effects can be seen in the blood glucose profile (Figure 1), blood glucose level, AUC\(_{0-180}\), and percentage of blood glucose reduction (Table 1).

In figure 1, the blood glucose of diabetic rats after extract administration was seen to increase in all treatments, which was seen in the erroneous measurement before glucose loading (0 minutes). The highest increase in blood glucose was found in the negative control treatment and the lowest in the positive control treatment (P0). After 30 minutes of loading glucose, there was an increase in blood glucose in all treatments. The lowest increase in blood glucose was obtained at a treatment dosage of 500 mg/kg. It was also seen that the dosage of 500 mg/kg body weight reached a lower value at 180 minutes when compared to before glucose loading. This means that a dosage of 500 mg/kg showed hypoglycemic activity when compared with other dosages.
In Figure 1, it is also known that blood glucose on glibenclamide treatment has sharp enough decreased from 60 to 120 minutes when compared with the dosage of 500 mg/kg. Thus it is known that the ethanol extract at a dosage of 500 mg/kg of *E. fluctuans* aerial extract affects reducing blood glucose and not better when compared with glibenclamide based on OGTT.

Table 1 shows the level of blood glucose, AUC<sub>0,180</sub> in stable hyperglycemia rats treated with *E. fluctuans* aerial ethanol extract. At Table 1, the mean blood glucose levels of diabetic rats at 0, 60, 120, and 180 minutes at doses of 250 and 500 mg/kg differed significantly (p<0.05) when compared with negative controls, but did not differ significantly (p >0.05) when compared with positive control. The mean blood glucose level at the treatment dosage of 500 mg/kg showed a blood glucose level that was almost close to the blood glucose treated with positive control. This is evidenced by the lower AUC<sub>0,180</sub> values and approached the AUC<sub>0,180</sub> of the positive control treatment. The percentage of blood glucose reduction at a dosage of 500 mg/kg reached 27.51±12.60%. Our data suggest that *E. fluctuans* aerial ethanol extract have antidiabetic activity in stable hypoglycemia rats.

Table 1. Hypoglycemic effect of *E. fluctuans* aerial ethanol extracts on the blood glucose level, AUC<sub>0,180</sub> values and the percentage of blood glucose reduction in stable hyperglycemia rats.

| Treatments          | Blood Glucose Level (mg/dL) (n=5) | AUC<sub>0,180</sub> (mM.mg/dL.) | Percentage (%) of glucose reduction |
|---------------------|----------------------------------|---------------------------------|------------------------------------|
|                     | 0 minute | 30 minute | 60 minute | 120 minute | 180 minute |                         |                                     |
| Negative control    | 649.20±  | 673.20±  | 659.20±  | 606.60±  | 641.80±   | 115248.00±          | -                                  |
| Positive control    | 39.89±   | 30.88±   | 17.51±   | 39.25±   | 34.23±    | 6297.34±           | 35.15± 8.13                        |
| Dosage of 250 mg/kg | 519.20±  | 572.60±  | 546.00±  | 528.00±  | 487.80±   | 95850.00±          | 23.99± 8.07                        |
| Dosage of 500 mg/kg | 512.80±  | 515.00±  | 503.40±  | 510.20±  | 453.20±   | 90819.00±          | 29.39± 6.72                        |
| Dosage of 1000 mg/kg| 592.00±  | 615.00±  | 634.60±  | 574.60±  | 510.80±   | 105687.00±         | 20.41± 8.47                        |
The different letters in the same column indicate that there are significantly different according to the Duncan test of 0.05 level.

3.2. Screening phytochemical

E. fluctuans aerial ethanol extract contained saponins, phenolic, terpenoids, steroids, and coumarin compounds.

4. Discussion

Prior work has documented the effectiveness of E. fluctuans aerial ethanol extract in controlling diabetes \[18\], for example, reports that administering ethanol extract for 21 days improved blood glucose status, including improving plasma lipid profile and lowering plasma creatinine levels. However, these studies used only one dosage, have not focused on diabetic male rats and have not used stable hyperglycemia rats. In this study, we tested hypoglycemic effects of the extract with different dosages using OGTT in stable hypoglycemia rats and its phytochemical screening.

We found that that E. fluctuans aerial ethanol extract have hypoglycemic effect based on blood glucose level, AUC\(_{180}\) value and percentage of blood glucose reduction (Figure 1 and Table 1). Our results are in general consistent with previous study \[18\]. The activity of reducing blood glucose is due to the addition of hypoglycemic substances in an appropriate amount of ethanol extract. The ability of the animal's body to reduce plasma glucose indicates the influence of the substances contained \[18\]. E. fluctuans aerial ethanol extract in this study are known to contain terpenoids, steroids, saponins, and phenolics compound. The compounds contained in plants that are known to be hypoglycemic include phenolic \[28\], saponin \[17, 18, 19\], terpenoids \[20, 21, 22\], and steroids \[35\]. Terpenoids and steroids stimulate insulin secretion and inhibit glucogenesis \[36\]. Terpenoid compounds are also involved in modulating insulin signals, improved oxidative stress status, decreased apoptosis, and decreased inflammatory response \[37\].

The hypoglycemic effect in our study used stable hyperglycemia rats. Rat models are said to have stable hyperglycemia prolonged period if their blood glucose levels after diabetogenic induction are above 350 mg/dL \[38\]. Stable hyperglycemia should be a major concern in testing the hypoglycemic potential of a drug substance \[39\]. Unstable hyperglycemia is not appropriate for the assessment of antidiabetic drugs. It also leads to illusory assumptions regarding the antidiabetic importance of the test compounds \[19\]. So, the hypoglycemic effect of aerial E. fluctuans previously reported by Hasan et al \[18\] is doubtful validity, because used unstable hyperglycemia rats (7.80 ± 0.44 mmol/ L (140.54 mg/dL) in their treatments. Thus, it is clear that our results highlight the weaknesses research of Hasan et al results.

This study also differs from Hasan et al in terms of the dose tested. They used a single extract dosage (200 mg/kg), whereas our study used three different dosages (250, 500 and 1000 mg/kg). Our study showed that a dosage of 500 mg/kg body weight exhibits the best hypoglycemic activity based on OGTT. The results of this study looked better in terms of reducing blood glucose when compared with Hasan et al results. The ability of substances contained in drug substances to reduce blood glucose increases with increasing dosage \[40\]. Dosage of 500 mg/kg also revealed hypoglycemic activity that was not significantly different (p<0.05) and longer if compared with glibenclamide (Table 1, Figure 1). This study, therefore, indicates that the E. fluctuans aerial has potential as a natural drug for treating diabetes.

Most notably, this is the first study to our knowledge to evaluate the hypoglycemic effect of E. fluctuans aerial ethanol extract used stable hyperglycemia rats. Our results suggest that E. fluctuans aerial uses to diabetes management. However, some limitations are worth nothing. Although our hypotheses were supported statistically, the study was not tested hypoglycemic effect for a long time. Future work should study to evaluate hypoglycemic activity in the long-term, more variables and also identification of active compound responsible for hypoglycemic agents.

5. Conclusion

The finding of these results indicated that E. fluctuans aerial have hypoglycemic activity in stable hypoglycemia rats. We have also shown that this had longer hypoglycemic activity compared with
glibenclamide. Our results suggest that *E. fluctuans* aerial uses to diabetes management. Nonetheless, more studies are required to evaluate hypoglycemic activity in the long-term, more variables and also identification of active compounds responsible for hypoglycemic agents.

**Acknowledgment**
The author acknowledges the financial support from DIPA Institut Agama Islam Negeri (IAIN) Batusangkar 2019 funds.

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