DOI: https://doi.org/10.31351/vol32iss3pp128-133

Ameliorative Effect of *Enhydra fluctuans* on Liver Damage in Diabetic Rats

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Abstract

Diabetes mellitus and insulin resistance cause harm to the liver. In this study, the ameliorative effect of *Enhydra fluctuans* on liver damage in alloxan-induced diabetic rats was examined. Thirty acclimatized male albino rats were divided into six groups (n = 5). Normal rats were fed a standard diet (Group 1); diabetic rats were provided standard feed (Group 2); diabetic rats were fed standard feed and glibenclamide (Group 3); and diabetic rats were fed standard food and fractions of n-hexane *E. fluctuans* 57.03, 114.06, and 171.09 mg/kg, respectively (Group 4, 5 and 6). For 21 days, rats were fed the n-hexane fraction orally. On day 1 and day 21 of treatments, blood sugar levels were measured. The histopathology of the liver, serum glutamic oxaloacetic transaminase (SGOT), and serum glutamic pyruvate transaminase (SGPT) level were estimated. A dosage of 57.03 mg/kg was substantially ($p < 0.05$) beneficial in decreasing blood sugar and SGOT. The n-hexane fraction of *E. fluctuans* ameliorated liver damage in diabetic rats and avoided diabetes complications, according to our findings. As a result, *E. fluctuans* could have a potential role as a novel diabetic therapy.

Keywords: Diabetes, Enhydra fluctuans, Insulin resistance, Serum glutamic oxaloacetic transaminase.

تأثير إنهيدرا فلكتوان على تلف الكبد في الفئران المصابة بداء السكري

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الخلاصة

يسبب داء السكري ومقاومة الأنسولين ضررًا للكبد. في هذه الدراسة ، تم فحص التأثير التحسني لـ يسبب داء السكري ومقاومة الأنسولين ضررًا للكبد. في هذه الدراسة ، تم فحص التأثير التحسني لـي إنهيدرافلكتوان على تلف الكبد في الفئران المصابة بداء السكري التي يسببها الألوكسان.

ثلاثون ذكور جرذان ألبينو متأقلمة مقسمة إلى ست مجموعات (ن = ٥). تم تغذية الفئران العادية بنظام غذائي قياسي (المجموعة ١) ؛ تم تزويد الجرذان المصابة بداء السكري بأعلاف قياسية (المجموعة ٢) ؛ تم تغذية الفئران المصابة بداء السكري بالأعلاف المعيارية والجليبينكلاميد (المجموعة ٣) ؛ تم تغذية الفئران المصابة بداء السكري بالغذاء القياسي وأجزاء من إنهيدرا فلكتوان جزء الهكسان ٥٧,٠٣ و ١١٤,٠٦ و ١٧١,٠٩ مجم / كجم على التوالي (المجموعة ٤ و ٥ و ٦). لمدة ٢١ يومًا ، تم تغذية الفئران بجزء الهكسان عن طريق الفم. في اليوم الأول واليوم الحادي والعشرين من العلاجات ، تم قياس مستويات السكر في الدم. في نهاية العلاج ، تم تقدير أنسجة الكبد وتركيز إنزيم ناقلة أمين الجلوتاميك أوكسالواسيتيك في الدم وترانس أميناز الغلوتاميك بيروفات المصلز.

بجرعة ٥٧,٠٣ مجم / كجم ، كان جزء الهكسان مفيدًا إلى حد كبير (ف < ٠,٠٥) في تقليل نسبة الجلوكوز في الدم و وتركيز إنزيم ناقلة أمين الجلوتاميك أوكسالواسيتيك في الدم . أدى جزء الهكسان من إنهيدرا فلكتوان إلى تحسين تلف الكبد في الفئران المصابة بداء السكري وتجنب مضاعفات مرض السكري ، وفقًا لنتائجنا. نتيجة لذلك ، قد يكون لتقلبات إنهيدرا فلكتوان محتمل كعلاج جديد لمرض السكري.

الكلمات المفتاحية : مرض السكري ، تقلبات إنهيدرا ، مقاومة الأنسولين ، تجديد خلايا الكبد.

Introduction

Diabetes is a chronic metabolic condition with an increasing incidence and prevalence [(1)]. International Diabetes Federation [(2)] and Saeedi et al [(1)] revealed that people with diabetes are expected to account for 10.2 % of the world's total population (578 million) in 2030 and 10.9 % in 2045 (700 million). It is estimated that 4.2 million people worldwide will die from diabetes and its complications [(2)], like fatty liver disease, cirrhosis, liver cancer, and liver failure. Epidemiological studies have shown that diabetes patients have an increased risk of getting liver disease [(3)]. The key indicator of liver health is liver function testing.

In human and animal models, biochemical measures of liver function such as SGOT, SGPT, and bilirubin have been used to assess liver damage. In addition, a low level of serum albumin suggests that liver is not working properly. A blood test may also look for signs of abnormal blood clotting, which can indicate significant liver damage [(4)].There is a positive correlation between liver damage and liver enzyme levels [(5)]. Oxidative stress is the main factors that could cause liver damage in diabetics caused by insulin resistance, which causes adipose lipolysis. Additionally, pro-inflammatory cytokines such as interleukin (IL)-1β, IL-6 and tumor necrosis factor (TNF)-α exacerbates the accumulation of oxidative

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Received: 18/10 /2022
Accepted: 28/12 /2022

damage products in the liver [6]. Accordingly, insulin resistance, adipose lipolysis, and the production of TNF-α and leptin cause liver cell inflammation and necrosis [7, 8]. As a result, insulin resistance, adipose lipolysis, and the production of TNF-α and leptin cause liver cell inflammation and necrosis [7, 8]. Hence, active compounds derived from traditional medicinal plants are one of the promising for the treatment of diabetes and diabetes complications [9], because contain compounds exerting various underlying mechanisms and relatively minimal side effects [10, 11].

Enhydra fluctuans (Asteraceae) is a traditional herb of the Minangkabau Tribe, West Sumatra, Indonesia. This herb commonly is known as Bufalo Spinach (English), Haruch (Hindi), *Godobos*, *Cikarau* (Indonesia). This plant is believed to possess a number of biological activities, including anti-diabetic activity [10, 11, 12, 13], anti-inflammatory [14], hepatoprotective [15], and anti-oxidants [16]. This plant is reported to be nutrient-dense, including triterpenoids, flavonoids, phenols, and steroids [17, 15].

Ethanol extract of *E. fluctuans* has anti-diabetic activity [10, 11, 12, 13]. It was discovered to reduce blood sugar levels in diabetic and prediabetic on animal model. Hasan et al. [13] discovered that *E. fluctuans* seemed to have no influence on SGOT. However, the possible effect of this plant against liver damage in diabetic animal model has not fully discovered. The present research is to investigate whether the n-hexane fraction of *E. fluctuans* could reduce liver damage in diabetic rats.

Materials and Methods

Reagent and Animals

The alloxan was supplied by Sigma-Aldrich (St. Louis, MO, USA). Alloxan was used to induce diabetes in rats. The SGOT and SGPT measurement kits were given by DiaSys TM, Germany. SGOT, SGPT (DiaSys TM, Germany). Wistar male rats were collected from animal house of the Biology Education Department, Faculty of Tarbiyah and the Teacher Training at UIN Mahmud Yunus Batusangkar. The utilization of rats was governed by the Committee on Research Ethics of Andalas University's Medicine Faculty in Padang, Indonesia Number 038/KEP/FK/2019).

Aerial fractionation of E. fluctuans

E. fluctuans aerial samples were taken in Lima Kaum, Tanah Datar District, West Sumatra, Indonesia, and plant taxonomists from Andalas University's Department of Biology's Herbarium validated it. Samples were dried for up to 15 days before being mashed into flour and processed. 532 g of flour were extracted using the maceration process with a 96% distillate alcohol solvent. A rotary evaporator was used to evaporate and concentrate the extract at 40°C. (Germany, Hei-Vab CRE), and obtained crude extract. In 96 percent alcohol, 100 g of crude extract was dispersed. After dispersing the viscosity extract, a graded liquid-liquid technique with n-hexane as a solvent was used for fractionation. The fractions were then concentrated and saved until being utilized in the study [12].

Diabetic treatment induction

Male rats weighing 180-250 g were adapted for three days before the intervention and afterwards fasted for 15 hours without meals but were given a constant intake of water. In addition, rats were administered a 0.9 percent sodium chloride solution of alloxan at 125 mg/kg. Blood sugar levels in diabetic rats were measured 96 hours after being injected intraperitoneally (ip). The animals used in this research had blood sugar levels above 300 mg/dL [11].

Animal grouping

Diabetic rats were randomly assigned to one of six groups (n = 5):

- Normal control group (GN): Normal rats were given standard feed
- Diabetes control group (G0): diabetes rats were given standard feed
- Glibenclamide (G1): diabetes rats were given standard feed and 0.45 mg/kg glibenclamide;
- Dosage 1 (G2): diabetes rats were given standard feed and 57.03 mg/kg n-hexane fraction
- Dosage 2 (G3): diabetes rats were given standard feed and 114.06 mg/kg n-hexane fraction
- Dosage 4 (G4): diabetes rats were given standard feed 171.09 mg/kg n-hexane fraction

The n-hexane fraction was given orally once a day at 8-9 a.m for 21 days. Rats had free acces of feed and water ad libitum.

Blood glucose testing

On days 0 and 21, fasting blood samples were taken straight from of the rats' tails. Blood sugar levels were measured using a glucometer (GlucoDr. AutoTM, Model AGM-4000, Korea).

Histopathology of liver tissue

Histological specimens of the liver were produced at Balai Veteriner Bukittinggi, Ministry of Agriculture Republic of Indonesia. Liver samples were fixed in 10% neutal buffered formalin for at least 24 hours before processing. Tissue samples were fixed in paraffin, sectioned at 5 m thickness, processed with hematoxylin-eosin (H&E), and pictures were captured using a photo microscope (Olympus DP 22, Tokyo, Japan). Preparation of the liver were examined at 400x magnifying in 5 distinct fields of view, with at least 20 liver cells within every field of view. Manja Roenigk's modified liver histology score has been used to determine liver cell damage. The preparations were read by two people to obtain valid and reliable examination results [18].

Liver damage score

The degree of damage was quantified, with a score of 1 indicating normal liver cells, a score of 2 indicating vacuolization (parenchymal degeneration), a score of 3 indicating degeneration, and a score of 4 indicating necrotic liver cells. Normal liver cells are approximately the same size as hepatocytes, the cytoplasm (hepatocyte plate) is flat, and one or two nuclei are located in the middle of the cytoplasm. Hydrophilic vaculorization causes liver cells to have unequal cell sizes, a wide cytoplasm, and white coloration. The nuclei of liver cells that undergo hydrophilic degeneration shrink or undergo pyknosis and blacken (dense color), but the nucleus still has a membrane [18]. Each treatment's percentage of liver damage was determined.

SGOT and SGPT

The SGOT and SGPT values were determined using a test kit. The based detection is used in according with the manufacturer guidelines, and the findings are reported in mg/dL.

Statistical analysis

The data is shown as mean standard deviation (S.E.M.). To determine statistical significance, SPSS 21 was utilized. Duncan's New Multiple Range Test (DNMRT) was employed after one-way ANOVA. If the p-value was less than 0.05, the difference was judged significant. Data on hepatic injury were examined descriptively and given in percentages. The percentage of reduction in blood glucose levels was determined by the formula:

$$\% \ \textbf{reduction} = \left(1 - \frac{\mathbf{We}}{\mathbf{Wc}}\right) \mathbf{x\ 100}$$

W_e is the blood glucose concentration in the glibenclamide or fraction treatment, and W_c is the control of blood glucose concentration [19].

Results and Discussion

Effect of E. fluctuans on blood glucose.

The treatment of n-hexane fraction of *E. fluctuans* at various dosages for 21 days leads in a decline in blood glucose concentrations in diabetic rats, with a high percentage reduction at 57.03 mg/kg (G2) (Figure 1B). The dosage of 57.03 mg/kg (G2) was statistically different ($p < 0.05$) compare to the negative control (G0), but not statistically different ($p > 0.05$) compare to glibenclamide. It implies that the *E. fluctuans* fraction has anti-diabetic efficacy comparable to glibenclamide (G1).

E. fluctuans is more effective than glibenclamide in reducing blood glucose because it contains compounds that can activate multiple pathways for pancreatic β-cell regeneration by modulating numerous genes or proteins involved in insulin signaling, inflammation, oxidative stress, and apoptosis. Terpenoids are a class of drugs that have been shown to regenerate and improve pancreatic β-cells function [20], by modulating numerous genes or proteins involved in insulin signaling, inflammation, oxidative stress, and apoptosis [21]. In our previous study, the n-hexane fraction contains terpenoids and steroids [10]. *E. fluctuans* is rich in terpenoid compounds such as saponins, sesquiterpene lactones and steroids such as sitosterol and stigmasterol [22]. Terpenoids have blood glucose lowering activity [23]. Steroid (stigmasterol) has blood glucose lowering activity by increasing the expression of GLUT 4 [24].

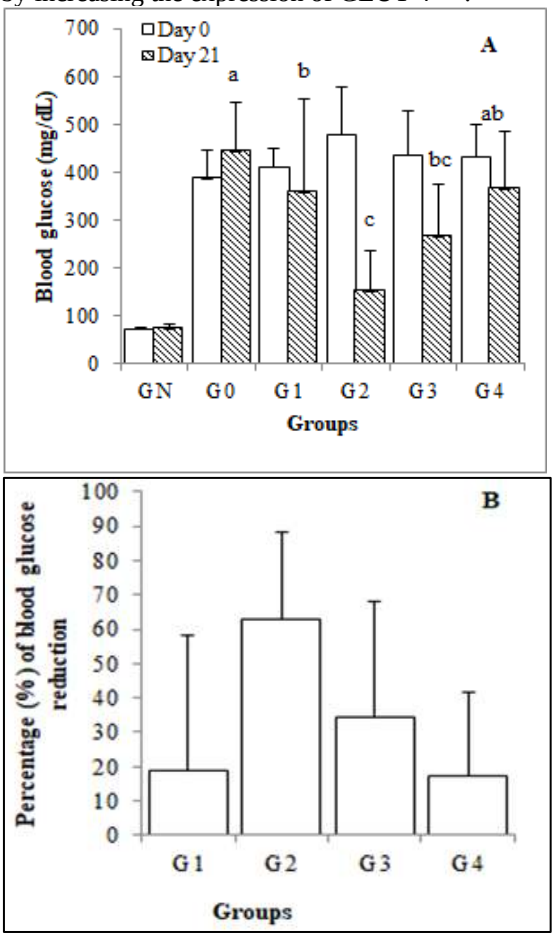

Figure 1. A = Mean blood glucose; B = percentage of blood glucose reduction . The several symbol indices (a, b, c) over each bar diagram demonstrate a significantly different ($p<0.05$) compared to diabetes control group based on DNMRT.

The finding of this study supported diabetes management, as claimed by traditional practitioners in India [25]. The findings also support the findings Hasan et al [11] and Delfita et al [10, 12, 13], who found that the ethanol extract has antidiabetic activity in prediabetic and diabetic rats. Most notably, the antidiabetic activity of n-hexane fraction of *E. fluctuans* is the first to be investigated in diabetic rats.

Effect of E. fluctuans on liver histology and liver score damage

Our data show that n-hexane fraction of *E. fluctuans* treatment ameliorate liver structure in alloxan-induced diabetic rats (Figure 2, Figure 3). In Figure 2A (GN), it is known that normal rat hepatocyte cells have a cell arrangement that is radial to the central vein, flat cytoplasm, one or two nuclei located in the center of the cell and normal cells are more numerous when compared to cells that experience hydrophic vacuolisation, hydrophic degeneration and necrosis. In diabetic rats, it is known that many cells experience vacuolization, necrosis, degeneration and few normal cells (Figure 2B; G0).

The results of this study are supported by research by Hasballah et al. [18] where diabetic rats experienced significant amounts of hydrophic degeneration and necrosis. Characteristics of hepatocytes that undergo vacuolisation are characterized by an enlarged hepatocyte volume, an average nucleus of only one and is located in the center of the cell, the hepatocyte cytoplasm is slightly concave, looks wide and is white in color. Vacuolated hepatocytes are found around the central venous region. Hepatocyte damage in the form of hydrophic degeneration is characterized by a shriveled and blackened nucleus but still has a cell membrane, hepatocyte damage in the form of necrosis is characterized by a blackened and fragmented nucleus.

The histopathological data of this study show that diabetic rats cause liver damage (Figure 2). Moreover, the results showed that the percentage of liver cells of diabetic rats (G0) that undergo vacuolisation, necrosis, and degeneration is higher compared with normal rats (GN), glibenclamide, and n-hexane fraction. Oxidative stress caused by insulin resistance is one of the main factors causing liver damage in diabetic rats [7]. Oxidative stress increases adipose lipolysis, as well as the production of TNF-α and leptin [6], all of which are factors that cause inflammation and liver cell necrosis [8].

In the administration of glibenclamide (G1), it was found that it did not protect liver cells from necrosis, whereas in the administration of *E. fluctuans* was known to protect liver cells in diabetic rats from damage. G2 treatment showed the best protective effect. Thus, the results of this study indicate that the fraction of *E. fluctuans* has activity to protect or reduce hepatocyte damage in diabetic rats as evidenced by a lower liver damage score when compared to G0 and G1.

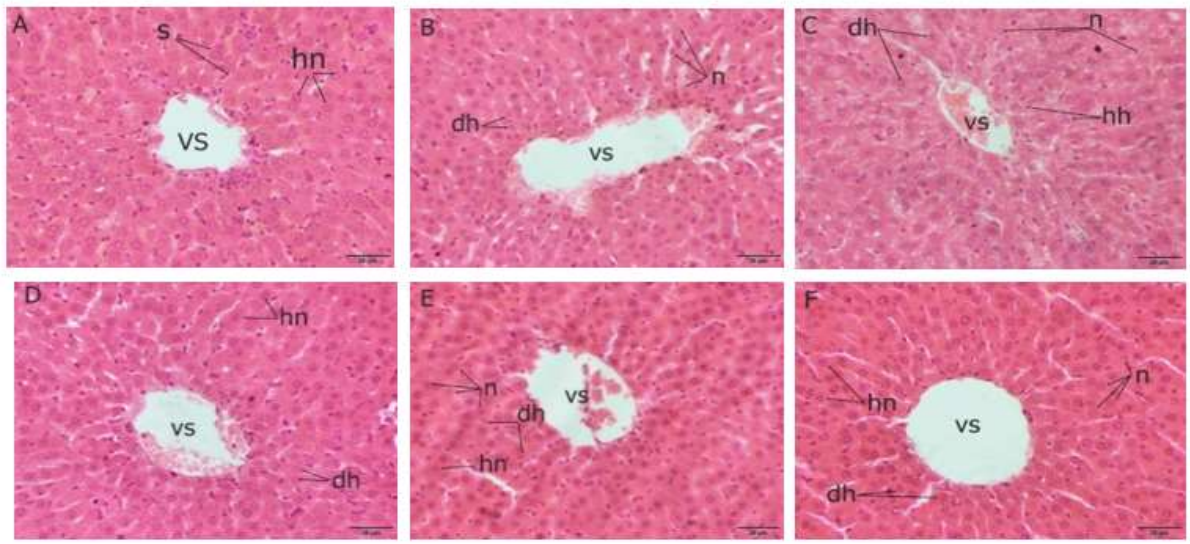

Figure 2 Histopathology of the liver of diabetic rats A = normal control group (GN); B = Diabetes control group (G0); C = Glibenclamide (G1); D = dosage 1 (G2); E = dosage 2 (G3); F = dosage 3 (G4); Vs = central vein; hn = normal hepatocytes; hh = hepatocyte vaculorization; dh = hepatocyte degeneration; n = necrosis.

Administration of *E. fluctuans* ameliorates liver damage in diabetic rats (Figure 2D, Figure 3). A dosage of 57.03 mg/kg (G2) results in the lowest percentage of liver cell damage and also more effective compare to glibenclamide (G1) (Figure 3). This suggests that *E. fluctuans* has ameliorative effect on liver damage in diabetic rats. The ameliorative effect of *E. fluctuans* aerial was attributed to the content of active compounds from the triterpenoid and steroid groups [10], which act as antioxidants. Exogenous antioxidants including terpenoids and steroids have been reported to reduce free radicals and prevent complications [26], regenerate liver cells, and prevent liver cell apoptosis [20]. Terpenoid groups such as swertiamarin inhibit translocation and accumulation of fatty acids into hepatocytes [27]. However, the influence of *E. fluctuans'* nhexane fraction on the amelioration of liver cell damage was the first to be reported.

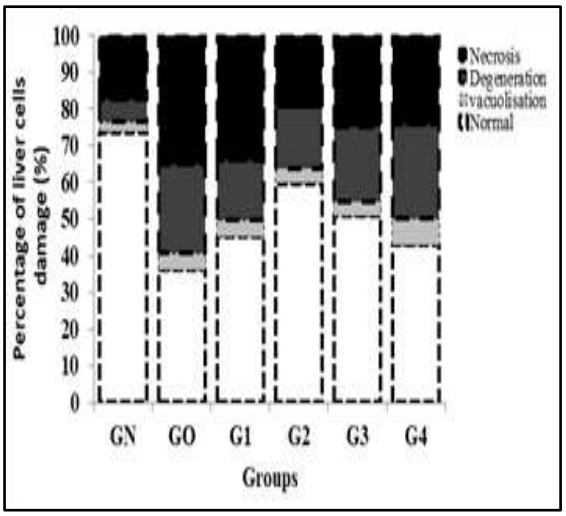

Figure 3. Percentage of liver cells damage in each treatment

Effect of E. fluctuans on SGOT and SGPT level

Effect of *E. fluctuans* on SGOT and SGPT activity show in Table 1. In Table 1 shows that diabetic rats have high SGOT and SGPT activity (G0). SGOT activity of diabetic rats was higher and significantly different ($p<0.05$) when compared to G1, G2, G3 and G4. SGPT activity in G2 was almost the same as SGPT activity in normal rats (GN). Meanwhile, the SGPT activity of diabetic rats that were given *E. fluctuans* was lower when compared to G0 and G1, although not significantly different ($p>0.05$). Increased levels of liver enzymes like SGOT and SGPT indicate cytosol leakage from liver cells into the bloodstream, which is an indicator of liver damage [(4)]. In addition, previous study revealed that there a positive correlation between liver damage and liver enzyme levels [(5)]. Udayakumar et al. [(28)] stated that if these enzymes have activities close to those of normal rat SGOT and SGPT, then the liver is said to be functioning normally. If the liver enzymes approach normal rat liver enzyme levels, the extract is said to have hepatoprotective activity [(29)].

Administration of *E. fluctuans* was able to reduce the activity of these two enzymes. *E. fluctuans* improve liver function via presenting a significant ($p<0.05$) reduction in the especially at 57.03 mg/kg (G2). This finding showed that *E. fluctuans* is hepatoprotective, so liver enzyme activity was normal. The results of this study support the research of Hasan et al. [(13)], who found that liver enzymes function normally with the administration of *E. fluctuans* etahnol extract in pre-diabetic rats. So, *E. fluctuans* not only improves the structure of liver tissue, but it also improves liver function. This result clearly indicates that *E. fluctuans* has an ameliorative effect on liver cell damage in diabetic rats.

Compounds involved in liver cell amelioration include those from the terpenoid and steroid groups [(30, 31)]. Terpenoid and steroid compounds have been shown to have high antioxidant activity and suppress inflammatory factors such as inflammatory factors like TNF-α, IL-6, and IL-1-β [(20)]. Steroids (28Nor-22(R)Witha 2,6,23-trienolide) improve blood glucose status and increase insulin levels [(30, 31)]. In other words, terpenoids and steroids are indirectly involved in the repair of liver damage due to high blood glucose. So, *E. fluctuans* aerial modulate liver cell regeneration.

The administration of the n-hexane fraction, especially at the dose of 57.03 mg/kg body weight (G2), has the activity of ameliorating liver cells and reducing SGOT and SGPT activity. The finding of this study indicates that *E. fluctuans* is potentially used for diabetes management. However, this study has limitations in terms of not using a single active substance, which means that other active compound contained in it might affect the results of this study.

Table 1. The mean SGOT and SGPT level

Groups	SGOT (U/L)	SGPT (U/L
GN	26.06 ± 4.22	27.44 ± 2.31
G0	42.98 ± 1.82[a]	42,34 ± 2.29
G1	35.44 ± 2.26[b]	39.10 ± 1.70
G2	30.80 ± 2.38[b]	35.04 ± 2.20
G3	32.96 ± 1.54[b]	33.60 ± 4.22
G4	32.44 ± 2.27[b]	34.00 ± 1.41

The several symbol indices (a, b, c) over each bar diagram demonstrate a significantly different ($p<0.05$) compared to diabetes control based on DNMRT.

Conclusion

In conclusion, the findings show that *E. fluctuans* has activity in lowering blood glucose, ameliorating damaged liver tissue structure, and lowering SGOT. Thus, *E. fluctuans* could be potentially prevented diabetes-related liver complications. However, further study is required to characterize the active compounds.

Acknowledgment

We are grateful to the head of Andalas University's Basic and Central Laboratories of the Integrated Service Division in effort to provide facilities.

Conflicts of Interest

No conflict of interest.

Funding

The research did not receive any financial support from institution.

Ethics Statements

The utilization of rats was governed by the Committee on Research Ethics of Andalas University's Medicine Faculty in Padang, Indonesia Number 038/KEP/FK/2019).

Author Contribution

The authors confirm contribution to the paper as follows: study conception and design: Rina, D., Djong HT; data collection: Rina D; analysis and interpretation of results: Rina, D, Djong, HT; draft manuscript preparation: Rina, D. All authors reviewed the results and approved the final version of the manuscript.

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