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Antihyperglycemic, Endothelial protection and Toxicity study of Basil Leaves Extract on Diabetic Rats

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Abstract

BACKGROUND: Diabetes Mellitus (DM) remains a serious debilitating global health problem in low- and middle-income countries with rising incidence of DM-related complications due to ineffective Diabetic control. Herbs of the Ocimum family, especially *Ocimum basilicum* or basil leaves, have been investigated for their antihyperglycemic properties.

AIM: This study aimed to demonstrate the antihyperglycemic effect, endothelial protection, and toxicity of basil leaves on Diabetes-induced Wistar rats *in vivo*.

METHODS: Streptozosin injections were used to induced diabetes in male Wistar rats. Basil leaves extracts 100, 300, and 1000 mg/kg BW were introduced to diabetic rats. Blood glucose levels (BGL), soluble Advanced Glycation End, tumor necrosis factor-α, interleukin (IL)-6, IL-2 were measured using enzyme-linked immunosorbent assay. Kidney and liver functions together with the histopathology reports were reported for acute, subacute, and chronic toxicity studies.

RESULTS: Basil leaves exposure significantly lowers BGL ($p < 0.00$), but yielded no statistically significant difference between extract doses. Hemostatic parametersshowed significantly reduced endothelial dysfunction markers for all doses compared to control. Toxicity study yielded no differences between control and any doses of basil leaves in all acute, subacute, and chronic toxicity studies. Histopathological findings exhibited no evidence of tissue damage on the liver, kidney, heart, pancreas, lung, and lymph tissues in either control or experiment rats.

CONCLUSIONS: Basil leaves exposure were positively associated with lower glucose level, lower endothelial activation markers on Diabetic rats. The toxicity and histopathological results of the extract are on par with control.

Introduction

Diabetes Mellitus (DM) remains one of the major contributors of preventable chronic disease with an alarming increase in incidence around the world within the past few decades [1], [2]. Type 2 DM (T2DM) compose 90% of DM cases in adults, imposing a substantial burden, especially in low-and middle-income countries [2], [3], [4]. The underlying key events for the development of T2DM are chronic hyperglycemia and insulin resistance [5]. There is evidence of endothelial dysfunction, macro and microvascular complications that associated with high mortality and morbidity of T2DM patients [6]. Endothelial dysfunction and hyperglycemia heightened vascular damage including the production of advanced glycation end (AGEs) products seen as alteration of Von Willebrand factor (vWF) as indication for endothelial function in diabetic microangiopathy [6], [7], [8]. The recommendation for T2DM management focus on achieving long-term good metabolic control through the improvement of lifestyle and pharmacological intervention, which can be seen from both macrovascular and microvascular complication endpoints [9], [10].

Dealing with diabetes and its complications requires very high patient compliance as the gold standard of treatment is the combination of diet, exercise, and medicine [11]. Standard drugs to control blood sugar levels and their complications have been circulating, but morbidity and mortality rates are still high, arguably, due to noncompliance with medication or fear of consuming drugs in the long term [12], [13]. Indonesia, particularly, with approximately 14 million T2DM patients, the fifth largest in the world, has been finding the importance of alternative treatments which are found abundant in herbal alternatives [14], [15].

Basil or *Ocimum basilicum* is a culinary herb of the Ocimum family or known as mints, abundantly found in Asia. The anti-diabetic properties have only been investigated in a few in vitro studies albeit showing a potential of lowering blood glucose level (BGL) through inhibition of the carbohydrate metabolizing enzyme, namely, amylase and glucosidase [16], [17], [18], [19].

Indonesia's archipelago and plethora of diverse medicinal plants have pioneered various studies on traditional medicines, including the usage of readily available basil leaves [20], [21], [22], [23]. Hence, it is imperative to further investigate basil leaves efficacy in the study using rats models.

Methods

This study was done after getting approved from the Health Research Ethical Committee Medical faculty of Universitas Sumatera Utara/H Adam malik General hospital number: 450/TGL/KEPK FK-USU-RSUPHAM/2018.

Preparation of basil extract

Basil extract was made using macerated dried *Ocimum sanctum.* The ground fine powder was extracted using 96% ethanol solution. The ethanol extract of basil leaves went through standardization using azeotropic distilation, gravimetry, and chromatography to determine water content, water solubility, and ethanol extract content, total ash content, acid insoluble content, as well as chromatogram profile. Afterward, suspension of each basil extract was made by mixing specific concentrations with carboxymethyl cellulose (CMC-Na) 2.0% solution.

Preparation of experimental animals, induction of diabetes, and introduction of extract

Male white Wistar rats of 180–200 g weight were prepared for two weeks to adapt to their surroundings, exposed to alternate 12 h of light and dark. Diabetes was induced by Streptozosin (STZ) injection. The STZ solution was prepared by dissolving STZ in a 50 mmol/L citrate buffer solution.

Wistar rats were fasted for approximately 18 hours, body weight (BW) weighed, and fasting blood sugar levels measured using the GlucoDr tool to obtain baseline data. STZ solution in citrate buffer 55 mg/kg BW was given through intraperitoneal injection. Rats blood sugar levels were measured on the 3rd and 7th days. On the 7th day, rats with BGLs higher than 250 mg/dl were grouped into tested group. Rats with blood sugar levels lower than 250 mg/dl were induced again. If on the 3rd day the BGL of the test animal is more than 250 mg/dl, then the rats would be grouped into treatment group.

Suspension of the test material (Basil extract) was administered orally and BGLs were measured on the 3rd, 7th, and 10th day after STZ induction. Each treatment group will be given basil extract dosage of 100, 300, 1000 mg/kgBW, respectively.

Antihyperglycemic effect of the basil extract

To evaluate the antihyperglycemic effect of basil extract, a total of 25 rats weigh 180–200 g were divided into five groups, which consist of three treatment groups of dosage (n = 5) of 100, 300, and 1000 mg, negative control group (normal rats), and positive control (diabetic rats without treatment). BGL was measured at days 0, 3, and 7 after STZ induction. Basil extract treatment started on day 7. The follow-up measurements of blood glucose was conducted at day 0, 3, 6, 9, 12, and 14 after treatment.

AGE, tumor necrosis factor (TNF)-α, vWF level assessment in rats

The values of the soluble AGE, TNF-α, interleukin (IL)-6, IL-2 were determined using a kit obtained from Fine Test and performed using the enzyme-linked immunosorbent assay method.

Toxicity study

A total of 50 rats induced with 50 mg/kg BW STZ solution underwent acute, subacute, and chronic toxicity study. Acute toxicity study utilizes lethal dose of 1500, 3000, and 5000 mg, subacute and chronic toxicity study at 100, 300, and 1000 mg. Evaluation of toxicity study comprised of BGL, liver function tests (Serum glutamic oxaloacetic transaminase [SGOT], serum glutamic pyruvic transaminase [SGPT], Alkaline Phosphatase, Gamma GT), kidney function tests (Urea and Creatinine), and endothelial activation (vWF and prothrombin fragment F1+2) of the STZ-induced rats.

Further, histopathological examination was conducted to evaluate toxicity through assessment of necrosis of liver cells, kidney cells, pancreas, heart, lung, brain parenchymal cells with H and E staining.

Statistical analysis

Statistical analysis was done on PRISM GraphPad version 8 and Statistical Package for Social Sciences. One-way analysis of variance and *Post-hoc* Tamhane test was done. The aqua dest control group was compared with the diabetic group. Data were expressed as mean ± Standard deviation. The results were rationally analyzed and $p < 0.05$ was considered statistically significant.

Results

Baseline data of rats

To obtain baseline value, twenty-five male Wistar rats were selected, with average weight of 184.7 ± 13.6 g and average BGL 78.8 ± 9.0 mg/dL.

Effect on BGL

There was a consistent reduction on the average BGL after being given 100 mg/kg BW basil extract from 423 mg/dL on day 0, to day 3, 6, 9, 12, and 14, respectively, at 384 mg/dl, 268 mg dl, 189 mg/dl, 126 mg/dl, and 102 mg/dl. Similar reduction was found after 300 mg/kg BW basil extract administration, starting from 487 on day 0, 401.6 mg/dL at day 3 to 283 mg/dl on day 6, 214 on day 0, 137 mg/dL on day 12, and 93.3 mg/dL on day 14 [Figure 1].

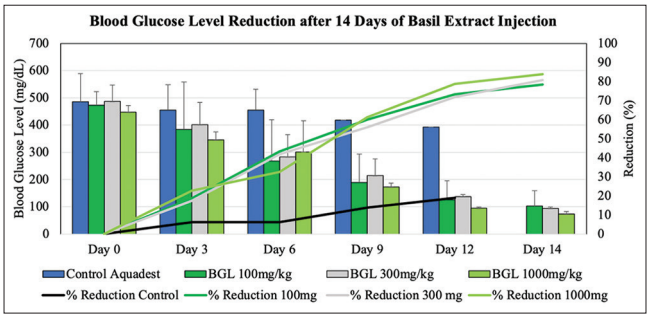

Figure 1: Comparison of antihyperglycemic effect between dose 100 mg/kgBW, 300 mg/kgBW, and 1000 mg/kg BW

Overall, the highest reduction of BGL was achieved with Basil extract 1000 mg/kg BW at 83.75% followed by dosage 300 mg/kg BW at 80.34%, and finally the least dosage 100 mg/kg BW at 78.43%. In addition, control group did not complete the 14 days test, 80% of control sample died before day 14. Statistically, through the one-way ANOVA *Post hoc* Tamhane test, the reduction of BGL showed significant difference ($p < 0.00$) compared to control. However, the end result of BGL between dosage groups at day 14 did not yield statistically significant difference.

Results of Hemostatic Parameters Assessment in Rats

A total of 18 male Wistar rats were tested for changes in inflammatory and endothelial activation markers such as AGE, TNF-α, and vWF levels. We found no statistically significant difference of AGE level between normal control and basil leaves extract on all three dosages of 100 mg ($p = 0,12$), 200 mg ($p = 0,33$), and 400 mg ($p = 0,26$). On the other hand, basil leaves extract showed significant reduction of endothelial activation shown by WF and TNF-α reduction compared to control.

Table 1: Blood chemistry markers on acute toxicity study obtained after lethal dose of basil extract

Parameter	SGOT (IU/L)	SGPT (IU/L)	Alkaline Phosphatase (IU/L)	Gamma GT (U/L)	Urea (mg/dL)	Creatinine (mg/dL)
Control	100	33	371	3.6	57.3	0.46
Basil 1500 mg	142	41	510	16	110	0.40
Basil 3000 mg	93	30	566	11	77	0.30
Basil 5000 mg	110	37	686	13	97	0.43

SGOT: Serum glutamic oxaloacetic transaminase, SGPT: Serum glutamic pyruvic transaminase.

Toxicity study

Effects on biochemical parameters

The acute toxicity study conducted did not show any morbidity or mortality, behavioral changes, or other symptoms. The blood chemistry markers on three dosages were within normal limits albeit slight elevation in SGPT, alkaline phosphatase, Gamma GT, and Urea levels, however, there was no statistically significant difference compared to control [Table 1]. The biochemical reference range for SGOT is 44-147 IU/L, SGPT 10–40 IU/L, ALP 50–150 IU/L, Gamma GT 1–5.3 U/L, urea 15–45 mg/dL, and creatinine 0.2–0.8 mg/dL [24], [25], [26], [27].

Table 2: Blood chemistry markers on subacute toxicity study

Parameter	SGOT (IU/L)	SGPT (IU/L)	Alkaline Phosphatase (IU/L)	Gamma GT (U/L)	Urea (mg/dL)	Creatinine (mg/dL)
Control	24	140	275	25	41	0.37
Basil 100 mg	69	165	255	30	53	0.43
Basil 300 mg	66	183	300	28	86	0.38
Basil 1000 mg	35	102	280	13	40	0.51

SGOT: Serum glutamic oxaloacetic transaminase, SGPT: Serum glutamic pyruvic transaminase.

As for the subacute toxicity study, throughout the period, there were no observed changes, symptoms, or behavioral and neurological alterations. There is no morbidity or mortality in all rate samples. The differences within and between groups are not significant [Table 2].

Table 3: Blood chemistry markers on chronic toxicity study

Parameter	SGOT (IU/L)	SGPT (IU/L)	Alkaline Phosphatase (IU/L)	Gamma GT (U/L)	Urea (mg/dL)	Creatinine (mg/dL)
Control	148.32	35	368	21.55	25.06	0.32
Basil 100 mg	88.99	35.66	377	11.8	30	0.43
Basil 300 mg	138.99	47.32	394	16.22	28.,5	0.35
Basil 1000 mg	70.33	32.66	302	13.8	49.2	0.4

SGOT: Serum glutamic oxaloacetic transaminase, SGPT: Serum glutamic pyruvic transaminase.

Chronic toxicity study of basil leaves extracts effect on the treatment batch yields no changes in morbidity and mortality. There were inconsistent variations and increases of liver and kidney function tests results between dosages, however, the treatment groups showed no statistical differences compared to control group [Table 3].

Despite showing elevated numbers of alkaline phosphatase, and gamma GT, evaluation of blood chemistry markers of liver damage (SGOT, SGPT, Alkaline phosphatase, and gamma GT), markers of kidney damage (urea and creatinine) did not show significant differences between treatment group and control as well as within group.

Histopathological findings

Histopathological assessment on sections of the liver, kidney, heart, pancreas, lung, and lymph using light microscopy to assess the effect of basil leaves extract in STZ-induced diabetic tissues. Pathologically, all organ sections in all dosage groups did not show anatomical abnormalities administration compared to control group [Figure 2].

Liver cells showed normal and uniform arrangements of hepatocytes. Neither hepatocytes destruction nor congested hepatic inflammation was seen. In regards to lymph biopsy, there is no congestion or necrosis indicative of lymph damage. In all treatment and control groups, the renal tissue showed normal glomeruli, the epithelial lining of Bowman's capsule and mesangial cells, and no necrotic tissue found. The tubules were preserved well with normal columnar epithelial cells. Normal lung biopsy was obtained, the alveolar sac was not clearly seen. There is no foamy macrophage seen despite increase of infiltrate in the rat lung tissue. The pancreas was found normal, there was no evident of necrosis, degranulation, or shrinkage particularly in Langerhans Islet.

Discussion

Basil leaves is one of the numerous medicinal herbs recommended for Diabetes treatment. Previously, basil extract was found to decrease the classic Diabetes symptoms, namely, polydipsia, polyphagia, and polyuria in 30 T2DM patients with consumption of 2 g basil leaf powder for 3 months daily [28]. In the present study, the effects, dosage, and toxicity of the basil leaves extract were evaluated in concordance to its anti-inflammatory properties.

Basil leaves, herbs from the Ocimum family have been proposed to possess antihyperglycemic properties in multiple studies [16], [17], [20], [29], [30], [31]. This study found significant blood glucose -lowering effect and endothelial protection of basil leaves. The findings are consistent with our previous study which also elucidates that basil leaves extract effectivity to reduce blood glucose was on par with Metformin 50 mg/kg BW control group [18].

Insulin-secretory effect of the Ocimum family was elucidated by Hannan *et al*., as the extract enhances effects of insulin secretion from the pancreas, isolated islets, and modulation of intracellular calcium [30]. Basil leaves also shown to possess insulinotropic effect through the enhancement of physiological pathways on pancreatic beta-cells [20]. Thus, increasing plasma insulin and decreasing BGL in rats with type 2 diabetes [30].

In regard to biochemical markers, there is still lack of in-depth studies on the toxicity effect and biochemical profile of basil leaves. One attempt, by Huq *et al*., in a basil-based tea, found that in addition to antidiabetic effect, there are no harmful effects on the liver by evaluation of SGPT [32], [33]. The anti-inflammatory effect of *Ocimum sanctum* was lacking as well. However, *Ocimum tenuiflorum* has been researched to possess pharmacological activities involving reduction of lipid peroxidation, increasing uptake of radical scavenging activity, and stimulation of antioxidant enzymes [33], [34].

Through histopathological biopsy, this study found normal results in the liver, kidney, lymph, lung,

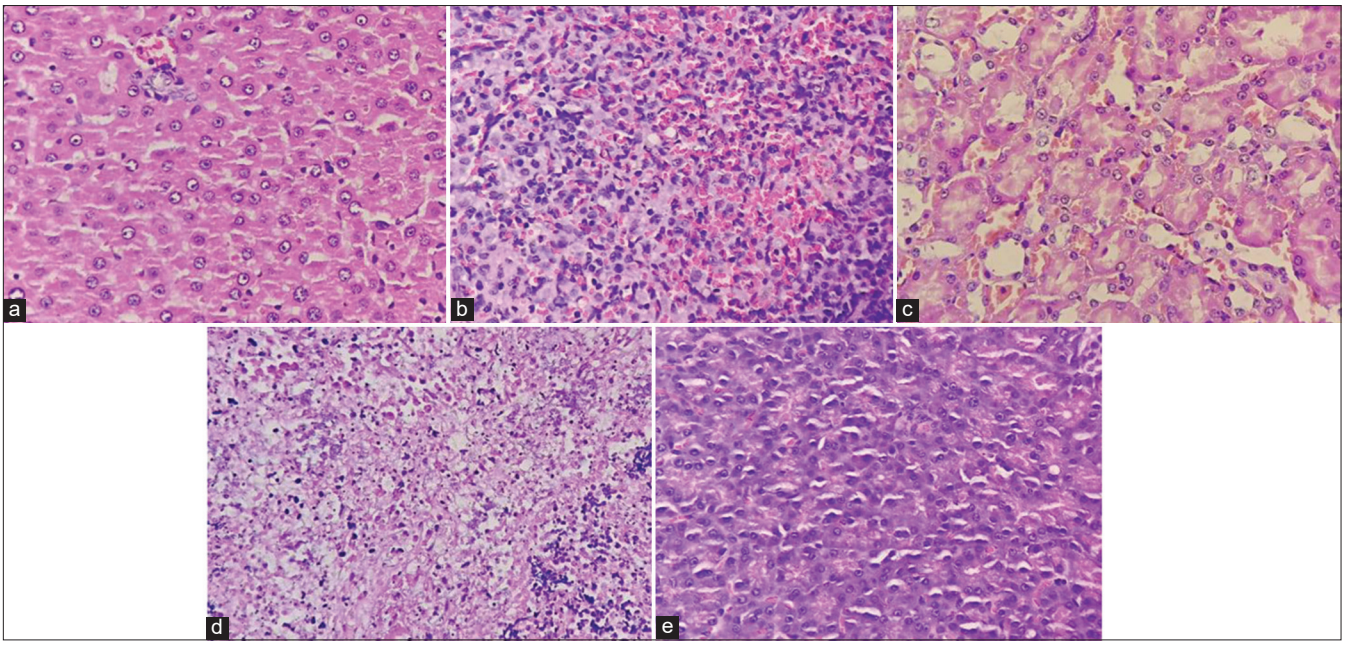

Figure 2: Comparison of microscopic representation of histological sections in toxicity study. (a) Liver (b) Lymph (c) Kidney (d) Lung (e) Pancreas

and pancreas which are in concordance with the biochemical markers from blood tests. SGOT and SGPT were slightly increased but none of which showed significance difference compared to their control sample counterparts. In addition, all of the Wistar rats results of SGPT were increased. It is known that SGOT is the least specific of the two because SGOT was not only produced by the liver but also by the heart. On the other hand, SGPT directly reflects liver damage as it is directly produced by liver cells. Therefore, SGPT is the more reliable marker along with liver enzymes such as alkaline phosphatase [35], [36]. The increase in SGPT, ALP, and Gamma GT might indicate liver damage, however, it is debunked as even the normal Wistar rats possess similar levels of SGPT, ALP, and Gamma GT.

Conclusions

This study confirmed the antihyperglycemic and antiinflammatory effects of basil leaves *(Ocimum sanctum)*, as well as safety in acute, subacute, and chronic setting. This study will be the pioneer to further unravel the potential of basil leaves extract for Diabetes treatment. In the future, more work needs to be directed in achieving characterization of active components and human trials to be further formulated into novel antidiabetic agents.

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