Antihyperglycemic capacity of basil (*Ocimum basilicum* L.) leaves extracts coated with the marine fish scales derived nanochitosan

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**Abstract.** Basil (*Ocimum basilicum* L.) is well-known as a medicinal plant and culinary herb because of its phytochemical contents. Crude extracts of basil leaves are of particular interest for health benefits, including in reducing blood glucose level. Nanochitosan is potentially applied to increase the efficacy of phytopharmaceuticals due to its low viscosity that easily absorbed by the tissues. This study was conducted to evaluate the antihyperglycemic capacity of basil leaf extracts when coated with nanochitosan. Nanochitosan was prepared from the parrot fish (*Scarus* sp) scales derived Chitosan by using the gelatin ionic method. Both chitosan and nanochitosan were characterized by Fourier Transform Infrared Spectroscopy (FTIR), SEM, particle sizing and heavy metals analyses. Extraction of basil leaves were performed by a maceration method using ethanol 96% and the extract was evaporated. Nanochitosan was then applied as coatings of the extracts, and the nanochitosan coated extract was separated from the solution by centrifugation. In vivo assay was applied using 24 male rats, which divided into extract treated groups and the control groups. All the animals were fasted for 12 h, but were allowed free access to water, before commencement of the experiments. Hyperglycemia condition was induced by a single intraperitoneal dose of 120 mg/kg. The rats were fasted for 12 h and blood was taken from the tail artery of the rats. The extract of basil leaves at the dose of 400, 800, 1000 mg/kgBW significantly lowered blood glucose level (*P*<0.05) of rat in hyperglycemic after 5 hours of extract administration. The efficacy of antihyperglycemic dose of basil leaves extracts coated with nanochitosan was found to be 400 mg/kgBW. Based on the result of the study, it is suggested that nanochitosan could increase the efficacy of phytopharmaceuticals.

1. Introduction

Diabetes mellitus is one of the most common metabolic disorders [1]. It is mainly characterized by a loss of glucose homeostasis by an interruption in carbohydrate, fat and protein metabolism and defects in insulin secretion, insulin action, or both [2]. Deficiency of insulin can cause some part in body fall short in using glucose from the blood circulation. This results in elevation of blood glucose level, which is known as hyperglycemia [3]. Hyperglycemia is directly related to increased hepatic glucose production. The ensuing β-cell dysfunction promotes decreased insulin synthesis and secretion, further perpetuating the associated hyperglycemia. As for the vascular endothelial, chronic hyperglycemia is strongly correlated with many Diabetes mellitus related microvascular complications, including retinopathy, nephropathy, and neuropathy [4]. Glycemic management of diabetes is needed
to avoid complications of the disease that will occur. Studies have conclusively determined that reducing blood sugars decreases the onset and progression of microvascular complications [5].

A possible antihyperglycemic capacity in *O. basilicum* has been reported in many studies [6][7]. *O. basilicum* is a common herb that is known for its ornamental and therapeutic importance. The chemical constituents which have been isolated from the plant include terpenoids, alkaloids, flavonoids, tannins, saponin glycosides and ascorbic acid [8]. These compounds have been related to reduce blood glucose level. However, the active compounds that provide pharmacological effects such as flavanoid compounds and polyphenols have weaknesses due to its unstable against influence temperature and high light intensity that make easily oxidized [9]. The solution to protect these compounds is to use coating method with nanoparticle. Nanoparticle is defined as a solid particle with size range of 1-1000 nm [10]. The superiority of chitosan such as biocompatible, biodegradable, and non-toxic can be developed as a coating for an extract.

Chitosan is obtained from the deacetylation of chitin, a naturally occurring and abundantly available (in marine crustaceans) biocompatible polysaccharide [11]. In this study, chitosan was extracted from parrot fish scales (*Scarus* sp) because it refers to a previous study which has successfully tested the active group of chitin in parrot fish scales that can be developed into chitosan [12].

Modification basic structure of chitosan to nanochitosan as a coating of an extract provide more benefits caused by its low viscosity properties so that it is more easily absorbed by the body and able to deliver nutrients to the target cell [13]. Nanochitosan coated on basil leaf extract also was gave increased the total phenolic compound [14]. This study aims to analyze the antihyperglycemic capacity of basil (*O. basilicum* L.) leaves extracts coated with nanochitosan derived the marine fish scales.

2. Materials And Methods

2.1. Plant Material

Basil plants varieties Minahasa were collected from market local in Minahasa. The basil leaf extraction process refers to the modified Sangi and Katja (2011) methods [15]. Wet basil (*O. basilicum*) leaves are sorted, sliced, dried. The dried simplicia is smoothed with a blender. Basil leaf powder was extracted by maceration with 96% ethanol. A total of 200 g of basil leaf powder was macerated in 2000 ml of 96% alcohol for 24 hours. The filtrate from maceration is then evaporated using a vacuum evaporator to remove the ethanol solvent to obtain a thick extract. The thick extract of basil leaves was heated in an oven at 40°C for 24 hours.

2.2. Extraction of chitosan

The fish scales of parrotfish (*Scarus* sp.) was collected from local markets in North Sulawesi. Preparation of chitosan fish scales refers to the modified Suptijah et al (1992) method [16], and starts with extraction chitin as reported by Rumengan et al (2017) [12]. Chitosan is obtained with deacetylation of chitin with add 40% NaOH (1:10), then heated at a temperature of 100-110°C for 1 hour. The solids obtained are washed with distilled water until neutral pH befordried in the sun. The chitosan obtained was weighed and stored at room temperature.

2.3. Preparation of nanochitosan and coating method

Preparation of nanochitosan using the ionic glass method [17]. Tween 80 as much as 0.1% added as homogenizer and tripoliposphate (TPP) 0.1% as stabilizer. Coating of leaf extract basil with nanochitosan refers to Kurniasari and Atun (2017) method which has been modified [18]. The coating process is carried out with add basil leaf extract coated with nanochitosan at extract ratio Chitosan: extract was 2 : 1. Then homogenized using a magnetic stirrer for 30 minutes. Solution of the basil leaf extract coated with nanochitosan were centrifuged.

2.4. Experimental design
In this experiment, a total of 24 adult male Wistar rats was used. All the animals were fasted for 12 h, but were allowed free access to water, before commencement of the experiments. The rats were divided into 8 groups of 3 rats each. The animal of group 1 served as negative control was given accolades; group 2 served as a positive control group was given glibenclamid at dose 0.45 mg/kgBw; group 3 to 5 were given basil leaf at dose 400 mg/kgBw (BLE 1); 800 mg/kgBw (BLE 2); and 1000 mg/kgBw (BLE 3) respectively. Group 6 to 8 were given extracts basil leaf coated with nanochitosan at dose 400 mg/kgBw (BLECN 1), 800 mg/kgBw (BLECN 2), and 1000 mg/kgBw (BLECN 3) respectively.

Diabetes mellitus was induced in overnight-fasted rats by single intraperitoneal injection of freshly prepared Alloxan monohydrate dissolved in sterile normal saline at a dose 150 mg/kg body weight. Diabetes mellitus was confirmed by measuring fasting blood glucose levels after 3 days. Rats with fasting blood glucose of more than 126 mg/dL were considered as diabetics and used for further experiment [19]. Treatment was given immediately after the determination of diabetes in rats. Measurement of fasting blood sugar was carried out every 1 hour after treatment was given. All blood samples were collected from the tail artery of the rats at the interval.

Determination of the blood glucose levels was done using a GlucoDr TM instrument. Blood glucose levels for each group were expressed in mg/dl as mean ± SD. The data were statistically analyzed using ANOVA with multiply comparisons versus control group and t test to see optimal dose of extract coated with nanochitosan. The values of p<0.05 were considered as significant [20].

3. Results and Discussion

| Group        | Fasting blood glucose pra alloxan (mg/dL) | Fasting blood glucose post alloxan (mg/dL) | Δ(%) |
|--------------|-------------------------------------------|---------------------------------------------|------|
| Control (-)  | 75 ±5.000                                 | 140 ± 9.165                                 | 46   |
| Control (+)  | 64 ±1.528                                 | 136 ± 1.32                                  | 53   |
| BLE 1        | 86 ±7.211                                 | 137 ± 15.716                                | 37   |
| BLE 2        | 69 ±3.215                                 | 136 ± 8.505                                 | 49   |
| BLE 3        | 67 ±15.275                                | 139 ± 11.533                                | 52   |
| BLECN 1      | 65 ±5.000                                 | 139 ± 35.726                                | 53   |
| BLECN 2      | 68 ±3.055                                 | 136 ± 15.177                                | 50   |
| BLECN 3      | 68 ±6.429                                 | 136 ± 6.506                                 | 51   |

Control (-) : aquades; Control (+) : glibenclamid at dose 0.45 mg/kgBw; BLE 1 : basil leaf extract at dose 400 mg/kgBw; BLE 2 : basil leaf extract at dose 800 mg/kgBw; BLE 3 : basil leaf extract at dose 1000 mg/kgBw. BLECN 1 : basil leaf extract coated nanochitosan at dose 400 mg/kgBw; BLECN 2 : basil leaf extract coated nanochitosan at dose 800 mg/kgBw; BLECN 3 : basil leaf extract coated nanochitosan at dose 1000 mg/kgBw.

The average of fasting blood glucose level in all groups before administration of alloxan according to Table 1 were in the range of 64-86 mg/dL. The highest fasting blood glucose level was found to be in BLE 1 group which was 86 mg/dL and the lowest was in the positive control group which was 64 mg/dL. After administration of alloxan, the rats' fasting blood glucose level in all groups were increased. The highest fasting blood glucose level was increased in the positive control group and BLECN 1 with an increase of 53%. The lowest increase was in the negative control group with a percentage increase of 46%.

Normal fasting blood glucose levels in rats was ≤100 mg/dL [21]. It means that the level of fasting blood glucose level before administration of alloxan was in the normal category. In pharmacology / bioactivity testing of experimental animals, the state of diabetes mellitus can be induced by pancreatectomy and the administration of chemicals. In this study was using alloxan as a chemical to make diabetic rats characterized by a hyperglycemic state. Alloxan is a hydrophilic compound and unstable. As diabetogenic, alloxan can be used intravenously, intraperitoneally and
subcutaneously. This study was used an intrapertitoneal injection method because it is considered more effective.

**Table 2. Change in fasting blood glucose level after treatment for five hours**

| Group          | Fasting glucose post alloxan | Time (Hours) |
|----------------|-----------------------------|--------------|
|                |                             | 1            | 2            | 3            | 4            | 5            |
| Control (-)    | 140 ± 9.165                 | 139 ± 19.553 | 148 ± 9.018  | 152 ± 6.110  | 153 ± 6.658  | 158 ± 26.633 |
| Control (+)    | 136 ± 1.732                 | 128 ± 6.807  | 119 ± 5.568  | 93 ± 2.887   | 90 ± 0.577   | 71 ± 8.544   |
| BLE 1          | 137 ± 15.716                | 136 ± 14.526 | 137 ± 13.50  | 126 ± 2.039  | 102 ± 4.509  | 94 ± 3.786   |
| BLE 2          | 136 ± 8.505                 | 129 ± 9.018  | 104 ± 7.371  | 84 ± 5.132   | 91 ± 19.757  | 79 ± 1.000   |
| BLE 3          | 139 ± 11.533                | 137 ± 6.928  | 101 ± 17.03  | 98 ± 9.000   | 84 ± 6.028   | 115 ± 15.011 |
| BLECN 1        | 139 ± 35.726                | 128 ± 32.047 | 100 ± 10.44  | 89 ± 9.644   | 80 ± 2.000   | 73 ± 6.506   |
| BLECN 2        | 136 ± 15.177                | 125 ± 18.028 | 142 ± 35.53  | 165 ± 44.44  | 130 ± 20.55  | 107 ± 12.014 |
| BLECN 3        | 136 ± 6.506                 | 132 ± 8.185  | 100 ± 9.609  | 87 ± 8.888   | 79 ± 8.327   | 72 ± 9.713   |

Control (-) : aquades; Control (+) : glibenclamid at dose 0.45 mg/kgBW; BLE 1 : basil leaf extract at dose 400 mg/kgBW; BLE 2 : basil leaf extract at dose 800 mg/kgBW; BLE 3 : basil leaf extract at dose 1000 mg/kgBW; BLECN 1 : basil leaf extract coated nanochitosan at dose 400 mg/kgBW; BLECN 2 : basil leaf extract coated nanochitosan at dose 800 mg/kgBW; BLECN 3 : basil leaf extract coated nanochitosan at dose 1000 mg/kgBW

According to Table 2, there were a changed of fasting blood glucose (FBG) level to all group after being treated with basil leaf extract with or without coated by nanochitosan and glibenclamide drug for five hours of observation. In the negative control group there was an increase in FBG level every hour during 5 hours of observation, while in the other groups had a decreased even though not all groups had a stable decreased. At the first hour after the treatment all groups didn’t show a significant change in FBG. It was changed occur at the second hour until the fifth hour after treatment.

![Graph of changes in blood glucose level during 5 hours of observation](image)

**Figure 1.** Graph of changes in blood glucose level during 5 hours of observation

A: early blood glucose post alloxan  C: blood glucose at 2nd hour  E: blood glucose at 4th hour
B: blood glucose at 1st hour  D: blood glucose at 3rd hour  F: blood glucose at 5th hour
In Figure 1 it is clear that the positive control group, BLE 1, BLECN 1 and BLECN 3 have a stable graph of FBG level reduction compared to the other groups for 5 hours of observation. In the negative control group there was a steady increase in FBG level. In the fifth hour most of the rats were in the normal FBG category in the range of 70-100 mg / dL except in BLE 3 group (115 mg / dL) and BLECN 2 (107 mg / dL) which were still in the category of impaired fasting blood glucose level which defined in the range of 100 - 125 mg / dL [22]. Based on One Way Anova test, indicate that there was an effect of giving basil leaf extract and basil leaf extract coated with nanochitosan on blood glucose of hyperglycemic mice, with a significance number = 0.004 (<0.05). This value shows that among treatment groups had different mean values significantly. Before testing ANOVA, the data has been tested for normality and homogeneity.

The comparison of each treatment group with the positive control group showed no significant difference. That means the administration of glibenclamide drug which is a commercial drug for diabetics was almost the same as giving basil leaf extract in its function to reduce high blood glucose levels (hyperglycemia). Glibenclamide is a second generation sulfonyluea drug which has the potential as an antihyperglycemic agent. The mechanism of action stimulates insulin secretion from beta langherhans cells, usually used for damage to langherans with less severe cells [23].

The results of this study are in line with the research of Mohammed et al (2007) who used basil species in Africa (Ocimumum gratissium) to test the antidiabetic effects of basil leaves in streptozocin-induced wistar rats [7]. The results of the study stated that at the 2nd hour of observation, there was a decrease in the group given the extract, but the increased in blood glucose level occurred after 4th hour of treatment. The group was given basil leaf extract at a dose of 500 mg /kgBW was reported to have the most effective effect with the presentation of glycemic changes of 81.3% for 8 hours of observation. While at doses of 250 mg /kgBW and 1000 mg / kg BW did not show a significant changes. Basil leaf extract at a dose of 400 mg /kgBW can increase antidiabetic effects and inhibit hepatic glucose mobilization and metabolism of carbohydrate enzymes for 2 hours of treatment [24]. In addition, basil leaf extract can also increase insulin secretion from the pancreas in 70 minutes of observation [25].

The effectiveness of nanochitosan in coating basil leaf extract was seen in the BLECN group at a dose of 400 mg /KgBW when compared to the BLE group with the same dosage (p = 0.023). The results of this study indicate that nanochitosan can increase the effectiveness of basil leaf extract as antihyperglycemic. A similar study was conducted by Malapermal et al (2017) using silver nanoparticles to increase the antidiabetic effect of basil leaves (O. basillicum) and successfully reduce blood glucose level by 79.74% [26].

Active compounds such as flavonoids have an activity in increasing insulin secretion by increasing the entry of Ca²⁺ ions through Ca channels [27]. Saponin compounds work by reducing absorption in the intestine by decreasing glucose absorption and modifying carbohydrate metabolism, increasing glucose utilization in peripheral tissues, glycogen storage and increasing sensitivity of insulin receptors in tissues. Tannin also acts as an antihyperglycemic with a mechanism to inhibit intestinal glucose uptake and inhibits adipogenesis and antioxidants beside its ability to regenerate damaged pancreatic β cells [28]. Basil leaf extract has antihyperglycemic activity caused by its phenolic components contained in it which play a role in lowering blood glucose levels [29].

4. Conclusion
The administrate of basil leaf extract in hyperglycemic diabetic rats was shown to reduce blood glucose levels in all basil leaf extract group. The effectiveness of nanochitosan coating on basil leaf extract was shown at BLECN 1 at dose of 400 mg/kgBW (p = 0.023). It is indicated that the basil leaf extract has an antihyperglycemic capacity and its phytopharmaceuticals increased caused by coated with nanochitosan.
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