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PHYTOCHEMICALS, ANTIOXIDANT, α-AMYLASE INHIBITORY AND ANTIHYPERGLYCEMIC POTENTIALS OF ETHANOL EXTRACT FROM *Nauclea orientalis* BARK

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ABSTRACT

Natural products are used as alternative medicines to treat various diseases including diabetes. *Nauclea orientalis* belongs to the family Rubiaceae and has been utilized as a traditional herbal for decades in many countries. The present investigation aimed to evaluate the antioxidant, α-amylase inhibitory, anti-hyperglycemic properties, and phytochemical contents in the ethanol (EtOH) Bark Extract of this plant. The EtOH Bark Extract showed that the IC_{50} value of DPPH scavenging activity was 20.69 ± 5.41 µg/mL whereas the standard ascorbic acid was 11.13 ± 2.81 µg/mL. The enzyme inhibitory potential of the extract against α-amylase was also determined with IC_{50} value of 242.2 ± 83.87 µg/mL. Compared to control animals, when administered at 400 mg/kg, the EtOH Bark Extract reduced blood glucose levels and body weight by 27.04% and 19.21%, respectively. *N. orientalis* bark extract reduced blood glucose and the area under the curve (AUC) after the oral glucose tolerance test. Phytochemical screening indicated the presence of carbohydrates, alkaloids, flavonoids, phenols, tannins, and saponins that might contribute to the biological activities from *N. orientalis*. In conclusion, this study demonstrates the EtOH Bark Extract of *N. orientalis* which possesses potent antioxidant, α-amylase inhibitory, and antihyperglycemic activity might be a potential candidate for biological and chemical analysis in pharmacy applications.

Keywords: Nauclea orientalis, antioxidant, α-amylase inhibition, antidiabetic, plant extracts.

1. INTRODUCTION

Diabetes mellitus is described as a metabolic disorder characterized by a chronic hyperglycemic condition because of impaired insulin secretion or insulin resistance [1]. It is one of the oldest diseases that have affected the global population due to the alteration of modern society such as sedentary lifestyle, aging, obesity, depression, and smoking. Technically, diabetes can alter carbohydrate metabolism leading to insulin resistance and thus producing a hyperglycemic state in the blood. As a result, hyperglycemia is one of the most physiological abnormal characteristics in diabetic patients [2]. The high blood glucose levels in diabetes have damaged many tissues and organs such as the brain, eyes, feet, kidneys, heart, and vascular system. Currently, several pharmaceutical therapies have approved for diabetic treatment based on the stimulation of insulin release, the increase of glucose transporters, or the reduction of glucose absorption via several molecular pathways [3]. Firstly, the beneficial therapy targets digestive enzymes such as α-amylase and α-glucosidase to inhibit carbohydrate digestion leading to the reduction of glucose absorption. Secondly, there are multiple resources for the generation of reactive oxygen species (ROS) including superoxide, hydroxyl, peroxyl, or hydroperoxyl under hyperglycemic conditions during the diabetic progression [4].

Therefore, the utilization of antioxidants has been applied to the strategy therapy of diabetes. In recent years, there are commercial antidiabetic medicines containing insulin, sulfonylureas, thiazolidinediones, and metformin [5]. These drugs can be utilized singly or in combination to promote the hyperglycemic state. However, there are serious adverse effects such as weight gain, hypoglycemia, and liver toxicity from these drugs [6]. Therefore, natural products from plant extract may be the alternative opportunity for novel therapeutic agents with fewer side effects to cure diabetes. Many medicinal plants have been investigated for hypoglycemic activities of diabetes mellitus and confirmed in animal models [7-9].

Nauclea orientalis (*N. orientalis*) belonging to the family Rubiaceae is known as the medicinal plant in Vietnam, Thai Land, India, and Australia [10, 11]. Recently, a study by Sandamali and colleagues reported that the aqueous bark extract of *N. orientalis* can protect the cardiomyocytes in rats against Dox-induced cardiotoxicity by the attenuation of oxidative stress, inflammation, and apoptosis [12]. The leaves of *N. orientalis* have shown the presence of tannins, phenols, saponins, flavonoids, and sterols as well as possessed the ability for antihelmintic activity on earthworms [10]. Additionally, the previous study revealed that the most abundant compositions from the leaf and stembark extracts were alkaloid glycosides and their derivatives such as strictosamide and other aglycones [13]. The indole alkaloids isolated from the stems, leaves, and roots of this plant were evaluated the cytotoxicity on cancer cell lines [14, 15]. However, there is no available report on the anti-hyperglycemic and α-amylase inhibitory potentials of the bark of *N. orientalis*. Therefore, the present study investigated the antioxidant, α-amylase inhibitory, anti-hyperglycemic properties, and phytochemical constituents of the EtOH Bark Extract from *N. orientalis* based on its traditional utilization.

2. MATERIALS AND METHODS

2.1. Material

The bark of *N. orientalis* was collected from natural resources at Road 54, Dinh Thanh hamlet, Dinh Hoa commune, Lai Vung district, Dong Thap province, Vietnam in August, 2022. Mature healthy plants over five years old were utilized for the collection of stem bark (50-80 cm in height) and chosen by the electric hand saw. The inner of the bark (the cork) from stems was selected with a carving knife and washed thoroughly with sterilized distilled water and then dried at room temperature for 24 h. The bark from the stems was stored at 4°C before utilization. All healthy Swiss albinos (20 - 35 g, 6-8 weeks old) were obtained from the Laboratory Animals of the Pasteur Institute Ho Chi Minh City, Vietnam. Chemical constituents including streptozotocin (STZ), diphenyl picryl hydrazine (DPPH), ascorbic acid, and α-amylase were purchased from Sigma Aldrich (St. Louis, MO, USA) whereas the glucose solution was acquired from the Fresenius Kabi Vietnam Company, Vietnam. Other chemicals used in this study were of analytical grade.

2.2. Animal Maintenance

Adult Swiss albino mice bred in the animal cage (23 × 17 × 18) cm with weights ranging from 20 to 35 gr at 6-8 weeks of age were used for this research. These animals were housed in groups of three mice per cage randomly. They were maintained on a 12-hour light-dark cycle at (28 ± 3)°C. These mice were acclimatized for a 2-week period before experiments and had free access to food and tap water. They were supplied the normal diet with commercial pellets containing 21% protein, 5% fat, 5% cellulose, 6% mineral mixtures from Laboratory Animals of the Pasteur Institute Ho Chi Minh City for the duration of the experiment. The mice were housed on bedding material with natural wood sawdust and changed once every two days. After two weeks of acclimatization, these baseline measurements consisting of blood glucose levels, body weight, food consumption, and water intake were assessed before the commencement of

all experiments. The animal procedures were performed according to the Guidelines for the Care and Use of Laboratory Animals of the Pasteur Institute Ho Chi Minh City, Vietnam.

2.3. Extraction of plant material

The bark of *N. orientalis* was sliced into smaller pieces (10 - 30 mm) and dried at 40 °C for 2 hours in a hot air machine (UF55 Memmert, Germany) and then powdered with a blender (OS-1500 Osako, Vietnam). Next, 200 g of the powder was macerated in one liter of 90% ethanol for 24 hours at room temperature. Thereafter, the ethanol extract was filtered using filter papers (Number 102, Newstar, China). The filtrate was concentrated by evaporation under reduced pressure using the rotavapor apparatus (CA-1115-CE Eyela, Japan) at 50 °C. *N. orientalis* Extract was lyophilized in a lyophilizer (MDF-C8V1 Series Panasonic, Japan) under reduced pressure and stored in the dark Eppendorf at -20 °C for further experiments.

2.4. Preliminary qualitative phytochemical analysis

The phytochemical constituents were determined on this *N. orientalis* extract to estimate the occurrence of carbohydrates, alkaloids, flavonoids, phenols, saponins, and tannins by using qualitative methods. For the presence of carbohydrates, the *N. orientalis* extract (10 mg/mL) was tested with Molisch's reagent and concentrated hydrochloric acid. The formation of a violet-colored ring showed the occurrence of carbohydrates [16]. A volume of 2 mL of the extract was mixed with 1% hydrochloric acid and detected by using Dragendorff's reagent. The appearance of a reddish-orange precipitate was considered for the positive alkaloids [17]. For the presence of flavonoids, the extract was mixed with the ammonia solution and concentrated sulphuric acid. The result was confirmed with the yellow coloration in the test tube [18]. For the phenol detection, the extract was added to 1% ferric chloride solution and the positive result was confirmed with the appearance of the green color [19]. A volume of 2 mL of the extract was added with an equal volume of distilled water and shaken vigorously for 15 min. The presence of saponins was indicated by the foam layer on the top of the test tube [20]. The extract was mixed with drops of ferric chloride and the appearance of blue-black precipitates indicated the occurrence of tannins [21].

2.5. Antioxidant Activity of Assay of EtOH Bark Extract

The ability of the *N. orientalis* extract to scavenge DPPH free radical was estimated according to the procedure described previously with slight modifications [22]. A volume of 3.94 mg of DPPH was dissolved in 100 mL ethanol to prepare 0.1 mM DPPH working solution and stored at 4 °C. For the test of *N. orientalis* extracts, 1 mg of the extract was mixed with 1 mL dimethyl sulfoxide (DMSO) and vortexed for 15 min. The diluted extracts in different concentrations at 6.25, 12.5, 25, 50, and 100 µg/mL were added to the DPPH working solution with the ratio 1:1 (v/v) in each well of the 96-well plate. The 96-well microplate was incubated in the dark for 20 min at room temperature. The absorbance was measured at 540 nm using the Microtiter plate reader (HumaReader HS, Germany). Ascorbic acid was used as the reference compound. The DPPH radical scavenging activity was expressed and calculated using the following formula: DPPH radical scavenging activity = $[(Abs_{control} - Abs_{samples})/ Abs_{control}] \times 100$, where $Abs_{control}$ is the absorbance of control without the plant extract and $Abs_{samples}$ are the absorbance of the various concentrations of *N. orientalis* extract. The IC_{50} values were determined and compared with the anti-scavenging activity of ascorbic acid. This assay was conducted in triplicate.

2.6. α-Amylase inhibitory activity of EtOH Bark Extract

The α-amylase inhibition assay was determined according to the previous study with little alterations [23]. The extract was dissolved in DMSO with the following dilutions at 31.25, 62.5, 125, 250, 500, and 1000 µg/mL for further processing. A volume of 100 µL of each concentration from the working solutions was added with the stock including 100 µL phosphate buffered saline PBS 0.1M (pH 6.9) and 100 µL of α-amylase enzyme solution (3 units/mL) and then the mixture was incubated at 37 °C for 10 min. After the incubation period, the mixture sample was mixed with 200 µL of soluble starch solution (1 mg/mL) and incubated at 37 °C for 15 min. The reaction was stopped by 500 µL of 1% dinitrosalicylic (DNS) yellow color reagent (3,5-dinitrosalicylic acid, 0.2% phenol, 0.05% sodium sulfite, and 1% sodium hydroxide). After boiling at 100°C for 10 min, the volume of 200 µL of 40% potassium sodium tartrate solution was added to the samples and these samples were cooled at room temperature. The negative control was DMSO. The blank with each concentration of the extract was prepared with the absence of the α-amylase enzyme solution. The positive control sample used for the standard reference was acarbose. The absorbance was measured at 540 nm. The percentage of the inhibitory effect of α-amylase was calculated using the following formula: Inhibition of α-amylase (%) = $[(Abs_{control} - Abs_{samples})/Abs_{control}] \times 100$. The IC_{50} values were determined from the dose-response curve by nonlinear regression analysis. These experiments were performed in triplicate.

2.7. Induction of diabetes by a high-fat diet (HFD) and streptozotocin (STZ)

Based on previous articles, the diabetes model for Swiss albino mice was induced by a high-fat diet (HFD) and multiple low-dose streptozotocin (STZ) injections [24]. The mice were divided into two groups containing the normal control group (NC) (n = 4) and the HFD group (n = 12). The normal control group received a standard diet with commercial pellets while the HFD group was fed a high-fat diet including 19.3% protein, 26.5% carbohydrate, and 45.6% fat. After 8 weeks of dietary manipulation, the HFD mice groups were fasted for 16 h and injected intraperitoneal (i.p.) with a 40 mg/kg dose of STZ dissolved in 0.05 M cold sodium citrate buffer (pH 4.5) for three consecutive days. The total mice had free access to feed pellets and water after administration. After three days of STZ injection, the blood glucose values were estimated by the glucometer (OGCare, Italy) with disposable test strips. Blood glucose levels, body weight, food consumption, and water intake were recorded at the end of each week in the morning during the experimental period. The mice depicting blood glucose levels ≥ 200 mg/dL confirmed the induction of diabetes in the study.

2.8. Experimental design

The model mice were randomly divided into three groups. Group I was treated with the ethanol extract 400 mg/kg (DM + *N. orientalis*) (n = 3) whereas group II used as the diabetic control group received 4 mL/kg sodium citrate buffer (DM) (n = 3). Group III which mice were fed with the standard diet was treated with 4 mL/kg distilled water (NC) (n = 3). The *N. orientalis* extract was administered at a dose of 400 mg/kg by oral gavage for six weeks. Blood glucose levels, body weight, food consumption, and water intake were also evaluated.

2.9. Oral glucose tolerance test

The oral glucose tolerance test (OGTT) was evaluated 6 weeks after the treatment [25]. In general, following the 18-hour overnight after fasting, the tail vein blood was collected to determine the glycemic status (time point 0). Then, the mice were given a dose of 2 g/kg of oral glucose and the tail vein blood was measured at 30, 60, and 120 min. The blood glucose was monitored utilizing the glucometer to calculate the OGTT values. The area under the curve (AUC) was determined by the following formula: AUC (mmol.min/L) = (BG_0 + 2 x BG_{30} + 3

x BG_{60} + 2 x BG_{120})/4, where BG_0, BG_{30}, BG_{60}, and BG_{120} were the blood glucose values at 0, 30, 60, 120 min after treatment, respectively.

2.10. Statistical Analysis

Statistical analyses were done by using Graphpad Prism 9 software (USA). Results were expressed as Mean ± Standard Deviation (SD). All results were analyzed by unpaired Student's t-tests and one-way or two-way analysis of variance (ANOVA) tests. The statistically significant differences were determined at $P \leq 0.05$ (*), $P \leq 0.01$ (**), $P \leq 0.001$ (***), and $P \leq 0.0001$ (****).

3. RESULTS AND DISCUSSION

3.1. Preliminary phytochemical analysis

After determining the phytochemical experiments, it was observed that the *N. orientalis* extract includes a variety of bioactive constituents which were summarized in Table 1. The ethanol extract of *N. orientalis* showed the presence of carbohydrates, alkaloids, flavonoids, phenols, tannins, and saponins in the current study. Phytochemical studies were in the line with other previous reports [14, 26]. For instance, ursane-type triterpenoids, steroids, and phenolics isolated from the stem bark and leaves of *N. orientalis* were identified [26]. Naucleaoral A and B which belong to alkaloid compounds have been found in the roots of this plant [14]. Also, Songoen reported that strictosamide isolated as the major component in the crude methanolic leaf extract was elucidated and modified the possible biosynthetic pathway towards several alkaloids [13]. Likewise, these bioactive constituents might be isolated effectively by ethanol solvent because this solvent may be extracted hydrophilic and lipophilic compounds. Various secondary metabolites can be affected by the solubility and polarity of different solvents [27, 28]. Consequently, the positive results of carbohydrates, alkaloids, flavonoids, phenols, tannins, and saponins could be considered promising natural medicinal agents for further investigation.

Table 1. Preliminary phytochemical constituents of EtOH *N. orientalis* bark extract

Metabolites	Name of Test	Results
Carbohydrates	Molisch's test	+
Alkaloids	Dragendorff's test	+
Flavonoids	Alkaline test	+
Phenols	Ferric chloride test	+
Tannins	Ferric chloride test	+
Saponins	Foam test	+

Where (+) indicates the presence of phytochemicals.

3.2. Antioxidant activity of *N. orientalis* extract

The antioxidant activity of *N. orientalis* extract was evaluated by scavenging the stable radical of DPPH with ascorbic acid as the reference standard. The half maximal inhibitory concentration (IC_{50}) of the extract and ascorbic acid obtained were present in Figure 1. The IC_{50} values of the *N. orientalis* extract and ascorbic acid were 20.69 ± 5.41 µg/mL and 11.13 ± 2.81 µg/mL, respectively (Figure 1A-1B). The highest percentage of the inhibition capability of this extract at 100 µg/mL was 94.07 ± 7.28% (Figure 1B). Then, the dose-dependent percentage inhibitory activity of the ethanol extract was reported in this study. According to these previous

standards, *N. orientalis* extract obtained the IC_{50} value ranging from 10 to 50 µg/mL possesses strong antioxidant activity from plant extracts [29]. Thus, the ethanol extract might consist of phytochemical constituents such as phenolics or flavonoids which are responsible for antioxidant properties. Similarly, the previous studies reported that the polyphenolic content from the aqueous bark extract of *N. orientalis* for a significant antioxidant capability, and this was also in agreement with our results [12, 30]. Moe et al. reported a similar IC_{50} value for DPPH inhibitory assay was 29.57 ± 1.4 µg/mL from the leaf extract of *N. orientalis* [31]. Moreover, the polarity of bioactive compositions has been proven a higher antioxidant potential compared to nonpolar compounds of natural product extracts [32]. The phenolic compounds from this plant isolated by utilizing the polar solvent such as ethanol could increase the antioxidant activity significantly. Overall, phytochemicals in the ethanol extract might contribute an important role as antioxidants by neutralizing the hydroxyl ions in the DPPH assay.

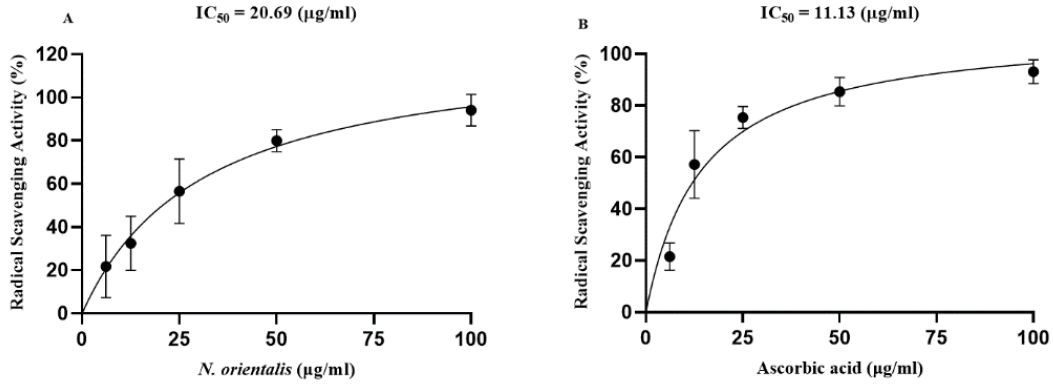

Figure 1. Antioxidant properties of *N. orientalis* extract. (A). DPPH free radical scavenging activity of *N. orientalis* extract from 6.25 to 100 µg/mL. (B). Antioxidant activity of ascorbic acid as the standard reference. Data were expressed as mean ± SD of three independent experiments.

3.3. Inhibitory effect of *N. orientalis* extract on α-amylase activity

The α-amylase inhibitory activity of this extract was estimated with the positive control acarbose (Figure 2). This extract at the concentration from 31.25 µg/mL to 1000 µg/mL revealed the inhibitory effect on alpha-amylase activity with the IC_{50} value of 242.2 ± 83.87 µg/mL (Figure 2A).

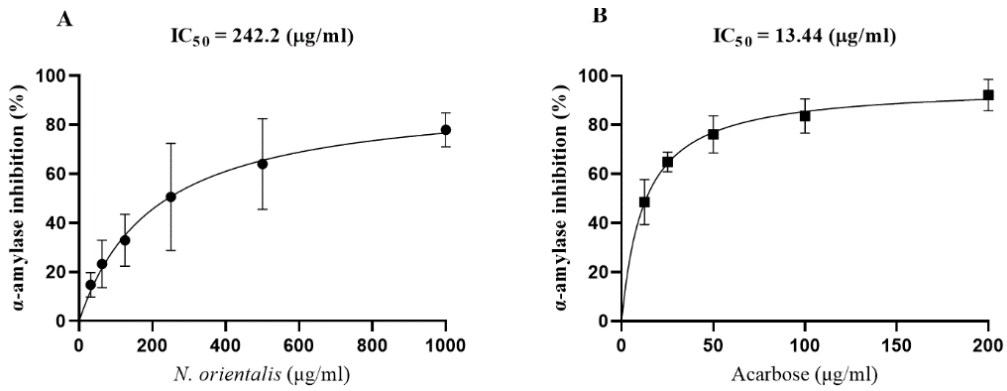

Figure 2. The percentage inhibition of α-amylase activity from *N. orientalis* extract. (A). α-amylase inhibitory activities of the ethanol extract from 31.25 to 1000 µg/mL. (B). Acarbose was used as a positive control. Data were expressed as mean ± SD of triplicate determinations.

The highest concentration of 1000 µg/mL showed a maximum inhibition of 77.98 ± 6.9% while the lowest concentration of 31.25 µg/mL showed a minimum inhibition of 14.8 ± 4.9%. The *N. orientalis* extract exhibited increased inhibition of the enzyme activity in a concentration-dependent manner. Acarbose used as the standard antidiabetic drug possessed the α-amylase inhibitory activity with the IC_{50} value of 13.44 ± 2.47 µg/mL (Figure 2B). Postprandial hyperglycemia is one of the most important factors of diabetes mellitus. The inhibition of digestive enzymes such as α-amylase and α-glucosidase has been one of the most effective ameliorating hyperglycemic conditions. The α-amylase inhibitors from natural products or synthetic chemicals catalyze the hydrolysis of starch by blocking the 1,4-glycosidic linkages into simple sugars. The α-amylase inhibitory property has been reported for the EtOH Bark Extract from *N. orientalis* for the first time although many previous studies evaluated the inhibition of α-amylase by the extract or pure compounds isolated from other species of this genus [33-35]. Thus, the present investigation demonstrated that *N. orientalis* extract possessed a low inhibition against α-amylase compared to acarbose. It could be possible that the different phytochemicals of the extract were combined and influenced the synergistic effects on the α-amylase inhibitory capacity.

3.4. Blood glucose, body weight, food intake, and water intake in diabetic mice by combining a high-fat diet and streptozotocin

The results from Figure 3 illustrated the development of HDF-induced obesity in mice. As shown in Figure 3A, the basal blood glucose level was 119 ± 7.4 mg/dL at the start of this study. After feeding in eight weeks, the blood glucose levels were 191 ± 7.21 mg/dL in HFD mice and 152.3 ± 7.5 mg/dL in NC mice, respectively. Meanwhile, the body weight was 32.5 ± 1.87 g in both groups in the beginning (Figure 3B). At the end of this experiment, the mice with HFD showed gradually increased body weight at 62.33 ± 9.71 g and were significantly higher than the normal control group (Figure 3B). Additionally, there was an increase in food and water intake in the HFD group (Figure 3C-3D). In the current study, HFD with a high percentage of 45.6% fat was considered to be the most important effect of obesity. After the induction with STZ, the blood glucose level in the HFD combined with STZ and the control group were 253.12 ± 42.17 mg/dL and 141.1 ± 11.13 mg/dL, respectively (Figure 4A). As shown in Figure 4B, the body weight of control animals was 41.5 ± 1.29 g while the body weight in HFD/STZ mice was 61.13 ± 4.58 g. These results illustrated there was a slight reduction of body weight in the treated mice compared to normal mice after STZ injection. Moreover, the food consumption and water intake were significantly higher in the HFD/STZ group (Figure 4C-4D). There are many elements associated with high blood glucose levels, for example, insulin resistance and impaired insulin production by β cells of the pancreas. Currently, the combination of HFD and multiple low doses of STZ can result in the characteristics of diabetes mellitus because the HFD feeding might develop insulin resistance and the multiple low doses of STZ intraperitoneal injection induce the mild impairment of insulin secretion in this research. The increased body weight in HFD mice might be owing to the consumption of a high-fat diet in energy in the form of fats. There have been many studies in the line with our research on high-fat diets in combination with low doses of STZ to generate a stable animal model for pharmaceutical screening [24, 36-38]. The present study demonstrated that the development of the diabetic mice model by feeding HFD and multiple low doses of STZ (40 mg/kg) could be easily accessible and practical for testing various constituents in diabetic therapy.

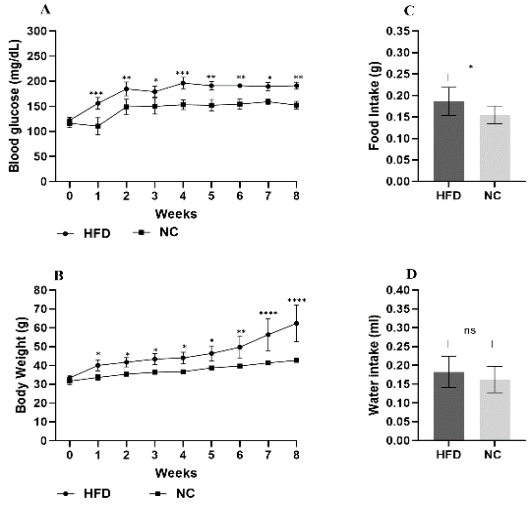

Figure 3. The development of obese Swiss albino mice with high-fat diet feeding. (A). Blood glucose levels. (B) Body weight. (C). Food intake. (D). Water intake. HFD: mice with high-fat diet (filled circles, n = 6), NC: mice with normal diet (filled squares, n = 3). Values were presented as mean ± SD. Data were analyzed by unpaired Student's t-test and two-way ANOVA, **P* ≤ 0.05, ***P* ≤ 0.01, ****P* ≤ 0.001, *****P* ≤ 0.0001, ns means *P* > 0.05.

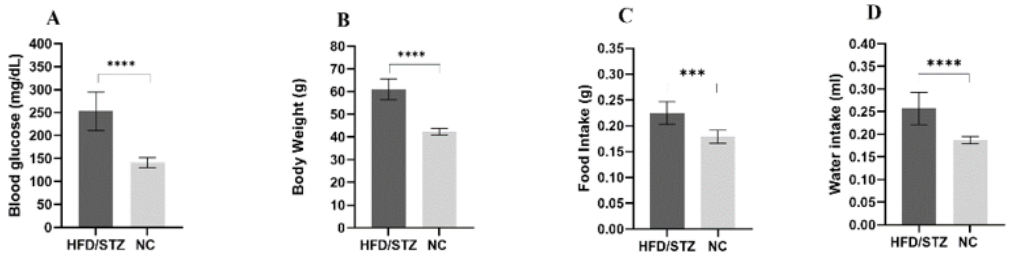

Figure 4. Blood glucose levels, body weight, food and water intake in diabetic mice after streptozotocin induction. (A). Blood glucose levels. (B). Body weight. (C). Food intake. (D). Water intake. HFD/STZ: mice with high-fat diet and streptozotocin-induced diabetes (n = 6), NC: mice with normal diet and sodium citrate buffer (n = 3). Data were presented as mean ± SD. Statistical differences were analyzed by unpaired Student's t-test, *****P* ≤ 0.0001, ns means *P* > 0.05.

3.5. Effect of *N. orientails* extract on blood glucose levels, body weight, food intake, and water intake

The effect of *N. orientalis* extract was presented in Figure 5 during six weeks of daily oral administration. As shown in Figure 5A, the blood glucose levels from the treated group decreased from 278.33 ± 37.65 mg/dL to 203 ± 6.08 mg/dL whereas the diabetic control group remained stable during the experimental time at 238 ± 17.78 mg/dL. Thus, the blood glucose levels of the treated diabetic group with the concentration of the extract at 400 mg/kg were reduced by 27.04%. Body weights were measured from 59 ± 5.29 g to 47.67 ± 2.08 g and presented a 19.21% reduction compared to the diabetic control mice and the baseline weight of the animals (Figure 5B). Besides, the food intake and water intake increased slightly in the treated mice (Figure 5C-5D). There are many medicinal plants have been utilized for the treatment of diabetes through the reduction of hyperglycemia [39-41]. The current results confirmed that the EtOH Bark Extract from *N. orientalis* decreased blood glucose levels remarkably after oral administration. This phenomenon might be attributed to the presence of phytochemicals that possesses the ability to regulate blood glucose levels through several signaling pathways. In addition, the tendency in body weight in diabetic mice should be

increased substantially due to the effect of antidiabetic drugs or plant extracts [42, 43]. Interestingly, there was a reduction of body weight in the treated mice during three weeks after oral administration, and then, a slight gain in body weight was observed later compared to the untreated diabetic group. The decreased body weight could result from the reduction of glucose metabolism associated with fat or protein metabolism to supply alternate energy in the body. Later, the increased body weight demonstrated the extract might improve the insulin production from β-cells to activate the hormones involved in fat storage. Hence, these results indicated *N. orientalis* extract might include hypoglycemic agents responsible for the reduction of blood glucose levels.

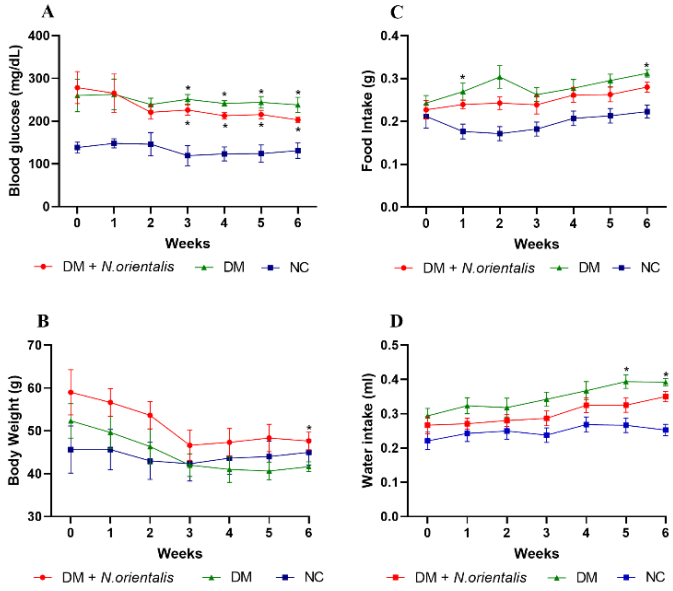

Figure 5. Effect of *N. orientalis* extract on blood glucose levels, body weight, food and water intake in diabetic mice. (A). Blood glucose levels. (B). Body weight. (C). Food intake. (D). Water intake. DM + *N. orientalis*: diabetic mice treated with *N. orientalis* extract (400 mg/kg), DM: diabetic mice treated with sodium citrate buffer, NC: normal control mice treated with vehicle (n = 3). Values were presented as mean ± SD. Statistical differences were evaluated by two-way ANOVA, $*P \leq 0.05$ (compared to the normal control group).

3.6. Effect of *N. orientalis* extract on oral glucose tolerance test

The blood glucose levels and area under the curve (AUC) of experimental groups were expressed in Figure 6. In negative control mice, the blood glucose level rose to a peak value of 266.33 ± 12.22 (mg/dL) after 30 min glucose administration and decreased to near normal level at 176 ± 7 (mg/dL) after 120 min (Figure 6A). In contrast, the values in diabetic control mice increased to a peak after 30 min and remained at high levels at 337.7 ± 22.05 mg/dL and 283.7 ± 6.43 mg/dL following the time point at 60 and 120 min, respectively. Compared to the normal and diabetic control group, the animals treated with *N. orientalis* extract showed a significant reduction in blood glucose levels after 120 min. In detail, the blood glucose level decreased from 386.7 ± 14.64 mg/dL to 248.7 ± 43.89 mg/dL (Figure 6A). Additionally, the AUC value in the treated mice was reduced by 14.18% compared with the untreated diabetic mice (Figure 6B). Glucose is one of the most important stimulators of insulin secretion and affects the initiation of glycolysis and fatty acid synthesis [44, 45]. During the experimental period, long-term HFD feeding can cause hyperglycemia and glucose intolerance. The gain in blood glucose was confirmed successful oral glucose loading after 30 min in three groups (Figure 6A). It can be explained that the glucose oral load was absorbed completely in

experimental mice. After that, the glucose might be utilized and as a result, it can improve glucose tolerance considerably in mice treated with the extract. The treatment of the extract at 400 mg/kg may promote glucose tolerance compared to the vehicle control.

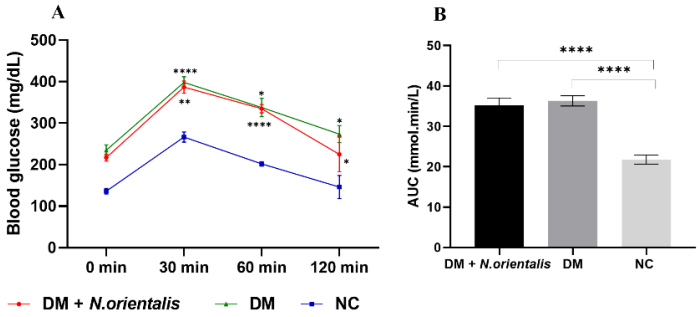

Figure 6. Effect of N. orientalis extracts on blood glucose after glucose loading. (A). Blood glucose levels after oral admininistration of glucose. (B). The area under the curve (AUC) of the glucose tolerance at six weeks. DM + *N. orientalis*: diabetic mice treated with *N. orientalis* extract (400 mg/kg), DM: diabetic mice treated with sodium citrate buffer, NC: normal control mice treated with vehicle (n = 3). Values were presented as mean ± SD. Statistical differences were analyzed by one- and two-way ANOVA, $*P \leq 0.05$, $**P \leq 0.01$, $****P \leq 0.0001$.

4. CONCLUSION

In conclusion, this study revealed that the ethanol extract of *N. orientalis* possesses the antioxidant capability and exhibits dose-dependent inhibitory potential against the α-amylase enzyme. The extract reduced blood glucose levels significantly in vivo in high-fat diet and low-dose STZ-induced diabetic mice. The bark extract also includes a diverse range of phytochemicals containing carbohydrates, alkaloids, flavonoids, phenols, tannins, and saponins. Overall, these results provide opportunities for future research of novel effective constituents for diabetics from *N. orientalis.*

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TÓM TẮT

SỰ HIỆN DIỆN CỦA MỘT SỐ HOẠT CHẤT SINH HỌC, HOẠT TÍNH KHÁNG OXY HÓA, KHẢ NĂNG ỨC CHẾ ENZYME α-AMYLASE VÀ HẠ ĐƯỜNG HUYẾT CỦA CAO CHIẾT EtOH TỪ VỎ CÂY GÁO VÀNG *Nauclea orientalis*

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Hợp chất tự nhiên được sử dụng như dược phẩm thay thế để điều trị nhiều loại bệnh nguy hiểm trong đó có bệnh tiểu đường. Cây gáo vàng *Nauclea orientalis* thuộc họ Rubiaceae và sử dụng như một loại dược liệu dân gian truyền thống tại nhiều quốc gia. Nghiên cứu này tập trung đánh giá khả năng kháng oxy hóa, ức chế enzyme α-amylase, hạ đường huyết của cao chiết ethanol từ vỏ cây gáo vàng và các nhóm hợp chất sinh học có trong đó. Khả năng bắt gốc tự do DPPH của cao chiết có giá trị IC_{50} là 20,69 ± 5,41 µg/mL so sánh với vitamin C là 11,13 ± 2,81 µg/mL. Khả năng ức chế enzyme α-amylase của cao chiết có giá trị IC_{50} là 242,2 ± 83,87 µg/mL. Cao chiết có khả năng giảm 27,04% đường huyết và 19,21% cân nặng của chuột tiểu đường tại liều lượng 400 mg/kg trọng lượng chuột bằng đường uống. Cao chiết cải thiện dung nạp glucose trong thử nghiệm dung nạp glucose. Các nhóm hợp chất sinh học hiện diện trong cao chiết bao gồm carbohydrate, alkaloid, flavonoid, phenol, tannin và saponin. Nghiên cứu này chứng minh cao chiết ethanol từ vỏ cây gáo vàng có khả năng kháng oxy hóa, ức chế enzyme α-amylase có thể là một đối tượng tiềm năng cho các nghiên cứu về sinh học và hóa học trong lĩnh vực y dược.

Từ khóa: Nauclea orientalis, kháng oxy hóa, ức chế α-amylase, tiểu đường, cao chiết thực vật.