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Original Article

HYPOGLYCEMIC AND HYPOLIPIDEMIC EFFECTS OF *OLDENLANDIA CORYMBOSA* AGAINST ALLOXAN INDUCED DIABETES MELLITUS IN RATS

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ABSTRACT

Objective: The present study was designed to demonstrate the antioxidant, hypolipidemic and hypoglycaemic potentials of aqueous extract of *O. corymbosa* against alloxan-induced diabetes in rats.

Methods: *O. corymbosa* extract was tested for phytochemical screening, total phenolic, flavonoids content and DPPH free radical scavenging activity. Diabetes was induced in Sprague Dawley rats by administration of alloxan monohydrate (65 mg/kg b. w i. v). The aqueous extract of *O. corymbosa* at a dose of 100 mg/kg and 200 mg/kg were administered through gavage feeding daily to diabetic induced rats for 14 d. The effect of aqueous extract of *O. corymbosa* was assessed on blood glucose, body weight, lipid peroxidation, catalase activity, glutathione and lipid profile. Pancreatic tissues were also examined by haematoxylin and eosin staining methods.

Results: Phytochemical screening shows the presence of tannins, saponins, phlobatannins and flavonoids. Total phenolic content was found to be 22.85±0.21 mg/g, IC_{50} is 450±1.39 µg/ml and total flavonoids content was found to be 4.25±0.09 mg/g of extract. The results of the present study showed that *O. corymbosa* can lower blood glucose and lipid parameters except for HDL. The levels of antioxidant enzymes CAT and GSH were increased along with the decreased in LPO level by the pre-treatment of animals with *O. corymbosa.* Microscopic examination of pancreatic sections revealed that diabetic rats treated with *O. corymbosa* extracts at either dose have normal architecture structure of islets.

Conclusion: These results indicate that *O. corymbosa* may be effective as a hypoglycaemic and antihyperlipidemic agent.

Keywords: *O. corymbosa,* Alloxan, Hypoglycaemic, Hypolipidemic, Antioxidative, Diabetes mellitus

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INTRODUCTION

Diabetes mellitus has affected the lives of many people from around the world suffering from it and Malaysia has 3.2 million cases of diabetes reported in the year 2014 alone [1]. The word diabetes means a condition of a person which secretes out "sweet urine" and also experiences a major loss of muscle which is due to a condition known as hyperglycaemia where the level of blood glucose increases, thus causes the spillage of glucose into the urine. Hyperglycaemia is caused by the absence or insufficient amount of insulin produced in the body [2] and this condition is the landmark of diabetes [3]. There are two types of diabetes which are type 1 and type 2. Patients with type 1 diabetes cannot produce insulin thus Type 1 diabetes is also known as insulin-dependent or juvenile-onset. On the other hand, patients with Type 2 diabetes or also known as non-insulin-dependent or adult-onset can produce insulin however the insulin produced cannot convert the food into energy [4].

Diabetes is currently treated by using insulin and oral hypoglycaemic agents which has recorded huge successes in addition to the adjustment of lifestyle [5]. However, treating diabetes with insulin or oral hypoglycaemic agents comes with several serious side effects such as recurrent cases of hypoglycaemic coma [5]. Besides that, diabetes patients are facing the risks of impairment of hepatic and other body functions with every shot of insulin they take [3]. These two medications are therefore not sufficient in preventing diabetic complications. Due to the problems associated with the current treatments, scientists have now looking for alternative remedies from potential plants. There are more than 800 plant species from around the world that have been used as a treatment for diabetes and proven to have antihyperglycemic activity [6]. Thompson Coon and Earnst [7] stated that there are several plants that have additional potentials such as ameliorate the abnormalities of lipid metabolism besides correcting the blood glucose levels in diabetes patients. The significant of research on medicinal plants is validated by the fact that many types of new drugs have been developed from plants, for example, *Galega officinalis* L. which is used to synthesis metformin as well as other bisguanidine-type anti-diabetic drugs [8]. Biologically active compounds in plants are recommended to be used to reduce the number of diabetes complications and also delaying their development [9].

O. corymbosa Linn or also known as *Hedyotis corymbosa* (L.) Lam is from the Rubiaceae family [10]. *O. corymbosa* is generally called as "Rumput mutiara" and it is said that the whole *O. coymbosa* plant (leaves, stem, roots, flowers and seeds) can be used as medicine [10]. According to Endrini [10], *O. corymbosa* has been reported to have many medicinal values such as for the treatment of jaundice [11]. Besides that, *O. corymbosa* has anticancer activity and high antioxidant activities and also contains flavonoids and phenolic compounds [10]. *O. corymbosa* is also used traditionally in India and China to treat hepatic disorders [12]. Therefore, it is worth conducting research work on *O. corymbosa* to find out whether or not it has anti-diabetic activity since it shows many potentially bioactive compounds that may play important roles in anti-diabetic activities.

MATERIALS AND METHODS

Chemicals and reagents

Alloxan monohydrate, 1,2-dithio-bis-nitrobenzoic acid (DTNB), bovine serum albumin (BSA), folin-ciocalteu reagent (FCR), thiobarbituric acid (TBA), hydrogen peroxide (H_2O_2), sulfosalicylic acid (SSA), gallic acid, catechin, 1,1-diphenyl-2-picrylhydrazyl (DPPH), haematoxylin, eosin and trichloroacetic acid (TCA) were purchased from Sigma-Aldrich Chemical Company, USA. All other solvents and chemicals used were either of analytical grade or of the highest purity commercially available.

Collection of plant

O. corymbosa plant was collected from Universiti Malaysai Sabah. The plant sample was identified based on morphological characteristics (voucher number: Caysandra 01) by Mr. Johnny Gisil, a botanist from Institut Biologi Tropika dan Pemuliharaan, Universiti Malaysia Sabah.

Preparation of plant extract

The whole plant of *O. corymbosa* (except roots) were washed under running tap water to remove dirt and foreign materials. The whole plant of *O. corymbosa* (except roots) were then dried under shade for 3 to 4 w. After the plant sample had been dried completely under a shade, the sample was then crushed into a coarse powder using heavy-duty blender and placed in air tight containers. Next, the aqueous extraction of plant sample was prepared following Yuan *et al.* [13].

Phytochemical screening

For the identification of the constituents present in the *O. corymbosa*, the extract obtained was subjected to six qualitative tests which are tannins, phlobatannins, saponins, flavonoids, terpenoids, and cardiac glycosides according to methods reported by Krishnaiah *et al.* [14].

Determination of total phenolic content

The total phenols in aqueous extract of *O. corymbosa* were estimated by Folin-Ciocalteu's phenol reagent according to Shingleton *et al.* [15] expressed as milligram gallic acid equivalents (GAE) per gram dry weight of the extract.

Determination of antioxidant activity

The ability of plant extract to scavenge DPPH free radicals was determined according to Hatano *et al.* [16] method.

Determination of total flavonoids content

Total flavonoids content in *O. corymbosa* plant extract was measured by aluminium chloride colourimetric assay [13]. Total flavonoid content was expressed in milligrams of catechin equivalents per gram of plant extract.

Sprague-Dawley rats

Animal study was approved by Animal Ethics Committee (AEC) of Universiti Malaysia Sabah. Twenty four male Sprague-Dawley rats weighing between 150 g-300 g and age between 6 to 12 w old were purchased from Fakulti Sains Makanan dan Pemakanan, Universiti Malaysia Sabah. All 24 rats were placed in Animal Biosafety Laboratory of Biotechnology Research Institute, Universiti Malaysia Sabah, in colony plastic cages and provided with water *ad libitum* and standard food pellet. The rats were put under 12-hour light and 12-hour dark cycles with 24-28 °C temperature. One week time was given to allow the rats to adapt to the environment.

Experimental design

After an overnight fast with water provided, the rats were then made diabetic by intravenously injecting alloxan monohydrate, 65 mg/kg body weight [17] dissolved in normal saline as reported by Kumar *et al.* [18] (Alloxan; Sigma Co., St. Louis, USA). Rats in the control group were only injected with normal saline. Blood from each rat was taken from the lateral veins of the tail and the blood sugar levels were then subjected to a glucometer to read the blood sugar level. Only rats with blood sugar level more than 200 mg/dl (diabetic) were used in the experiment. The rats were put into five groups randomly with three rats per group (n=3). The blood glucose levels and body weight of each rat in each group were measured weekly for two weeks. The rats were sacrificed at the 15th day by first anaesthetizing them, 24 h after their respective daily dosages.

Group 1: Normal rats administered normal saline (normal control)

Group 2: Diabetic rats administered with normal saline (diabetic control)

Group 3: Normal rats administered with *O. corymbosa* (200 mg/kg b. wt)

Group 4: Diabetic rats treated with *O. corymbosa* (100 mg/kg b. wt)

Group 5: Diabetic rats treated with *O. corymbosa* (200 mg/kgb. wt)

The weight of all treated rats was determined by using a physical balance (Sartorius, 6202-1S) before and after treatment. The rats were sacrificed at the 15th day after their last doses of plant extracts by cervical dislocation and were first anaesthetized by using light ether. After that, the rats' pancreases were dissected out quickly. Extraneous material was cleaned from the pancreases and washed with ice-cold saline (0.85% w/v, sodium chloride) and then dipped in 0.1M, pH 7.4 phosphate buffer and later stored at-80˚C for further biochemical and histopathological investigations.

Collection of blood

The blood sample of each rat was collected by using cardiac punctures technique after cervical dislocation before their heart-beats stop. The blood samples were collected in separated BD Vacutainer® Blood Collection Tubes and later centrifuged at 1375 x g for 30 min and the serum was separated.

Post mitochondrial supernatant preparation

The pancreases of the animal in every group were washed immediately in ice-cold saline (0.85% w/v, sodium chloride). After that, the pancreases of rats were homogenized in a chilled phosphate buffer (0.1 M, pH 7.4) containing KCI (1.17 % w/v, potassium chloride) using a homogenizer (Polytron PT 1200E). The homogenate sample was centrifuged at 2000 x g for 10 min at 4 °C to separate nuclear debris. Next, the supernatant obtained was centrifuged again at 10,000 x g for 30 min at 4 °C to obtain post-mitochondrial supernatant which was used for determination of malondialdehyde and reduced glutathione content. The supernatant obtained was kept in-80 °C before further analysis.

Determination of reduced glutathione

Reduced glutathione was determined by using the method described by Jollow *et al.* [19]. The yellow colour was developed indicating reduced glutathione and was read at 412 nm using a spectrophotometer. The result was expressed as micromoles of GSH/gram of tissues by using a molar extinction coefficient of 13.6 x $10^3 M^{-1}$ cm^{-1}.

Determination of lipid peroxidation

Lipid peroxidation was measured using the method reported by Buege and Aust [20]. The absorbance was measured using a spectrophotometer with a wavelength of 535 nm. The results were expressed as the amount of MDA formed in each of the samples by using a molar extinction coefficient of 1.56 x 10^5 M^{-1} cm^{-1}.

Determination of catalase activity

Catalase activity was carried out using the Claiborne [21] method with slight modification. The changes in absorbance were recorded at 240 nm for every 30 seconds for 3 min using a spectrophotometer and the enzyme activity was calculated as nmol H_2O_2 consumed/min/mg protein by using a molar extinction coefficient of 6.4 x 10^3 M^{-1} cm^{-1}.

Determination of total protein content

Total protein contents were determined by Lowry assay using Folin-Ciocalteau reagent [22].

Determination of total cholesterol

The serum concentration of total cholesterol was measured using Chod-Pap method as described in instruction provided with the kit (BIOLABO, France).

Determination of high-density lipoprotein cholesterol

The serum concentration of high-density lipoprotein cholesterol was measured using the direct method as described in instruction provided with the kit (BIOLABO, France).

Determination of low-density lipoprotein cholesterol

The serum concentration of low-density lipoprotein cholesterol was measured using the direct method as described in instruction provided with the kit (BIOLABO, France).

Histopathological examination

The pancreases of sacrificed rats were removed and immediately immersed in 10% phosphate-buffered formalin solution. Before the

embedding process in paraffin wax, the tissues were first processed using a tissue processing machine with the standard procedure. The tissue sample was first embedded with paraffin wax. After that, the specimens were cut into 5 µm thick sections and then immediately mounted on the slide for haematoxylin and eosin staining (H and E). The specimens were evaluated for degree of haemorrhage, edema, necrosis, inflammation and other changes.

Statistical analysis

All results were expressed as mean±SD. The results were analysed for statistical significance by one-way analysis of variance (ANOVA) followed by Dunnett t-test multiple comparison using SPSS statistical analysis software. A p-value less than 0.05 is considered statistically significant.

RESULTS

Phytochemical screening (qualitative analysis)

O. corymbosa showed the presence of tannins, saponins, phlobatannins and flavonoids. Terpenoids and cardiac glycosides were not detected. The results obtained from phytochemical screening are summarized in table 1 where (+) sign indicates the presence of the bioactive compound and (-) sign indicates the absence of the bioactive compound.

Table 1: Phytochemical constituents of *O. corymbosa*

Constituents	Test	*O. corymbosa*
Tannins		+
Phlobatannins		+
Saponins		+
Flavonoids		+
Terpenoids		-
Cardiac glycosides		-

Key = (+) present; (-) absent

Total phenolic content

The total phenols of *O. corymbosa* which was found to be 22.85±0.21 mg/100 g expressed as milligram gallic acid equivalents (GAE) per gram dry weight of the extract.

Antioxidant activity using 2, 2-diphenyl-2-picrylhydrazyl (DPPH)

The antioxidant activity of free radical scavenging activity of *O. corymbosa was* determined using DPPH assay as shown in table 2. Ascorbic acid was used as a reference standard to determine the antioxidant potential of *O. corymbosa*.

Total flavonoid content

The concentration of flavonoids in *O. corymbosa* was found to be 4.25±0.09 mg/g expressed in terms of catechin equivalent, mg/g of extract.

Blood glucose levels in rats

Blood glucose levels in rats (5 groups; 3 rats per group) were monitored and recorded since day-4 for pre-treatment until day 15, the last day of treatment. Blood glucose levels in rats are summarized in table 3. The blood glucose level in diabetic rats showed significant $P<0.05$ increment as compared to normal control rats.

Table 2: IC_{50} values of *O. corymbosa* by free radical scavenging method

Sample	Radical scavenging activity (DPPH) IC50 (µg/ml)
Ascorbic acid	15.52±0.59
O. corymbosa	450±1.39

Data are presented as mean±SD of three replicates.

Table 3: Effect of aqueous extract of *O. corymbosa* on blood glucose levels (mg/dl) in rats

Group (n=3)	Treatment	Blood glucose levels (mg/dl)				
		-4 d	0th d	3rd d	7th d	14th d
I	Normal control	64.33±13.61	62.67±7.09	76.67±8.5	70.33±10.02	75±19.08
II	Diabetic control	63.67±16.86	60±9.85	241±24.56*	248.67±26.5*	313.33±27.47*
III	Normal+*O. corymbosa* (200 mg/kg)	73±17.52	68.33±5.69	70.67±4.73**	69.33±7.77**	79.33±10.26**
IV	Diabetic+*O. corymbosa* (100 mg/kg)	63±12.12	56±7.55	293±26.91*	250.33±27.15*	120.67±26**
V	Diabetic+*O. corymbosa* (200 mg/kg)	74±18.73	53.67±32.88	296±23.39*	122.33±31.09**	93±14.42**

Data are presented as mean±SD, Group 2 was compared with Group 1, Groups 3-5 were compared with Group 2, *Significant value at $P<0.05$ compared to Normal control, **Significant value at $P<0.05$ compared to Diabetic control

Table 4: Body-weight changes in rats

Group (n=3)	Treatment	Body-weight (Kg)				
		-4 d	0th d	3rd d	7th d	14th d
I	Normal control	104.2±11.58	111.03±5.5	122.03±7.8	131.03±5.72	145.6±9.96
II	Diabetic control	136.6±30.31*	146.07±23.29*	139.1±19.56*	141.47±16.85*	133.59±18.99*
III	Normal+*O. corymbosa* (200 mg/kg)	116.23±20.99	110.83±19.88	116.2±20.69	124±19.18	125.43±19.69
IV	Diabetic+*O. corymbosa* (100 mg/kg)	129.97±32.16**	128.83±28.29**	121.97±30.00**	126.6±24.2**	131.2±25.29**
V	Diabetic+*O. corymbosa* (200 mg/kg)	127.8±27.5**	132.03±19.51**	126.47±16.97**	128.5±19.85**	132.1±22.6**

Data are presented as mean±SD, *Significant value at $P<0.05$ compared to Normal control, **Significant value at $P<0.05$ compared to Diabetic control

Body-weight changes in rats

Body-weight of rats (5 groups; 3 rats per group) was monitored and recorded since day-4 for pre-treatment until day 15, the last day of treatment. Body-weight changes of the rats are summarized in table 4. Body-weight of all rats showed steady increment from day-4 till day 0. All rats that were induced with alloxan monohydrate, 65 mg/kg b.w., intravenously showed a decrease in appetite and weight loss after 3 d of induction.

Lipid peroxidation

As shown in table 5, the lipid peroxidation value was the highest in the diabetic control group (97.77±2.23 nmol MDA/g of tissue). Alloxan-induced diabetic rats in group IV and V which received *O. corymbosa* extracts (100 mg/kg and 200 mg/kg respectively) showed the lipid peroxidation levels were significantly reduced and no indicated-any significant difference with the rats in the normal control group.

Table 5: Effect of treatment of alloxan-induced diabetic rats with aqueous extract of *O. corymbosa* for 14 d on lipid peroxide

Group (n=3)	Treatment	Lipid peroxidation (nmol MDA/gram of tissue)
I	Normal control	70.03±2.08
II	Diabetic control	97.77±2.23*
III	Normal+*O. corymbosa* (200 mg/kg)	75.01±1.61**
IV	Diabetic+*O. corymbosa* (100 mg/kg)	73.49±1.53**
V	Diabetic+*O. corymbosa* (200 mg/kg)	72.52±1.24**

Data are presented as mean±SD, *Significant value at P<0.05 compared to Normal control, **Significant value at P<0.05 compared to Diabetic control

Reduced glutathione

Glutathione (GSH) acts as a free radical scavenger and also involved in the repairmen of damages caused by free radical. GSH is the heart of all immune functions where it acts as the body's master antioxidant. The values of GSH in alloxan treated and animals that were treated with *O. corymbosa* treated with alloxan are summarized in table 6. Glutathione value was found lowest in the diabetic control group 5.22±0.24 µmol GSH/g of tissue. Alloxan-induced diabetic rats in Group IV and V which received *O. corymbosa* extracts (100 mg/kg and 200 mg/kg respectively) showed the GSH levels increased slightly from the GSH value of diabetic control group.

Table 6: Effect of treatment of alloxan-induced diabetic rats with aqueous extract of *O. orymbosa* for 14 d on glutathione

Group (n=3)	Treatment	GSH (µmol GSH/g of tissue)
I	Normal control	10.62±1.49
II	Diabetic control	5.22±0.24*
III	Normal+*O. corymbosa* (200 mg/kg)	9.83±0.07**
IV	Diabetic+*O. corymbosa* (100 mg/kg)	6.43±0.19*
V	Diabetic+*O. corymbosa* (200 mg/kg)	7.52±0.36**

Data are presented as mean±SD, *Significant value at P<0.05 compared to Normal control, **Significant value at P<0.05 compared to Diabetic control

Catalase activity

The values of catalase activity on the treated rats are summarized in table 7. Catalase activity was found to be lowest in the diabetic control group was found to be 10.72±0.99 U H_2O_2 consumed/min/mg protein. Alloxan-induced diabetic rats in Group IV and V which received *O. corymbosa* extracts (100 mg/kg and 200 mg/kg respectively) showed the catalase activities were significantly increased and not indicated any significant difference with the rats in the normal control group.

Table 7: Effect of treatment of alloxan-induced diabetic rats with aqueous extract of *O. corymbosa* for 14 d on catalase

Group (n=3)	Treatment	CAT (U H_2O_2 consumed/min/mg protein)
I	Normal control	25.17±0.65
II	Diabetic control	10.72±0.99*
III	Normal+*O. corymbosa* (200 mg/kg)	22.18±0.28**
IV	Diabetic+*O. corymbosa* (100 mg/kg)	21.87±0.18**
V	Diabetic+*O. corymbosa* (200 mg/kg)	25.35±0.27**

Data are presented as mean±SD, *Significant value at P<0.05 compared to Normal control, **Significant value at P<0.05 compared to Diabetic control

Total cholesterol, LDL and HDL

Total cholesterol, LDL and HDL are expressed in mg/dl and the results obtained are shown in table 8. Total cholesterol was the highest in the diabetic control group with a total cholesterol value 152.49±0.95 mg/dl. Groups IV and V which received treatment of plant extracts of 100 mg/kg and 200 mg/kg respectively showed lower total cholesterol as compared to the diabetic control group. Similarly, LDL was the highest in the diabetic control group with LDL value of 165.83±1.00 mg/dl. Groups IV and V which received treatment of plant extracts of 100 mg/kg and 200 mg/kg respectively showed significantly lower LDL values as compared to the diabetic control group. In contrast, HDL was the lowest in the diabetic control group with HDL value of 23.61±2.34 mg/dl. Groups IV and V which received treatment of plant extracts of 100 mg/kg and 200 mg/kg respectively showed significantly higher HDL values as compared to the diabetic control group.

Histopathology examination

Sections of the pancreas of rats from each group were stained with haematoxylin and eosin (H and E) and examined under the microscope (FSX 100 fluorescence microscope, Tokyo, Japan). Islets of Langerhans displaying degenerative and damaged acinar cells were seen in diabetic control fig. 1 C, D. However, most of the changes were alleviated by pretreatment of animals with *O. corymbosa* as shown in fig. 2 C, D, E and F.

Table 8: Effect of treatment of alloxan-induced diabetic rats with aqueous extract of *O. corymbosa* for 14 d on total cholesterol, LDL and HDL

Group (n=3)	Treatment	Total cholesterol (mg/dl)	LDL (mg/dl)	HDL (mg/dl)
I	Normal control	98.09±1.56	51.97±1.59	35.48±3.13
II	Diabetic control	152.49±0.95*	165.83±1.00*	23.61±2.34*
III	Normal+*O. corymbosa* (200 mg/kg)	112.93±4.64**	65.49±3.16**	34.19±2.52**
IV	Diabetic+*O. corymbosa* (100 mg/kg)	127.9±6.52**	123.35±4.50**	32.42±1.25**
V	Diabetic+*O. corymbosa* (200 mg/kg)	92.56±5.49**	115.71±3.20**	34.47±1.67**

Data are presented as mean±SD, *Significant value at P<0.05 compared to Normal control, **Significant value at P<0.05 compared to Diabetic control

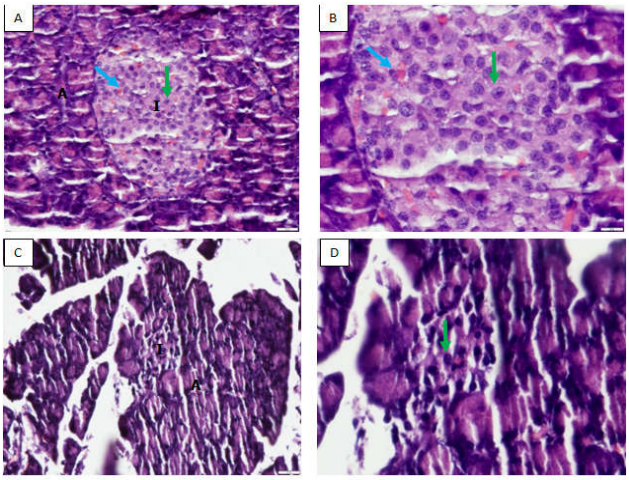

Fig. 1: Haematoxylin and eosin (H and E) stained histopathological sections of the pancreas after 14 d of treatment. (A) Normal control with 20X magnification: Native architecture of islets and pancreatic tissue. (B) Normal control with 40X magnification. (C) Diabetic control with 20X magnification on Islets of Langerhans displaying degenerative and damaged acinar cells can be seen. (D) Diabetic control with 40X magnification, I: Islets of Langerhans; A: Acinar cells; Blue arrow: Red blood cells; Green arrow: β-cells

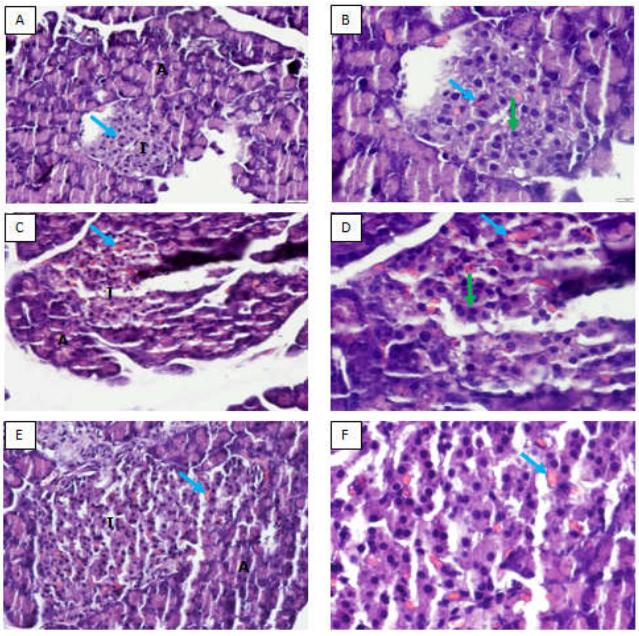

Fig. 2: Haematoxylin and eosin (H and E) stained histopathological sections of the pancreas after 14 d of treatment. (A) Plant control with 20X magnification: Native architecture of islets and pancreatic tissue. (B) Plant control with 40X magnification. (C) Diabetic+100 mg/kg *O. corymbosa* extract with 20X magnification: Islets of Langerhans showing evenly distributed β-cells with an increased number of β-cells. Red blood cells were spotted and the acini cells appeared to be normal. (D) Diabetic+100 mg/kg *O. corymbosa* extract with 40X magnification. (E) Diabetic+200 mg/kg *O. corymbosa* extract with 20X magnification. Islets of Langerhans showing evenly distributed β-cells with an increased number of β-cells. Red blood cells were spotted and the acini cells appeared to be normal. (F) Diabetic+200 mg/kg *O. corymbosa* extract with 40X magnification, I: Islets of Langerhans; A: Acinar cells; Blue arrow: Red blood cells; Green arrow: β-cells

DISCUSSION

Phytochemicals can be defined as a large group of plant-derived compounds which can benefit the human-kind in health improvement and diseases prevention against several diseases such as diabetes and cancer [23, 24]. Based on the results obtained in this study, *O. corymbosa* showed the presence of tannins, phlobatannins, saponins and flavonoids as of its phytochemical constituents and possesses antioxidant activity and this result is also supported by previous studies conducted by Fatema and Hossain [12]. Various studies have been shown that medicinal plants possess anti-diabetic properties in search of cure or treatment for diabetes that has little side effects. Thus, the present study was undertaken to demonstrate the anti-diabetic effect of *O. corymbosa* in alloxan-induced diabetic rats.

In the present study, a significant elevation of blood glucose and a decrease in body weight were observed after alloxan injection. The decrease in body weight in diabetic induced rats was due to the process known as catabolism of fats and proteins [25] and may be caused by stress and deranged metabolism which leads to anorexia and reduced digestion [26]. The administration of aqueous extract of *O. corymbosa* to diabetic rats showed increased in body weight as compared to diabetic control rats which according to Balamurugan *et al.* [25] this is a positive indicator in which the plant has a preventive effect on the degradation of structural proteins [25] and also the plant studied in this current research might have the ability to control muscle wasting [27]. The elevation of blood glucose level in diabetic induced rats after administration of alloxan may be caused by the reduction of insulin production by β-cells of pancreas or insulin resistance in peripheral tissues [25, 26]. In the present study, the aqueous extract of *O. corymbosa* was found to reduce the blood glucose levels in diabetic rats and showed no effect on blood glucose of normal rats. The hypoglycaemic action of *O. corymbosa* may be due to the increase in the activities of the enzymes which responsible in the utilization of glucose by insulin-dependent pathway or regeneration of damaged β-cells in the pancreas [28]. The hypoglycaemic effect of *O. corymbosa* may be attributed to the bioactive compounds present in it. Khan *et al.* [26] suggested that the presence of bioactive ingredients in plant extracts is responsible in the anti-hyperglycaemic effect of herbal plants which causes restoration of pancreatic tissues function.

Increase in the levels of LPO was observed in the diabetic induced rats. An increase in the levels of LPO in the diabetic induced rats may be due to the increase in oxidative stress in the cells which resulted from depletion of antioxidant scavenger systems [29]. In the current study, a significant decrease in LPO levels was observed in diabetic rats that received 100 mg/kg and 200 mg/kg of *O. corymbosa* extracts respectively as compared to the diabetic control group. This might be due to the antiperoxidative effect of bioactive compounds present in *O. corymbosa* which inhibit the oxidative damage. Concomitantly, a decrease in GSH and catalase activity was observed in the diabetic rats. The results presented in this study are in agreement with the other authors where they also reported decreased levels of GSH in diabetic rats [29, 30]. A significant elevation of GSH levels and catalase in diabetic induced rats were observed after treatment with *O. corymbosa* extracts might be due to its protective efficacy.

Lipids play an important role in the pathogenesis of diabetes [31]. Significant elevation of total cholesterol (TC) and low-density lipoprotein (LDL) with a decreased level of high-density lipoprotein (HDL) are usually observed in diabetes cases and such conditions represent a risk factor for coronary heart disease [32]. The significantly high levels of TC and LDL as compared to normal control rats, may be due to the deficiency or absence of insulin as insulin functions to disturb in the regulation of the activity of the hormone-sensitive enzyme, lipase which is responsible in converting triglycerides to free fatty acids and glycerol [33]. On administration of aqueous extract of *O. corymbosa* to the diabetic rats, a significant reduction on the TC and LDL levels were observed. Khan *et al.* [26] reported that the presence of phenolic and flavonoids compounds in the extracts have a positive correlation with the reduction in blood glucose level as well as a lipid profile. The restoration of the TC, LDL and HDL values near to or at normal values could be attributed with bioactive compounds present in *O. corymbosa*. The histopathological examination undertaken on the pancreas has also shown recovery of the damaged and ruptured islet as well as an increase in the number of β-cells after treatment with *O. corymbosa* extracts.

CONCLUSION

Our data suggest that aqueous extract of *O. corymbosa* at a dose of 100 mg/kg and 200 mg/kg body weight lowers blood glucose and lipid parameters except HDL. Significant restoration in GSH, LPO and CAT was observed in dose dependent manner. Histopathological examination of pancreatic sections revealed that diabetic rats treated with *O. corymbosa* extracts at either dose have normal architecture structure of islets. These results indicate that *O. corymbosa* may be effective as a hypoglycaemic and antihyperlipidemic agent.

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AUTHOR CONTRIBUTIONS

Caysandra Audrey Michelle Elwon conducted the experiment and prepared the manuscript. Associate Professor Dr. Mohammad Iqbal helped in designing and conducting the experiment. Dr Muhammad Dawood Shah proofread and edited the manuscript.

CONFLICT OF INTERESTS

The authors declare that there are no conflicts of interest.

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