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Original Article

Anti-diabetic Effects of Alcoholic Extract of Coscinium fenestratum through Pancreatic Cell Protection and Partial Regeneration induction in Streptozotocin-induced Diabetic Rats

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ABSTRACT

The study aims to evaluate the effects of the stem ethanolic extract of Coscinium fenestratum on Streptozotocin-induced diabetic rats. Rats were divided into 4 groups. Group 1 and 2 which served as normal and diabetic controls, respectively, did not receive the extract. A prevention group (group 3) was daily orally fed with the extract (250 mg/kg BW) for 2 weeks prior to diabetic induction. A therapeutic group (group 4), received the extract after the onset symptoms of diabetes. Blood glucose and body weight were measured every 3 days within further period of 6 weeks after the extract feeding. Pancreases were collected and histological evaluated. The results revealed the extract has the anti-diabetic effects by protection the severe weight loss due to diabetes, and reduced hyperglycemic. Morphometric analyses of the islets showed no changes of the mean area of the islets and the distances between the adjacent islets, implied atrophy was not obvious. Histological profile showed less damage and more compact arrangement of the islets cells in prevention group compared to the diabetic control group, whereas in therapeutic group also found the partial regeneration of the islets cells. The results suggest the compounds in the extract exert their effects through the protection of cells and stimulation of cell regeneration. The balanced of damaged and regenerated cells should improve insulin production and secretion.

1. Introduction

Diabetes mellitus (DM) is a group of metabolic disorders characterized by the classical symptoms of hyperglycemia, polyuria, polydipsia and polyphagia. These are either because of the body does not produce enough of insulin or cells do not respond to the insulin that is produced. Since the varieties of therapeutic drugs have been introduced, all forms of diabetes have been treatable. Based on the mechanism of actions, those therapeutic drugs are classified into two groups; drugs enhancing the effectiveness of insulin, i.e. Metformin, Troglitazone, Acarbose; and drugs increasing the supply of insulin, i.e. Sulfonylurea, Repaglinide. Improper treatment of diabetes, however, can cause many acute implications; hypoglycemia, diabetic ketoacidosis, or nonketotic hyperosmolar coma, for instance. Serious long-term complications may include cardiovascular disease, chronic renal failure, and retinal damage. Proper treatment is thus important.

Despite the availability of anti-diabetic drugs, the treatment outcome is not satisfactory in most cases due to the fact that oral anti-diabetic drugs cannot cure the disease. On the other hands, these drugs may not be effective in preventing the secondary complications of diabetes. New agents that are able to control and even to treatment diabetes are therefore of great interest. Attempting to find better therapeutic options for diabetes is in progress in many laboratories around the world, and lots of data has been generated as a result. Among those, using of natural products for the treatment of diabetes is one of alternatives since varieties of pharmacological aspects of their phytoconstituents has been approved with varieties of animal models [1, 2]. Using of natural products to treat the disease, however, still need to be explored in depth.

In South-East Asian countries, varieties of botanical products have been widely used in diabetes treatment [3, 4]. Among those, Coscinium fenestratum has been proved effective in diabetic and anti-hyperglycemic treatment Lines of evidence from many research data have been proved berberine is the main bioactive alkaloid isolated from the stem of Coscinium fenestratum [3, 5, 6].

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The multiple pharmacological effects of berberine, such as anti-microorganisms [7], anti-inflammation [8], anti-oxidant properties [9, 10], and anti-tumor [11] have been demonstrated. Berberine has also been reported to improve glucose metabolism through induction of glycolysis [12] and inhibition of hepatic gluconeogenesis [13].

A reduction of plasma insulin reflects a reduction of the beta-cell mass in the pancreas, and is the critical clinical event in the development of diabetes. The acute onset of disease is preceded by a period of progressive destruction of pancreatic islets eventually leading to decreased insulin production and subsequent hyperglycemia. Generally speaking, the total number of beta-cells reflects the balance between the lost and the proliferation of the cells. Investigations of the factors that prevent or promote replacement the lost β -cells of the islets are therefore very interesting aspects.

The present study is going to undertake on the basis of several reports in the literature that pancreatic beta-cells are capable of replication/regeneration and also being afforded protection against damage produced by streptozotocin. Nicotinamide and Thiazolidines were reported to protect streptozotocin-induced damage of the islets in rats [14]. Since berberine and its derivatives have been earlier reported have anti-oxidant activity, plausible mechanisms of action are by reducing oxidative stress in pancreas and might protect the cells from degeneration. Alternative approach is possible for diabetes treatment through formation of new β -cells or even induction of regeneration as it has been reported to induce proliferation of hepatocyte [15]. These would increase the mass of islets and increase the release of insulin. Under such assumptions, this work is therefore focused on assessment, both quantitative and qualitative analysis, the efficacy of Coscinium fenestratum alcoholic extract under pathological investigations.

2. Materials and Methods

2.1. Chemicals

Streptozotocin (STZ) (Lot # 119K1591) was purchased from Sigma. Commercial kits of blood glucose test strip, AccuCheck® Advantage II, were purchased from Roche. All other chemical reagents used in this study were of analytical grade.

2.2. Experimental animal

The study performed on mature normoglycemic male Wistar rats weighing between 200-250 g, which were separated housed in cages. Animals were purchased from The National Experimental Animal Center, and maintained in an air-conditioned room in Department of Biology, Naresuan University with a 12-h dark and light cycle. All animals were free access ad libitum with standard pellet rodent diet and tap water, and received humanely care. Animal care and animal experimental protocol were approved by the University Ethical Committee.

2.3. Plant materials

The dried *C. fenestratum* stem slices were purchased from local herbal markets in Ubon Ratchathani Province. The origins of the samples were collected from the border of Thailand and Laos. All materials were re-authenticated by Dr. Chaichan Maneeratanarungrot, an expert botanist of Department of Biology, Naresuan University. Samples were dried in a hot air oven (50 ºC) for 1 h, ground to powder. Approximately 100 g of powdered samples were extracted by maceration with 500 ml of 80% ethanol for 72 h. After centrifugation to remove the pellets, the supernatant was evaporated in a rotary evaporator under reduced pressure. The extract powder was kept in airtight bottle in desiccators for further use.

2.4. Experimental design

Streptozotocin (STZ) dissolved in 0.01 M citrate buffer (pH 4.5) was injected intraperitoneally (i.p.) to rats fasted overnight in the dose of 70 mg/kg BW. To avoid the effect of hypoglycemic shock, 1% glucose solution was substituted tap water for at least 24 h. Fasting blood glucose collected from the tail vein was measured by oxido-reductase reaction commercial kits on the starting day and every 3 days until finish a course of treatment in 6 weeks. Rats which showed a fasting glucose of >250 mg/dl are considered as diabetes. Fasting blood glucose was checked every 3 days until 6 weeks of experiment.

Rats were divided into 4 groups: (1) normal rats, which needed to be concurrently used as negative control, (2) diabetic group as positive control, (3) prevention group with *C. fenestratum* extract feeding prior to diabetic induction and (4) therapeutic group with C. fenestratum extract feeding after the onset of diabetes. The numbers of rat for each group are 5-8. The normal control and the diabetic control groups were orally administered with distilled water daily, while the third groups were administered with alcoholic extract of *C. fenestratum* dissolved in distilled water with a dose of 250 mg kg-1 for 2 weeks prior to diabetes induction, and blood glucose was monitored for further 4 weeks. The fourth group was fed with the extract after 2 weeks of the clearly onset of diabetes, and blood glucose was measured for further 4 weeks. During the course of experiment, bodyweight of rats were also recorded every 3 days. All rats were sacrificed on the day after the last dose of the extract feeding with the excess of ether followed by cervical dislocation. Pancreases were removed, and prepared for histological studies.

2.5. Histological investigation

The tissues were fixed with Bouin's solution and subsequently processed using traditional paraffin embedding techniques. Thin slide of 5-6 µm were cut with a Leica microtome, and placed on glass slides. Pancreas sections were stained with hematoxylin and eosin. For histological evaluation, islets of Langerhans were identified and studied for size, distance between the adjacent islets, atrophy, cell death, and regeneration of islet cells under microscope.

Morphometric analyses of islets of Langerhans were evaluated with the aiding software, ImagePro Plus®. A researcher blind to the treatments did the histological evaluation.

2.6. Statistical Analyses

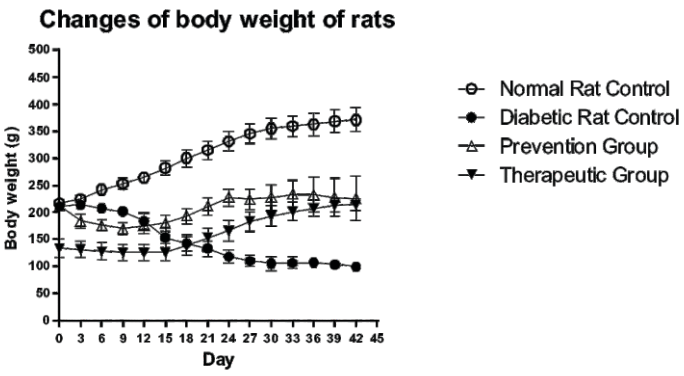
Statistical analyses of results were done by using a computer-based program (Prism GraphPad Software version 6). The different between groups of experiment was analyzed by one-way ANOVA. P-value < 0.05 is considered as statistically significant.

3. Results

3.1. Effects of the extract on body weight:

Diabetes usually characterized by weight loss and it is also seen in this study. There were significant differences in body weight between normal and diabetic rats ($p < 0.05$). Normal rat showed the age-related increasing of body weight with the average was 371.6 ± 22 g at the end of the experiment, while diabetic rats were changing trends in respect of the amount of blood glucose accompanied by severe loss of weight with the average was 100 ± 5 g (figure 1). Although the pattern of age-related increasing of body weight is similar to the non-diabetic control, rats of prevention and therapeutic group which both of them were fed with the extracts, were slightly increasing of body weight and less than the non-diabetic control rats. Consideration on the aspect of anti-weight loss due to diabetes, the data shown the body weight of rats in group 3 (prevention group) and group 4 (therapeutic group) were nearly the same in average of body weight by the end of experiment with 203 ± 41 and 203 ± 12 g, respectively. The potential of the extract was therefore effectively in the protection of the severe weight loss up to 54.7%. In addition, some remark on the effects of the extract has been found is, it decreases the body weight of the rats at the beginning state of the extract feeding as shown in figure 1.

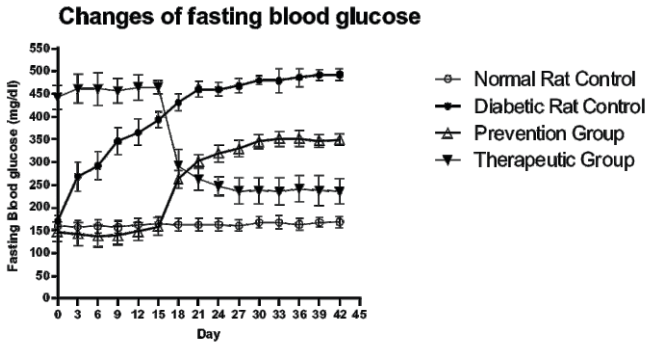
Figure 1. Changes of average rat body weight during the time course of diabetic induction for 6 weeks. The prevention group has been fed with the alcoholic extract of C. fenestratum for 2 weeks prior to diabetic induction, while the therapeutic group was induced after the clear onset of diabetes. Data are expressed as mean ± SEM, n = 5 for each group.



3.2. Effects of the extract on blood glucose:

Selective destruction of β–cells in the islets of the pancreas has been proved to bring an increase in blood glucose levels. It is evident from the present investigation shown the success of diabetes induction by streptozotocin. As shown in figure 2, in diabetic control group (group 2), the amount of blood glucose was almost stable in the late stage of diabetic induction with the average of 492 ± 12.3 mg/dl indicates irreversible destruction of Langerhans islet cells, compared to the normal rats in group 1 which have the blood glucose in average of 168.8 ± 12.7 mg/dl. Feeding the extracts of C. fenestratum lowered the blood glucose of the rats in both prevention group (group 3) and in the therapeutic group (group 4) was observed (figure 2). Although blood glucose levels of the rats in group 3 were higher than in the normal group (349.8 ± 13.8 mg/dl), and considered diabetes, their blood glucose level still were much less than of the diabetic group. The results implied the severity of diabetes in this group was much less than the diabetes group, and the extract reversed their diabetes to nearly normal. The statistic approved this was significant ($p < 0.05$). In therapeutic group (group 4), the extract lowered the blood glucose to nearly normal level (235.2 ± 27.8 mg/dl) also has been observed in this investigation. These revealed the extract effectively protected the increasing of blood glucose in both groups.

Figure 2. Average level of rats fasting blood glucose in the various treatments during 6 weeks of experiment. Data of the therapeutic group were plot on the same scale with other groups starting from the onset of diabetes symptom. Data are expressed as mean ± SEM, n = 5 for each group.



3.3. Morphometric profile of the islets:

By the assumption that destruction of β–cells in the islets of the pancreas leads to the islets atrophy. Presumably, the size of the islets reduced, consequence, increasing the distance between the neighboring islets should be clearly observed. Thus, morphometric analyses of both aspects had been done by the aid of Image Pro software. Surprisingly, the results revealed there was no significant different, either of the mean area of islets in all groups (figure 3) or the distance between the islets (figure 4).

Figure 3. Mean area of islets of Langerhans in various treatments. Data are expressed as mean ± SEM, the numbers of islet observed were 20, randomly sampling from 5 rats of each group.

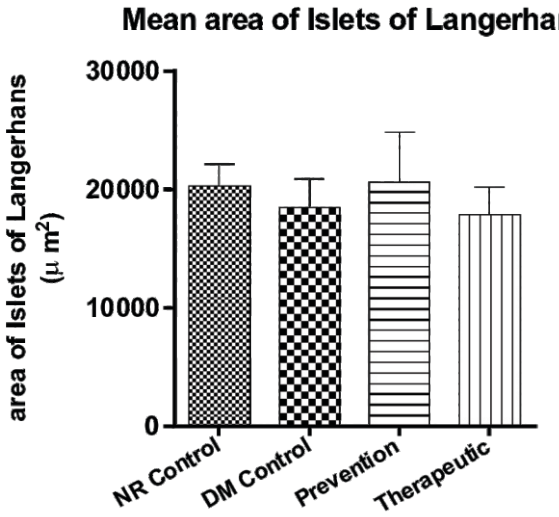
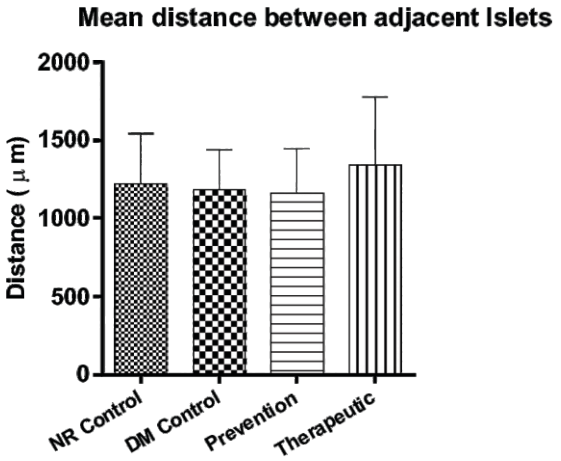


Figure 4. Mean distance between neighboring islets of Langerhans in various treatments. Data are expressed as mean ± SEM, the numbers of islet observed were 20, randomly sampling from 5 rats of each group.



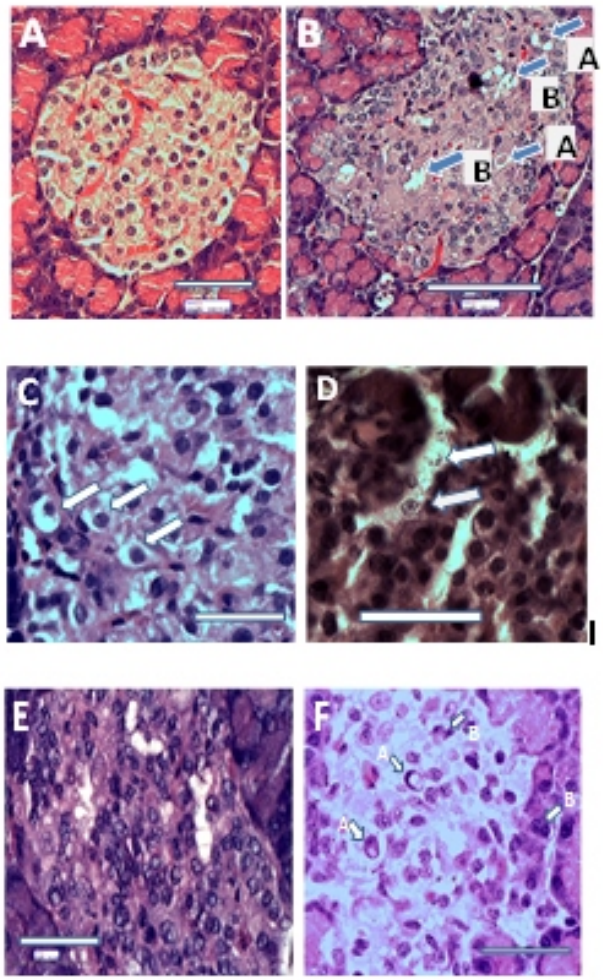
3.4. Histopathology of the islets:

Histologically, the islets of Langerhans of the normal control group (group 1) are clearly defined, prominent and well circumscribed as shown in plate A in diabetic control group (group 2) the islets appear with distortion architecture indicating cytotoxic action of streptozotocin (plate B, C and D). Toxicity is characterized by swollen of the islets cells and cytoplasmic vacuolation (arrow A of the plate B, and white arrow of the plate C) resulting in increasing of the intercellular spaces (arrow B of the plate B). Pyknosis and the necrotic cells are also observed indicating the cell death (white arrow of the plate D).

In the prevention group (group 3) which had been fed the extract for 2 week prior to diabetic induction, the results are quite similar to the diabetic group. Some remarkable differences, however, is the severity of cell damage is much less than of the diabetic group, and the cells are much more in number and are compactly arranged (plate E) with existing of the intercellular spaces in lower frequencies.

The death cells and remnants of death cells are observed in the pancreas of therapeutic group (group 4) which had been treated with the extract of *C. fenestratum* for 6 week after the clear onset of diabetes (arrow A of the plate F). However, the islets cells are still present. Interestingly, some evidence indicating the regeneration of the formerly destroyed β–cells is the existing of newly formed β–cells characterized by their smaller in size and densely nuclei (arrow B of the plate F).

Figure 5. Pancreatic histology of normal rats (plate A) compared to the diabetic rats that confirms the destruction of islets and cells due to the effect of streptozotocin (plate B, C and D). Severity of cell damage is less in prevention group (plate E). Although cell death still occurs in therapeutic group (arrow A of plate F), newly formed cells are also observed (arrow B of plate F). (Scale bars = 50 μm)



4. Discussion and Conclusion

The results from this study revealed significant loss of weight in diabetes rats compared to normal control rats. Either insulin deficiency or insulin resistant results in the depletion of glucose which is the first energy source of cells. Consequence, excessive breakdown of proteins and fatty acids as a result of gluconeogenesis occurs instead. Loss of muscle and adipose tissues are proposed the main causes of weight loss in diabetic rats [16]. The effects of feeding the C. fenestratum extract in this study agree with the studies of Akpaso et.al. [17], which previously showed that treatment with the extracts of root of Vernonia amygdalina resulted in appreciation weight gain of the animals. Makimattila et.al. [18] reported that improved glycemic control by insulin promotes weight gain by decreasing both metabolic rate and glycosuria. Severe weight loss was prevented in the rats treated with the alcoholic extract of C. fenestratum is possibly due to the action of its bioactive compounds to improve glycemic physiology of the rats.
Since the role of β-cell of the pancreas is very crucial in glucose homeostasis, the destruction or degeneration of β-cell is direct reflect the severity of diabetes. The secondary complication of the disease leading to the metabolic derangements has been found parallel to the β-cell function is severely reduced.

As reviewed by Patel et.al. [2] there are various types of phytoconstituent present in crude extract of plant materials belonging to different chemical classes with different mechanisms to reverse hyperglycemia: the groups of alkaloids inhibit alpha-glucosidase and decrease glucose transport through the intestinal epithelium; imidazoline compounds stimulate insulin secretion in a glucose-dependent manner; polysaccharides increase the level of serum insulin, reduce the blood glucose level and enhance tolerance to glucose; flavonoids suppress the glucose level, reduce plasma cholesterol and triglycerides significantly and increase hepatic glucokinase activity probably by enhancing the insulin release from pancreatic islets; saponin stimulates the release of insulin and blocks the formation of glucose in the blood stream, for instance.

Analysis of the phytoconstituents from many research data have been proved berberine is the main bioactive alkaloid isolated from the stem of Coscinium fenestratum [3, 5, 6]. The multiple pharmacological effects of berberine have also been reported [19]. On the aspects of anti-diabetic action, berberine has been reported to improve glucose metabolism through induction of glycolysis [12] and by inhibition of hepatic gluconeogenesis [13].

Berberine enhances glucose stimulated insulin secretion in rat islets and probably exerts the insulinotropic effect via a pathway involving NHF4α and glucokinase activity, which is distinct from sulfonylureas [20]. In addition, berberine reduces insulin resistance through protein kinase C-dependent up-regulation of insulin receptor expression [21, 22].

Free radicals may play an important role in the causation and complication of diabetes. The increased oxidative stress and accompanying decrease in anti-oxidants may be related to the causation of diabetes. In diabetes mellitus, alterations in the endogenous free radical scavenging defense mechanisms may lead to ineffective scavenging of reactive oxygen species, resulting in oxidative damage and tissue injury. It has been proposed that streptozotocin acts as a diabetogenic owing to its ability to destroy pancreatic beta-islet cells, possibly by a free radical mechanism. It is of interest, therefore, on the point that anti-oxidative stress properties of berberine would have the protective effects on the many high metabolism and vulnerable organs. On this aspect, berberine showed its protective effect on radiation-induced of lung injury in patients with lung cancer treatment [23] and also been found that it has neuroprotective effects both in vitro and in vivo [24, 25]. Moreover, recently research has proved that berberine decreased chemical-induced hepatotoxicity, such as, liver damage induced by acetaminophen [26] or diethylnitrosamine and phenobarbital [15]. The result from this study, the extract of C. fenestratum has the ability to ameliorate or reverse pancreatic lesions that results in hyperglycemia [27]. This means that the plant extract must possess antioxidant properties which would reverse the cytotoxic cycle of streptozotocin in the pancreas or get rid of the reactive oxygen species similar to the previous reports of other kinds of plant extract [17, 28].

Pancreatic beta-cells capable of regeneration have been reviewed [29], and some kind of plants has been reported to induce regeneration of islets in streptozotocin-induced diabetic rats. In the group treated with the extract of C. fenestratum, there was regeneration of the islets cells. This agrees with the previous report of regeneration of the islets treated with the extract of Centaurium erythrea of Sefi et.al., [28] and the work of Sabash-Babu et.al., [30] who did on Nymphaol, a sterol isolated from Nymphaea tellata. Plausible explanation on morphometric profile that the size of islets of Langerhans, and the distance between them were not alter might be, because of the balance between the damaged and the newly proliferated cells. Instead, the alterations occur inside the islets, by increasing the intercellular spaces due to cell death, changes of cyto-architecture of the islets cells, for instance. The balance of islets cells could increase in insulin production and secretion and reverse the diabetes in the extract treated rats.

In summary, from the results the alcoholic extract of *C. fenestratum* may exert their anti-diabetic effects either through the actions of the islets cells protection or via pancreatic islet regeneration, or even the combination of both. However, viability of the regenerated cell with respect to the increase of insulin production and secretion is still needed to be validated.

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