Assessment of Anti-Diabetic Activity of Vinca Rosea Extract on Induced Diabetic Mice

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Abstract

Background and Objective: Vinca rosea is a medicinal plant that has been investigated and applied as an alternative treatment for disorders. This study aimed to investigate the anti-diabetic activity of ethanolic extract of Vinca rosea on induced diabetes in mice.

Methods: Forty male albino mice were randomly divided into four groups, including control that was administered a standard physiologic saline and served as control, diabetic group, Streptozotocin & Vinca rosea, and only Vinca rosea; each group contained ten mice.

Results: No significant lesions were observed in pathological sections of the control; however, the lesions in diabetic group showed massive lesions in the islets of Langerhans and reduced dimensions of islets due to the damage of beta-cells. Furthermore, the Pancreas in Streptozotocin & Vinca rosea demonstrated a moderated vacuolation and Langerhans of islet cells. In contrast, the histopathological sections of the pancreas, which treated by ethanolic extracts of Vinca rosea, illustrated increasing number of beta-cells hypercellularity. The values of blood glucose were increased in the diabetic group; however, the significant decreases ($P<0.05$) in blood glucose detected in treated by ethanolic extract as represented by increased Beta-cells. While, the blood glucose levels were tolerated in treated Streptozotocin & Vinca rosea; thus, these values were closely for the control results.

Conclusion: The Vinca rosea extracts induced a significant impact on diabetic mice, and they can improve and protect pancreatic cells in diabetic mice. Therefore, ethanolic extracts of Vinca rosea could be beneficial for the diabetes.

Keywords: *Anti-diabetic, Streptozotocin, Vinca rosea, diabetic mice.*

Introduction

Plant materials contain different characteristics such as chemical and biological functions, and these features could play important roles in the animal diet that include some of the plant parts, e.g. stems, leaves, flowers and seeds [1]. Plants also contain bioactive compounds, which are secondary metabolites. Those have a significant contribution to the physiological processes of the animal body. Furthermore, plant extracts are often applied as pharmaceuticals in the animal industry that have multiple conditions [2].

Vinca rosa (*Catharanthus roseus L.*) is one of the medicinal plants that have been investigated for decades. The investigations have concentrated on the isolation of bioactive compound from this plant, such as flavonoids and alkaloids, which is the most dominated in different parts of the plant [3]. Many kinds of these alkaloid compositions, including vinblastine and vincristine, have been discovered in this plant and they have been used as alternative remedy and treatment of diseases e.g.

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antimicrobial, anti-inflammatory and anticancer [4; 5; 6].

Several studies have focused recently on the alkaloid impacts that extracted from Vinca rosea plan, although the mechanism of their effects as therapeutical agents is still unclear [7]. Therefore, many researches have been done to this plant and its extract as the natural source of treatment [8].

One of the biggest endocrine disorders is diabetes that influences several people over the world). The patients with diabetes have been treated orally with different medicinal plants. However, researches are still looking for anti-diabetic alternative medicine that could extract from natural materials [9]. For decades, an aqueous extract of Vinca rosea is applied to control the diabetic disorders, as well as many countries are used for diabetes the whole plant or its parts, e.g. leaves or flowers, as household treatment [5]. Furthermore, Vinca rosea extracts were indicated to show a significant effect on blood glucose of lab animals [10].

Materials and Methods

Plant description and extraction:

Vinca rosea plant was obtained locally from Baghdad gardens; they have been shed and dried at room temperature. These plants were deposited to be identified and authenticated at the National Herbarium of Iraq Botany Directorate in Abu-Ghraib. The analytical dose of 70% ethanol applied to extract the Vinca rosea plant, which was described by ([11]) plus more modification. Each 100g of these dried leaves were washed under tap water and ground using a coffee blender then adding 250 ml of 70% ethanol into thermal stirrer for 2.5 h at room temperature. Afterwards, the mixture were filtered and centrifuged to make it as clear solution, and around 20% of dried leaves of Vinca rosea in 50 ml were applied for this research. The LD50 dose of Vinca rosea extract was used at 1000mg extract for each a Kg.

Animals:

Forty male albino mice, weighing 28.0 ±7.0 g and ageing 10-12 weeks, were randomly divided into four equal groups. They were obtained from the Institute of Sera and Vaccines/ Baghdad. Mice were housed in plastic cages with water provided ad libitum and fed standardized commercial pellet. This experiment was carried in the Pathological Laboratory, Department of Veterinary Pathology, College of Veterinary Medicine, the University of Baghdad for the period extended from 1st July 2019 to the end of December 2019.

Experimental design:

The 1st group was administered normal physiologic saline and served as control. The 2nd group was injected intraperitoneally with a single dose 200-250 mg/kg of Streptozotocin® (STZ, Santa Cruz-USA) diluted in saline with mixing [12]. Dissolved STZ was kept at 4 ºC for 1-2 hours and then mixed again before injection. Diabetes is confirmed by sustained blood glucose levels above 350 mg/dl or more consecutive days by using glucose meter® (Gluco Lab-Korea) [13]. A similar dose of STZ was given to the 3rd group by the same administration route that followed by a single dose per week (a long period of experiment) of 1mg/kg BW of ethanol extract of Vinca rosea (I/P for 60 days). The 4th group was only administered of Vinca rosea extract with similar dose and same administration route to the 3rd group.

Histopathological analysis:

At the end of the experiment, the experimental animals were sacrificed using 'Euthanasia technique' that was done by cervical dislocation in rabbits, and the postmortem examination was achieved. The macroscopic appearance was recorded and any abnormal gross changes in internal organs were observed. Specimens were taken from all internal organs; moreover, the organ tissues have been kept in 10% formalin buffer phosphate immediately after removal. After 48 hours of fixation, the tissue slide processing was made routinely using a set of increasing alcohol concentrations. The tissue sections were embedded in paraffin blocks and sectioned by microtome at 5µm. All tissues were stained with hematoxylin and eosin stain; then, the histopathological changes were examined under a light microscope [14].

Determination of insulin levels:

The insulin ELISA KIT (Raybiotich, USA) was applied according to manufacturer recommendation (US-Biological, USA). The glucose measurement in the blood level has been done by biochemistry methods [15] with modification by using a commercially available kit

(Cypress diagnostic, Belgium).

Statistical Analysis

The values of blood glucose in experimental mice were expressed as (Mean ±SEM) and analyzed for ANOVA and using student's t-test by SAS software (V. 0.19). The variance values among these groups were considered significantly ($p<0.05$).

Findings

Pathological examination:

No significant histopathological lesions were observed in the control group; however, the histopathological lesions of pancreas gland of mice, which were treated with Streptozotocin, showed focal acinar necrosis and atrophy with vacuolar degeneration of Langerhans islet that occurred due to the damage of β-cells (Figure 1 and 2). Moreover, the Pancreas in 3rd group, which was treated with Streptozotocin & Vinca rosea extract, showed a moderated vacuolation in islet and moderated Langerhans islet, as well as a moderated regeneration of pancreatic islet cells (Figure 3). Further, the histopathological sections of the pancreas, which treated only with Vinca rosea extract, showed increases in the number of β-cells hypercellularity (Figure 4); while in the control group, the pancreatic islet of Langerhans seemed to be normal.

Glucose and insulin levels:

The values of blood glucose were dramatically increased at the group of diabetes that reached to (266.20 ±1.29) at day 60, while the treated group with Streptozotocin & Vinca rosea the values closely for the control group that recorded (at day 60) 91.20 ±0.37 and 94.20 ±0.58, respectively as compared with the control, so there was no significant differences. In contrast, the values of blood glucose in treated group with extract of Vinca rosea only was decreased significantly ($P<0.05$) compared to control at day 60 (81.93 ±0.75) (Table 1).

The levels of serum insulin were decreased in the group of diabetes that reached to 0.194 ±0.036 at day 60; while in the treated group with Streptozotocin & Vinca rosea, the values closely to the control (1.944 ±0.063 and 1.760 ±0.088 respectively), so there was no significant differences between these groups. However, the treated group of Vinca rosea extract only showed significant increases ($P<0.05$) in insulin level at day 60 that recorded to 0.194±0.018 as represented by increased inducing the β cells. This increase tolerated the effect of Streptozotocin & Vinca rosea group (Table 2).

Discussion

Histo-pathological lesions:

The present study indicated that Vinca rosea extracts exhibited significantly increasing in the number of β-cells hypercellularity. Aside from that, the group that treated with Streptozotocin & Vinca rosea showed moderated vacuolation and Langerhans of the islet; pancreatic islet cells were also moderated and regenerated. These findings indicated that Vinca rosea extracts could exhibit significant activities as anti-hyperglycemic in diabetic induced mice. These results were also observed the anti-diabetic activity of Vinca rosea extracts. On the other hand, the affected β cells in diabetic animals were reported in many studies [16] that suggested the regeneration of islet ß cells in induced diabetic lab animals, such as rats and guinea pigs, could be influenced Vinca rosea extracts. In the current study also illustrated that diabetic control mice had necrosis and atrophy in the Langerhans islet and vacuolar degeneration, which happened due to the damage of β cells; this could play a critical role by a protective impact when it was induced a diabetic effect in mice, which could have a 'hormetic effect' that indicates stimulation effect in a low dose, and inhibitory impact in high dose [17]. Therefore, these findings revealed, in general, that Vinca rosea extract was more effective on lesions and closed results showed to Streptozoitine & Vinca rosea treatment.

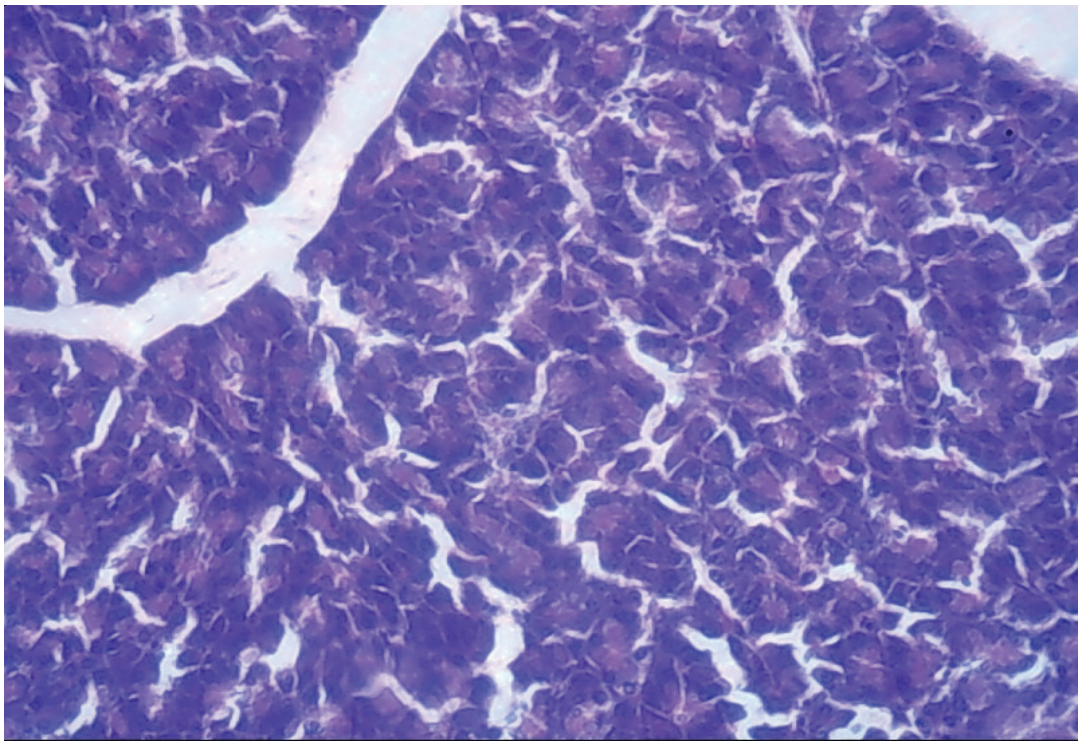

Figure 1 shows the severe vacuolation in the pancreatic islet of Langerhans with the disappearance of luminal acinar lobules (H&E X200).

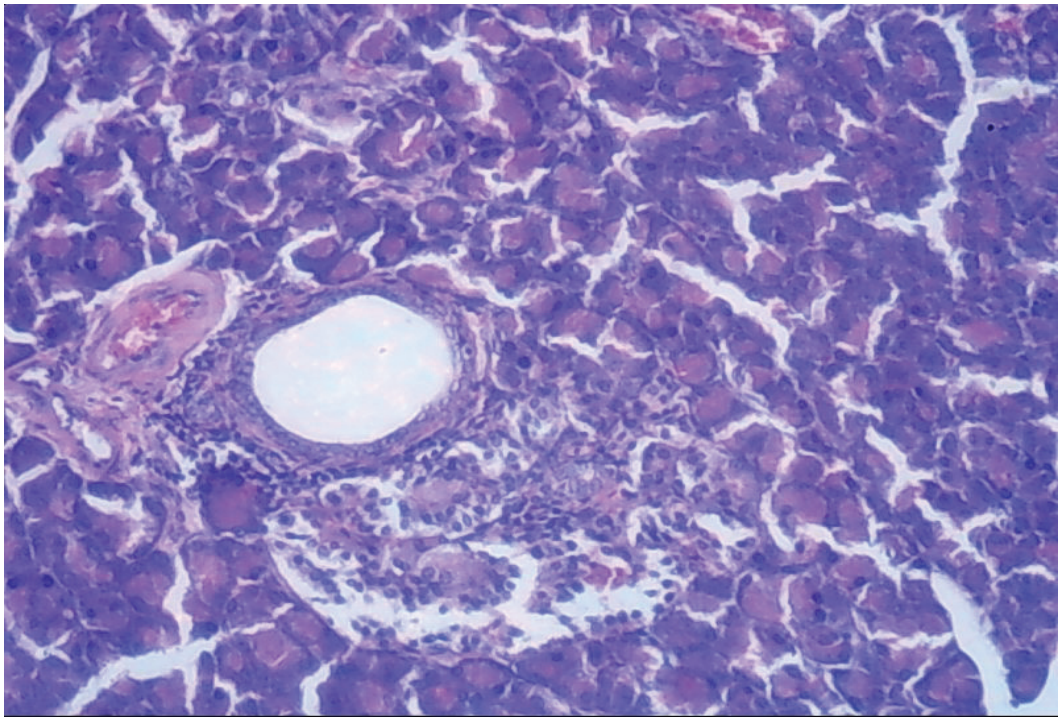

Figure 2 shows the necrosis of acini and atrophy with vacuolar degeneration of pancreatic islet of Langerhans with congested vascular capillaries (H&E X200).

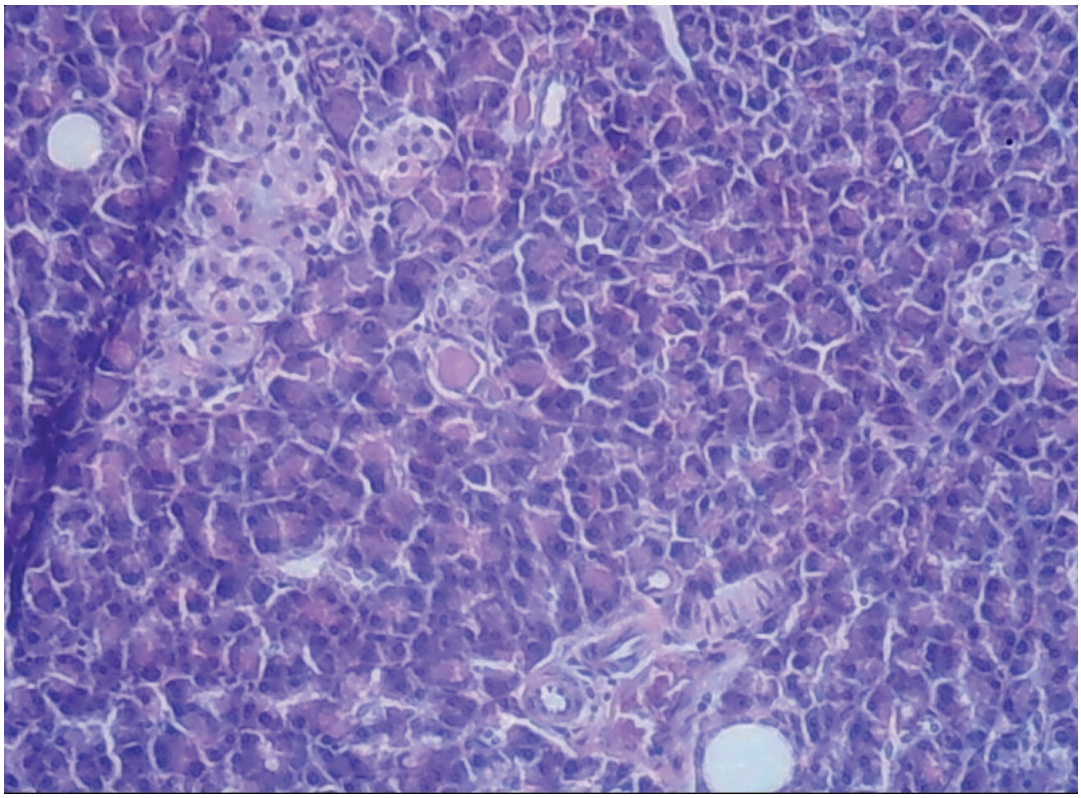

Figure 3 shows moderate vacuolation in the islet of Langerhans (H&E X200).

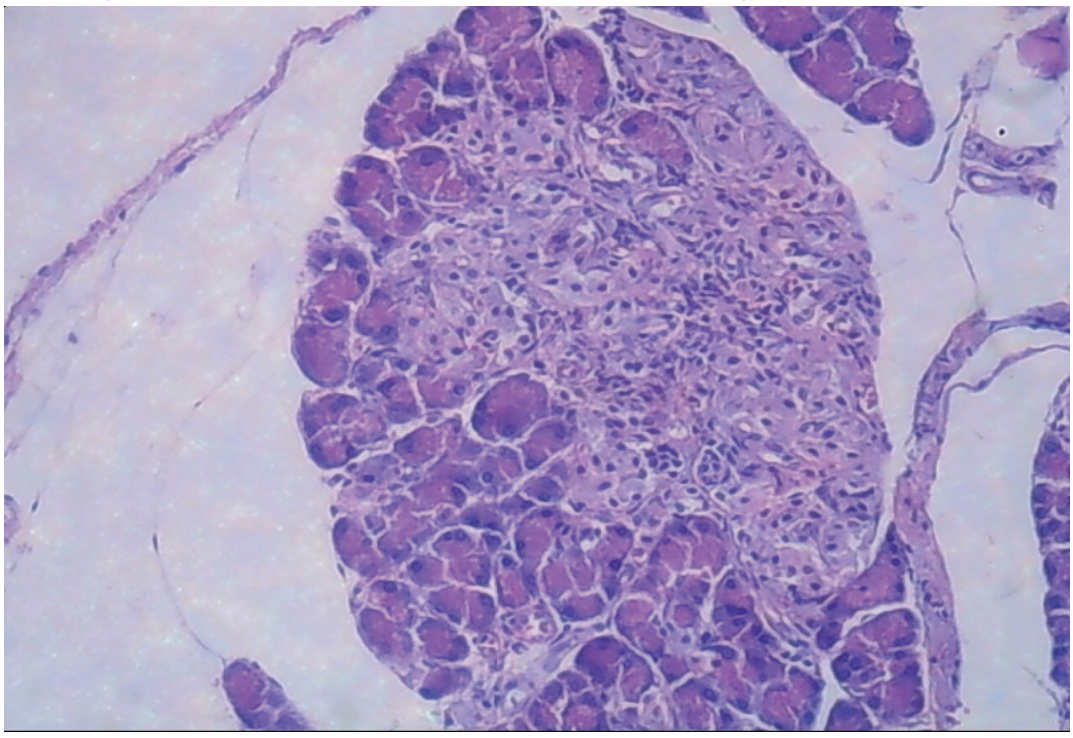

Figure 4 shows an increasing number of β cells resulting in hyperplasia of the pancreatic islet (H&E X200).

Glucose and insulin finding:

Although there are several drugs available to treat diabetes, these medicines have side effects on the pancreas [3]. Thus, there is a demand to search for an effective and safe alternative medicine [4]. This study tried to support β cells that could be possibility survived by using Vinca rosea extract, which could be exerted the insulin-releasing. Moreover, histopathological findings indicated the healing of pancreas using Vinca rosea treatment, which demonstrated the mechanism of anti-diabetic activity.

The glycogen was increasingly breakdown, as gluconeogenesis mechanism, which acts as a decreasing amount of insulin in diabetic animals that might be responsible for a reduction in cellular glucose as well. A study revealed that the vascular complications of the pancreas could lead to pathogenic changes in diabetic animal [18].

In contrast, the diabetic control mice reported increasing of blood glucose level. This group was statistically significant (P<0.05) compared to treated animals. This study recorded that the reduction in blood glucose level in Vinca rosea group compared to control. This finding could be due to the normal metabolism of glucose mechanism by plant extract.

Furthermore, the increasing of β cells of the pancreas could be detected in diabetic mice, which treated with Streptozotocin & Vinca rosea and diabetic group. The findings of this research revealed that daily treatment by Vinca rosea extracts for 60 days illustrated a significant decrease of the blood glucose level of induced diabetic mice; these results have matched with [19].

Table 1: Effect of Vinca rosea extracts on levels of blood glucose (mg/dL) in diabetic mice.

Group	Description	Day 0	Day 7	Day 30	Day 60
1st	Control	93.91 ±0.48 Aa	94.80 ±0.32 Aa	95.10 ±0.66 Aa	94.20 ±0.37 Aa
2nd	Diabetes	94.56 ±0.31 Aa	218.00 ±2.27 Bb	251.00 ±1.89Bc	266.20 ±1.29 Bd
3rd	Streptozotocin & Vinca rosea	94.83 ±0.64 Aa	96.20±1.28 Aa	93.65 ±0.84Aa	94.20 ±0.58 Aa
4th	Vinca rosea only	95.04 ±0.24 Aa	90.40 ±0.84 Cb	81.20 ±0.88 Cc	81.93 ±0.75 Cc

The data expressed as (mean ±SEM). The different small letters indicates to significant differences between column ($p<0.05$), and different capital letters indicates to significant differences between rows ($p<0.05$).

Table 2: Effect of Vinca rosea extracts on levels of serum insulin (mg/dL) in diabetic mice.

Group	Description	Day 0	Day 7	Day 30	Day 60
1st	Control	1.853 ±0.112 Aa	1.871 ±0.097 Aa	1.726 ±0.101 Aa	1.760 ±0.088 Aa
2nd	Diabetes	1.768 ±0.093 Aa	0.752 ±0.083 Bb	0.526 ±0.091 Bc	0.194 ±0.036 Bd
3rd	Streptozotocin & Vinca rosea	1.926 ±0.089 Aa	2.100 ±0.163 ACa	1.854 ±0.094 Aa	1.944 ±0.063 Aa
4th	Vinca rosea only	1.816 ±0.059 Aa	2.246 ±0.073 Cb	2.230 ±0.082 Cb	2.270 ±0.058 Cb

The data expressed as (mean ±SEM). The different small letters indicates to significant differences between column ($p<0.05$), and different capital letters indicates to significant differences between rows ($p<0.05$).

Conclusion

The present study revealed that Vinca rosea extracts significantly influenced on blood sugar levels by releasing more β cells of the pancreas that led to an increase of insulin levels in treated group competed with diabetic mice. In contrast, the Streptozotocin & Vinca rosea treatment showed similar values to the control group as there were no significant differences.

In contrast, the histopathological lessions of the pancreas, which treated by Vinca rosea only, showed increasing number of beta-cells hypercellularity. Therefore, Vinca rosea extract might help to prevent diabetes as using in traditional medicine. In the future, further study is needed to determine the action of the whole plant on other organs and detected the toxicity on diabetes.

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