

Isolation of 1, 2 di-substituted idopyranose from *Vitex negundo* and its effects on diabetic rats

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Abstract- The aim of this study was to investigate the protective effect of plant compound idopyranose is evaluated against streptozotocin-induced diabetics Wistar rats. The animals were divided into four groups consisting of 6 rats each. Group I contained the normal control were treated with olive oil, group II was served as a negative control (diabetic control), group III comprising normal rats were administered with idopyranose alone for 21 days and group IV diabetic rats were administered with idopyranose (50 mg /kg body wt/ day orally) respectively. Diabetic rats were given idopyranose isolated from the plant, *Vitex negundo* showed significant reduction in blood glucose, alanine aminotransaminase and aspartate aminotransaminase activities. Serum urea, creatinine and cholesterol were also significantly reduced when compared to diabetic control. Apart from these parameters, idopyranose showed improvement in parameters like body weight, lipid profile such as triglyceride, high density lipoprotein (HDL) and low density lipoprotein (LDL). In diabetic rats changes like coarsening of acinar cells of endoplasmic reticulation, destruction of beta cells and alternation in their secretory function were observed in the histomorphology of the pancreas. The changes like dilation of vein, loss of unusual concentric arrangement of hepatocytes and liver fibrosis were observed in the liver. Thickening of tubules and expansion of glomerulus were observed in kidneys. All these altered parameters were reversed close to normal condition by the treatment of idopyranose. According to biochemical and histological results obtained, it can be concluded that the idopyranose helps in regeneration of damaged pancreas and protects pancreatic β cells and hyperglycemic nature against streptozotocin-induced diabetes.

Key words: *Vitex negundo*, Verbanaceae, anti-diabetic compound, idopyranose, diabetes, Wistar albino rats

1. Introduction

Diabetes mellitus (DM) is a chronic disease caused by inherited and/or acquired deficiency in the production of insulin by pancreas, or by ineffectiveness in the production of insulin. Such a deficiency results in increased concentration of glucose in blood which, in turn damages many body systems and in particular the blood vessels and nerves. As the number of people with diabetes mellitus world wide is increasing, the disease has taken an ever increasing share of national and international health care budgets. The world health organization (WHO) reported that by the year 2025, 300 million peoples would suffer from diabetes mellitus [20]. India is one of the principal countries for the number of people with diabetes and it is estimated that it affects approximately 57 million people by the year 2025 [4]. Apart from currently available drugs sulphonyl urea, biguanides, α -glucosidase inhibitor and thiazolidinediones, many herbal medicines have been recommended for treatment of diabetes. Traditional plant medicines are used throughout the world for a range of diabetic medicine due to their merits of efficacy, low incidence of side effects and low cost [1]. *Vitex negundo* Linn (Verbanaceae) was found throughout India and also called as "nochi" (local vernacular). The extracts of leaves, flower, seed, bark and root of the plant have been reported to be antibacterial [19], anti-diabetic [12] anti-inflammatory [9, 25, 26], anti-oxidant [27], anti-HIV [29] and snake

venom neutralization [2]. Considering its value as a traditional medicine the active compound against anti-diabetic was isolated from the leaves of *Vitex negundo* which was named as 1, 2 di-substituted idopyranose followed by its effect against diabetic rats.

2.0. Materials methods

Healthy male albino of Wistar rats, weighing 170-200 g was procured from National Institute of Nutrition, Hyderabad, India. They were given sufficient quantity of water and fed with standard rat feed *ad libitum* (Hindustan Lever Limited, Mumbai, India). The rats were housed in polypropylene cages over husk bedding and a 12 hour light and dark cycle was maintained throughout the experimental period. The animals were allowed to acclimatize in the laboratory condition for four days before conducting each experiment. The experiments were conducted according to the ethical norms approved by Government of India and Institutional Animal Ethics Committee guidelines (Approval No.360/01/a/CPCSEA).

2.1. Extraction, isolation and purification of idopyranose

Vitex negundo belongs to the family of Verbanaceae were collected from Pallikaranai, Chennai, Tamil Nadu, India. The leaves of *Vitex negundo* were allowed to shade dry for two to three weeks. The shade dried leaves were

pulverized and used for extraction. Pulverized leaves (500 gram) were extracted in a Soxhlet extractor with methanol. Methanolic concentrate was fractionated with hexane, diethylether, chloroform and n-butanol. The n-butanol concentrate was charged on silica gel column or alumina (neutral) column. Further purity check was performed by using precoated TLC plate (silica gel GF₂₅₄, EtoAc100/MeoH, 15, Pristamatic needle like crystal from ethyl acetate and methanol). The compound was further analyzed using IR and NMR (Regional Sophisticated Instrumentation Centre, Indian Institute of Technology, Chennai, India) for the elucidation of its structure.

2.1. Preparation of Streptozotocin

Streptozotocin was purchased from Upjhon Company, Kalamazoo, USA. One vial contains 1 g of streptozotocin and 220 mg of citric acid anhydrous. A 0.1% solution of streptozotocin was freshly prepared in 0.1 M citrate buffer (pH 9.0) and each ml of prepared solution contains 1 mg of streptozotocin and 0.22 mg of citric acid.

2.3. Experimental design

The animals were divided into four groups consisting of 6 rats each. Group I contained the normal control were treated with olive oil, group II was served as a negative control (diabetic control), group III comprising normal rats were administered with idopyranose alone for 21 days and group IV diabetic rats were administered with idopyranose (50mg/kg body wt/ day orally) respectively. After the experimental period, the animals were fasted overnight and then sacrificed by cervical decapitation. Blood was collected from the rats. Serum was obtained after blood coagulation and centrifugation at 5000 rpm for 15 min to obtain a clear supernatant (serum) which was stored at -70°C, until its use for further biochemical analysis.

2.4. Blood glucose estimation and serum biochemical profiles

After 21 days all the treated and control rats were used to collect blood by cardiac puncture under mild ether anesthesia and blood glucose was estimated by Sasaki and Matsui [23]. Serum was separated and analyzed for cholesterol [18], triglyceride [15], HDL [3], LDL [11], urea [16], creatinine [7] and AST and ALT [13].

2.5. Histology

Paraffin sections of pancreas, liver and kidney were made and stained using haematoxylin and eosin stain, while the pancreas sections were subjected to aldehyde fuchsin light staining followed by the method of Bora et al [6]. The histological observations were made under a binocular Carl Zeiss microscope.

2.6. Statistical analysis

The data were expressed as mean \pm SD. Statistical comparisons were performed using one way analysis of variance. The results were considered statistically significant of the p values at 0.05 or lesser levels.

3. Results and Discussion

The compound pyranose was eluted with 100:25 of CHCl₃:MeoH. General molecular formula of this compound was calculated as C₂₃ H₂₈ O₁₂ and its specified molecular formula derived was C₆ H₁₂ O₆ in which hydrogen (H) and hydroxyl group (OH) were arranged in controversial to glucose molecule. It contained both terpene and sugar molecule. Terpenes found generally in plants were known for its defense function. Attachment of both sugar and terpene molecule was found in the same compound and hence the compound was named as 1, 2, di-substituted idopyranose. The following data were the results of IR and NMR. IR (KBr) λ_{\max} 1697 cm⁻¹ (C=O), 3408 cm⁻¹ (-OH), 1600 cm⁻¹ (C=C). MS m/z 496, M.p. 110, NMR (CD₃OD) 1.31(s, 3H), 1.36-1.38 (m, 1H), 1.60-1.72 (m, 3H), 2.0 (bs, 4H), 2.15-2.18 (m, 1H), 2.23 (dd, 1H), 3.40 (t, 1H), 3.54 -3.60 (m, 2H), 3.74-3.76 (m, 1H), 4.0 (t, 1H), 4.47 (t, 1H), 5.0 (s, 1H), 5.34 (d, 1H), 5.54 (d, 1H), 6.84 (d, 2H), 7.38 (s, 1H), 7.82 (d, 2H), 11.0 (s, 1H) "Fig. (1)", "Fig. (2)". The body weight of the control animals and idopyranose alone treated animals were constantly increased in three weeks over experimental diabetic rats. The body weights of diabetic rats were significantly decreased. However the loss of body weight was regained in diabetic animals treated with idopyranose (Table 1). The level of blood glucose was normal in control rats. But it was significantly increased in diabetic animals, while in the case of idopyranose alone treated group showed no change in their blood glucose. However, the increased blood glucose was returned to normal in diabetic rats treated with idopyranose (Table 2). The levels of serum cholesterol, triglyceride, HDL, LDL, urea, creatinine, aspartate and alanine aminotransaminase activities of experimental animals were increased when compared to control except the rats treated with drug alone where all the profiles were similar to control. The results clearly indicated that the levels of all biochemical profiles were returned to normal in diabetic rats treated with idopyranose (Table 3). Diabetic rat given idopyranose showed significant reduction of blood glucose, alanine aminotransaminase and aspartate aminotransaminase activities were also decreased. Serum urea, creatinine and cholesterol were significantly reduced in diabetic rats given with idopyranose when compared to diabetic control. Apart from these parameters, idopyranose treated diabetic rats were showed improvement in parameters like body weight and

lipid profile. In this context, the crude and non-characterized extracts of other medicinal plants such as *Phaseolus vulgaris* [28], *Scoparia dulcis* [14] and *Gymnema montanum* [22] have also been reported to have a similar effect as observed in the present investigation. The light microscopic examination by specific staining of pancreatic beta cells in control tissues showed abundant patches of beta cells which was not observed in diabetic pancreas "Fig. (3a)", "Fig. (3b)". Pancreatic section of rats treated with test compound (idopyranose) showed no notable histological changes "Fig. (3c)". Pancreatic section of diabetic rats treated with idopyranose showed beta cells were stained purple in colour similar to the control "Fig. (3d)". Liver tissues of control animals showed no changes in its histology "Fig. (3e)". Liver tissues of diabetic rat showed distortion in the arrangement of cells around the central vein, enlargement and thickening of the wall veins, capillaries and development of fibrosis in the degenerated cells "Fig. (3f)". Liver tissues of rat treated with idopyranose showed no notable changes "Fig. (3g)". In addition liver sections of diabetic rat treated with idopyranose brought back the cellular arrangement around central vein and reduced the fibrosis and also helped to bring blood vessels to normal conditions "Fig. (3h)". Kidney section of control rats showed no variation "Fig. (3i)". Kidney section of diabetic animal showed thickening on the wall of the nephrons filling their lumen and glomerulopathy "Fig. (3j)". Kidney section of rat treated with idopyranose also showed no notable changes as like that of control "Fig. (3k)". Diabetic rats treated with idopyranose showed no thickening on the wall of the nephrons and looks normal and glomerulus expansion was completely reduced "Fig. (3l)". The histological studies revealed that the diabetic rat without any treatment showed no pancreatic islets regeneration. However, the regeneration of beta cells was observed in diabetic rat treated with idopyranose. The pancreatic section from the drug alone treated rats also showed no changes in the histology of islets of beta cells as shown in the control animals. Histology of liver in diabetes condition showed that there was structural alternation in the liver as a result of absence of insulin. The major alternation was in the structure of blood vessels and capillaries. The fibrosis observed in liver showed that there was extensive damage to liver cells which was replaced by fibrous tissues [5]. This damage was totally reversed by the idopyranose treatment. Diuresis was known as a common feature associated with diabetes which may be the reason for structural changes observed with glomerulus. However these changes were fully reversed by the treatment of idopyranose. The anti-hyperglycemic activity of idopyranose probably due to stimulation of insulin

secretion from remnant pancreatic beta cells which in turn enhances the glucose utilization by peripheral tissue of diabetic rats. Several research works reported that the regeneration of beta cells in pancreas [8, 21], distortion in arrangement of cells around the central vein, enlargement and thickening of the wall of the vein, capillaries and development of fibrosis in the degenerated cells in the liver and enlargement of glomerulus in kidney were all reversed partially to normal state due to the administration of crude extracts of various medicinal plants, *Tinospora crispa* [17], *Gymnema sylvestre* [24], *Aegle marmelose* [10].

4. Conclusions

Our findings in this study, the effects of idopyranose on diabetic rat proved strongly the reduction of blood glucose. The enzyme activities of aspartate and alanine aminotransaminase were significantly decreased. Serum urea, creatinine and cholesterol were also brought back to normal condition. Apart from these parameters, idopyranose showed improvement in parameters like body weight, triglyceride, LDL, and HDL. The observed structural alternations in the histology of pancreas, liver and kidney tissues of diabetic rats were brought close to normal condition by the treatment of idopyranose and hence it would serve as an alternative drug for diabetes.

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Fig. (1): 1, 2 disubstituted idopyranose

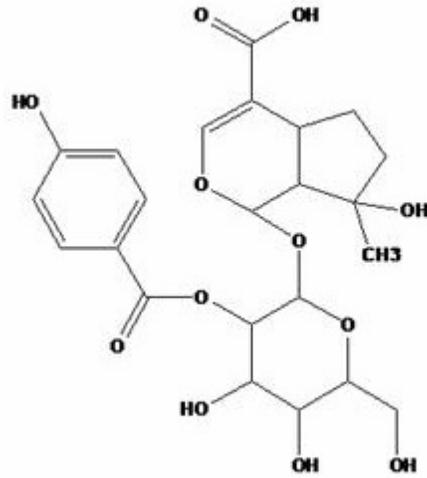


Fig. (2): Single crystal XRD (idopyranose)

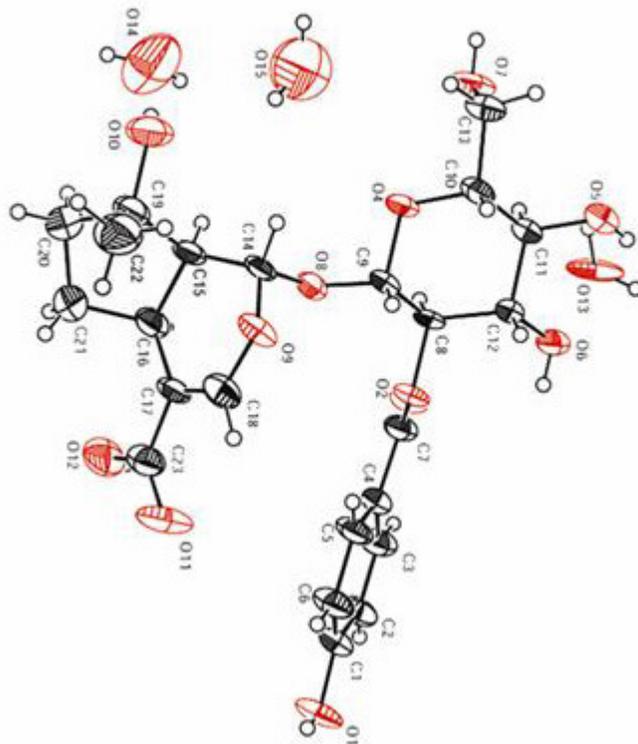
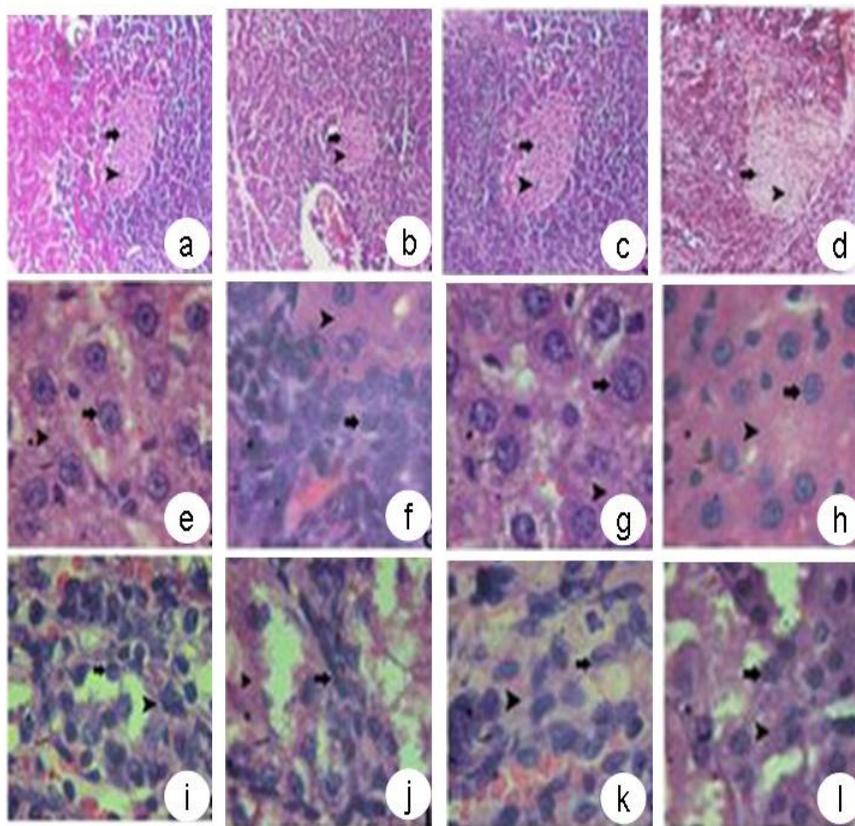


Fig 3: Haematoxylin and eosin stained pancreas, liver and kidney sections were obtained from the control and treated group of Wistar rats



Legends to Fig 3:

- [a] Pancreatic section from normal albino rat showing the islets of langerhans (arrow 1) and well-defined granulated dark beta cells (arrows 1 & 2)
- [b] Pancreatic section from diabetic induced albino rat showing the islets of langerhans with imperfect atrophied beta cells (arrows 1 & 2)
- [c] Pancreatic section from idopyranose alone treated albino rat indicating no changes on beta cells and resembled like control pancreas (arrows 1 & 2)
- [d] Pancreatic section from diabetic rat after idopyranose treatment indicating the islets of langerhans with well-organized beta cells (arrows 1 & 2; 20 X)
- [e] Normal control liver section showing regular arrangement of hepatic cells and no fibrosis
- [f] Diabetic liver section showing irregular appearance of degenerated cells due to liver fibrosis
- [g] Idopyranose alone treated liver sections showing no changes in the appearance of cells as like that of control
- [h] Diabetic liver section after treatment with idopyranose showing reversal of altered parameters to normal condition (arrows; 20 X)
- [i] Renal cortex of normal kidney section showing Bowman's capsule and renal tubules (arrow)
- [j] Diabetic kidney section showing expanded glomerulus and also thickening of walls of renal tubules
- [k] Kidney section of idopyranose alone treated rat showing no glomerulopathy like control
- [l] Diabetic kidney section after treatment with idopyranose showing no expanded glomerulus and thickening of wall of the renal tubules (arrow; 20 X)

Table-1: Effects of idopyranose on diabetic rats maintained for 3 weeks

Groups	Initial weight	Final weight
I Control	173.3 ± 6.05	182.5 ± 7.14
II Diabetic	194.1 ± 4.91	183.6 ± 6.05
III drug	184.1 ± 7.35	192.5 ± 9.35
IV diabetic + drug	187.5 ± 5.24	198.3 ± 6.83

Table-2: Blood glucose level in control and experimental groups after 21 days

Groups	Blood glucose (mg/dl)
Control	87.4 ± 2.6
Diabetic	211.4 ± 5.1*
Drug	86.6 ± 2.5
Diabetic + Drug	112.8 ± 4.7*

**P* < 0.05 significant

Table-3: Effects of idopyranose on serum biochemical profiles in diabetic rats after 21 days

Group	Cholesterol	Triglyceride	HDL	LDL	Urea	Creatinine	AST	ALT
Control	184 ± 4.03	86.53 ± 5.5	37.23 ± 1.5	95.23 ± 5.4	41.77 ± 1.2	1.05 ± 0.12	31.85 ± 2.3	31.98 ± 2.0
Diabetic	336.49 ± 2.3*	200.83 ± 11.1*	29.67 ± 1.4*	210.41 ± 15.3*	68.29 ± 1.0*	2.81 ± 0.08*	80.42 ± 1.5*	74.10 ± 1.75*
Drug	185 ± 3.23	90.23 ± 2.4	39.37 ± 1.2	98.23 ± 4.6	40.62 ± 1.3	1.01 ± 0.14	31.60 ± 2.6	30.96 ± 1.62
Diabetic + Drug	232.63 ± 5.4*	120.21 ± 3.4*	35.22 ± 1.5*	148.23 ± 4.3*	42.90 ± 0.67*	1.46 ± 0.22*	43.83 ± 3.3*	40.50 ± 0.75*

**P* < 0.05 significant