RESEARCH ARTICLE

Investigation of Antidiabetic Activity of Isolated Molecule from the Bark of *Olax scandens*

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Received: 30th June, 2023; Revised: 14th July, 2023; Accepted: 05th August, 2023; Available Online: 25th September, 2023

ABSTRACT

Hyperglycemia caused by defects in insulin synthesis and/or activity characterizes diabetes mellitus (DM), which is categorized as a metabolic condition. Some patients may develop side effects or drug interactions when treating these disorders with synthetic drugs. Due to this, there is an increased interest in researching the medical properties of plants and herbal substances while also considering synthetic medications' interactions and negative effects. To isolate and identify the flavonoid 5-hydroxy-apigenin-7-O-Dglucopyranoside, the bark of *Olax scandens* was extracted with methanol in a soxhlet system (5HAG). The current study set out to examine 5HAG's potential ability to treat albino wistar rats with STZ-induced diabetes. The chemical component significantly lowered rats' blood glucose levels and improved lipid markers. Hepatic enzymes improved significantly, including SGOT, SGPT, ALP, plasma insulin, and total proteins. Overall, this study exposes new avenues for researching how 5HAG reduces blood sugar levels so drastically, but according to the aforementioned study, the flavonoid possesses antidiabetic properties because of its antioxidant activity. In addition, the isolated flavonoid may significantly impact the reduction of blood sugar and its complications by acting as a lead molecule in the identification of novel antidiabetic drug candidates.

Keywords: *Olax*, Standardization, Antioxidant activity, Phenols, Extraction.

International Journal of Pharmaceutical Quality Assurance (2023); DOI: 10.25258/ijpqa.14.3.04

How to cite this article: Johnsymary F, Komala M. Investigation of Antidiabetic Activity of Isolated Molecule from the Bark of *Olax scandens*. International Journal of Pharmaceutical Quality Assurance. 2023;14(3):481-486.

Source of support: Nil.

Conflict of interest: None

INTRODUCTION

A metabolic disorder, diabetics cause hyperglycemia caused by deficiencies in insulin production and/or action. Unbalanced circumstances that aren't treated for an extended period eventually result in hyperglycemia, one of the main causes of diabetes.[1] There are four or five leading causes of death worldwide associated with diabetes, which is the most common communicable disease.[2] India has twice as many diabetic patients as it did in 1995, according to the IDF. By 2025, 69.9 million people are expected to live in the country. Not just those with diabetes, but everyone in society. Approximately $210 billion is spent annually on diabetes-related deaths in the Indian population, and the figure is expected to reach $335 billion within ten years, according to the WHO. An important analysis parameter for these estimations is the probability of premature death.[3]

Some people who take synthetic medications to treat such diseases may experience adverse effects or drug interactions. As a result, there is a renewed interest in investigating the medicinal potential of plants and herbal ingredients while taking into account the interactions and adverse effects of synthetic drugs.[4] We aimed to identify chemical components in *Olax scandens* bark powder and test its antidiabetic potential against streptozotocin-mediated insulin resistance in albino wistar rats as part of this study.

MATERIALS AND METHODS

Chemicals Used

In this study, Rankem and Himedia Laboratories Ltd., Mumbai, provided all solvents and reagents and all solvents and reagents were analytical grade.

Extraction and Isolation of Active Chemical Constituents

Fresh bark of *O. scandens* Roxb was harvested locally in Chennai, Tamil Nadu. During the fat-freeze extraction process, 1.5 kg, of bark was air-dried in the shade, coarsely ground (sieve-40) and bathed in Pet. ether at 60–80°C. Rotary vacuum evaporators were used to evaporate the extracted sample to dryness. Using the dry weight of the powdered bark as a basis, the final extract yield was determined to be 24.32%. The thick methanol extract (MEO) was weighed out to be around 2 gm, and the correct amount of silica gel medium was added

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(100–200 mesh size). Chloroform (100%), a combination of chloroform and methanol in a range of ratios (90:10, 70:30, 50:50, 30:70, and 10:90), and then methanol (100%) were used to elute the extract. Fractions from the column were collected in 200 mL portions. Based on the Rf values of TLC, the resultant fractions were combined with comparable fractions. The fractions' Rf values are shown below. The chloroform: Methanol (70:30) fraction 18–176 produced a chemical with a pale-yellow hue. A recrystallized product was obtained from methanol. This substance was identified as a flavonoid using FTIR, NMR, and UV analysis.

In-vitro Antidiabetic Activity of Isolated Flavonoid (5HAG)

In-vitro alpha-amylase inhibitory activity

The starch solution (one mL of 1% starch dissolved in 25 mL of sodium phosphate and 6.9 mM of NaCl diluted in 100 mL of deionized water) was conducted with 1-mL of escalating inhibitor concentration solutions (MEO and 5HAG 100 to 1000 l per mL and acarbose 5 to 50 gram per mL). The samples were then treated with 1-mL of solutions of enzymes for three minutes at 25ºC. After that, 1-mL of colorimetric reagent was added. Approximately 10 to 15 minutes were spent to cool the mixture. In order to measure the production of maltose, 3,5-dinitro salicylic acid was converted into 3-amino-5-nitro salicylic acid. In comparison with the reagent blank, the reaction is quantified at 540 nm. According to Conforti *et al.,* (2005), 5HAG inhibited the activity of amylase *in-vitro* using a slightly modified sigma-aldrich bioassay.[5] The inhibition percentage was calculated using the formula,

$$Percentage\ of\ inhibition = \frac{Positive\ control - test}{Positive\ control} \text{x}100$$

Alpha-glucosidase inhibition assay (In-vitro)

Using a slightly modified approach from Dahlqvist, the *in-vitro*-glucosidase inhibitory effect of 5HAG and MEO was evaluated.[6] Typically, yeast-glucosidase was utilized to test for -glucosidase inhibitory activity. However, when compared to glucosidase derived from rats, the findings of this approach did not display accurate data. However, as the mouse small intestine produced superior results and more closely reflected the *in-vivo* condition, it was dissected, homogenized, and utilized as a -glucosidase solution. The mouse was held for a 20-hour fast before being sacrificed. A gentle incision was made just above the ceacum and below the pylorus sphincter. The small intestines were removed, cleaned with cooled ice salt solution and homogenized in 12 mL of maleate buffer (pH-6). From the homogenate, the glucosidase solution was prepared. Each sugar substrate was dissolved in 2% weight per volume solution of a buffer, acarbose was dissolved in 10 to 50 µg/mL, 5HAG was dissolved in 100 mL, and acarbose was dissolved in 100 mL of 5HAG. Then, the samples were pre-incubated for five minutes at 37°C. Using glucose oxidase, we measured freed glucose in the reaction mixture using the kit mentioned above. The absorbance of the reagent was calculated and compared with a blank at 540 nm. The rate of inhibition was calculated using the following formula,

$$Percentage\ of\ inhibition = \frac{Positive\ control - test}{Positive\ control} \text{x}100$$

Experimental Protocol

In this study, we selected male healthy wistar rats having body weights 240 to 260 grams were kept at a temperature of 25 ± 2°C and relative humidity of 50 ± 5% in a light and dark cycle for 12 hours. During this, animals were fed with chow pellets and water ad libido. Before 7 days of the commencement of the experiment, the animals were assimilated. The Institutional Animal Ethics Committee gave the approval for conducting animal study under guidelines and the Committee for the purpose of control and supervision of experiments on animals, New Delhi.

(AOT) acute oral toxicity study

A group of animals weighing 160 to 200 g was used in the study. In view of the high concentrations of lethal dose-50 values in most extracts, it was recommended that the isolated compound (5HAG) be administered at an initial dosage level of 500 mg/kg body weight. While toxicity indicators were monitored, food was delayed a further three to four hours following administration in accordance with OECD 423 standards.

OGT test

In this study, animals were fasted overnight following the method described by a researcher.[7] There were five groups of six animals each.

Animal protocol

Animal group	*Treatment given*
G-1	Animals receiving vehicle only (Positive control)
G-2	Animals receiving vehicle only (Negative control)
G-3	Animals receiving 5HAG (50 mg per kg) of body weight
G-4	Animals receiving metformin. Hcl (5 milligram per kg) of body weight
G-5	Animals receiving crude methanol extract (500 mg per kg) body weight

This experiment used CMC: carboxy-methyl cellulose (0.5% w/v in saline) as a vehicle. Oral glucose two gm/kg was administrated. Then, the sample extract was administered to the animal after half an hour. Blood was collected from the tail vein at 0 to 120 minutes and the blood sugar level was identified by using a glucometer.

Anti-hyperglycaemic assay

Type II diabetes was induced in animals by injecting STZ (55 mg per kg in vehicle) via i.p. respectively, once after fasting overnight.[8] A 72 hour time interval after STZ administration increased blood glucose levels by 200 to 300 mg per/deci litre. Oral treatments were administered once a day through a cannula for 28 consecutive days. The glucose levels were measured at days 0, 7, 14, 21 and 28 using blood drawn from

a tail vein. Blood sugar levels were measured using a digital glucometer.[9]

Insulin, proteins and enzymes determination

A 12 hour starvation period was followed by anesthesia and beheading on the 28th day. Blood samples were taken from the heart puncture and placed in an Eppendorf tube. A 30-minute waiting period at 37°C allowed it to coagulate.[10-12] As part of the key enzyme measurements as well as liver glycogen measurement, serum was centrifuged at a speed of 2k revolutions for ten minutes. In order to estimate plasma insulin, a radio-immuno-assay tool was used from BARC (Mumbai, India). Protein totals were estimated using standard procedures.[13]

Assay on free radical scavenging property

All animal groups were anesthetized with diethyl ether on the 28th day. Following this, a portion of the liver was dissected and preserved in 10% formalin for further histopathological studies. An ELISA reader was used to measure malondialdehyde (MDA) production to determine lipid peroxidation levels (LPO). Lipid peroxidation can be measured directly in liver samples using this method. Tris-HCl buffer was used to homogenize the liver's remaining section. Various components were then determined using this homogenate. Malondial is caused by super-oxide dismutase, catalase, reduced glutathione and glutathione peroxidase.[14]

Study on histo- pathological character

The animals fasted for 12 hours on the 28th day of treatment. Diethyl ether was used to anesthetize them, and cervical dislocation was used to euthanize them. An ice-cold saline solution was used to maintain the integrity of the pancreas after dissection and excising it. Hematoxylin and eosin staining were performed on sections of the pancreas to examine the histological changes. Cellular structures and abnormalities can be visualized with these staining techniques. Finally, 40X magnification was applied to the histopathological observations. Fine details can be observed and any changes to histology can be accurately assessed through this magnification. To assess any potential histopathological changes, the aforementioned procedure was followed.[15]

Statistics

GraphPad Prism 5.0, developed in San Diego, United States was used to analyse the data in this study. To ensure accuracy, the results were calculated by multiplying the mean by the standard error of the mean in three studies. An ANOVA followed by a Dunnet's test was used to evaluate the significance of the data. Comparing to the negative control, statistical differences were observed for biochemical estimations, with a significance level of $p < 0.05$.

RESULTS AND DISCUSSION

In-vitro Alpha-amylase Inhibitory Assay

Amylase inhibitory percentage was calculated by comparing reduced maltose production with the control. Compared to the reference drug acarbose, the isolated molecule showed substantial efficacy dose-dependently, with a 75% inhibition rate and inhibitory concentration -50 value of 270.56 µg/mL at a maximum dosage of 1000 µg/mL.

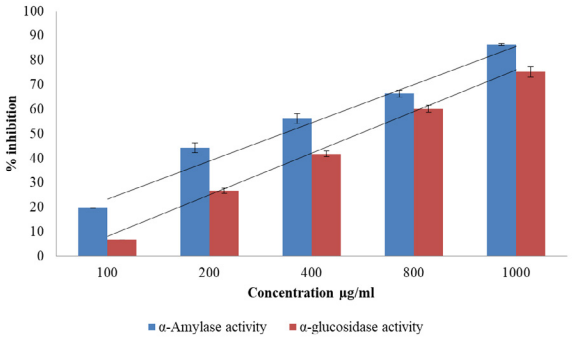

Figure 1: Invitro antidiabetic activity of 5-hydroxyapigenin 7-O-β-D-glucopyranoside, 5HAG

In-vitro α- glucosidase Inhibitory Activity

The isolated compound (5HAG) demonstrated substantial efficacy in a dose-dependent manner when compared to the reference medication acarbose, with the maximum dosage of 1000 g/mL showing 86% inhibition and an IC_{50} value of 346.01 µg/mL (Figure 1).

Acute Oral Toxicity studies

In acute toxicity tests, animals treated with 500 mg per kg of 5HAG showed no adverse effects. In comparison to vehicle-treated animals, body weight and food intake were normal. Haematological and biochemical (liver enzymes and serum lipids) indicators did not alter significantly. Therefore, the dose was taken at 10% of the acute toxicity study's highest dose, or 50 mg per kg, and further research was conducted.

Effect of 5-hydroxyapigenin 7-O-β-D-glucopyranoside (5HAG) on STZ induced animals

Table 1 and Figure 2 show that 5HAG group significantly reduced blood glucose load in OGT ($p<0.05$) compared with G-1 group and reference medication metformin (5 mg per kg).

When diabetic rats received STZ treatment, their blood glucose levels increased abnormally. Diabetes rats' blood glucose levels dropped significantly following treatment with (5HAG) (50 mg per kg). 5HAG is equivalent to metformin (five mg per kg) and significantly different from methanol extract of *Olax* 500 mg per kg compared to the conventional metformin medication. In Table 2, we summarize the effects on blood glucose levels of an isolated molecule in rats with and without diabetes. In diabetic rats, insulin and glycogen levels were significantly lower. After 21 days of therapy with isolated molecule (50 mg per kg), body weight, insulin level, and glycogen content significantly increased compared to diabetic animals. Control and experimental animals showed statistically significant differences ($p < 0.05$). The isolated molecule behaves similarly to metformin (5 mg per kilogram), one of the most common medications globally.

SGOT, SGPT, and ALP activity increase in diabetic rats because STZ leaks into the bloodstream from the liver cytosol.[16]

Table 1: Effect of isolated molecule (5HAG) and methanol extract of *Olax* bark (MEO) on oral glucose tolerance of albino wistar rats

Groups	*0 minutes*	*30 minutes*	*60 minutes*	*90 minutes*	*120 minutes*
Normal	96.61 ± 1.28	177.05 ± 1.51	146.61 ± 2.77	117.52 ± 2.58	95.25 ± 0.57
STZ induced	96.03 ± 0.40	96.24 ± 1.66	92.08 ± 1.34	194.66 ± 2.37	230.63 ± 1.25
5HAG (50 mg/kg.p.o)	95.54 ± 0.45	98.54 ± 1.32	98.10 ± 1.39	92.91 ± 0.60	91.93 ± 0.68
Metformin (5 mg/kg, p.o.)	95.08 ± 0.85	120.02 ± 1.28	100.08 ± 1.17	97.67 ± 1.33	95.29 ± 1.37
MEO (500 mg/kg p.o.)	94.43 ± 0.89	116.81 ± 1.43	102.32 ± 1.47	99.82 ± 1.11	97.22 ± 0.54

Values are mean ± SEM of 6 replicate experiments

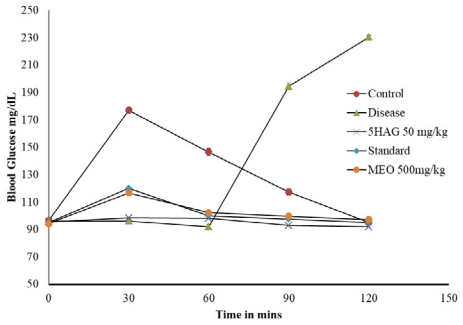

Figure 2: Oral glucose tolerance of albino wistar rats after administration of isolate molecule

Several enzymes in the liver of diabetic rats were decreased by 5HAG treatment. In diabetic conditions, it demonstrates that flavonoids protect liver enzymes. The plasma levels of TPR and CRTN were also lower in diabetic rats. This is because diabetic kidneys have a specific metabolic, renal dysfunction that results in a negative nitrogen balance, an increase in proteolysis, and a decrease in protein synthesis. Table 3 shows that plasma protein levels in diabetic rats treated with 5HAG improved significantly. Flavonoids prevented diabetic rats from worsening their kidney damage.

Effect of 5-hydroxyapigenin 7-O-β-D-glucopyranoside (5HAG) on lipid profile in diabetic rats

An analysis of the serum lipid profile of diabetic rats using an isolated molecule (5HAG) is presented in Table 4. In diabetic rats, triglycerides, total cholesterol, and low-density lipoprotein (LDL) levels all increased, whereas triglycerides, total cholesterol, and LDL levels decreased. A significant increase in HDL values was observed in isolated molecule (5HAG) (50 mg per kg) treated groups when compared with diabetic control groups. As compared to normal rats, the isolated molecule (5HAG) is statistically significant (p 0.05) and equivalent to metformin (5 mg per kg).

Effect of 5-hydroxyapigenin 7-O-β-D-glucopyranoside (5HAG) on antioxidant enzymes

As shown in Table 5 and Figure 3 below, SOD, CAT, GSH, and GR activities were different between diabetic and normal rats. In diabetic rats treated with STZ, SOD, CAT, GSH, and GR activities were abnormally elevated. An isolated molecule (5HAG) significantly reduced several antioxidant enzymes' activity in diabetic rats ($p<0.05$) compared to control rats. The elevated MDA levels of diabetic rats treated with 5HAG (50 mg per kg) were significantly lower than those of normal rats. The impact of the isolated molecule (5HAG) is equivalent to that of the common medication metformin (5 mg/kg) on antioxidant enzymes.

Histopathological Observations

In diabetic rats given STZ treatment, the pancreas showed widespread necrotic alterations, damaged-cell populations, and reduced islet size. These changes were followed by fibrosis and atrophy (B). Rats treated with an isolated molecule (5HAG)

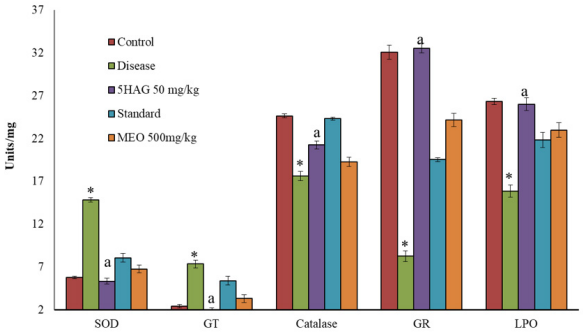

Figure 3: Effect of isolated molecule on the antioxidant parameters

Table 2: Effect of isolated molecule (5HAG) and methanol extract of *Olax* bark (MEO) in streptozotocin induced diabetic animals

Animal Group	*Days*				
	0^{th}	*7^{th}*	*14^{th}*	*21^{st}*	*28^{th}*
Normal	97.31 ± 0.80	99.31 ± 0.78	98.32 ± 0.70	95.84 ± 1.42	95 ± 0.52
STZ induced	97.62 ± 0.61	194.96 ± 3.26*	220.09 ± 4.70*	237.81 ± 2.65*	247.72 ± 1.36*
5HAG (50 mg/kg.p.o)	96.36 ± 0.55	98.69 ± 0.88[a]	97.45 ± 2.01[a]	94.58 ± 0.30	91.60 ± 0.63
Metformin (5 mg/kg, p.o.)	96.26 ± 0.86	119.85 ± 1.73	103.42 ± 2.69	99.36 ± 0.76	95.63 ± 0.66
MEO (500 mg/kg p.o.)	95.66 ± 0.59	117.18 ± 1.37	107.09 ± 3.73	101.13 ± 1.29	97.9 ± 0.73

Values indicate mean ± SEM (n=6); Induction was statistically significant at *$p<0.001$, induced group compared to normal control group; a activity significant compared to the induced group at $p<0.05$.

Table 3: Effect of isolated molecule (5HAG) and methanol extract of *Olax* bark (MEO) on hepatic enzymes and plasma insulin in STZ induced diabetic rats

Group	*SGOT (IU/dl)*	*SGPT (IU/dl)*	*ALP (IU/dl)*	*Liver glycogen (mg/g of tissue)*	*Plasma insulin (µU/ml)*	*Total protein (g/dl)*
Normal	58.23 ± 4.84	70.28 ± 1.94	120.04 ± 8.5	55.17 ± 5.25	13.33 ± 0.86	9.97 ± 0.75
STZ induced	191.51 ± 7.26*	150.69 ± 4.61*	219.44 ± 6.35*	16.54 ± 3.92*	7.37 ± 1.65*	6.05 ± 1.45*
5HAG (50mg/kg.p.o)	60.57 ± 5.18[a]	75.16 ± 6.66 [a]	127.81 ± 5.84 [a]	58.21 ± 3.6 [a]	11.24 ± 2.07 [a]	8.93 ± 1.49 [a]
Metformin (5mg/kg, p.o.)	61.27 ± 8.23 [a]	76.25 ± 5.33 [a]	124.05 ± 2.25 [a]	51.63 ± 1.39 [a]	12.07 ± 1.7 [a]	8.57 ± 1.82 [a]
MEO (500mg/kg p.o.)	65.91 ± 7.01 [a]	84.74 ± 4.61 [a]	135.28 ± 7.68 [a]	39.85 ± 4.74 [a]	10.25 ± 2.02 [a]	7.88 ± 1.78 [a]

Values indicate mean ± SEM (n=6); Induction was statistically significant at *p<0.001, induced group compared to normal control group; [a] activity significant compared to the induced group at p<0.05.

Table 4: Effect of isolated molecule (5HAG) on lipid profile in STZ induced diabetic rats

Group	*TG (mg/dL)*	*TC (mg/dL)*	*LDL (mg/dL)*	*VLDL(mg/dL)*	*HDL (mg/dL)*
Normal	75.78 ± 2.86	88.48 ± 1.99	34.70 ± 0.84	23.24 ± 0.53	32.45 ± 0.87
STZ induced	215.96 ± 2.85*	138.26 ± 1.11*	149.46 ± 1.54*	30.14 ± 0.91*	22.11 ± 0.73*
5HAG (50mg/kg.p.o)	74.49 ± 2.16 [a]	86.42 ± 1.39 [a]	34.88 ± 2.05 [a]	21.84 ± 0.67	33.41 ± 0.80
Metformin (5mg/kg, p.o.)	105.61 ± 1.70	125.60 ± 1.98	80.19 ± 0.93	26.06 ± 0.99	29.67 ± 1.05
MEO (500mg/kg p.o.)	89.40 ± 1.58	95.97 ± 1.10	64.10 ± 1.59	27.85 ± 0.71	27.07 ± 1.05

Values indicate mean ± SEM (n=6); Induction was statistically significant at *p<0.001, compared to other groups; [a]significant compared to another groups p<0.05.

Table 5: Effect of Isolated molecules on antioxidant enzymes in STZ induced diabetic rats

Treatment	*SOD (U/mg protein)*	*GT (U/mg protein)*	*Catalase (U/mg protein)*	*GR (U/mg protein)*	*LPO (U/mg protein)*
Normal	5.77 ± 0.16	24.64 ± 0.19	2.42 ± 0.25	32.06 ± 0.83	26.33 ± 0.37
STZ induced	14.82 ± 0.24*	17.61 ± 0.45*	7.35 ± 0.55*	8.26 ± 0.62*	15.84 ± 0.71*
5HAG (50mg/kg.p.o)	5.31 ± 0.34[a]	21.23 ± 0.65 [a]	1.56 ± 0.47 [a]	32.55 ± 0.57 [a]	26.01 ± 0.73 [a]
Metformin (5mg/kg, p.o.)	8.07 ± 0.54	24.31 ± 0.52	5.39 ± 0.19	19.53 ± 0.22	21.82 ± 0.89
MEO (500mg/kg p.o.)	6.74 ± 0.46	19.26 ± 0.45	3.30 ± 0.56	24.16 ± 0.75	22.99 ± 0.87

Values are mean ± SEM of 6 replicate experiments. Induction was statistically significant at *p<0.05, compared to other groups.

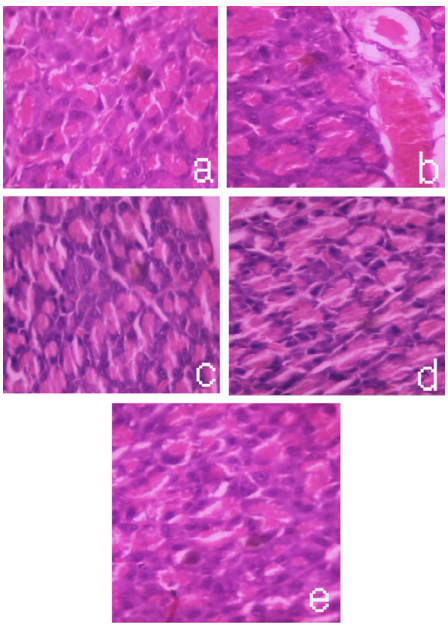

Figure 4: Histology of pancreas of experimental rats of a. normal group; b. STZ induced group; c. 5HAG treated group; d. Metformin-treated group; MEO treated group

(50 mg/kg) and metformin had their necrotic and fibrotic alterations repaired, and their islets' number and size had grown as well (C). In the pancreatic cells of the G-1 animals, normal acini and cellular structures were seen (A). Similar to 5HAG-treated rats, the metformin-treated group (D) saw alterations in pancreatic morphology. Figure 4 is an attachment showing how 5HAG affects the pancreatic region in both normal and negative groups.

CONCLUSION

Using the methanolic extract of *O. scandens* Roxb bark, the isolated flavonoids 8-hydroxyapigenin 7-O-D-glucopyranoside. It is a novel flavonoid chemical compound. The flavonoid compound exhibits strong hypoglycemic (antidiabetic) and antioxidant (radical scavenging) activities in diabetic rats treated with streptozotocin. Molecules from *O. scandens* Roxb bark are the first to contain flavonoids. Further studies must be conducted to better understand why the isolated flavonoid is antidiabetic. In contrast, the isolated flavonoid may be exploited to develop innovative antidiabetic medication candidates to treat insulin-resistant diabetes mellitus or may substantially reduce diabetes mellitus with little harm to the host.

ACKNOWLEDGMENT

We acknowledge the management of Vels Institute of Science, Technology and Advanced Studies (VISTAS), Pallavaram, Chennai-600 117, Tamil Nadu, India, for providing a research facility.

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