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Article

Metabolomic profiling and antidiabetic potential of *Rumex vesicarius* seed extract in high-fat diet and streptozotocin-induced diabeticrat

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Rumex vesicarius hasbeen extensively used for the management of diabetes in the traditional system of medicine. The current study was designed to investigate antidiabetic and antihyperlipidemic effects of *R.vesicarius* and also to explore metabolomic profiling using UPLC-QTOF-MS. The effect of extracts was observed by checking the biochemical and histopathological parameters in diabetic rats. The results had shown a significant dose-dependent inhibition potential of aqueous extract of *R. vesicarius* seed against α-amylase and α-glucosidase along with significant inhibition in DPPH free-radical scavenging activity. Oral administration of *R. vesicarius* to diabetic rats significantly ($p< 0.05$) ameliorated blood glucose level. It also improved the function of the liver and kidney as well as ameliorated dyslipidemia in diabetic rats. Histopathological examination of the treatment groups reversed the damage of the pancreas, liver, and kidney tissues confirming the antidiabetic efficacy of *R. vesicarius*. UPLC-QTOF-MS analysis of the extract revealed a total of 42 bioactive compounds, which might contribute to the antidiabetic activity. Based on our findings, we can conclude that *R. vesicarius* might be a promising candidate for the management of diabetes.

Keywords: Diabetes.*Rumex vesicarius*.Antioxidant.UPLC-QTOF-MS.

INTRODUCTION

Diabetes mellitus (DM) is a chronic metabolic disorder characterized by hyperglycemia due to insufficiency of secretion or action of endogenous insulin, oxidative stress, and inflammation. Globally, DM affects a population of approximately 424 million adults worldwide in 2017, andit an estimated to rise to 642 million by 2040 (Cho *et al.*, 2018). Asia is a major area of the rapidly emerging DM, India and China are the top two epicenters worldwide (Zheng,Ley, Hu,2018).

The etiology of DM is complex and associated with hyperglycemia, dyslipidemia, reactive oxygen species (ROS), and inflammation, resultingin long-term damage and complications (Chen *et al.*, 2018). The previous studies claim that oxidative stress occurs might be due to increased generation of reactive oxygen species and decreased antioxidant enzymes like superoxide dismutase catalase, and glutathione peroxidase(Incalza *et al.*, 2018). Moreover,patients havediabetic dyslipidemia; characterized by a higher level of triglycerides (TG), total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), and lower-level high-density lipoprotein cholesterol (HDL-C),and it is commonly associated with cardio-metabolic disorders(Husain *et al.*, 2015). Dyslipidemia and elevated oxidative stress exert harmful impacts on

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biomolecules to create diabetes probably due to the dysfunction of pancreatic β-cells.

Good glucose homeostasis delays the progression of diabetic complications but does not completely ameliorate diabetes (Joseph *et al.*, 2016). Many effective synthetic antidiabetic agents such as thiazolidinediones, sulfonylureas, and α-glucosidase inhibitors have been used either alone or in combination with the effective treatment of DM, but these agents produce several side effects, and harmful impacts of synthetic drugs is a key concern among consumers (Shah,Khan, Ahmed, 2020).

Together with the aforementioned limitation, demand for natural products is rapidly increasing (Al-Ishaq *et al.*, 2019). The number of benefits like manipulating carbohydrate metabolism by various mechanisms such as insulin-releasing activity, boosting insulin secretion, improving glucose uptake utilization, anti-inflammatory, and antioxidant properties present in medicinal plants provide exemplary options to develop novel therapeutics (Unuofin, Lebelo, 2020). Hence, for better safety and potential therapeutic value, the search for novel molecules has been extended to herbal drugs that offer better protection with lesserside effects and lower cost (de Medeiros *et al.*, 2020).

Recent studies have confirmed the bioactive potential of medicinal plants mediated by polyphenols and flavonoids (Khan *et al.*, 2020). *Rumex vesicarius* Linn. (Polygonaceae) is a highly reputed medicinal plant used in the traditional system of medicine for the management of several diseases (Beddou *et al.*, 2015). Previous studies suggested that *R. vesicariusis* prominently used in the therapy of hyperglycemia and to have direct insulinotropic activities (Reddy *et al.*, 2017). Despite the traditional use of this plant in Asian countries, no systematic study has been done to substantiate its acclaimed antidiabetic property. The current study aimed to evaluate the antidiabetic potential of *R. vesicarius* along with the antilipidemic, and antioxidant activities, and also to reveal phytochemicals present in the plant extract using ultra-performance liquid chromatography equipped with quadrupole and mass spectrometer (UPLC-QTOF-MS).

MATERIAL AND METHODS

Collection of plant material and preparation of Extract

The seeds of *R. vesicarius*were collected and authentication was done as per the protocol. 100 g of seeds werecoarsely powdered and extracted through maceration using distilled water. Thereafter, the extract was filtered and subjected to lyophilization and stored in a suitable container at 4°C until its use.

Estimation of total phenolic and flavonoid contents

Total phenolic and flavonoid contents in the aqueous extract of *R. vesicarius*were determined according to the described procedure (Gaurav *et al.*, 2020). Gallic acid and rutin were used to quantify total phenol and flavonoid contents in the sample. All the experiments were performed in triplicate.

In vitro antioxidant activity

The DPPH assay was used to evaluate the antioxidant activity of *R. vesicarius*(Fahim *et al.*, 2019). In brief, 200 mL of different concentrations of the sample (100-500 mg/mL) were mixed with 3.8 mL of DPPH solution and kept in a dark place. After1 h, the absorbance was recorded at 517 nm. Ascorbic acid was used as a positive control.

In vitro alpha-amylase and alpha-glucosidase inhibition assay

The activity of α-amylase and α-glucosidase was carried out as per the described method (Gaurav *et al.*, 2020). For α-amylase, 1.0 mL of sample and 1.0 mL α-amylase were mixed and incubated for 30 min at 37ºC. The starch solution was added to the incubated mixture and again incubated for 1 h at 37ºC. Further, 100µL of supernatant was taken out and glucose concentration was measured by glucose reagent. Whereas for α-glucosidase inhibition activity, 120 µL of sample and 20 µL of α-glucosidase in potassium phosphate buffer were incubated for 15 min at 37ºC. The reaction

was carried out by adding 20 µL of para-nitrophenyl-α-D-glucopyranoside and the final solution was further incubated for 15 min. The reaction was terminated by adding 80 µL of sodium carbonate. Absorbance was measured at 545 and 405 nm for α-amylase and α-glucosidase, respectively.

Phytochemical analysis of extract by UPLC-QTOF-MS

The extract was chromatographically separated in the mobile phase consisting of 0.5% v/v formic acid in water (A) and acetonitrile (B) in gradient elution mode. Water's ACQUITY BEH C18 column was used and the flow rate of the mobile phase was 0.5 mL/min. About 0.5 µL of the sample was injected with the split mode of 5:1 with the help of an auto-injector and the pressure of the system was set to 15000 psi. The separated metabolites were detected by the MS detector. The separated compounds were identified based on their m/z value through a literature survey (Parveen *et al*., 2019).

Oral glucose tolerance test (OGTT)

The OGTT was employed to evaluate the ability to respond appropriately to the glucose challenge. The blood glucose was monitored using glucometers to calculate the OGTT values after the administration of the tested drug. After an overnight fast, all rats were fed with glucose (2 g/kg, b.w.). The blood was collected at different time intervals such as 0, 30, 60, 90, and 120 min after being fed with glucose (Belgacem *et al*., 2019).

Experimental animal design and diabetic model induction

The *in vivo* study was conducted at Jamia Hamdard, India. Healthy Wistar albino rats with an average body weight of 175-200 g, as per the standard protocol. The study was approved by the Institutional Animal Ethics Committee, Jamia Hamdard, Constituted by the Committee for the Purpose of Control and Supervision of Experiments on Animals. A total of 30 rats were used in this study and randomly divided into five groups (n=6): Group I served as normal control (NC); Group II served as diabetic control (DC),treated with a high-fat diet and multiple doses of streptozotocin 35 mg/kg/b.w.; Group III and Group IV, diabetic rats treated with low and high doses of *R. vesicarius*extract at 300 mg/kg/b.w. (RVLD) and 500 mg/kg/b.w. (RVHD), respectively. Whereas, Group V diabetic rats were treated with standard drug metformin with a dose of 40 mg/kg/b.w.) and considered as a positive control (PC). All the tested drugs were administered orally to diabetic rats for 28 days. Every week, blood glucose levels were measured using glucometers. At the end of the experiment, rats were sacrificed by carbon dioxide anesthesia after fasting. After sacrifice, the animals, tissues,and serum were collected for future analysis.

Mean body weight

The bodyweight of all the animals was measured on the day of initiation of the experiment and every seventh day throughout the experimental periods.

Biochemical analysis

The blood was collected and serum was separated by centrifugation at 3000xg for 10 min and stored at -20°C until use. Estimation of serum glucose, lipid profile such as triglyceride (TG), total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), and high-density lipoprotein cholesterol (HDL-C), liver function test such as aspartate aminotransferase (AST) alanine aminotransferase (ALT) and alkaline phosphatase (ALP) while kidney function tests like urea, uric acid, and creatinine were carried out(Ahangarpour *et al*., 2016).

Histopathological studies

At the end of treatment, the animals have fasted for 12 h, anesthetized using carbon dioxide, and sacrificed by cervical dislocation. The pancreas, liver, and kidneys were instantly dissected out and processed as follows. A portion of the pancreas, liver, and kidney tissue was fixed in formalin solution for 4 days. After fixation, tissues were dehydrated in ethanol, cleared in xylene, and embedded in paraffin. The solid transverse sections of

2-4 mm thickness were obtained by a rotary microtome. The sections were stained with hematoxylin-eosin and histopathological observations were carried out under the microscope (Mohamed *et al*., 2020).

Statistical analysis

Results were conducted by using GraphPad Prism 5, software. All the experiments were performed in triplicate and recorded the results as mean ± SD (standard deviation). One-way ANOVA and Turkey's test were used for *in vivo* analysis. The $p<0.05$ was considered to be statistically significant.

RESULTS

Estimation of total phenolic and flavonoid contents

Total phenolic and flavonoid contentsoflant extract weredetermined from the calibration curve of gallic acid (r^2=0.9969) and quercetin (r^2=0.9921), respectively. The total phenolic and flavonoid contents were found to be 67.57±0.20 and 85.67±0.30 mg equivalents per gram of gallic acid and rutin, respectively. Our results revealed that *R. vesicarius* extract is enriched with phenolics and flavonoids.

Free radical scavenging assay

The potential to scavenge DPPH radical was calculated in terms of percentage inhibition. Inthe present study, DPPH screening of *R. vesicarius*had clearly shown the dose-dependentantioxidant activity at different concentrations tested (62.5, 125, 15, 250, 500, and 1000 µg/mL). It inhibited DPPH free radical, and the highest concentration showed 94.87% inhibition and the lowest concentration showed 32.56%, while that of the reference compound, i.e. ascorbic acid showed98.29 and 37.47% inhibition of DPPH free radicles at highest used concentration and lowest used concentration, respectively.The obtained results revealed antioxidant inhibition by the extract comparedto the reference compound.

In vitro alpha-amylase and alpha-glucosidase inhibition activity

The inhibitory potential of *R. vasicarius*was found in a dose-dependent manner with different concentrations taken (62.5, 125, 15, 250, 500, and 1000 µg/mL). In the case of α-amylase, maximum inhibition potential was found at 95.35%, whereas in the case ofα-glucosidase, maximum inhibition potential was found at 89.26% at the highest tested concentration i.e. 1000 µg/mL.The inhibition potential of standard acarbose was found at 97.53% at a higher concentration.The results clearly showed that *R. vesicarius*possessesamylase and glucosidase inhibition activity.

UPLC-QTOF-MS analysis

UPLC-QTOF-MS was used for metabolite profiling of seed extract of *R. vesicarius*.The full chromatogram of the sample is shown in Figure 1, whereas the mass spectrums and group of major metabolites were depicted in Figures 2and 3. The detail of compounds with their m/z value, compound name, and the molecular formula with corresponding IDs weregiven in Table I.

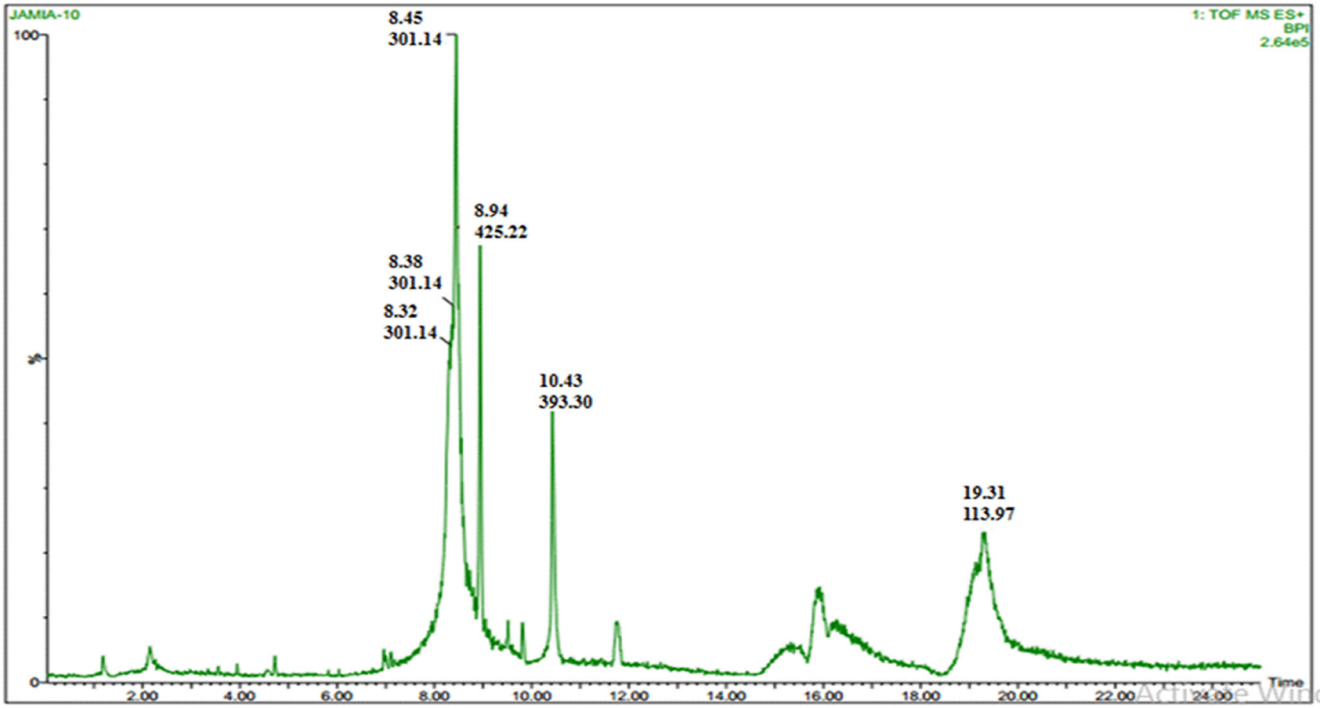

FIGURE 1 - Full chromatogram of aqueous extract of *R. vesicarius* seed using UPLC-QTOF-MS in negative ion mode.

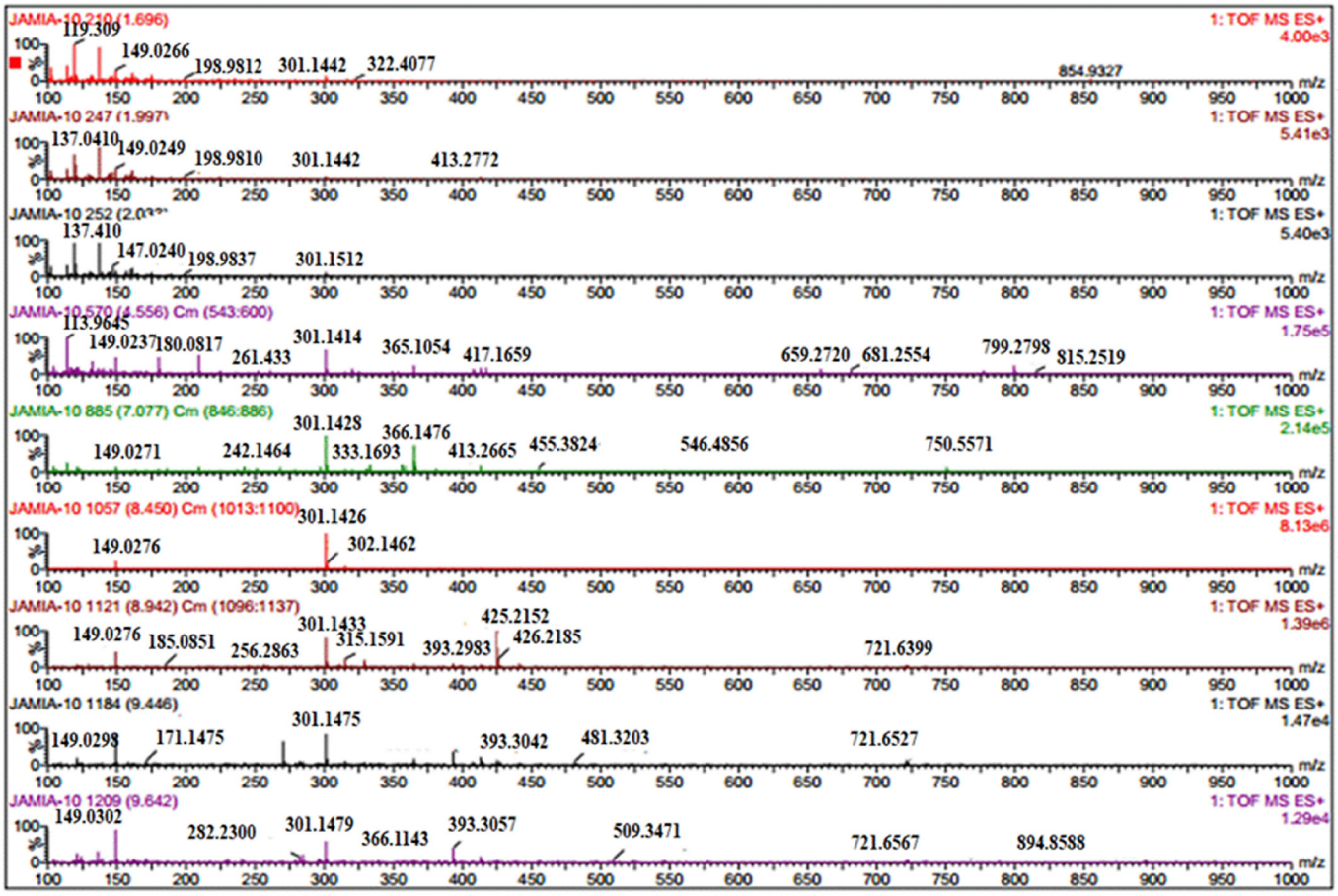

FIGURE 2 - MS spectrum of major metabolites presents in aqueous extract of *R. vesicarius* seed in positive mode of UPLC-QTOF-MS in negative ion mode.

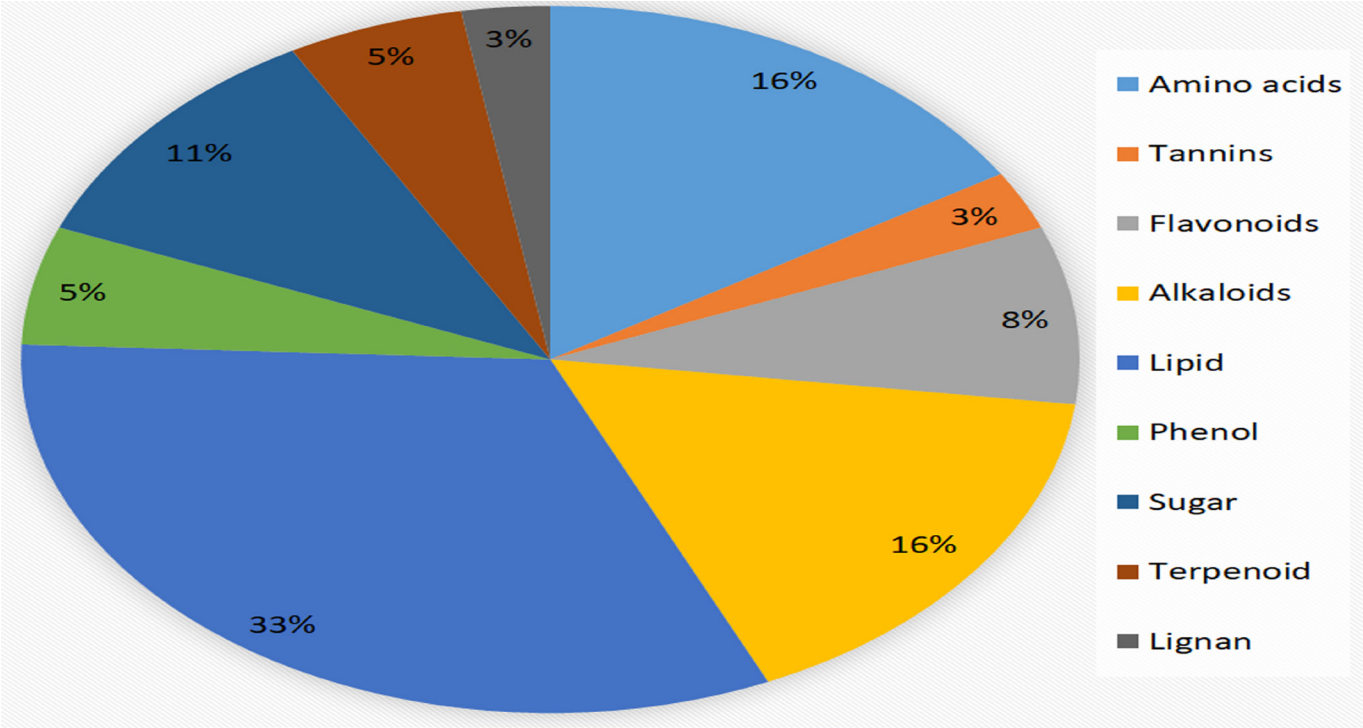

FIGURE 3 - Group of major groups of metabolites present in aqueous extract of *R. vesicarius* seed.

TABLE I - Mass spectrometric data of metabolites present in seed extract of *R. vesicarius*

Metabolites	Compounds name	Tentative mass	Formulae	Reference/Mass IDs
M1	Betaine	119.03	$C_5H_{12}NO_2$	FIO00887
M2	L- (+)-Tartaric acid	149.02	$C_4H_6O_6$	KO001899
M3	Syringic acid	198.98	$C_9H_{10}O_5$	KO001814
M4	Quercitin	301.14	$C_{15}H_{10}O_7$	PR040053
M5	Copticine	322.40	$C_{19}H_{14}NO_4$	TY000044
M6	2-Aminobenzoic acid	137.04	$C_7H_7NO_2$	SM800901
M7	Phosphatidylserine 20:4-22:6	854.93	$C_{48}H_{74}NO_{10}P$	UT001508
M8	1-Methylnicotinamide	137.04	$C_7H_9N_2O$	KO00344
M9	3-5 Dimethoxy-4-hydroacetophenon	198.98	$C_{10}H_{1204}$	BS003072
M10	Peonidine	301.14	C16H13O6+	Pubchem CID: 441773
M11	1-Decanoyl-2-hydroxy-sn-glycero-3-phosphocholine	413.27	C18H39NO7P+	PR100341
M12	1-Methylnicotinamide	137.04	$C_7H_9N_2O$	KO003442
M13	Glutamate	147.02	C5H9NO4	CE000662
M14	DErySphinganine	301.15	C18H39NO2	CE000605
M15	1-Methylhydantoin	113.96	C4H6N2O2	KO001343
M16	Mannose	180.08	C6H12O6	CE000286

TABLE I - Mass spectrometric data of metabolites present in seed extract of *R. vesicarius*

Metabolites	Compounds name	Tentative mass	Formulae	Reference/Mass IDs
M17	DL-Hexanoylcarnitine	261.04	$C_{13}H_{26}NO_4$	MT000135
M18	Isopentenyl-Adenine-glucoside	365.10	C16H23N5O5	CE000239
M19	Juglanin	417.16	$C_{20}H_{18}O_{10}$	TY000222
M20	Enniatin A	681.25	C36H63N3O9	AC000444
M21	Phosphatidylcholine 19:0-18:2	799.27	$C_{45}H_{86}NO_8P$	UT001058
M23	Phosphatidylserine	815.25	$C_{44}H_{82}NO_{10}P$	UT001496
M24	13-methylmyristic acid	242.14	C15H30O2	RP024601
M25	Beta-Nicotinamide mononucleotide	333.16	C11H15N2O8P	PR100219
M26	GlcNAcThrNAc	365.14	$C_{14}H_{24}N_2O_9$	FU000263
M27	Stigmasterol	413.26	C29H48O	Pubchem CID: 5280794
M28	Riboflavin-5-monophosphate	455.38	$C_{22}H_{18}O_{11}$	TY000083
M29	Phosphatidylcholine lyso 20:2	546.48	C28H54NO7P	UT002350
M30	Triacylglyrerol 12:0-16:0-16:0	750.55	$C_{47}H_{90}O_6$	UT000534
M31	O-Phospho-L-serine	185.08	C3H8NO6P	PR100594
M32	Pinocembrine	256.26	C3H8NO6P	BML00145
M33	Capillarisin	315.15	$C_{16}H_{12}O_7$	TY000038
M34	Brucin	393.29	$C_{23}H_{26}N_2O_4$	FIO00814
M35	Isovitexin	430.21	$C_{23}H_{26}N_2O_4$	PN000123
M36	rac-Glycerol 3-phosphoate	171.14	C3H8NO6P	PR100603
M37	Glucomalcomiin	481.32	C17H22NO11S2-	CE000406
M38	Phosphatidylethanolamine alkenyl 16:0-20:5	721.65	$C_{41}H_{72}NO_7P$	UT001903
M39	Emodin	268.23	$C_{15}H_{10}O_5$	AC000130
M40	Gibberellin A8	365.11	C19H24O7	PR020148
M41	HXGXA	509.34	$C_{23}H_{39}N_7O_6$	MT000093
M42	Man2GIcNAcFucGIcNAc	894.85	$C_{34}H_{58}N_2O_{25}$	FU000249

Effect of *R. vesicarius* on OGT test in normoglycemic rats

Hyperglycemia is the key symptom of diabetes and the OGT test is an important indicator of diabetes alleviation. In the OGT test, RVLD and RVHD significantly ($p< 0.05$) reduced plasma glucose levels as compared to the normal control group (Figure 4A). The metformin-treated group showed significantly higher ($p< 0.01$) reduction activity compared to the normal control group as well as treatment groups.

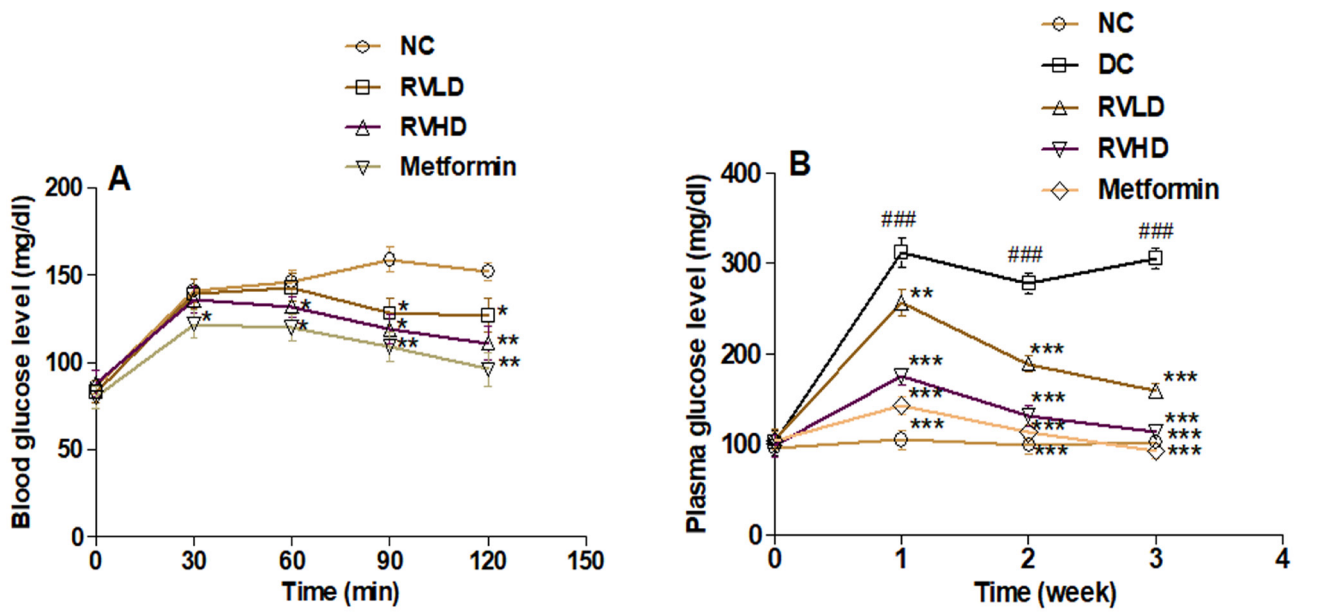

FIGURE 4 - (A) Effects of RVLD and RVHD on oral glucose tolerance test (OGTT) and (B) Effects of RVLD and RVHD on fasting plasma glucose level in experimental groups. Data are expressed as mean ± SD (n=6).

Effect of *R. vesicarius* on blood glucose levels in experimental rats

Throughout the study, diabetic rats exhibited a significant increment in the level of blood glucose as compared to the normal rats. After the administration of RVLD and RVHD to diabetic rats for 28 days, the blood glucose levels were significantly reduced to normal as compared to the diabetic control rat at doses of300 and 500 mg/kg (Figure 4B). The standard metformin-treated rats also showed a marked reduction in plasma glucose levels when compared to diabetic control rats.

Effect of *R. vasicarius* on mean body weight

As shown in Table II, the HFD rats significantly ($p < 0.05$) increased in body weight compared to the normal control group and showed characteristics of obesity. However, after STZ injection to the diabetic group, the body weight sharply decreased. Treatment groups resulted in significant ($p < 0.05$) elevation in the body weight gain towards normal after 28 days as compared with the diabetic control group.

TABLE II - Effect of *R. vesicarius* extract on body weight in experimental rats

Days	Experimental Group				
	NC	DC	RVLD	RVHD	Metformin
0 day	181.07 ± 11.50	188.14 ± 16.00	180.88 ± 12.86	188.98 ± 11.31	184.49 ± 13.60
7th day	204.65 ± 9.00	212.45 ± 10.44	209.48 ± 12.12	205.48 ± 11.78	212.71 ± 12.09
14th day	249.52 ± 10.79	237.26 ± 9.39	182.90 ± 9.00	234.63 ± 8.96	229.16 ± 9.22
21st day	276.29 ± 12.52	196.86 ± 11.21###	216.22 ± 13.44	245.32 ± 14.00**	238.02 ± 12.44**

Effects of *R. vesicarius* on serum lipid profiles in diabetic rat

Table IIIdisplays the serum levels of TG, TC, LDL-C, and HDL-C in normal and diabetic rats. Compared with the normal control group, the diabetic group exhibited higher levels of serum TG, TC, LDL-C, and lower levels of HDL-C. In this study, after oral administration of *R. vesicarius* for 28 days, the level of TC,TG, and LDL-C ($p< 0.05$) were significantly restored to normal, while, HDL-C amelioration ($p < 0.05$) was only found in a higher dose of *R. vesicarius* level.

TABLE III - Effect of *R. vesicarius* on lipid profile, liver function, and kidney function in experimental rats

	Experimental Group				
	NC	**DC**	**RVLD**	**RVHD**	**Metformin**
Lipid profile					
TC (mg/dl)	87.15 ± 8.79	189.66 ± 10.01$^{###}$	164.86 ± 7.02*	145.00 ± 7.21***	91.33 ± 7.50***
TG (mg/dl)	92.19 ± 7.04	165.63 ± 6.02$^{###}$	141.82 ± 7.63**	134.03 ± 6.95**	97.63 ± 6.06***
LDL (mg/dl)	18.99 ± 2.17	32.46 ± 1.84$^{###}$	27.29 ± 2.05*	21.34 ± 1.40***	19.49 ± 1.78***
HDL (mg/dl)	32.72 ± 1.62	21.45 ± 1.54$^{###}$	23.48 ± 1.80	26.85 ± 2.32*	23.12 ± 1.10
Liver function test					
AST (U/L)	51.64 ± 6.69	128.95 ± 8.03$^{###}$	96.33 ± 9.50*	56.31 ± 6.88***	74.61 ± 6.66***
ALT (U/L)	27.79 ± 3.49	60.94 ± 6.55$^{###}$	46.96 ± 5.50***	37.38 ± 4.74***	35.14 ± 3.50***
ALP (U/L)	73.44 ± 5.85	145.61 ± 14.02$^{###}$	125.13 ± 7.08*	65.15 ± 8.50**	74.61 ± 5.35***
Kidney function test					
Urea (mg/dl)	59.16 ± 6.39	201.40 ± 11.42$^{###}$	173.48 ± 10.69*	95.82 ± 11.4^{1***}	102.88 ± 7.8^{4***}
Uric acid (mg/dl)	8.54 ± 0.98	13.38 ± 1.33$^{##}$	11.37 ± 1.51	8.91 ± 0.79**	10.20 ± 0.59*
Creatinine (mg/dl)	0.72 ± 0.14	0.86 ± 0.13	0.82 ± 0.11	0.72 ± 0.11	0.69 ± 0.08

Effects of *R. vesicarius* on serum liver profiles in diabetic rats

The effects of *R. vesicarius* on liver abnormalities in diabetic rats are summarized in Table III. Compared with the normal control group, the diabetic control group exhibited notably higher levels of serum ALT, AST, and ALP. However, *R. vesicarius* intake for 28 days resulted in a significant ($p< 0.05$) decrease in the level of ALT, ALP, and AST. The higher dose of *R. vesicarius* was found more significant as compared to a lower dose.

Effect of *R. vesicarius* on serum kidney profiles of diabetic rat

In the diabetic control group, a significant elevation of urea and uric acid was recorded as compared with the normal control group. Upon oral administration of *R. vesicarius*for 28 days, the level of urea was significantly ($p < 0.05$) ameliorated to normal levelsof uric acid in a higher dose of *R. vesicarius*. There is no significant difference was observed in the case of the creatinine level. The high dose treated group shows an almost similar ameliorative effect as standard metformin Table III.

Effect of *R. vesicarius*on the histopathology of the pancreas, liver and kidney

Figure 5 illustrates the histopathology of the pancreas of the experimental groups. Observation of pancreatic tissues showed normal acini and β-cells with no structural changes in the normal control group. Histopathology of pancreatic tissues in the diabetic control group showed degeneration of β-cells followed by atrophy in comparison with the normal control group. Treatment with *R. vesicarius* showed visible positive changes in the histo-architecture of pancreatic β-cells and the islet tissue section of the metformin-treated group also showedthe better appearance of β-cells as evidenced by the histopathological observation. Hepatic cells showed radially arranged hepatocytes around the central vein with well-defined nucleoli in the normal control group. The

section of the hepatic cells of the diabetic control group showed an increase in apoptotic hepatocytes (shrunken and dark-stained cells with small degenerated nuclei. *R. vesicarius* treatment groups ameliorated the pathology of hepatocytes through alleviation of distorted central vein, hepatocyte structure, and apoptotic cells in diabetic ratsas shown in Figure 6. In kidney histopathology, we found that HFD/STZ damagedtissues in the form of disarrangement and atrophy of tubular epithelium and inflammatory infiltration with widened Bowman's space in the diabetic control group as compared to the normal control group. The diabetic rats treated with *R. vesicarius* displayed no glomerular or tubular pathological alterations (Figure 7).

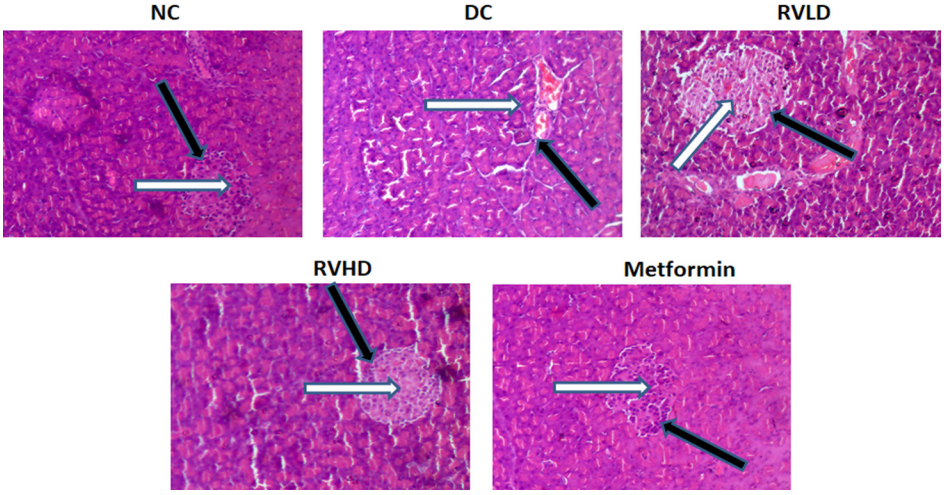

FIGURE 5 - Photomicrograph of the pancreas stained with HE. Normal control rats showednormal β-cells (white arrow) surrounded by deeply stained pancreatic exocrine cells (black arrow). Diabetic control groups showing destructed β-cells (white arrow) and pancreatic exocrine cells (black arrow). Treatment with *R. vesicarius* and metformin showing well-rejuvenated β-cells (white arrow) surrounded by deeply stained pancreatic exocrine cells (black arrow).

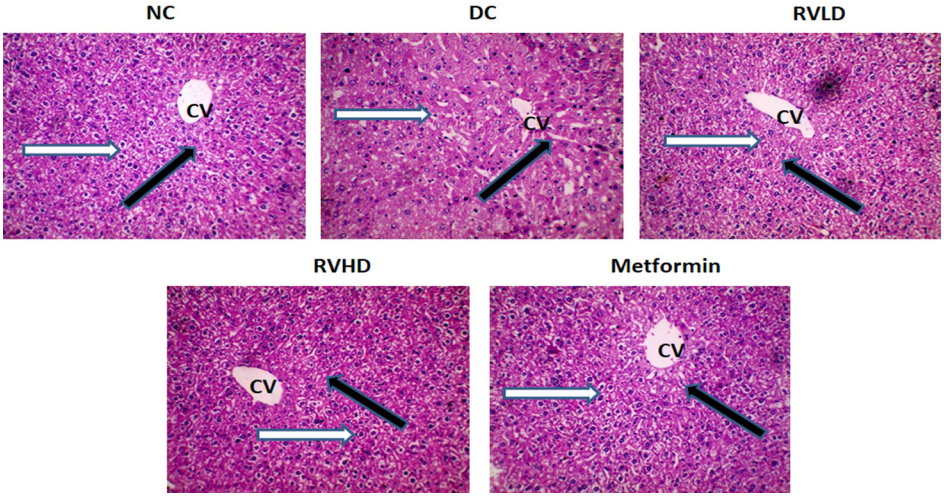

FIGURE 6 - Photomicrograph of the liver stained with HE. Normal control rats showednormal portal triad (black arrow) along with normal hepatocytes (white arrow) with the central vein (CV). Diabetic control groups showeda destructed portal triad along (black arrow) with disarranged hepatocytes (white arrow) with the disarranged central vein (CV). Treatment with *R. vesicarius* and metformin showing well-rejuvenated hepatocytes (black arrow) and portal triad (white arrow).

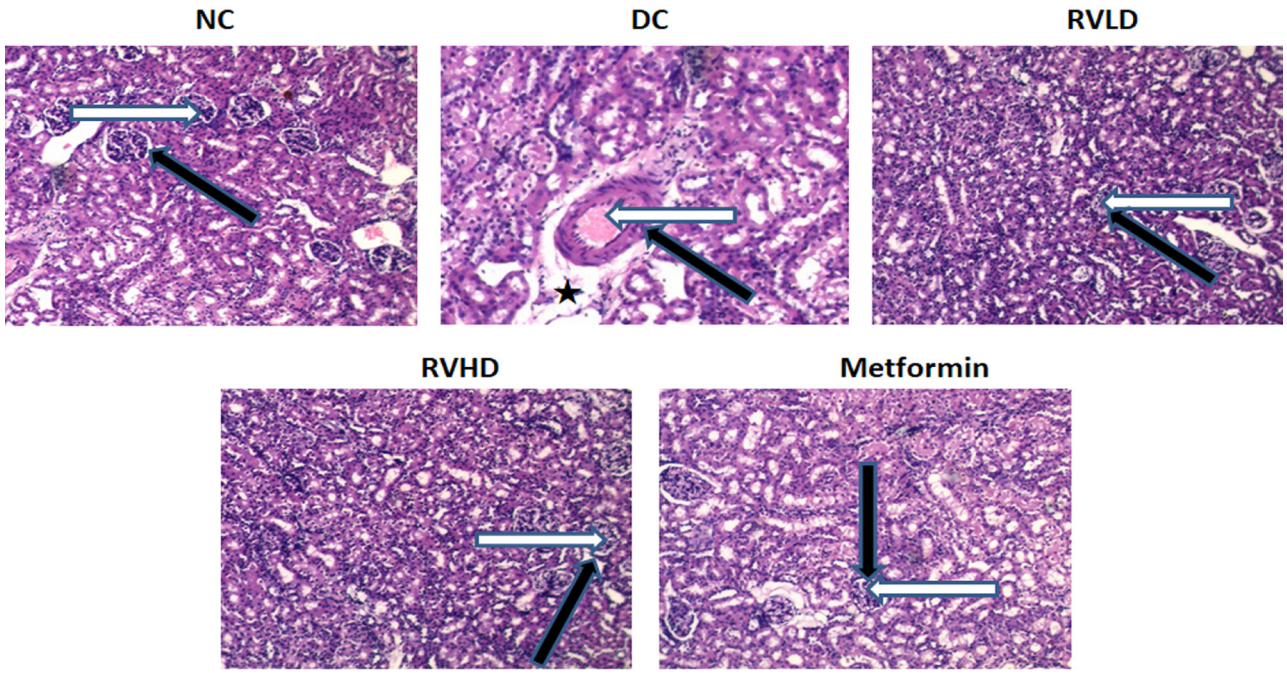

FIGURE 7 - Photomicrograph of the kidney stained with HE. Normal control groups rats showednormal histoarchitecture with well-developed normal glomerulus (black arrow) and Bowman's capsule (white arrow). Diabetic control groups showed degenerated glomerulus (black arrow), inflammations (star), and Bowman's capsule (white arrow). Treatment with *R. vesicarius* and metformin showing well-rejuvenated glomerulus (black arrow) and Bowman's capsule (white arrow).

DISCUSSION

Quantitative analyses indicated that *R. vesicarius* is enriched with polyphenols.Polyphenols are the most abundant antioxidants in medicinal plants,which are mainly responsible for therapeutic potential. Polyphenols are one of the important bioactive leads that play a key role in diabetes management by regulating postprandial glucose levels, protecting the deleterious effects of hyperglycemia-induced oxidative stress, and also to havean additive effect on the endogenous scavenging compounds (Panche, Diwan, Chandra, 2016). Therefore, biologically and pharmacologically it isbelieved that phenolic and flavonoid-rich extract may reduce the risk of diabetes (Syiem, Warjri, 2015).

The DPPH assay is the most simple and reliable method for the assessment of the antioxidant properties of herbal products.*R. vesicarius,*rich in polyphenols (ArOH), which reduces the rates of oxidation of organic matter by transferringa hydrogen atom to the chain-carrying ROO* radicals (Gaurav *et al.*, 2020). Through this mechanism,polyphenols inhibit the formation of free radicals and play an important role in ROS metabolism in the biological system.

Previous experimental and clinical evidence suggested that inhibition of carbohydrate hydrolyzing-enzymes, such as α-amylase and α-glucosidase reduced the progression of diabetes (Franco *et al.*, 2020). The main source of carbohydrates for human and animal species is starch. Salivary and pancreatic α-amylase cleaves the starch into simple saccharides at random sites, and form smaller molecules such as glucosethat are absorbed into the bloodstream. The α-amylase and α-glycosidase inhibitors block the conversion of starch into simplersaccharides or slow down the absorption of sugar in the gastrointestinal tract (Gaurav *et al.*, 2020). Our results revealed that *R. vesicarius*may delay the digestion of carbohydrates.

Herbal products contain a number of bioactive compounds with different chemical natures, and it is very difficult to find out a particular compound to which the complete biological activity of the product can be attributed (Sasidharan *et al.*, 2011). WHO has issued guidelines to validate the natural products that are used

for medicinal and therapeutic purposes (Parveen *et al*., 2015). As we know that India is one of the largest exporters of herbal raw materials, which are used as food products as well as for therapeutic purposes. In such cases, the chromatographic profile is very useful and widely can be used for its identity, quality control analysis, and regulatory bodies to assure its quality and safety. Results obtained from the UPLC-QTOF-MS fingerprint revealed that *R. vesicarius* extract enriched with bioactive metabolites such as alkaloids, flavonoids, glycosides, lipids, phenols, and terpenoids,are responsible for its therapeutic potential. Thus, for the analysis of varied metabolites, the LCMS method seems to be the best method of analysis.

The OGT test is used to determine the altered carbohydrate metabolism during post glucose administration. *R. vesicarius* treated rats reduced dose-dependent glucose level, which indicated that the increased glucose tolerance might be due to secretion of sufficient insulin from β-cells of the pancreatic islets and increased glucose utilization by the tissues. Our findings matched with previously reported studies (Kim *et al*., 2016).

The hypoglycemic effect of the extract was evident due to the stimulation of insulin release from pancreatic β-cells,which acts directly or indirectly on the liver to lower glucose production as well as acts on the gut to increase glucose utilization (Rena,Hardie, Pearson, 2017). The possible mechanism of *R. vesicarius* brought about by its hypoglycemic action might be by increasing insulin secretion from regenerated β-cells of the pancreas. It was further supported by histological observations, which revealed a damaged β-cell population in the pancreas in diabetic rats. The diabetic treated with low and high doses of *R. vesicarius* (RVLD and RVHD) animals showed an increase in the number of islets, lesser degree of shrinkage, and restoration of necrosis of β -cells of the pancreas. Our finding is consistent with an earlier report by Junejo *et al*.(2017).

The control and HFD fed rats constantly increased their body weight, whereas after administration of STZ injection to diabetic control group significantly dropped bodyweight probably because of decreased glucose metabolism and increased fat metabolism(Guo *et al*., 2018). The body-weight loss is considered to be the typical characteristic of diabetes induced by HFD/STZ. The results indicated that *R. vesicarius* could ameliorate the decrease in body weight of diabetic rats thereby improvingthe quality of life in diabetic rats.

Dyslipidemia is featured with an increase in serum TG, TC, LDL-C, and a decrease in serum HDL-C level in the case of diabetic rats. Elevated serum triglycerides herald the development of diabetes mellitus and also accelerated cardiovascular diseases (Zheng, Ley, Hu, 2018). It is recognized as a complication of diabetes mellitus owing to an increased breakdown of lipids and free fatty acids from peripheral deposits. Indeed, insulin deficiency or resistance during the hyperglycemic state, which in turn could lead to the free fatty acid mobilization from adipose tissues mediated by hormone-sensitive lipase (Schofield *et al*., 2016). Lipoprotein lipase located on vascular endothelium largely determines the rate of removal of triglycerides from the circulation. In contrast to intracellular hormone-sensitive lipase, this lipoprotein lipase may be down-regulated in states of insulin resistance or deficiency (Taskinen, 2003). Therefore, based on the finding we can say that *R. vesicarius* possesses dose-dependent activity on lipid profile. Obtained results revealed that*R. vesicarius* showscomparative ameliorative potential as metformin.

The liver is the most important organ that plays a key role in regulating various physiological processes in the body including glucose homeostasis by storing glucose as glycogen, breaking this down to glucose when needed, and forming glucose from non-carbohydrate sources such as amino acids (Rui, 2014). In the liver, amino acids are converted to keto-acids by AST, ALT, and ALP, and their levels could be increased because of damage to the liver leading to their leakage into the blood. The significant increase in the serum levels of ALT, AST, and ALP in the diabetic control group showed the extent of liver injury, which indicates that the impaired liver function might be due to hyperglycemia (Shibabaw *et al*., 2019). Moreover,histopathological changes in the liver tissues have well supported the leakage of enzymes in the blood. Distorted central vein and hepatocytes were reinstated to normal in the liver of HFD/STZ-induced diabetic rats treated with various doses of *R. vesicarius* when compared to diabetic control.

The physiological efficiency of the kidney was analyzed by measuring the expression of renal injury biomarkers such as urea, uric acid, and creatinine. High serum urea, uric acid, and creatinine indicate kidney malfunction. This is clear evidence that a chronic hyperglycemic causes dysfunction of renal and vascular cells mediated by altered metabolic pathways in a self-perpetuating manner(Al-Daghri *et al.*, 2017). The significant reduction in the level of kidney biomarkers treated with various doses of *R. vesicarius* to diabetic rats indicated that the *R. vesicarius* prevented the progression of renal damage in diabetes (Sagbo *et al.*, 2018). Further, in this study histopathological observation revealed deterioration of the glomerulus structures and cellular inflammatory infiltration was at the forefront. The findings of the treatment groups were to ameliorate the deterioration of the glomerulus structures and cellular inflammatory infiltration in diabetic rats. Our results strongly matched the findings of Ibrahim *et al.* (2021).

CONCLUSION

The present study demonstrated that *R. vesicarius* excellently inhibits enzymes α-amylase and α-glucosidase as well as possessesexcellent antioxidant potential by scavenging DPPH. Further, *R. vesicarius* significantly ameliorated hyperglycemia in HFD/STZ-induced diabetic rats with significant improvement in levels of blood glucose, serum lipids, liver, and kidney biochemical markers. The histopathological observation showed that *R. vesicarius* treatment could protect against the HFD/STZ-induced deterioration of the pancreas, liver, and kidney. These effects might be due to the presence of bioactive leads in the extract. However, we suggest that extensive experimental and clinical studies are required to reveal the exact mechanism of *R. vesicarius* against diabetes and its complications.

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ETHICAL ISSUE

All experiments were performed according to the guidelines of the Institutional Animals Ethics Committee, Jamia Hamdard (Approval Number: JH/2019/1622).

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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