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Phytochemical Screening, *In-silico* Toxicity Profiling, and Antidiabetic Potential of the Flowers of *Rosa alba* L.

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ABSTRACT

Rosa alba L. is a perennial flowering shrub belonging to the Rosaceae family. Diabetes is treated in Assam, India, with a decoction of its flower. But to date, there is no scientific evidence for its antidiabetic potential. Therefore, in addition to toxicity investigations, the current study aimed to determine its antidiabetic potential to support conventional claims. *In-silico* toxicity profiling for the phytoconstituents of the plant was predicted as non-toxic, non-mutagenic, and non-carcinogenic in mice and rats. The Total phenolic and total flavonoid contents were determined and found to be higher in the ethanolic extract than in the aqueous extract of the flower. Antidiabetic activity of orally administered aqueous and alcoholic extracts (200 mg/kg body weight) was evaluated against the high-fat diet fed with a low dose of streptozotocin-induced diabetic rats. Treatment of these extracts significantly ($p<0.001$) reduced the blood glucose level and lipid profile in induced-diabetic rats without affecting their liver and kidney functions. It was also shown that the liver and pancreatic tissues of animals treated with extracts had enhanced histopathologic architecture. The findings of the study revealed that the flowers of *R. alba* are a safe herbal remedy for controlling diabetes.

Keywords: Diabetes, Histopathology, *In-silico*, *Rosa alba*, STZ.

INTRODUCTION

Rosa alba L. is a perennial flowering shrub that belongs to the family Rosaceae, popularly known as a white rose. The height of the shrubs is up to 1.8 m with wide branches and thorns. Its white velvety flowers spread a pleasant smell, therefore attracting insect pollination [1-4]. Traditionally *R. alba* L. is used as a rubefacient, lactagogue, insecticidal, antioxidant, anthelmintic, and laxative, for the treatment of piles, diarrhea, cardiovascular diseases, eye troubles, and vaginal candidiasis [5, 6]. The hydrodistillate product of the flowers is used to prevent cardiovascular diseases. Rose oil is also used in the preparation of various skin creams/ointments as a fragrance or perfuming agents. Flowers of *R. alba* and its essential oil were reported various biological activities like- antioxidant, antimicrobial, [7-9] memory enhancing [10, 11], antistress [12] and antiallergic [13,14].

Diabetes mellitus (DM) is one of the most significant public health problems in the world. It is a metabolic disorder characterized by an elevation in the blood glucose level due to defects in either insulin secretion or insulin resistance or both. Type-1 DM (T1DM) is insulin-dependent diabetes in which the human immune system destroys the insulin-secreting β-cells of the pancreas. While type-2 DM (T2DM) is noninsulin-dependent diabetes that begins with insulin resistance until the pancreas slowly loses its ability to secret insulin. All around the world almost 90% of people suffer from type-2 diabetes; therefore, it is the most common and very prevalent disorder in both developed and developing countries [15,16]. Daimari M. *et al*. have found in their ethnobotanical survey of the Bodo tribe of Kokrajhar district, Assam that people use the decoction of *R. alba* flowers for the treatment of diabetes [17] and to date, there is no scientific evidence for its antidiabetic potential. Therefore, in the present research, flowers of *R. alba* have been preferred to investigate their antidiabetic effects against a high-fat diet (HFD) and low-dose STZ-induced diabetes model of rats to verify its traditional claims. The combination of HFD and low-dose STZ injection model of rat mimic the natural history and metabolic characteristics of T2DM in humans. This model is cheap, easy to develop, and most suited for studying the pathophysiology of T2DM and screening the therapeutic compounds for the treatment of T2DM [18].

MATERIALS AND METHODS

Collection and authentication of plant

Fresh flowers of *R. alba* were collected from the Garden of Baba Raghav Das Medical College, Gorakhpur, Uttar Pradesh, India. The whole plant with flowers was identified and authenticated by Professor (Dr.) V. N. Pandey, Department of Botany, Deen Dayal Upadhyaya Gorakhpur University,

Gorakhpur. Herbarium specimen of the same has been submitted to the Department vide reference no."Bot/11017".

All the chemicals and solvents used in the present study were of analytical grade. Streptozotocin (STZ) extra pure,a diabetes-inducing agent, was procured from Sisco Research Laboratories Pvt. Ltd. (SRL), Mumbai (Maharashtra), India.

Preparation of ethanolic extract (ERA)

Accurately weighed (200g) quantity of the powder of *R. alba*was extracted with ethanol (85% v/v) by continuous hot extraction in soxhlet apparatus (Borosil®). The solvent was filtered and evaporated to dryness (concentrated) using a rotary vacuum evaporator (IKA-RV 10 basic) to obtain the dry extract. The dried extracts were kept in desiccators for further use.

Preparation of aqueous extract (ARA)

Aqueous extract of the flower of *R. alba* was prepared by cold maceration method. Accurately weighed 200 gm of dried-coarse powder of the *R. alba* flowers in a large beaker and distilled water was added in sufficient quantity to immerse the powdered drug completely. A small amount of chloroform was added to the beaker as a preservative. The beaker was set aside for 72 hours with occasional stirring. After that, the extract was filtered out to get a brownish-red filtrate. Then the filtrate was concentrated under a high vacuum and completely dried in a desiccator.

Phytochemical investigation

The above-prepared extracts of the powdered drug were subjected to preliminary phytochemical analysis for determining the various phytoconstituents like alkaloids, glycosides, steroids, saponin, flavonoids, tannins, carbohydrates, etc. by routine methods of analysis [19-22].

Total phenolic content

The total phenolic content of the ethanolicand aqueous extracts of *R. alba* was determined by the Folin–Ciocalteu method [23]. The method is described briefly-200 µL of crude extract (1 mg/mL) was taken in the test tube, the volume of the extract in the test tube was made up of 3 mL with distilled water then 0.5 mL of Folin–Ciocalteu reagent was added and mixed thoroughly for 3 min. After that 2 mL of 20% (w/v) sodium carbonate solution was added to the mixture and kept aside the test tube for 60 min in the dark for incubation. After incubation, the absorbance of the mixture was measured at 650 nm with a UV/Vis spectrophotometer in triplicate. A similar procedure was adopted with the standard solutions of gallic acid (05, 10, 15, 20, 25, 30, 35, 40, 45, and 50µg/mL) in triplicate and prepared the calibration curves between concentration vs absorbance. The total phenolic content was calculated by calibration curve and expressed as the mg of gallic acid equivalents (GAE).

Total flavonoid content

The total flavonoid content of the ethanolic and aqueous extracts of *R. alba* was determined by the aluminium chloride colorimetric method [23]. In brief, 50µL crude extract of the above plants was taken in a test tube of 10 ml capacity and made up its volume 1mL with methanol. After that 4 mL distilled water and 0.3 mL of 5% sodium nitrite solution were added to this mixture and incubate it for 5 minutes. 0.3 mL of 10% aluminium chloride solution was then added to it and again kept aside for 6 minutes. Subsequently, 2 mL of 1 molar NaOH solution was added to it and made up its volume to 10 ml with distilled water. Finally, the mixture was kept at room temp for 15 minutes and then checked its absorbance at 510 nm by UV/V is spectrophotometer in triplicate. A similar procedure was adopted with the standard solutions of rutin (05, 10, 15, 20, 25, 30, 35, 40, 45, and 50µg/mL) in triplicate and prepared the calibration curve between concentration vs average absorbance. The total flavonoid content was calculated by calibration curve, and the results were expressed as the mg of rutin equivalents (RE).

***In-silico* toxicity profiling**

The *in-silico* toxicity profiling of major chemical constituents of *R. alba*was determined using "LAZAR toxicity predictions" property explorer program. The chemical structure of the compound under examination was drawn or entered its SMILES string in the LAZAR property explorer program. After that, the parameters of toxicity like-acute toxicity, carcinogenicity in rats, carcinogenicity in mice, and mutagenicity had been selected and clicked on the prediction icon to find out the result.

Antidiabetic activity

Preparation of 0.1 M sodium citrate buffer

0.1 M sodium citrate buffer was prepared by mixing 1.475 gm of sodium citrate in 50 ml of ice-cold distilled water which was adjusted to pH 4.45 with the help of 1 M Citric acid solution obtained by dissolving 1.92 gm citric acid in 10 ml of distilled water.

Animals

Male Sprague Dawley rats (150-180g) were procured from the Laboratory Animal Service Division of Central Drugs Research Institute (CDRI), Lucknow. The experimental protocol was duly approved by the Institutional Animal Ethics Committee (IAEC, No. BRD/IAEC/23/03/2022) and the experiments were performed as per the guidelines. All the animals were housed in polypropylene cages under standard conditions of temperature (23 ± 2 °C), 12 h light/dark cycles in the Central animal house, BRD Medical College, Gorakhpur, and fed with normal pellet diet (NPD)/High-fat diet (HFD) and water *ad libitum*. All animals were acclimatized in the laboratory conditions for 7 days before the experiments.

After the acclimatization period, rats were divided into two groups. One group of 6 animals was considered as a normal control group fed with NPD. The rest of the animals are considered as the second group, fed with HFD for four weeks.

Induction of diabetes

Type-2 diabetes was induced in all the animals of the second group by injecting a low dose of streptozotocin (35mg/kg body weight) intraperitoneally while the respective normal control rats were given vehicle citrate buffer. After the injection, all the animals were closely observed for 7 days to determine their body weight and blood glucose level (BGL) by glucometer (Dr. Morpen® Gluco One Model: BG-03). The rats with random (non-fasting) BGL above 250 mg/dl were considered diabetic and selected for further antidiabetic screening.

Grouping of diabetic animals

Diabetic rats were divided into the following groups with oral treatments for further 28 days and all the groups were allowed to continue their respective diet until the end of the study. 6 rats were taken in each group. A dose of extracts was prepared with 2% gum acacia as suspension and each timehas shaken well before dosing.

Group-1= Normal control (NC) and received only vehicle (2% gum acacia suspension).

Group-2= Diabetic control (DC) and received only vehicle (2% gum acacia suspension).

Group-3= Extract treated group received 200 mg/kg body weight of ethanolic extract of the flower of *Rosa alba* (ERA).

Group-4= Extract treated group received 200 mg/kg body weight of water extract of the flower of *Rosa alba* (ARA).

Group-5= Metformin (Standard antidiabetic drug) treated group and received 100mg/kg body weight.

The body weight and fasting blood glucose (FBG) levels of all the animals of each group were estimated weekly. The oral glucose tolerance test (OGTT) of all the animals of each group was determined on the fourth week of the study.

Fasting blood glucose

FBG level of all the overnight fasting animals was estimated by glucometer (glucose oxidase-peroxidase method using strips) weekly.

Oral glucose tolerance test

All the animals of each group were given glucose (2g/kg body weight) orally 60 min after the treatments. The blood samplewas withdrawn through the tail vein at 0, 30, 60, 90, and 120 min after glucose loading.Blood glucose levels were estimated by glucometer [24].

At the end of the study (after 4 weeks), all the animals were anesthetized and euthanized. Blood was collected in a vial by intracardiac puncture and kept aside for 15 minutes to separate the serum for estimating biochemical parameters. The pancreas and liver of each animal were carefully dissected, weighed, and preserved in formalin for histopathological analysis.

Biochemical parameters

Lipid profile[serum total cholesterol (TC), total triglyceride (TG), low-density lipoprotein cholesterol (LDL-C), and high-density lipoprotein cholesterol (HDL-C)], liver function tests [serum total bilirubin, Serum Glutamate Pyruvate Transaminase (SGPT), Serum Glutamate Oxaloacetate Transaminase (SGOT)] and renal function tests[serum urea and creatinine] of all the animals of each group were measuredby a semi-autoanalyzer (Mindray, BA-88AShenzhen Mindray Bio-Medical Electronics Co. Ltd., China) using procedure, recommended in commercial assay kits (Beacon diagnostics Pvt. Ltd. and TRUEchemie Athenese-Dx Pvt. Ltd.).

Lipid profile

Serum total cholesterol (TC) of the treated animals was determined by taking 1 ml of cholesterol reagent (enzyme reagent) with 10µl test serum and cholesterol standard each in the separate test tubes. 1 ml of cholesterol reagent was taken in another test tube as blank. All the tubes were incubated at 37°C for 5 minutes and then checked their absorbance at 505 nm.

$$\text{Cholesterol (mg/dl)} = \frac{\text{(Abs. of sample - Abs. of blank)}}{\text{(Abs. of standard - Abs. of blank)}} \text{ X200}$$

The total triglyceride (TG) level of the treated animals was determined by the same procedure as TC, except only triglyceride reagent and triglyceride standard was taken in the place of cholesterol reagent and cholesterol standard respectively [25, 26].

Low-density lipoprotein cholesterol (LDL-C) was determined using a direct LDL cholesterol test kit. In this method, two liquid reagents (R1 and R2) were used in a 3:1 ratio and a calibrator reagent. 375µl of R1 was taken in a test tube as blank, 375µl of R1 with 5 µl calibrator reagent in a second test tube as calibrator, and 375µl of R1 with 5 µl test serum in a third test tube as a test. The contents of all the test tubes were mixed thoroughly and incubated at 37°C for 5 minutes. After that 125 µl of R2 was added in all three test tubes and incubated again at 37°C for 3 minutes then measure the absorbance of the calibrator and test serum was against the reagent blank at 578 nm [27]. The concentration of the LDL cholesterol was calculated as under-

$$\text{LDL (mg/dl)} = \frac{\text{(Absorbance of test serum)}}{\text{(Absorbance of calibrator)}} \text{ X Calibrator concentration}$$

High-density lipoprotein cholesterol (HDL-C) was also determined using a direct HDL cholesterol test kit. In this method, two liquid reagents (R1 and R2) in a 3:1 ratio and a calibrator reagent were used. 450µl of R1 was taken in a test tube as blank, 450µl of R1 with 5 µl calibrator reagent in a second test tube as a calibrator, and 450µl of R1 with 5 µl test serum in a third test tube as a test. The contents of all the test tubes were mixed thoroughly and incubated at 37°C for 5 minutes. After that 150 µl of R2 was added in all three test tubes and incubated again at 37°C for 3 minutes then the absorbance of the calibrator and test serum were measured against the reagent blank at 578 nm [28].

Liver function tests

The total bilirubin content in the serum was measured by taking 1.0 ml total bilirubin reagent, 20 µl total bilirubin activator, and 50 µl test serum in a test tube as a test sample. 1.0 ml total bilirubin reagent and 50 µl test serum in a test tube as a blank. Mixed well the contents of both test tubes, incubated at 37°C for five minutes, and read the absorbance at 546 nm [29]. The concentration of total bilirubin was calculated as under-

Total bilirubin (mg/dl) = Abs. of test-Abs. of blank x Factor

SGPT and SGOT in the serum were determined by taking 800 µl enzyme reagent (R1), 200 µl substrate reagent (R2), and 100 µl test serum in a test tube. The contents of the test tube were mixed well and incubated at 37°C for one minute, and read the absorbance at 340 nm. The absorbance of the mixture was again measured after 1, 2, and 3 minutes. Distilled water had been used as blank [30]. The concentration of SGPT and SGOT was calculated as under-

ΔE= Initial Absorbance-Absorbance after $1^{st}/2^{nd}/3^{rd}$ min.

ΔE/min. was calculated for each reading and taken average.

SGPT/SGOT (U/L) = (Average ΔE/min.) x 1768

Kidney function tests

Urea in the serum was determined by the Berthelot method. In this method, 1.0 ml of working reagent was taken in a test tube as blank, 1.0 ml of working reagent with 10 µl standard reagent in a second test tube as standard, and 1.0 ml of working reagent with 10 µl test serum in a third test tube as a test. The contents of all the test tubes were mixed thoroughly and incubated at 37°C for 5 minutes. After that 1.0 ml of chromogen reagent was added to all three test tubes. The absorbance of the standard and test were measured against the reagent blank at 600 nm [31]. The concentration of the urea was calculated as under-

$$\text{Urea (mg/dl)} = \frac{\text{(Abs. of test- Abs. of blank)}}{\text{(Abs. of standard- Abs. of blank)}} \text{ X 40}$$

Creatinine in the serum was determined by the alkaline picrate method. In this method, 1.0 ml of working reagent was taken in a test tube as blank, 1.0 ml of working reagent with 50 µl standard reagent in a second test tube as standard, and 1.0 ml of working reagent with 50 µl test serum in a third test tube as a test. The contents of all the test tubes were mixed thoroughly. Absorbance (A_1) for the standard (A_1S) and test (A_1T) were measured after 30 seconds at 505 nm. Read another absorbance (A_2) of standard (A_2S) and test (A_2T) exactly 120 seconds later [32]. Change in absorbance (ΔAS/ ΔAT) for both standard and test was calculated as-

$\Delta AS = A_2S - A_1S$; $\Delta AT = A_2T - A_1T$

Creatinine (mg/dl) = $\Delta AT / \Delta AS \times 2$

Histopathological studies

For histopathological studies, the pancreas and liver were identified, isolated, weighed, and preserved in 10% formalin. The tissues were sectioned at 8-10 micron thickness by Microtome Cryostat and stained with Hematoxylin and Eosin (H&E). All the sections were observed and examined under the microscope (Leica DM500).

Statistical analysis

All the data were analyzed by two-way analysis of variance (ANOVA) followed by Bonferroni-post tests using GraphPad Prism 5 software. $p<0.05$ values were considered statistically significant. Values are represented as mean ± SD. Six animals were taken in each group.

RESULTS

Preliminary phytochemical screening results revealed that *R. alba* extracts included a variety of phytoconstituents, like protein, amino acids, carbohydrates, tannins, terpenoids, glycosides, saponin, and flavonoids (Table 1).

Total phenolic content

The total phenolic content of the aqueous and ethanolic extracts of *R. alba* flower (ARA and ERA) were calculated from the calibration curve (Figure 1) of standard gallic acid and was obtained higher than the ARA (Table 2).

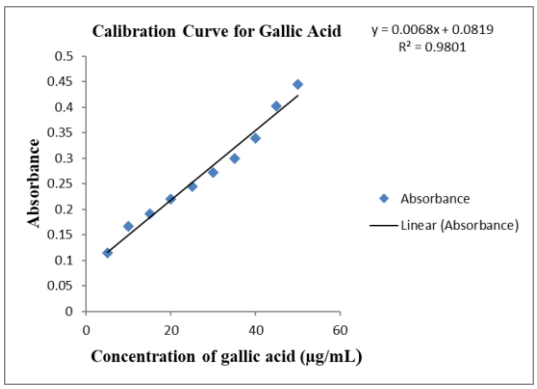

Figure 1: Calibration curve of gallic acid for determination of total phenolic content

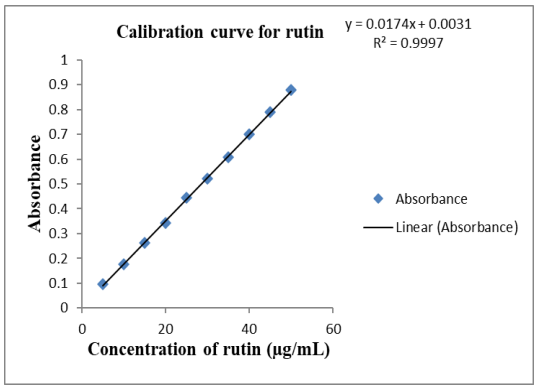

Figure 2: Calibration curve of rutin for determination of total flavonoid content

Total flavonoid content

The total flavonoid contents in ARA and ERA were calculated from the calibration curve (Figure 2) of standard rutin and was obtained higher than the ARA (Table 2).

***In-silico* toxicity profiling**

The major chemical constituents of the plant were evaluated by the LAZAR property explorer and most of them were predicted non-toxic, non-mutagenic, and non-carcinogenic in mice and rats. The acute toxicity dose of each compound was predicted in milligrams/liter or millimoles/liter (Table 3).

Antidiabetic activity

The antidiabetic effects of the flowers of *R. alba* were evaluated against a high-fat diet with the low dose of streptozotocin induced diabetic model of rat. Body weight of the normal control, diabetic control, extract-treated and standard drug-treated animals were determined weekly for a period of further four weeks (Table 4). Comparing the FBG levels of all the animals injected with STZ to the control group, there was a significant three-fold rise. During the second week of the study, the FBG levels of the animals treated with the extracts and metformin, started to decrease, and by the end of the fourth week, they were nearly within the normal range (Table 5). In oral glucose tolerance test the blood glucose level of the extracts treated animals were reached almost to the normal range at the end of the test (Table 6).

Lipid profiling

The effects of *R. alba* extracts on the lipid profile parameters in all the treated groups were studied, the level of total cholesterol, triglycerides, and low density lipoproteins were significantly reduced in induced diabetic rats treated with *R. alba* extracts and standard drug compared with the untreated diabetic control group (Table 7).

Liver and kidney function tests

The effects of *R. alba* extracts on the liver and renal functions in all the treated groups were observed at the end of the study. The diabetic control group that was not receiving extracts had somewhat higher levels of total bilirubin, SGOT, and SGPT than the groups which received metformin, extracts treatments, and normal control group. Serum urea and creatinine levels, which are indicative of renal function, were found to be almost same in the treated and DC groups compared to the NC groups. Thus, as compared to the normal control group, none of the treated rats' liver or kidney functions showed any appreciable abnormalities (Table 8).

Histopathological studies

At the end of the study liver and pancreas of rats were isolated and weight after the dissection of animals. Physically both the organs of each animal were found in normal weights and appearance (Table 9). Figures 3 and 4 depict the histological manifestation of the pancreatic and liver tissues of each animal, respectively.

Table 1: Preliminary phytochemical screening of the ARA and ERA

Phytoconstituents	Extracts of the flowers of *R. alba*		Name of test
	ARA	ERA	
Carbohydrate	+	+	Molisch test
	+	+	Benedict's test
	+	+	Fehling's test
Alkaloids	-	-	Mayer's test
	-	-	Dragendorff's test
Tannins	+	+	Gelatin test
	+	+	Ferric chloride test
Protein Amino acids	+	+	Millon's Test
	+	+	Ninhydrin Test
Sterol and Terpenoids	-	+	Liebermann-Burchard test
	-	+	Salkowaski's test
Flavonoids	++	++	Shinoda's test
	++	++	Pew's Test
Glycosides	+	-	Baljet's test
	+	-	Legal's test
Saponin	+	+	Foam test

+ (present), - (absent), ++ (intense positive test for presence)

Table 2: Total phenolic content and total flavonoid content in the extracts of *R. alba* flower

S No	Material	Total phenolic content (Gallic acid equivalents/g) ± SD	Total flavonoid content (Rutin equivalents/g) ± SD
1	Aqueous extract of *R. alba* (ARA)	32.676±0.294	23.13±0.200
2	Ethanolic extract of *R. alba* (ERA)	39.245±0.224	18.34±0.087

Table 3: *In-silico* toxicity profiling of *Rosa alba* L.

Chemical Constituents	Acute toxicity	Mutagenicity	Carcinogenicity in Mouse	Carcinogenicity in Rat
Citral	1.18 (mg/L)	Non-mutagenic	Non- carcinogenic	Non-carcinogenic
Citronellol	Not Predicted	Non-mutagenic	Not Predicted	Not Predicted
Geraniol	Not Predicted	Non-mutagenic	Non- carcinogenic	Non-carcinogenic
Linalool	Not Predicted	Non-mutagenic	Not Predicted	Not Predicted
Nerol	Not Predicted	Non-mutagenic	Non-carcinogenic	Non-carcinogenic
Neral	1.18 (mg/L)	Non-mutagenic	Non-carcinogenic	Non-carcinogenic
Geranial	1.18 (mg/L)	Non-mutagenic	Non-carcinogenic	Non-carcinogenic
Lonone	Not Predicted	Non-mutagenic	Not Predicted	Not Predicted
Benzyl benzoate	20.5 (mg/L)	Non-mutagenic	Non-carcinogenic	Non-carcinogenic
Fenchyl alcohol	0.238 (mg/L)	Non-mutagenic	Non- carcinogenic	Not Predicted
Ocimene	Not Predicted	Non-mutagenic	Not Predicted	Not Predicted
Eudesmol	Not Predicted	Non-mutagenic	Not Predicted	Not Predicted
Farnesol	Not Predicted	Non-mutagenic	Non-carcinogenic	Non-carcinogenic
Geranyl acetate	10.0 (mg/L)	Non-mutagenic	Non-carcinogenic	Non-carcinogenic
Squalene	2.53 (mg/L)	Non-mutagenic	Non-carcinogenic	Non-carcinogenic
Quercetin	4.53 (mg/L)	Non-mutagenic	Non-carcinogenic	Non-carcinogenic
Rutin	Not predicted	Non-mutagenic	Non-carcinogenic	Non-carcinogenic

Table 4: Effects of the treatments on body weight of different animal groups

Groups	Dose (mg/kg)	Body wt. (gm) On 0 day	Body wt. (gm) On 7th day	Body wt. (gm) On 14th day	Body wt. (gm) On 21st day	Body wt. (gm) On 28th day
NC	Vehicle	151.6±3.57$^{###}$	161.8±2.95$^{###}$	166.6±3.43$^{###}$	173±3.536	181.4±3.64$^{##}$
DC	Vehicle	180.7±1.96***	179±3.09***	174.0±1.09***	171.4±2.51	175±2.70**
ERA	200	176.7±2.94***	174.3±4.22$^{***\#}$	176.7±2.94***	183.3±2.87$^{***###}$	189.7±2.73$^{***###}$
ARA	200	177.5±1.51***	180±1.26***	182.5±1.04$^{***###}$	187.7±1.96$^{***###}$	193.3±2.13$^{***###}$
Metformin	100	177±1.63***	185.5±2.38$^{***##}$	196.8±2.21$^{***###}$	214.8±3.50$^{***###}$	221.5±3$^{***###}$

The comparisons of NC and DC vs. treated groups were performed by two-way ANOVA and Bonferroni post-tests. ***/ ### p< 0.001, **/ ## p< 0.01 and */ # p< 0.05. * (compared with NC) and # (compared with DC). Values are Mean ± SD.

Table 5: Effects of the treatments on fasting blood glucose of different animal groups.

Groups	Dose (mg/kg)	FBG (mg/dL) On 0 day	FBG (mg/dL) On 7th day	FBG (mg/dL) On 14th day	FBG (mg/dL) On 21st day	FBG (mg/dL) On 28th day
NC	Vehicle	102.8±5.80$^{###}$	103.6±4.21$^{###}$	98.4±2.60$^{###}$	101.6±1.81$^{###}$	100.4±3.97$^{###}$
DC	Vehicle	338.8±61.14***	377.3±35.82***	374.4±31.52***	354±26.53***	317.8±7.72***
ERA	200	303.8±50.13***	304.2±45.11$^{***###}$	279.2±16.18$^{***###}$	233.3±10.07$^{***###}$	135.5±7.76$^{###}$
ARA	200	286.8±20.13$^{***\#\#}$	269.8±16.89$^{***###}$	261.3±12.66$^{***###}$	214.3±11.52$^{***###}$	174.5±11.55$^{***###}$
Metformin	100	286±30.47$^{***\#}$	246±14.49$^{***###}$	233±14.70$^{***###}$	175±4.967$^{***###}$	122.5±7.32$^{###}$

The comparisons of NC and DC vs. treated groups were performed by two-way ANOVA and Bonferroni post-tests. ***/ ### p< 0.001, **/ ## p< 0.01 and */ # p< 0.05. * (compared with NC) and # (compared with DC). Values are Mean ± SD.

Table 6: Effects of the treatments on oral glucose tolerance test of different animal groups.

Groups	Dose (mg/kg)	Blood glucose (mg/dl) at 0 min.	Blood glucose (mg/dl) at 30 min.	Blood glucose (mg/dl) at 60 min.	Blood glucose (mg/dl) at 90 min.	Blood glucose (mg/dl) at 120 min.
NC	Vehicle	102.4±4.561$^{###}$	143.8±6.09$^{###}$	165±4.18$^{###}$	178.4±3.84$^{###}$	123.2±3.83$^{###}$
DC	Vehicle	318.6±5.36***	421.6±12.44***	534±31.68***	399.4±21.22***	339.2±28.07***
ERA	200	135.8±6.91$^{***###}$	366.2±10.26$^{***###}$	391.3±7.20$^{***###}$	257.7±6.56$^{***###}$	143.5±5.32$^{*###}$
ARA	200	122.7±4.63$^{*###}$	324.8±16.52$^{***###}$	402.2±10.53$^{***###}$	260.7±21.60$^{***###}$	133.3±4.59$^{###}$
Metformin	100	123.3±7.50$^{###}$	138.5±7.76$^{###}$	234.3±6.07$^{***###}$	253.8±7.41$^{***###}$	133.8±8.69$^{###}$

The comparisons of NC and DC vs. treated groups were performed by two-way ANOVA and Bonferroni post-tests. ***/ ### p< 0.001, **/ ## p< 0.01 and */ # p< 0.05. * (compared with NC) and # (compared with DC). Values are Mean ± SD.

Table 7: Effects of the treatments on the lipid profile of different animal groups

Groups	Dose (mg/kg)	Total Cholesterol (mg/dl)	Total Triglycerides (mg/dl)	HDL-C (mg/dl)	LDL-C (mg/dl)
NC	Vehicle	122 ±1.58$^{\#}$	69.6±2.07$^{\#}$	42±1.58$^{\#}$	63.8±2.68$^{\#}$
DC	Vehicle	210.6 ± 3.84*	191.4±1.81*	22.4±1.51*	153.6±2.30*
ERA	200	178.3±1.96$^{*\#}$	163.3±2.16$^{*\#}$	25.17±2.13*	131.2±2.13$^{*\#}$
ARA	200	190.8±2.31$^{*\#}$	173.8±3.06$^{*\#}$	22.83±1.94*	142.7±2.16$^{*\#}$
Metformin	100	136.5±1.29$^{*\#}$	82±1.82$^{*\#}$	41.5±1.29$^{\#}$	56.75±1.70$^{*\#}$

The comparisons of NC and DC vs. treated groups were performed by two-way ANOVA and Bonferroni post-tests. */ # p< 0.05 * (compared with NC) and # (compared with DC). Values are Mean ± SD.

Table 8: Effects of the treatments on liver and renal functions of different animal groups

Groups	Dose (mg/kg)	Bilirubin total (mg/dL)	SGOT (U/L)	SGPT (U/L)	Serum Urea (mg/dL)	Serum creatinine (mg/dL)
NC	Vehicle	0.33±0.020	33.4±0.894	35.2±2.387	27.4±1.51	0.67±0.032
DC	Vehicle	0.39±0.015	35.0±1.870	36.4±1.341	27.8±1.92	0.65±0.034
ERA	200	0.35±0.010	34.1±1.471	34.4±1.211	29.0±2.19	0.66±0.029
ARA	200	0.34±0.025	34.1±1.940	34.5±1.870	28.3±2.06	0.64±0.027
Metformin	100	0.30±0.022	33±0.816	34.5±1.290	26.75±2.22	0.62±0.020

No significant changes were found when compared with NC and DC vs. treated groups by two-way ANOVA and Bonferroni post-tests. Values are Mean ± SD.

Table 9: Weights of isolated liver and pancreas of different treatments groups

Groups	Dose (mg/kg)	Weight of the liver (gm) (Mean ± SD)	Weight of the pancreas (gm) (Mean ± SD)
NC	Vehicle	8.87±0.639	0.647±0.050
DC	Vehicle	8.96±0.411	0.734±0.042
ERA	200	8.62±0.483	0.715±0.024
ARA	200	9.15±0.399	0.718±0.024
Metformin	100	9.29±0.308	0.720±0.034

The comparisons of NC and DC vs. treated groups were performed by one-way ANOVA and Bonferroni post-tests. ***/ ### $p< 0.001$, **/ ## $p< 0.01$ and */ # $p< 0.05$. * (compared with NC), and # (compared with DC). Values are Mean ± SD.

DISCUSSION

Flavonoid contents in both the extracts were found prominent; subsequently their total phenolic and flavonoid contents were quantified. *In-silico* toxicity studies of the major chemical constituents present in *Rosa alba* L. were predicted by "LAZAR" property explorer. LAZAR (lazy structure-activity relationships) is a modular skeleton for the prediction of toxicological parameters. Similar to the reading process in toxicological risk assessment studies, the LAZAR builds a local QSAR (quantitative structure-activity relationship) model for each of the predicted compounds. Those who develop the model can prefer many algorithms for descriptor calculation and selection, chemical similarity indices, and model building [33]. The regression analysis of similar compounds with similar pharmacological effects predicted and the data compiled by those similar molecules is called a training data set. When the insufficient training data set is generated due to the fewer number's availability of similar compounds, the parameters of the compound under examination are not predicted. As in the present research, the carcinogenicity of the lonone, ocimene, eudesmol, and rutin were not predicted due to insufficient numbers of similar substances and neighbors for the regression model, using the weighted average of similar substances (Table 3). Type-2 diabetes mellitus (T2DM) is recognized to be an indication of chronic low-grade inflammation. Pro-inflammatory cytokines increased in persons with T2DM contribute to pancreatic beta cell apoptosis and insulin resistance, in this way exerting a significant role in the initiation and augmentation of T2DM [34]. The T2DM model was optimized by feeding HFD to produce insulin resistance followed by the low dose of STZ injection to cause mild β-cell dysfunction. In the antidiabetic screening, the body weight of HFD-fed animals was significantly increased, compared to the animals of the normal control group after 4 weeks, and revealed characteristics of obesity. Body weights of extracts (ERA and ARA) and standard drug-treated animals were significantly increased with time when compared with normal control and diabetic control groups. Body weights of the diabetic control animals were also found to be significantly decreased gradually when compared with normal control groups during the study after STZ injection (Table 4). This happened may be due to the decreased glucose metabolism and increased fat metabolism. [35] The FBG level of all the STZ injected animals was dramatically increased about threefold, compared to the control group. The FBG level of the extracts and standard drug-treated animals began to decline during the second week of the study and were almost within the normal range by the end of the fourth week. Looking at the FBG results of the fourth week of the study, it can be said that the ethanolic extract of *R. alba* is more effective than the aqueous extract in normalizing blood glucose levels. Animals of the diabetic control group have remained at high FBG levels up to the end of study (Table 5). That is possibly due to the combined effect of STZ-induced severe damage to the pancreas and the development of insulin resistance by high-fat diet feeding. At the end of the study, overnight fasted animals from each group were subjected to an OGTT. The blood glucose levels were found to increase in diabetic control, extract-treated groups, and normal control, metformin-treated groups at 60 and 90 min respectively. After that, it was found in decreasing pattern up to120 minutes of the study. At the 120th minute of the study, the blood glucose levels of the extract and standard drug-treated animals were almost equal to the glucose levels at the beginning of the study. The blood glucose level of the extracts treated animals at the end of the test was significantly different (P<0.001) from the blood glucose level of the diabetic control group (Table 6). From these observations, it can be said that both the extracts of *R. alba* were found to be as effective as the standard drug in this test. The effects of *R. alba* extracts on the lipid profile parameters in all the treated groups were studied at the end of the study. The concentrations of TC, TG, and LDL-C were found to be significantly high and less the HDL-C in the untreated DC group when compared to the NC group. This is suggested that rats of the DC group were more susceptible to developing hyperlipidemia after STZ injection than the animals of the NC group. While the concentration of TC, TG, and LDL-C were significantly reduced in induced diabetic rats treated with *R. alba* extracts and standard drug compared with the untreated DC group. Due to continuous intake of HFD for 8 weeks, the lipid profile of rats of all the groups deviated from the lipid profile of the NC group. Although this deviation was found to be lower in the extract-treated groups as compared to the untreated DC group. It is reflected that the extracts of *R. alba* are effective in controlling lipid profile deviation along with diabetes. The ERA was found to be more effective in controlling the lipid profile than the ARA (Table 7). Levels of total bilirubin, SGOT, and SGPT were found slightly elevated in the untreated diabetic control group in comparison to the normal control, extracts and metformin-treated groups. Levels of renal functions like serum urea and creatinine in all the treated and DC groups were found almost similar to the NC groups. Thus, there were no significant changes found in the liver and kidney functions of all the treated rats in comparison to the normal control group (Table 8). At the end of the study liver and pancreas of rats were isolated and weight after the dissection of animals. The weights and physical characteristics of each animal's organs were confirmed to be normal (Table 9). The hepatic architecture with distinct normal strands of hepatocytes, nuclei, sinusoidal space with fenestrated endothelial cells, and central vein (CV) were found in the histology of liver tissues of the NC group. The hepatocytes with vacuolar degeneration were observed in the hepatic tissues of the DC group. While in the liver tissues of *R. alba* extracts-treated rats and metformin-treated rats were found normal hepatocytes with normal architecture (Figure 3). The slides of pancreatic tissues of normal control groups showed normal pancreatic architecture. Islets of Langerhans were embedded in the exocrine portion of the pancreas and formed by closely packed pyramidal-shaped acinar cells. Cells were uniformly distributed inside the islets of Langerhans with centrally located round to oval nuclei inside their cytoplasm. In the pancreatic tissues of the DC group, the islets of Langerhans were observed shrunken with partial to complete degeneration and necrosis of cells. Moreover, the nucleus of the cells of Langerhans appeared densely basophilic and karyolysis. In the pancreatic tissues of ERA-treated animals the cells of islets of Langerhans appeared as partly regenerated with closely packed pyramidal-shaped acinar cells of the exocrine portion of the gland. The Islets of the ARA-treated animals contained less number of cells at the center and were densely placed at the periphery with centrally located round to oval nuclei inside their cytoplasm. The exocrine portion of the pancreas was found normal in architecture. Overall histologic results of the pancreatic tissues of all the induced diabetic

rats showed the atrophic changes in acinar cells were less severe than the endocrine portion (Figure 4).

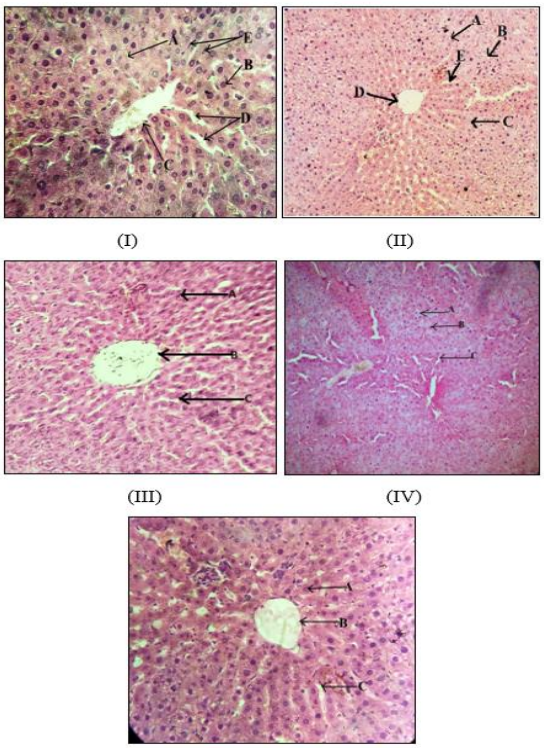

Figure 3: Liver histology of normal control group (I): A-Hepatocyte, B-Nucleus, C- Central vein, D- Liver sinusoid endothelial cells, E- Kuffer cell; Diabetic control group (II): A-Vacuolation of hepatocyte, B- Vacuolation of hepatocyte, C- Disappearance of nuclei, D-Central vein, E- Liver sinusoid endothelial cells; ERA-treated group (III): A-Hepatocyte, B- Central vein, C-Liver sinusoid endothelial cells; ARA-treated group (IV): A-Hepatocyte, B-Nucleus, C- Liver sinusoid endothelial cells; Metformin- treated group (V): A-Hepatocyte, B- Central vein, C- Liver sinusoid endothelial cells.

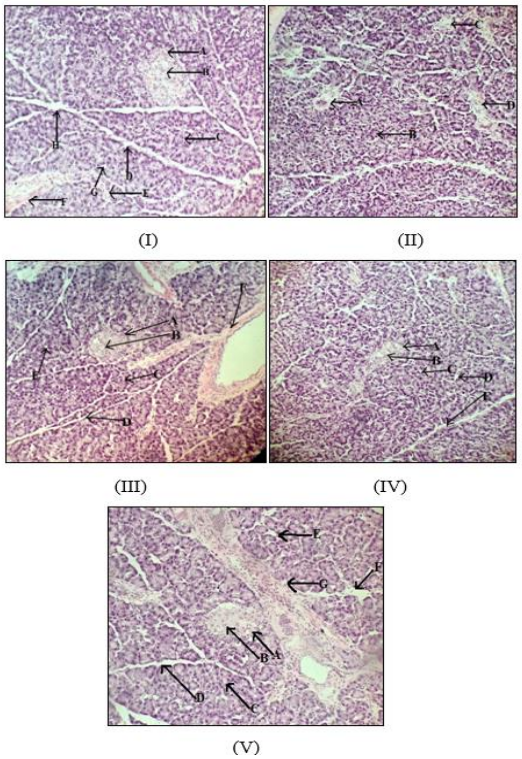

Figure 4: Normal control group (I): A-Normal sized islet of Langerhans, B-β-cells, C-Acinar cells, D-Pancreatic duct, E-Interlobular duct, F-Blood vessel, G-Pancreatic serous acini, H-Interlobular connective tissue; Diabetic control group (II): A-Shrinkage of islet of Langerhans, B- Karyolitic nucleus, C-Vacuolization, D-Degeneration and necrosis of cells; ERA-treated group (III): A- Islet of Langerhans, B-β-cells, C-Acinar cells, D-Pancreatic duct, E-Interlobular duct, F-Blood vessel; ARA-treated group (IV): A- Islet of Langerhans, B-β-cells. , C-Acinar cells, D- Interlobular duct, E-Pancreatic duct; Metformin- treated group (V): A-Normal sized islet of Langerhans, B-β-cells, C-Acinar cells, D-Pancreatic duct, E-Pancreatic serous acini, F-Interlobular duct, G- Blood vessel.

CONCLUSION

It is concluded that the toxicological profiling and antidiabetic effects of *R. alba* flowers are reported for the first time in the present research. Results of the *In-silico* toxicity study exposed that the plant is safe for its preclinical/clinical studies and formulation development as its major metabolites are predicted to be nontoxic, non-mutagenic, and non-carcinogenic. Both extracts also revealed significant antidiabetic effects in the rat model of HFD fed with a low dose of STZ injection. This model is cheap, easy to develop, and probably induced type-2 diabetes effectively in rats similar to humans with natural history and metabolic characteristics. Based on the findings of antidiabetic screening, the plant extract verified its traditional claim of antidiabetic potential. Thus, the plant extract can be used as an herbal antidiabetic agent to cure/control diabetes.

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Conflict of interest

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Abbreviations

ANOVA Analysis of variance

ARA	Aqueous extract of *Rosa alba*
BGL	Blood glucose level
DC	Diabetic control
DM	Diabetes mellitus
ERA	Ethanolic extract of *Rosa alba*
FBG	Fasting blood glucose
GAE	Gallic acid equivalent
H&E	Hematoxylin and Eosin
HDL-C	High-density lipoprotein cholesterol
HFD	High fat diet
LAZAR	Lazy structure-activity relationships

LDL-C	Low-density lipoprotein cholesterol
NC	Normal control
NPD	Normal pellet diet
OGTT	Oral glucose tolerance test
QSAR	Quantitative structure activity relationship
RE	Rutin equivalent
SGOT	Serum glutamate oxaloacetate transaminase
SGPT	Serum glutamate pyruvate transaminase
STZ	Streptozotocin
T1DM	Type-1 diabetes mellitus
T2DM	Type-2 diabetes mellitus
TC	Total cholesterol
TG	Total triglyceride

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