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RESEARCH ARTICLE

Evaluation of Antioxidant, Antibacterial and Antidiabetic Activity of *Juglans regia* Root Extract: *In Vitro* and *In Vivo* Studies

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Abstract

Background: Diabetes mellitus is a long-term metabolic illness that occurs due to a malfunction in the production or action of insulin. Prolonged hyperglycemia may lead to dysfunction and failure of several organs, such as the diabetic nephropathy, retinopathy and neuropathy. The incidence of diabetes and the number of patients has consistently risen over the last several decades. *Juglans regia*, a member of the Juglandaceae family, has been used by practitioners of folklore to treat number of illnesses. **Aim:** To Evaluate the antioxidant and anti-bacterial effects of a root extract from *Juglans regia* in *in vitro* studies and anti-diabetic effect Streptozotocin (STZ) induced Wistar albino rats. **Methods:** Qualitative phytochemical analysis, antioxidant assay were done using DPPH, antibacterial assay have been performed by using agar well diffusion method and anti-diabetic effect tested by inducing rats with STZ followed by administering methanolic root extract of *Juglans regia* alone and with adjuvants metformin and glipalamide. **Result:** The root extract of *Juglans regia* can decrease free radicals, possess antibacterial property in agar well diffusion method and in in-vivo studies group 2 (diabetic control) showed elevated FBS and HbA1C from day 3 to day 28 while group 3, 4, 5, 6, 7 and 8 showed significant reduction in FBS and HbA1C levels at the end of study. Safety profiles such as CBC, RFT and LFT did not show any significant difference between the groups from baseline to end of the study. Thus, methanolic root extract of *Juglans regia* was safe and efficacious against STZ induced rats. **Conclusion:** *Juglans regia* can reduce hyperglycemia in STZ induced diabetic rats alone and can be used as an adjuvant to Metformin and Glibenclamide. Also, it possesses anti-oxidant and anti-bacterial activity in *in vitro* studies.

Keywords: Anti-bacterial, Antioxidant, *Juglans regia,* Hyperglycemia, Streptozotocin

1. Introduction

Diabetes Mellitus (DM) is a long-term metabolic illness characterized by a malfunction in the production of insulin, the action of insulin, or both. Persistent elevation of blood glucose levels, known as chronic hyperglycemia, may lead to impaired function and eventual failure of vital organs including the kidneys, eyes, heart, nerves, and blood vessels[1]. DM is one of the most formidable health issues in the globe. There are about 537 million individuals worldwide who have diabetes, which impacts people of all genders

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and age groups, including men, women, and children. Diabetes and its consequences cause 1.5 million fatalities annually, mostly among individuals from low-income and developing nations. Presently, there are around 77 million individuals in India who are afflicted with diabetes. The incidence of diabetes and the number of patients has consistently risen over the last several decades[2]. Insufficient levels of insulin result in a persistent state of high blood sugar, which disrupts the body's ability to properly process carbs, fats, and proteins. As the condition advances, it causes harm to the tissues and blood vessels, resulting in significant micro and macrovascular problems. Type 1 DM, also known as insulin dependent diabetic mellitus or juvenile diabetes, is caused by a complete lack of insulin owing to the autoimmune death of pancreatic beta cells[3]. Type 2 DM, often known as non-insulin dependent diabetic mellitus. T2DM arises due to either reduced insulin production or heightened resistance to insulin[4].

Juglans regia is classified in the kingdom *Plantae*, division *Tracheophytes*, subdivision *Angiosperms*, order *Fagales*, family *Juglandaceae*, and genus *Juglans*. The tree is usually referred to as a walnut tree. *Juglans regia* is a commonly cultivated plant due to the widespread use of its nuts, known as walnuts. They are mostly found in temperate locations and are grown throughout Asia, western South America, the United States, and central and southern Europe[5].The leaves of *Juglans regia* have been widely used in traditional medicine around the globe for its antibacterial, antidiabetic, antihelmintic, astringent, keratolytic, antidiarrheal properties, as well as for treating sinusitis, colds, and stomach aches. The kernels of *Juglans regia* are essential for human nutrition because to their high protein and oil content. As a result, it is categorized as a strategic species for human nutrition and is listed as a priority plant by the FAO[6]. The roots of *Juglans regia* have been investigated for their anti-candidal activity, anti-proliferative effects, antioxidant properties, antimicrobial properties, and anti-inflammatory benefits[7-9].

An analysis of a methanol extract of *Juglans regia* revealed the existence of alkaloids, flavonoids, and saponin via phytochemical research. This research aims to assess the antioxidant, antibacterial and antidiabetic properties of the methanolic extract derived from the roots of *Juglans regia*.

2. Materials and Methodology

2.1 Collection and Preparation of Plant Material

The roots of *J. regia* were obtained at CHRI, Kelambakkam, Tamil Nadu - 603103. The roots were meticulously rinsed under flowing water, air-dried in a shaded area for a duration of 5 days, then finely pulverized using an electric mixer. The pulverized botanical substance was then treated to extraction by immersing it in 1 liter of methanol for a duration of 72 hours, with intermittent agitation. The liquid portion was passed through a filter made of Whatman filter paper. The resulting filtrate was evaporated using a water bath. Ultimately, a viscous dark concentrate was obtained. The achieved yield was 2.5%. The extract was thereafter placed in an airtight container and kept in a refrigerator at a temperature range of 2-8° C for future use.

2.2 Material Characterization[10]

The Fourier Transformation Infrared (FTIR) spectroscopy was conducted using a PerkinElmer 400 FT-IR/FIR spectrometer from the United States. The spectroscopy covered a range of 400 cm^{-1} to 4000 cm^{-1}. The spectral resolution was maintained at 0.4 cm^{-1}.

2.3 Antioxidant Assay

2.3.1 DPPH Free Radical Scavenging Assay

The DPPH free radical scavenging capability of *J. regia* root extract was assessed using a conventional approach with minor adjustments[11]. In summary, 100 microliters of *J. regia* root extract was diluted in a serial manner on a 96 well plate, with concentrations ranging from 6.75 to 100 mg/ml. Subsequently, a solution of DPPH with a concentration of 0.2 M was added to each well, with a volume of 100 ml. Subsequently, the constructed well plate was placed in a dark environment and incubated for a duration of 30 minutes. The absorbance (Abs) was measured using a spectrophotometer at a wavelength of 517 nm. The experiment was conducted in triplicate using a *J. regia* root extract. The DPPH and ascorbic acid were used as the negative and positive control, respectively. Methanol was used as a blank for the experiment. The calculation of radical scavenging activity was performed using the following method:

$$Free\ Radical\ Scavening\ Activity(\%) = \left(\frac{Control(Abs) - Test\ Sample(Abs)}{Control(Abs)}\right) * 100$$

2.4 Qualitative Phytochemical Analysis

The extract underwent testing to determine the presence of bioactive chemicals using established standard procedures[12].

2.5 Antibacterial Activity

2.5.1 Bacterial Strains

The gram-negative bacteria *Escherichia coli* (*E. coli* ATCC 25922), *Salmonella enterica subsp*, *Enterica serovar Typhi* (*S. typhi* ATCC 6539), *Shigella sonnei* (*S. sonnei* ATCC 25931), *Pseudomonas aeruginosa* (*P. aeruginosa* ATCC 27853) were obtained through an acquisition from Hi Media Laboratories Pvt. Ltd. in Mumbai, India. The different strains of bacteria were cultivated in nutrient broth (Hi Media, Mumbai, India) at a temperature of 37 °C for a duration of 24 hours with continuous agitating at 200 rpm.

2.6 Agar-Well Diffusion Method

The Agar-well diffusion technique was used to test the antibacterial activity of *J. regia* root extract that were generated by biosynthesis with some modifications. Using sterile cotton swabs, the bacterial strains were distributed on Mueller-Hinton Agar (MHA) (Merck, Germany). The agar was punched by using a sterile pipette tip. Each well was loaded with 100 mg/mL of 10 µl *J. regia* root extract, 100 mg/mL of10 µl ampicillin (positive control), and 10 µl of ethanol (negative control) individually. The whole setup was incubation at a temperature of 37°C for a duration of 24 hours. The presence of a zone of inhibition was found after 24 hours of incubation.

2.7 *In-vivo* Studies

2.7.1 Animal Model

The research was conducted after obtaining animal ethical clearance from the institutional animal ethical committee IAEC Ref No-(IAEC3/Proposal:94/A.Lr:67/ Dt:22.08.2022) of Chettinad academy of research and education, Kelambakkam, Tamil Nadu 6030103. Forty male Wistar albino rats were obtained from TANUVAS, Chennai. The newly received animals were quarantined for a period of one week for development of physiological, psychological and nutritional stabilization. All the animals were housed in individual clean polypropylene cages. Paddy husk was used for bedding. They were maintained at a temperature of 23-25 °C, humidity 50-60% in alternate light and dark cycle. The animals were fed with synthetic pellet feed and clean water adlibitum.

2.7.2 Induction of Hyperglycemia

The 40 animals have been divided into 8 groups, with each group consisting of animals. The induction of hyperglycemia based upon on different groups had been mentioned in the Table 1.

Table 1. Induction of hyperglycemia based upon each group

Groups	No of rats	Baseline (day 0)	Day 3 to day 28
I (control)	5	No Treatment	No treatment
II (Disease control)	5	STZ (55 mg/ kg) single IP dose	Distilled water 0.5 ml orally once daily
III	5	STZ (55 mg/ kg) single IP dose	Methanolic root extract of *J. regia* (200mg/kg) once daily orally
IV	5	STZ (55 mg/ kg) single IP dose	Methanolic root extract of *J. regia* (200mg/kg) with Metformin(50 mg/kg) once daily orally
V	5	STZ (55 mg/ kg) single IP dose	Methanolic root extract of *J. regia* (200mg/kg) with Glibenclamide (10 mg/kg) once daily orally
VI	5	STZ (55 mg/ kg) single IP dose	Methanolic root extract of *J. regia* (400mg/kg) once daily orally
VII	5	STZ (55 mg/ kg) single IP dose	Methanolic root extract of *J. regia* (400mg/kg) with Metformin(50 mg/kg) once daily orally
VIII	5	STZ (55 mg/ kg) single IP dose	Methanolic root extract of *J. regia* (400mg/kg) with Glibenclamide (10 mg/kg) once daily orally

2.7.3 Experimental Model

The animals in Group 1 were dismembered as a control. Diabetes was induced in groups 2-8 of rats by administering a single intraperitoneal injection of Streptozotocin (STZ) at a dosage of 55 mg/kg on day 0. 72 hours after causing diabetes, blood was extracted from the rats' tail vein and blood glucose levels were measured using a glucometer. Rats having a glucose concentration over 200 mg/dl were classified as diabetic and were used in the investigation. Bodyweight and fasting blood glucose levels were measured on day 3, 11, 19, and day 28. On day 0, before inducing diabetes, a complete blood count, HbA1c test, kidney function test, liver function test, and fasting lipid profile were conducted. The same tests were repeated on day 28. Neither Group 1 nor Group 2 were given any kind of therapy. Group 3 was administered a dosage of 200mg/kg of methanolic root extract of *J. regia*. Group 4 received the same dosage of methanolic root extract along with 50 mg/kg of metformin. Group 5 received the same dosage of methanolic root extract along with 10mg/kg of glibenclamide. Group 6 received a higher dosage of 400 mg/kg of methanolic root extract. Group 7 and group 8 were given the same higher dosage of methanolic root extract along with 50 mg/kg of metformin and 10 mg/kg of glibenclamide, respectively. Following a 28-day treatment period, rats were subjected to an overnight fast and then euthanized under halothane anesthesia. The important organs were then collected and submitted for histological investigation.

2.8 Statistical Analysis

The data was statistically evaluated using GraphPad prism. The experiment was conducted three times for each test, and the results were calculated using the formula for the average value plus or minus the standard deviation. The data was analyzed using One Way ANOVA, and Bonferroni's multiple comparison test ($p < 0.05$) was used to detect any significant differences across the samples.

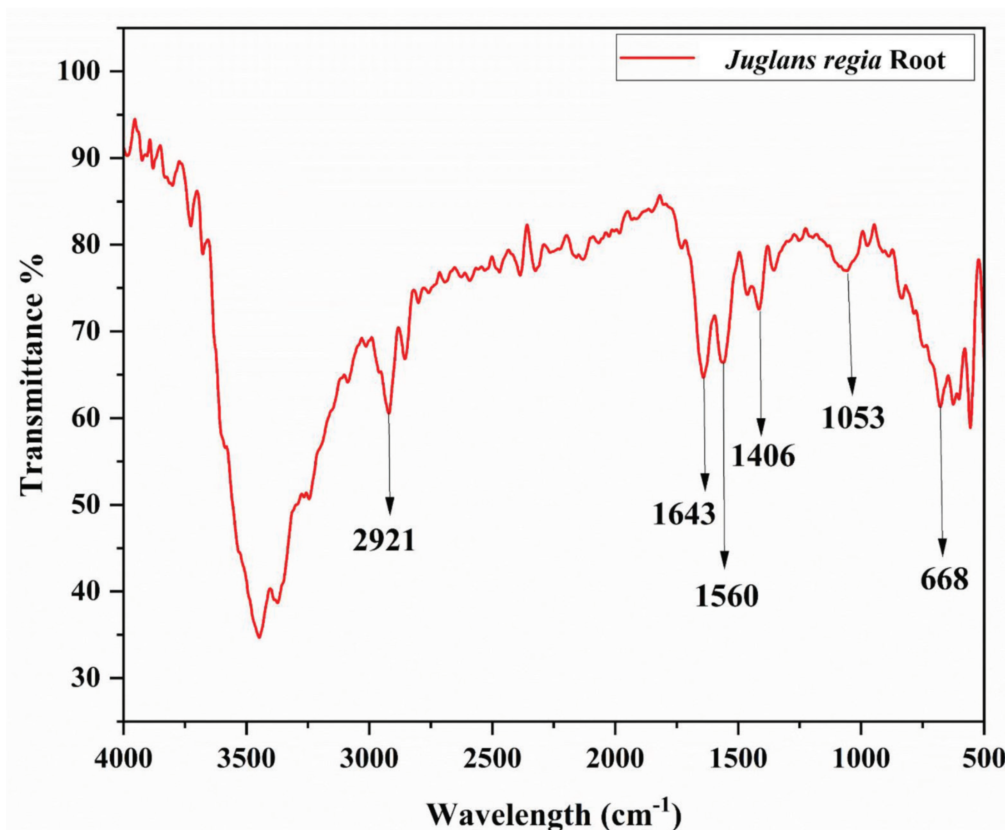

Figure 1. FTIR spectrum of *Juglans regia* root extract.

3. Result

3.1 Characterization

3.1.1 FTIR

The Fourier Transform Infrared (FTIR) approach was used to get information about the emission spectrum by analyzing the vibrational mode characteristics of the plant extract. The FTIR spectrum analysis reveals distinct peak locations for the samples, as seen in Figure 1. The band at 2921 cm^{-1} indicates the presence of O-H stretching presence of alcohol group, band at 1643 cm^{-1} indicates the presence of C=C stretching presence of conjugated alkene, the band at 1560 cm^{-1} indicates the presence of N-O stretching presence of nitro compound, the band at 1406 cm^{-1} indicates the presence of O-H bending presence of carboxylic acid, the band at 1053 cm^{-1} indicates the presence of C-O stretching presence of primary alcohol group and the band at 668 cm^{-1} indicates the presence of C=C bending presence of alkene group[13].

3.2 Antioxidant Activity

3.2.1 DPPH Assay

The DPPH free radical inhibition activity of several substances at different doses is shown in Figure 2. The inhibitory effect observed with these compounds was much greater than that of the other compounds and, to a certain degree, was equivalent to the standard, namely ascorbic acid. Figure 2 depicts the influence of different concentrations of plant extract on the antioxidant activity of DPPH radicals. The results of our investigation indicated that the plant extract exhibited comparable antioxidant activity to ascorbic acid. The antioxidant activities in the DPPH test rely on the plant extract's capacity to transfer electrons to the unpaired electron in the DPPH solution[14]. As a result, the strength of the n → π* transition at 517 nm decreases, creating a change in color from deep purple to light yellow[15]. The observed change in color indicates the existence of antioxidant action.

3.3 Qualitative Phytochemical Analysis

The phytochemical properties of the methanolic extract of *J. regia* roots were summarized in Table 2. The

Table 2. Phytochemical Screening from methanolic extract of *J. regia* roots

Name of the Test	Methanolic Extract
Fehling	+
Iodine	+
Phenol and Tannin	+
Shinoda	+
Alkaline	+
Saponin	+
Liberman	+
Salkowski	+
Keller-Kilani	+
Steroid	+
Terpenoid	+
Alkaloid	+

Where '+' represent the presence and '-' represent the absence.

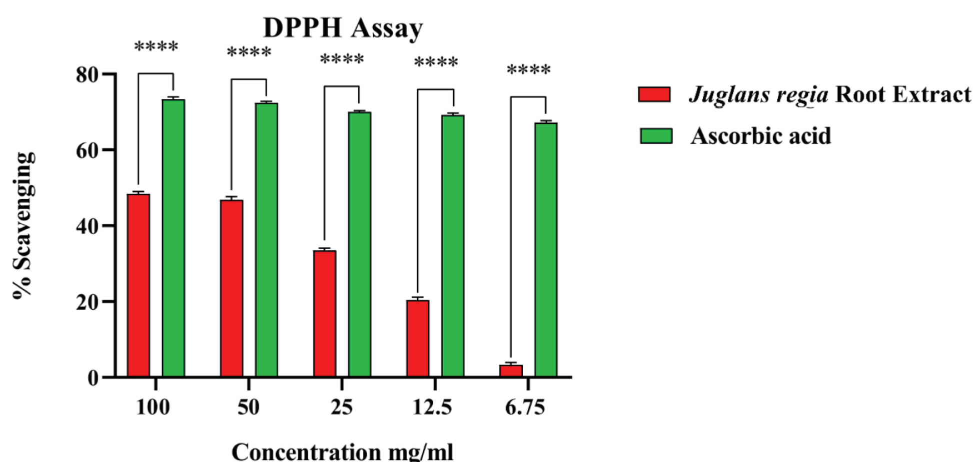

Figure 2. DPPH free radical inhibition at various concentration. Values are presented as mean ± SD (n=3). ****p<0.0001.

analysis showed the existence of pharmacologically active substances in the methanolic extract of *J. regia* roots. The table reveals the presence of carbohydrates, phenols and tannins, flavonoids, and saponins methanolic extract of *J. regia* roots.

3.4 Antibacterial Activity

Methanolic extract of *J. regia* roots have been examined using the agar well diffusion technique assay versus specific pathogens (Figure 3 I - II). In comparison to the usual medication, methanolic extract of *J. regia* roots

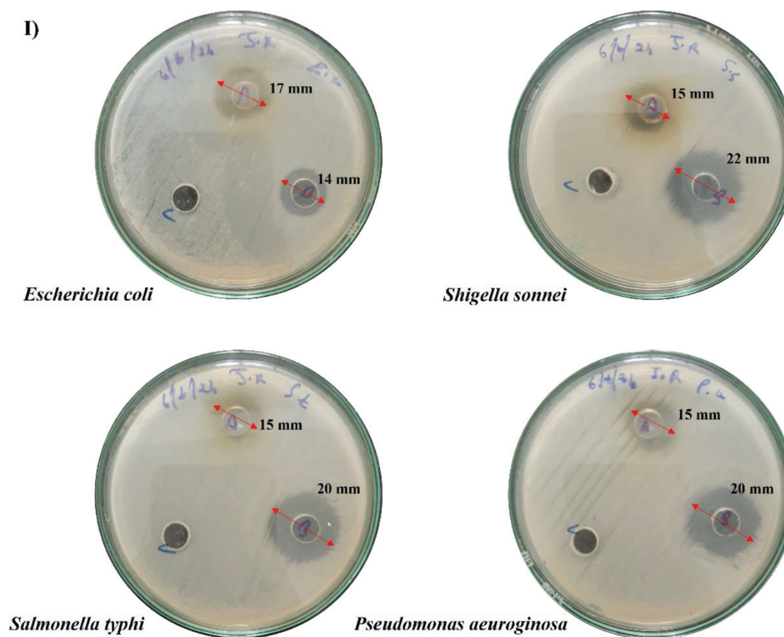

Figure 3 I. Antibacterial activity of methanolic extract of *J. regia* roots against pathogenic bacteria, **(A).** Methanolic extract of *J. regia* roots, **(B).** Ampicillin (+ve control) and **(C).** ethanol (Negative control).

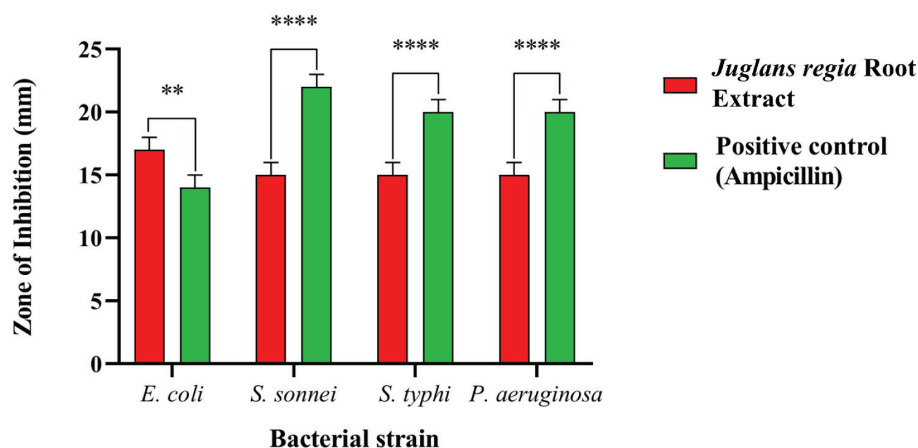

Figure 3 II. Zone of inhibition of methanolic extract of *J. regia* roots against pathogenic bacteria. Every value signifies the average ± standard deviation of three duplicates. Values are presented as mean ± SD (n=4). ****p<0.0001, **p<0.01 and ns p value is no significant value (negative control).

demonstrated a similar zone of inhibition versus the pathogenic bacteria tested. Based on the results, our plant extract demonstrated superior performance compared to conventional antibacterial agents in terms of its sensitivity to both gram-positive and gram-negative bacteria. Both gram-negative and gram-positive bacteria possess a negative charge, which results in an electromagnetic attraction with the Methanolic extract of *J. regia* roots due to the presence of the lipopolysaccharide layer. The methanolic extract of *J. regia* roots deactivates biological enzymes and DNA by interacting with electron-donating compounds such as thiols, amides, polysaccharides, indoles, and hydroxyls. Pitting in bacterial cell walls leads to increased permeability and consequent cell death.

3.5 *In Vivo* Studies

3.5.1 Effect on Body Weight

Body weight was assessed at the beginning of the study, as well as on day 3, 11, 19, and 28. The animals' end body weight exhibited a substantial augmentation compared to their beginning body weight in groups I, V, VI, VII, and VIII. There was a substantial reduction in body weight compared to the beginning body weight in group II (P<0.021) (Table 3).

The control group saw a notable rise in body weight from the first measurement to day 3, 11, 19, and 28. In contrast, the disease control group exhibited a considerable decline in body weight from the initial measurement to day 28. Group 8 exhibited a substantial increase in weight from the beginning to the conclusion of the trial, but groups 3 to 7 did not demonstrate any weight gain over the same period. On the 28th day, animals in group 8 exhibited a higher rate of weight increase compared to groups 2-7, but a lower rate compared to group 1.

Body weight was assessed at the beginning of the study, as well as on days 3, 11, 19, and 28.

The body weight of group 1 considerably increased from the beginning to the conclusion of the trial (P<0.021). Starting from day 3 to the completion of the trial, the body weight of group 2 decreased, and this decrease was determined to be statistically significant. The p-value is less than 0.035. There was no significant improvement in body weight from group 3 to 7, however group 8 exhibited a substantial increase in weight from the beginning to the conclusion of the trial (P<0.025). The baseline body weights were comparable across all groups (P=0.99). On the third day, there was a drop in bodyweight among those in groups 2 to 8. However, this reduction was not regarded to be significant. The probability is 0.97. By day 11, there was a substantial decrease in body weight from the first measurement (P<0.0001). Significant increases in bodyweight were seen on day 19 and 28 (P<0.0001).

3.5.2 Effect on FBS

At baseline, FBS was similar in all the groups. After induction of diabetes with STZ in groups 2 to 8, the FBS showed a striking increase in day 3, whereas the normal healthy rats in group 1 remained constant from baseline to day 28. The treatment groups (Groups 3 to 8) showed a reduction in FBS from day 3 to day 28, whereas the diabetes control group (Group 2) showed a significant increase in FBS until day 28.

When the difference in FBS from baseline to day 28 was compared among the groups using One-way

Table 3. Effects of methanolic extract of *J. regia* roots on body weight in STZ-induced rats

Body weight	baseline	Day 3	Day 11	Day 19	Day 28	P value	
						Within group	Between group
Group 1	199.60±10.89	202.20±10.42	229.60±11.99	255.20±12.61	291.60±11.23	0.021*	<0.001*
Group 2	202.40±6.58	200.60±7.06	183.60±4.61	169.40±5.17	153.80±5.35	0.035*	
Group 3	199±18.26	197±18.37	183.80±15.51	179.20±9.14	186.60±8.41	0.24	
Group 4	200.60±17.85	199±16.38	181.60±17.12	190.80±16.76	197.20±18.32	0.186	
Group 5	198.40±9.94	196.80±9.62	191±8.21	197.20±8.46	204.80±7.69	0.43	
Group 6	196.20±9.65	194.20±9.54987	186.80±9.28	196±9.11	203±7.90	0.96	
Group 7	201.80±6.38	199.40±5.89	193.60±4.61	199.60±6.02	209.60±7.63	0.24	
Group 8	198.20±13.53	195.60±13.95	188.40±14.01	197.60±16.60	208±14.98	0.025*	

ANOVA with post hoc comparisons, rats treated with *J. regia* extract high dose, 400 mg/kg + Glibenclamide exhibited significant reduction in FBS, when compared to diabetic control and other treatment groups. Though rats belonging to groups 3 to 7 did not have similar reduction in FBS as group 8, the FBS values are significantly reduced when compared to diabetic control (group 2).

This FBS comparative analysis shows that *J. regia* alone has a dose dependent effect on reduction in the FBS. In addition, it enhances the efficacy of both Metformin and Glibenclamide in reducing the FBS levels in a dose dependent manner. (Table 4 and Figure 4).

3.5.4 Effect on HbA1c

There is no change in HbA1c levels from baseline to day 28 in group1 (normal control), whereas the diabetic control rats showed significantly elevated HbA1c on day 28 compared to baseline. Among the treatment groups, except group 8 rats treated with high dose extract (400 mg/kg) + Glibenclamide which did not show an increase in HbA1c on day 28, all the other groups (groups 3 – 7) had elevated HbA1c on day 28, though the values are not as high as diabetic control rats (Table 5).

Intergroup comparison of HbA1c levels showed that group 8- high dose extract (400 mg/kg) + Glibenclamide performed better compared to other treatment groups in terms of significant reduction in HbA1c on day 28 compared to baseline. When the extract was used alone, high dose extract showed higher reduction in HbA1c, compared to low dose extract (200 mg/kg). Similarly, low and high doses of the extract added to the standard drugs Metformin and Glibenclamide exhibited dose dependent reduction in HbA1c levels, as evident from Table 5 and Figure 5.

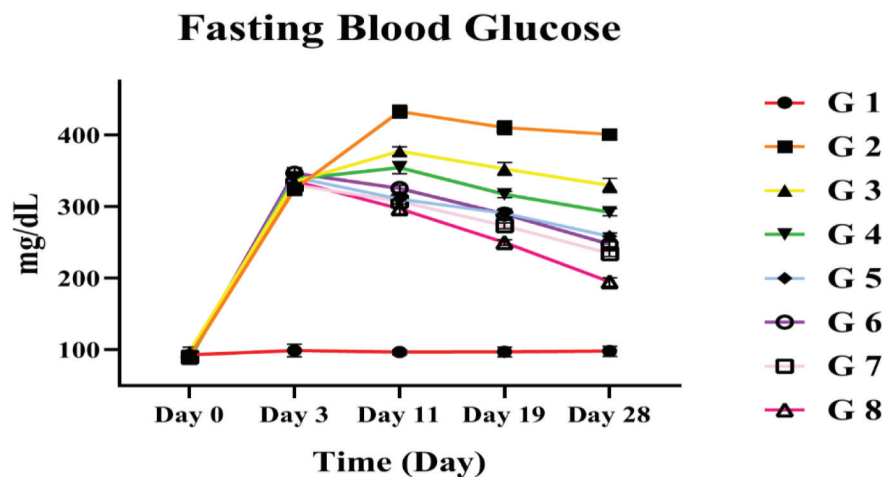

Figure 4. Effect of FBS level in methanolic extract of *J. regia* roots extract on STZ induced rats.

Table 4. Effect of FBS levels in methanolic extract of *J. regia* roots extract on STZ induced rats

	Baseline	Day 3	Day 11	Day 19	Day 28	P Value	
						Within group (Repeated measures ANOVA)	Between groups (One way ANOVA)
Group 1	92.80±7.59	98.80±9.12	96.60±3.50	96.80±6.94	98.00±7.17	0.914	<0.0001*
Group 2	90.60±4.03	323.80±8.37	432.20±8.87	410±9.46	400.40±6.94	0.024*	
Group 3	96.40±7.05	334.40±8.87	377.20±6.30	352.40±9.07	329.40±9.81	0.003*	
Group 4	88.20±3.96	338.20±10.10	354.20±8.34	316.80±4.08	291.80±4.96	0.034*	
Group 5	88±4.30	340.60±6.98	310.20±10.20	290.40±4.87	258.20±4.91	0.017*	
Group 6	89.80±3.34	346.40±7.63	325±4.69	289.20±7.49	247±3.53	0.032*	
Group 7	89.60±3.20	330±7.07	306.80±3.56	273±3.80	234.20±3.96	0.022*	
Group 8	89.20±5.63	335.20±7.01	296.80±6.83	249.60±3.84	194.60±5.89	0.003*	

When we look at the above analysis, it is evident that *J. regia* extract at higher dose (400 mg/kg), when added to *Metformin* and *Glibenclamide*, has significantly reduced the HbA1c levels like the baseline values. We could infer that 400 mg/kg of *J. regia* extract potentiates the HbA1c lowering effect of the standard anti-diabetic drugs Metformin and Glibeclamide. (Table 5 and Figure 5).

The blood parameters, including hemoglobin, hematocrit, WBC, platelet count, BUN, creatinine, Total bilirubin, AST, and ALT, were examined at the beginning and conclusion of the trial. The results were almost identical across all groups at both time points.

Furthermore, in group 1, TC, TG, and LDL levels were unchanged both at the beginning and at the conclusion of the trial (Table 6). However, in group 2, there was a considerable rise in these levels. Conversely, in groups 3 to 8, there was a notable decrease in TC, TG, and LDL levels as the dosage rose.

3.5.5 Histopathological Examinations

The histopathological examination of key organs, including the brain, kidney, and liver, showed no notable alterations. HPE of pancreas are as follows.

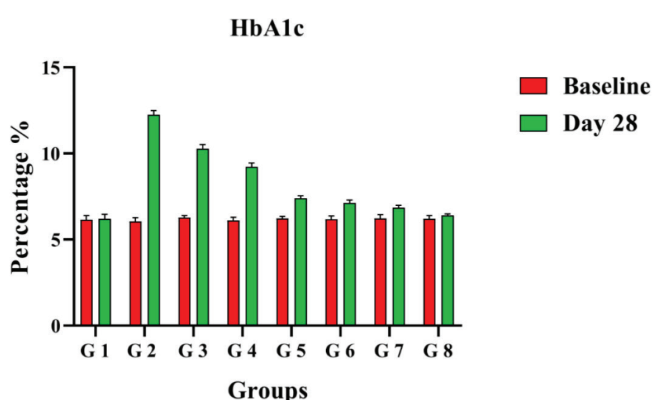

Figure 5. Effect of HbA1c level in methanolic root extract of *J. regia* roots extract on STZ induced rats.

Table 5. Effect of HbA1c level in methanolic root extract of *J. regia* roots extract on STZ induced rats.

	Baseline	Day 28	P value	
			Paired t test	One way ANOVA
Group 1	6.16±0.23	6.22±0.25	0.697	<0.001*
Group 2	6.06±0.20	12.26±0.23	0.000*	
Group 3	6.28±0.13	10.28±0.23	0.000*	
Group 4	6.12±0.19	9.24±0.21	0.000*	
Group 5	6.24±0.11	7.40±0.15	0.000*	
Group 6	6.18±0.19	7.14±0.15	0.000*	
Group 7	6.24±0.20	6.86±0.15	0.06	
Group 8	6.22±0.19	6.42±0.08	0.103	

Table 6. TG, TC and LDL level in methanolic extract of *J. regia* roots extract on STZ induced rats

	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6	Group 7	Group 8	P value
TG	77.80±3.76	94.60±3.64	78.80±1.92	71.20±1.48	63.20±1.92	67.40±1.51	63.80±1.48	58.60±1.14	0.001
TC	77±5.47	94.60±7.63	78.40±3.57	79.20±3.83	70.20±2.38	70.60±3.64	65±1.20	60.60±2.07	0.001
LDL	33.40±3.04	69.20±4.32	44.80±2.86	34.40±1.14	27.80±2.86	27.40±3.13	26.20±2.38	24.80±1.64	0.001

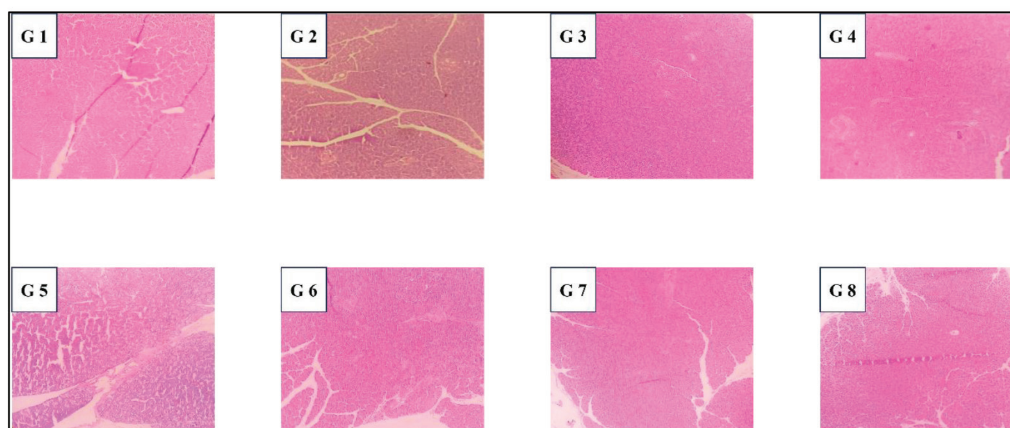

Figure 6. HPE of the pancreas treated (G 3 – G 8) with methanolic extract of *J. regia* root extract on STZ induced rat model.

- •Group 1 exhibited normal islet cells without any notable alterations.
- •Group 2 had sporadic islet cells with congestion and hyalinized blood vessels.
- •Group 3 and 4 exhibit few islet islands.
- •Group 5, 6, and 7 show the presence of typical islet cells characterized by big, pale, and ovoid beta cells.

Group 8 shows islet cell hyperplasia with big pale islet cells. (Figure 6).

4. Discussion

For our investigation, we split 40 male Wistar albino rats weighing from 190 grams to 210 grams was divided into 8 groups, each group containing 5 animals. Diabetes was induced in rats allotted from group 2 to group 8 (whereas group 1 was kept as control group), by providing 55 mg/kg body weight of streptozotocin injected intraperitoneally. Rats having fasting blood sugar levels greater than 200 mg/dl were then added to the trial. The study was carried out for 28 days. The parameters were carried out before starting the study and at the end of the study were HbA1c, CBC, serum BUN, creatinine, ALT, AST, Total bilirubin, Total cholesterol, triglycerides and LDL and the histopathology of vital organs were done after sacrificing the animal using halothane anesthesia. The body weight and fasting blood sugar was seen on day 0, 3, 11, 19, and 28.

In this study, we evaluated the effect of methanolic root extract of *Juglans regia* on streptozotocin induced diabetes in Wistar albino rats. In this study, there was a significant increase in fasting blood sugar and HbA1c in group 2. A significant reduction in fasting blood sugar and HbA1c was observed in groups 3, 4, 5, 6, 7 and 8 compared to the disease control group. Particularly groups 6, 7 and 8 with *J. regia* root extract 400mg/kg, 400 mg/kg + metformin 50 mg/kg and 400 mg/kg + glibenclamide 10mg/kg respectively decreased the fasting blood sugar and HbA1c close to that of control group ($p<0.05$).

Group 8 rats treated with *J. regia* extract high dose, 400 mg/kg + Glibenclamide exhibited significant reduction in FBS, when compared to diabetic control and other treatment groups. Though rats belonging to groups 3 to 7 did not have similar reduction in FBS as group 8, the FBS values are significantly reduced when compared to diabetic control (group 2).

This study findings showed increase in body weight in *J. regia* root extract treated groups compared to disease control group. Group 1 as control showed increase in weight gain. Group 2 as disease control decreased in body weight at the end of 28 days. Groups 3 to 8 treated with *J. regia* showed improvement in body weight despite diabetes induction. Of which *J. regia* along with glibenclamide group showed significant weight gain close to control.

A similar study was conducted by Mohammadi *et al.*,[16], by using thirty-two Wistar rats irrespective of sex which was divided into four groups: nondiabetic rats, streptozotocin-induced diabetic rats with no treatment, streptozotocin-induced diabetic rats treated with ethanolic leaf extracts of *J. regia* (200 mg/kg), and streptozotocin -induced diabetic rats treated with ethanolic leaf extracts of *J. regia* (400 mg/kg). Fasting blood sugar and HbA1C decreased meaningfully in diabetic rats treated with *J. regia* 400 mg compared to 200 mg/kg and with no treatment group.

In comparison with Mohammadi *et al.*, the leaf extract 400mg/kg was more effective in reducing Fasting blood sugar in diabetic rats than the root extract 400 mg/kg. However, *J. regia* root extract along with adjuvant glibenclamide decreases fasting blood sugar like the leaf extract.

Similarly, Sedigheh Asgary *et al.*,[17] demonstrated the hypoglycemic effect of *J. regia* leaves ethanolic extract in twenty-four male Wistar rats. Fasting blood sugar was decreased significantly in diabetic rats treated with *J. regia* and in diabetic rats treated with glibenclamide. Glycosylated hemoglobin decreased significantly in the diabetic groups receiving either glibenclamide or *J. regia*, compared with the untreated diabetic group When comparing with our study, *J. regia* root extracts 400mg/kg alone and in combination with adjuvants Metformin and Glibenclamide demonstrated a similar reduction in HbA1C levels as the leaf extract.

Aashaq *et al.*,[18] conducted research on clinically isolated bacteria that cause urinary tract infections (UTIs) using an extract from the root of *J. regia*. The study demonstrated that extracts derived from *J. regia* had notable antibacterial properties against uropathogenic microorganisms. Emira *et al.*,[19] assess the antioxidant activity of *J. regia* leaf extracts. The extract from *J. regia* had the most effective capacity to scavenge DPPH, as shown by the lowest IC_{50} value of 3 μg/ml.

Ara *et al.*,[20] investigated the antibacterial properties of two distinct extracts from *J. regia* tree bark. The zone of inhibition measures 12-15 mm for *E. coli* and 15 mm for *B. subtilis*. Muzaffer and Paul[21] assessed the antibacterial properties of the male flowers of *Juglans regia* L., *Staphylococcus aureus* and *Escherichia coli* had the greatest sensitivity, while significant activity was also shown against both fungal strains tested, particularly against *Candida albicans*.

Elouafy *et al.*,[22] evaluated the triterpenoid saponin extracts of *Juglans regia* kernels for their antioxidant, anti-diabetic, and antimicrobial properties. The butanoic extract exhibited the strongest antioxidant activity against DPPH, with an IC_{50} of 7.74 ± 1.49 μg/mL, while the precipitated extract had the most significant scavenging activity against the ABTS free radical, with an IC_{50} of 33.14 ± 2.96 μg/ml. Moreover, butanoic and hydroalcoholic extracts of *Juglans regia* kernels exhibited α-glucosidase inhibitory activity that surpassed that of synthetic medicines like acarbose, with IC_{50} values of 11.66 μg/mL and 16.17 μg/mL, respectively, compared to 18.01 μg/mL for acarbose. The assessment of antibiotic efficacy against *Bacillus subtilis, Escherichia coli,* and *Klebsiella pneumonia* strains shown that some extracts might suppress these bacteria. Abdoli *et al*.,[23] evaluated the anti-hyperglycemic impact of the aqueous extract of *Juglans regia* L. leaf on patients with type 2 diabetes. The findings demonstrated the anti-hyperglycemic action of the aqueous extract of *Juglans regia* L. leaf on type 2 diabetic patients.

The present investigation demonstrated that the *J. regia* roots methanolic extract can decrease free radicals, have the antibacterial properties against the pathogenic bacteria and have antidiabetic effect of methanolic extract of *J. regia* root extract. On day 28, the hemoglobin, hematocrit, WBC, and platelet levels were almost identical to the baseline values. There were no significant variations in the levels of Bun, creatinine, AST, ALT, ALP, and total bilirubin between the baseline and at the end of the trial. This contributes to the safety of the extract. The histopathological examination of essential organs, including the brain, kidney, and liver, in the present research showed no significant alterations. HPE of pancreas showed group 1 exhibited normal islet cells without any notable alterations. Group 2 had sporadic islet cells with congestion and hyalinized blood vessels. Group 3 and 4 exhibit few islet islands, Group 5, 6, and 7 show the presence of typical islet cells characterized by big, pale, and ovoid beta cells. Group 8 shows islet cell hyperplasia with big pale islet cells.

Based on research conducted by Schreck K *et al*., it was shown that the methanolic extracts of Pumas boldus and *Juglans regia* were able to reduce fructose transport in Caco2 cells by around 30 to 40 %. The study elucidated that the leaves of *Juglans regia* were found to possess flavonoids and phenolic acids, including 3- and 5-caffeoylquinic acids, quercetin-3-galactoside, and quercetin-3-arabinoside. These compounds were associated with a reduction in human fasting blood glucose, HbA1c, and fasting blood lipids[24].

Juglans regia extracts can help lower blood glucose levels due to the presence of bioactive compounds like polyphenols, flavonoids, and other phytochemicals. It may also enhance insulin sensitivity by improving the function of insulin receptors or modulating pathways involved in glucose metabolism.

Considering the current study findings as well as the available evidence for anti-diabetic, antioxidant and anti-bacterial activity, *J. regia* root extract can be used to treat hyperglycemia in diabetes. However further studies are needed to determine the exact mechanism of such activity by isolating bioactive compounds and assessing their effect.

5. Conclusion

Based on the outcome of evaluation, our study found that the methanol root extract of *J. regia* have both antioxidant and antibacterial properties in *in vitro* analysis. In phytochemical screening of the *J. regia* root extract indicated the presence of phytoconstituents such as iodine, phenol, tannin, flavonoids, saponin, glycosides, alkaloids, terpenoids and steroids. *J. regia* extract possesses dose- dependent anti-diabetic activity when used alone. *J. regia* extract augments the anti-diabetic activity of the standard drugs metformin and glibenclamide in a dose-dependent manner. Hence, it could a potential treatment option as a monotherapy and adjuvant to standard drugs metformin and glibenclamide.

6. Reference

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