Physicochemical characterization and pharmacological evaluation of *Grewia multiflora* leaf extracts: In vitro and in vivo assessment of antimicrobial and antidiabetic potentials.

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ABSTRACT

Grewia multiflora Juss. leaves were evaluated for physicochemical characteristics, antimicrobial activity, and antidiabetic potential using in vitro assays and an HFD-STZ-induced diabetic rat model. Leaf extracts prepared with water, n-hexane, methanol, and ethyl acetate were characterized for physicochemical properties, antibacterial activity (disc diffusion and minimum inhibitory concentration), and in vitro antidiabetic effects, including α-glucosidase inhibition, glucose uptake, and GLUT4 translocation. Methanol and ethyl acetate extracts showed higher total phenolic content (9.21% and 6.92% w/w, respectively) and significant antibacterial activity against Escherichia coli, Pseudomonas aeruginosa, Bacillus subtilis, and Staphylococcus aureus (MIC: 31.25–250 µg/mL; $p < 0.001$), while antifungal activity was weak. In vitro assays demonstrated dose-dependent α-glucosidase inhibition (IC_{50} = 526.43 µg/mL), enhanced glucose uptake, and moderate translocation of GLUT4. In vivo, HFD-STZ-induced diabetic rats exhibited hyperglycemia, impaired glucose tolerance, weight loss, and elevated hepatic markers, which were significantly ameliorated by G. multiflora treatment (200–400 mg/kg) in a dose-dependent manner. These findings indicate that G. multiflora leaves possess notable antibacterial activity and moderate antidiabetic and hepatoprotective effects, supporting their therapeutic potential in diabetes management.

***Keywords*:** Antimicrobial, diabetic, ethnopharmacology, *Grewia multiflora*, *in vivo*, α-glucosidase inhibition

INTRODUCTION

Infectious diseases and antimicrobial resistance continue to pose a serious public health challenge, particularly in developing countries. Increasing prevalence of drug-resistant bacterial infections has significantly reduced the effectiveness of conventional antibiotics, necessitating the exploration of alternative therapeutic agents from natural sources (Reta *et al*., 2019; Hemeg *et al*., 2020; Mangalagiri *et al*., 2021). Common pathogenic microorganisms such as *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Bacillus subtilis*

are responsible for a wide range of infections and exhibit increasing resistance to existing antimicrobial drugs (Ismail *et al*., 2016; Hemeg *et al*., 2020). Medicinal plants, owing to their diverse phytochemical constituents, represent a promising source of novel antimicrobial and metabolic regulatory agents (Eloh *et al*., 2024).

Grewia multiflora Juss is traditionally used as a coolant, anti-inflammatory, demulcent and hypoglycaemic agent, and is recognized for its ecological and medicinal value in India (Krishna *et al*., 2024). The present study was undertaken to evaluate the phytochemical composition and pharmacological potential of *Grewia multiflora* Juss, with particular emphasis on its antimicrobial and antidiabetic activities using *in vitro* and *in vivo* experimental models (Ismail *et al*., 2016; Eloh *et al*., 2024). This study aimed to investigate the antidiabetic and antimicrobial properties of *Grewia multiflora* Juss leaf extracts from Northern India through a combined physicochemical, *in vitro*, and *in vivo* pharmacological evaluation. The work involved standardization and phytochemical profiling of the leaf material, *in vitro* screening for antidiabetic and antimicrobial activities, and confirmation of antidiabetic efficacy in an experimental animal model, providing scientific validation for the traditional medicinal use of *Grewia multiflora*.

MATERIALS AND METHODS

Leaves of *Grewia multiflora* Juss. were collected from Almora and Ranikhet regions of Uttarakhand, India, during July–August 2018. The plant material was washed, shade-dried, powdered and sieved (100-mesh). Botanical authentication was performed at NISCAIR, New Delhi (Voucher No. NISCAIR/RHMD/consult/20**18**/3290-91-2). Experiment performed in School of Pharmacy and Research Centre, Sanskriti University, Semri, Mathura, Uttar Pradesh, India-281401, from 2018 to 2023.

Powdered leaves were subjected to hot and cold extraction using n-hexane, methanol, ethyl acetate and water following standard extraction procedures. Extracts were filtered, concentrated under reduced pressure and stored at 8 °C. Percentage yield was calculated as percentage yield was calculated as: Yield (%) = (ER / RM) × 100, where ER represents the extract residue weight and RM the raw material weight. Dried extracts were dissolved in Dimethyl sulfoxide (DMSO) for antimicrobial evaluation (Hossain *et al*., 2018; Auwal *et al*., 2014)

Moisture content, total ash, acid-insoluble ash and water-soluble ash were determined using standard pharmacopoeia methods to assess quality and stability of the plant material (Krishna *et al*., 2024).

Antibacterial activity was evaluated against *E. coli*, *P. aeruginosa*, *B. subtilis* and *S. aureus*, while antifungal activity was assessed against *Penicillium* sp. and *Aspergillus niger*. disc diffusion and minimum inhibitory concentration (MIC) assays were performed using Mueller–Hinton agar. Ampicillin served as the positive control, and 10% dimethyl sulfoxide as the negative control. Zones of inhibition were measured after incubation, and MIC values were determined using serial dilution techniques (Ismail *et al*., 2016; Hemeg *et al*., 2020; Mangalagiri *et al*., 2021).

Qualitative phytochemical analysis was conducted to identify major secondary metabolites using standard chemical tests (Vakte and Nehete., 2025; Togola *et al*., 2020). α-Glucosidase inhibitory activity was assessed using a 96-well microplate assay. Voglibose was used as the standard inhibitor, and half maximal inhibitory concentration (IC_{50}) values were calculated (Li *et al*., 2023; Singh and Kumar, 2024; Rahman *et al*., 2024).

Glucose uptake in HepG2 cells was evaluated using the fluorescent glucose analogue 6-NBDG. Extract-treated cells showed enhanced glucose uptake compared to untreated controls (Hung *et al*., 2022; Abruscato *et al*., 2024).

GLUT4 translocation in L6 skeletal muscle cells was analysed using flow cytometry. Treatment with *Grewia multiflora* extract resulted in increased mean

fluorescence intensity, indicating insulin-mimetic activity (Kumar *et al*., 2023; Li and Chen, 2025).

A high-fat diet and low-dose streptozotocin-induced type-2 diabetes model was employed in Wistar rats (Racine *et al*., 2024; Obidah *et al*., 2025) for *in vivo* antidiabetic study.

The experimental protocol involving animals was reviewed and approved by the Institutional Animal Ethics Committee (IAEC) of PBRI. The study was conducted in accordance with CPCSEA guidelines (Government of India). The approved IAEC reference number was PBRI/IAEC/2019/12-21/012**.** Experimental diabetes was induced in rats, followed by random allocation into groups using computer-generated randomization to minimize selection bias. The study design included a normal control group, a diabetic control group, two extract-treated groups (200 and 400 mg/kg), and a standard drug-treated group receiving metformin (250 mg/kg) (Gawali *et al*., 2025). Treatments were administered for seven weeks. Throughout the experimental period, body weight, fasting blood glucose levels, and oral glucose tolerance were systematically monitored for changes (Singh *et al*., 2024; Huang *et al*., 2024).

Respectively, dual doses of 200 mg/kg and 400 mg/kg were selected based on the assessment of a primary acute oral toxicity study performed as per Organization for Economic Co-operation and Development Guideline 423 (Saleem *et al*., 2024; Singh *et al*., 2024). The extracts were shielded to 2000 mg/kg, as there were no toxic effects observed during the 14 days. The doses of 200 mg/kg and 400 mg/kg were selected as $1/10^{th}$ and $1/5^{th}$ of the maximum non-toxic dose and are supported by previous studies demonstrating efficacy within the 100–500 mg/kg range without toxicity (Pradhan *et al*., 2025).

All data were presented as mean ± SD (n = 6). Statistical analysis was performed using one-way ANOVA and followed it with Bonferroni's post hoc test. Comparisons were made against the HFD/STZ-induced diabetic control group. Value of *P < 0.05 was considered significant, **P < 0.001 highly significant, and P > 0.05 not significant (NS) (Brito *et al*., 2025).

RESULTS AND DISCUSSION

Plant extraction yield

The percentage extractive yield (% w/w) of leaf extracts from *Grewia multiflora* Juss. varied with solvent and extraction method. Hot methanol extraction produced the highest yield (9.21%), followed by ethyl acetate (6.92%) and hexane (4.95%), while cold extraction gave lower yields, with methanol (6.67%) being the highest (Table 1). Physicochemical evaluation revealed a total ash of 17.96 ± 0.18% w/w, acid-insoluble ash of 3.12 ± 0.01% w/w, water-soluble ash of 96.87 ± 0.30% w/w, and sulfated ash of 3.51 ± 0.03% w/w. The loss on drying was 7.81%, and the aqueous solution exhibited a pH of 6.65. These results confirm that the plant material complies with pharmacopoeia standards for purity, quality, and acceptable physicochemical characteristics (Table 2).

Antibacterial activity of plant extracts

The extracts were tested against two fungal and four bacterial strains (*E. coli*, *P. aeruginosa*, *B. subtilis*, and *S. aureus*). Methanol, ethyl acetate, and n-hexane extracts showed strong antibacterial activity, likely due to efficient extraction of bioactive metabolites such as flavonoids, alkaloids, and phenolics. The aqueous extract exhibited weaker activity, possibly because it extracts fewer non-polar antimicrobial compounds. No antifungal activity was observed, which may be due to the distinct structural and defense mechanisms of fungal cell walls (Table 3 and Table 4).

Assessment of the phytochemical profiles of extracts

Phytochemical profiling indicated that methanolic extracts were rich in phenolic compounds, while hexane and ethyl acetate extracts predominantly contained carbohydrates with fewer phenolics. *Grewia multiflora* Juss. is rich in these compounds, while terpenoids, saponins, and cardiac glycosides were absent. Polar solvents like

Hexane extract (hot), methanol extract (hot), methanol extract (Cold) extracted more phenolics, and less polar solvents, hexane extract, ethyl acetate extract (Hot), ethyl acetate extract (cold), favoured carbohydrates, showing solvent polarity drives selective phytochemical extraction (Table 1).

The minimum inhibitory concentration (mic) of the plant extracts

The minimum inhibitory concentrations of *Grewia multiflora* extracts against four bacterial strains are presented in Table 4. For *Escherichia coli (E.colli)*, values ranged from 62.5 to 250 µg/mL, with the hot hexane extract showing the highest value (250 µg/mL) and the cold hexane, cold methanol, and cold ethyl acetate extracts the lowest (62.5 µg/mL). *Pseudomonas aeruginosa* was most sensitive to the hot ethyl acetate extract (31.25 µg/mL) and less sensitive to the hot and cold methanol extracts (62.5–125 µg/mL). The minimum inhibitory concentrations for *Staphylococcus aureus* and *Bacillus subtilis* were between 31.25 and 62.5 µg/mL, except for the hot hexane extract against *S. aureus* (62.5 µg/mL) (Table 4). Overall, the extracts demonstrated antibacterial activity comparable to or slightly higher than ampicillin.

Assessment of alpha-glucosidase inhibition activity

Grewia multiflora Juss. water-ethanol extract exhibited a concentration-dependent inhibition of α-glucosidase activity, ranging from 0.85% at 62.5 µg/mL to 92.42% at 1000 µg/mL (Table 5). The IC_{50} value of the extract (526.43 µg/mL) also revealed that it has moderate inhibitory potency relative to the positive control drug Voglibose, with an IC_{50} of 290.15 µg/mL. These results indicate that the extract of *Grewia multiflora* possesses a potent α-glucosidase-inhibitory action, and can be a natural antidiabetic.

Evaluation of glucose transport in skeletal muscle cells

Based on the Mean Fluorescence Intensity (MFI) of 6-NBDG, *Grewia multiflora* Juss. (S2) increased glucose uptake in C2C12 myotubes. A high basal uptake of glucose was also observed in the untreated cells (cell control) with a value for MFI of 9.98, compared with the standard drug (metformin), which resulted in an increased MFI score of 55.21 (Table 6). *Grewia multiflora* extract treatment induced an MFI of 35.58, which was between metformin but significantly modulated glucose transport as compared to untreated cells. Based on these results, the extract might possess insulin-mimetic features for the stimulation of GLUT-mediated glucose uptake in skeletal muscle cells.

Measurement of GLUT4-mediated glucose uptake and translocation

MFI was used to analyze Glucose Transporter Type 4 (GLUT4) expression level in L6 skeletal muscle cells. The positive control metformin led to a high level of GLUT4 translocation (37.42 MFI), whereas untreated cells showed low expression levels (7.04 MFI). *Grewia multiflora* Juss. (S1) yielded an intermediate increase of MFI value for GLUT4 (21.82), suggesting that glucose uptake on cells was only partially induced through GLUT4 stimulation (Table 7).

Comparative body weight variations among groups

Body weights on the 0th, 1st, 3rd, 5th and 7th weeks were measured. The normal control group (Group I) revealed a progressive increase from 198.98 ± 4.18 g to 206.42 ± 4.42 g, while HFD + STZ-induced diabetic rats (Group II) exhibited a gradual decrease from 193.85 ± 2.47 to 182.16 ± 3.29 g Metformin-treated rats (250 mg/kg, Group III and IV) partially rescued body weight compared with Group II at weeks ≤5 and week ≤7 (*p <0:001). *Grewia multiflora* given in 200 mg/kg (Group IV) was effective in reversing weight loss at all time points ($p < 0.05$; **$p < 0.001$), while the dose of 400 mg/kg (Group V) did show a significant difference in body weights at week 7 only ($p < 0.001$). Data are expressed as mean ± SD ($n = 6$) and analysed using one-way ANOVA

followed by the Bonferroni test, showing dose-dependent prevention of body weight reduction against the high-fat diet and streptozotocin-induced diabetes (Table 8).

Assessment of test sample-induced changes in fasting blood glucose

FBGL was determined at 0, 1, 3, 5, and 7 weeks. Mild increases were observed in the normal controls (Group I, 87.66 ± 3.08 to 93.00 ±1.79 mg/dL) and high-fat diet and streptozotocin-induced diabetes (HFD + STZ-induced rats) by gradual hyperglycaemia from day ~0 weeks to 10 weeks in Group II rats (270.17±2.40 - 309.16±7.36mg/dL). Significant decrease in FBGL with metformin (250 mg/kg, Group III) ($p < 0.001$) was to 95.83 ± 3.66 mg/dL at the end of week 7. The extract of G. multiflora 200 mg/kg (Group IV) and 400 mg/kg (Group V) also significantly decreased FBGL ($p < 0.001$) to reach 145.50 ± 5.21 and 167.74 ±4.02 mg/dL, respectively, showing dose-dependent anti-hyperglycaemic activities. Values are mean ± SD (n = 6) and analyzed by one-way ANOVA followed by Bonferroni post-test. *Grewia multiflora* extract as 200 mg/kg (Group IV) and 400 mg/kg (Group V) also significantly reduced FBG at week 1 till end (**$P < 0.001$), in comparison to diabetic control, dose-dependent effects were noticed. Statistical calculations were carried out with one-way ANOVA followed by Bonferroni's post-test; NS, non-significant from the diabetic one (Table 9).

Evaluation of test sample-induced changes in oral glucose tolerance

OGTT was carried on day 0, and at 30th days, 60th days, 90th days, and 120th days to estimate glucose metabolism. Using normal controls (Group I) transient rise in glucose up to 185.66 ± 4.95 mg/dL at 30 min and then declined to near baselines levels (101.66 ± 2.16 mg/dL) by 120th min were observed. In HFD + STZ-treated diabetic rats (Group II), the plasma glucose levels were persistently higher with peak at 420.16 ± 4.45 mg/dL and still remained high at 120 min (339.66±11.26 mg/ dL), suggesting an impairment in glucose tolerance. Glucose clearance was significantly enhanced ($p < 0.001$) on giving Metformin (250 mg/kg, Group III), and the value of glucose level after 120 minutes dropped to 109.00 ± 6.13 mg/dL. *G. multiflora* extract 200 mg/kg (Group IV) and 400 mg/kg (Group V) also significantly decreased the blood glucose level in a dose-dependent manner (**$p < 0.001$), to attain a level of 170.52 ± 6.70 mg/dL and 136.62 ± 8.57 at an interval span of T = 120 min, respectively. Results are presented as the mean ± SD (n = 6) and analysed by one-way ANOVA with Bonferroni post hoc test (Table 10).

***Grewia multiflora* extracts on liver functions**

The diabetic control mice (Group II) had significantly higher hepatic markers levels (Aspartate Aminotransferase, alanine aminotransferase, alkaline phosphatase and total bilirubin) than the normal control group ($P < 0.001$), indicating liver damage. The levels were significantly diminished upon pretreatment with metformin (Group III) and *Grewia multiflora* 200 mg/kg (Group IV) and 400 mg/kg (Group V; $P < 0.001$), the maximum protection being afforded by the high dose of 400 mg/kg, pointing out to a drug-dependent hepatoprotection (Table 11).

CONCLUSION

Grewia multiflora Juss. exhibited notable pharmacological potential, particularly in methanol and ethyl acetate extracts rich in flavonoids, alkaloids, glycosides, and sterols. The extracts showed significant antibacterial and antidiabetic activities, including enhanced 6-NBDG uptake and GLUT4 translocation, along with antihyperglycemic and hepatoprotective effects in HFD/STZ-induced diabetic rats. Further studies focusing on bioactive compound isolation, mechanistic evaluation, long-term toxicity, and clinical validation are required, as the present study was limited by the lack of compound characterization and mechanistic evaluation of isolated bioactive compounds.

List of abbreviations list

HFD – High-Fat Diet; STZ – Streptozotocin; HFD/STZ – High-Fat Diet and Streptozotocin-Induced Diabetes; FBGL – Fasting Blood Glucose Level; OGTT – Oral Glucose Tolerance Test; MIC – Minimum Inhibitory Concentration; MFI – Mean Fluorescence Intensity; ALT – Alanine Aminotransferase; AST – Aspartate Aminotransferase; ALP – Alkaline Phosphatase; DMSO – Dimethyl Sulfoxide; HEX – Hexane Extract (Hot); MET – Methanol Extract (Hot); ETA – Ethyl Acetate Extract (Hot); cHEX – Hexane Extract (Cold); cMET – Methanol Extract (Cold); cETA – Ethyl Acetate Extract (Cold); IU/dl – International Units per Decilitre; mg/dL – Milligram per Decilitre; µg/mL – Microgram per Millilitre; n – Number of Animals or Replicates; SD – Standard Deviation; NS – Not Significant; FITC – Fluorescein Isothiocyanate

CONFLICT OF INTEREST STATEMENT

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Table 1: The percentage yield of leaf extracts and phytochemical screening of *Grewia multiflora* Juss. obtained using various solvents.

Extract	Extraction Method	Major constituents	Extractive yield (% w/w)
HEX	Hexane (Hot)	Carbohydrates (higher), fewer phenolics	4.95
MET	Methanol (Hot)	Phenolics (higher)	9.21
ETA	Ethyl Acetate (Hot)	Carbohydrates (moderate)	6.92
cHEX	Hexane (Cold)	Carbohydrates (moderate)	2.78
cMET	Methanol (Cold)	Phenolics (moderate)	6.67
cETA	Ethyl Acetate (Cold)	Carbohydrates (lower)	1.87

HEX, MET, and ETA denote hot extracts prepared with hexane, methanol, and ethyl acetate, respectively, while cHEX, cMET, and cETA represent the corresponding cold extracts.

Table 2: Physicochemical evaluation of *Grewia multiflora Juss.*

Sl.No.	Test	Value (% w/w)
1	Total Ash	17.96 ± 0.18
2	Acid-Insoluble Ash	3.12 ± 0.01
3	Water-Soluble Ash	96.87 ± 0.30
4	Sulfated Ash	3.51 ± 0.03
5	Loss on Drying	7.81
6	pH (in Water)	6.65

Data are presented as mean ± SD of three independent experiments. Ash values were determined using standard pharmacopoeia methods, moisture content was assessed by loss on drying, and pH was measured in distilled water.

Table 3: Zone of inhibition of *Grewia multiflora* Juss. extracts against microorganisms.

Extract	*E. coli millimeter (mm)*	*P. aeruginosa (millimeter)*	*B. subtilis (millimeter)*	*S. aureus (millimeter)*
n-Hexane (Hot)	14.6 ± 0.5	13.8 ± 0.4	16.9 ± 0.6	17.2 ± 0.5
Methanol (Hot)	15.8 ± 0.6	14.9 ± 0.5	18.4 ± 0.7	18.9 ± 0.6
Ethyl acetate (Hot)	17.3 ± 0.7	16.5 ± 0.6	19.8 ± 0.8	20.4 ± 0.7
n-Hexane (Cold)	13.9 ± 0.4	13.2 ± 0.3	15.8 ± 0.5	16.4 ± 0.4
Methanol (Cold)	15.1 ± 0.5	14.3 ± 0.4	17.6 ± 0.6	18.2 ± 0.5
Ethyl acetate (Cold)	16.7 ± 0.6	15.9 ± 0.5	19.1 ± 0.7	19.6 ± 0.6
Aqueous extract	9.4 ± 0.3	0	0	0
Ampicillin (Standard)	22.6 ± 0.5	21.8 ± 0.4	24.3 ± 0.6	23.9 ± 0.5
DMSO (Negative control)	0	0	0	0

Data are presented as mean ± SD of three independent experiments

Table 4: Minimum inhibitory concentration (MIC, µg/mL) of *Grewia multiflora* Juss. extracts against bacterial strains

Microorganism	cHEX (Cold)	cMET (Cold)	cETA (Cold)	HEX (Hot)	MET (Hot)	ETA (Hot)	Ampicillin
E. coli	*62.5	62.5	62.5	250	125	125	51
P. aeruginosa	62.5	125	62.5	31.25	62.5	31.25	46
B. subtilis	62.5	62.5	62.5	31.25	62.5	62.5	43
S. aureus	–	62.5	62.5	62.5	62.5	31.25	38

**All values in µg/mL unit.*

Hot extracts were prepared using hexane, methanol, and ethyl acetate, while cold extracts were obtained using the same solvents. Minimum inhibitory concentration (MIC) values are reported in µg/mL.

Table 5: In vitro analysis of antidiabetic potential of *Grewia multiflora* extracts via α-glucosidase enzyme assay

Test (ug/ml)	62.5	125	250	500	1000	IC 50 (ug/ml)
Voglibose	29.4905	37.8454	52.9361	68.696	94.3221	290.15
Aqueous-alcoholic extract of *Grewia multiflora juss*	0.8544	11.9791	27.027	48.0558	92.415	526.43

Table 6: Assessment of glucose uptake in skeletal muscle cells via 6-nbdg mfi following treatment with hydroalcoholic *Grewia multiflora* extracts

Test	6-NBDG Mean Fluorescence intensity
Cell control	9.98
STD control	55.21
Aqueous-alcoholic extract of *Grewia multiflora* juss	35.58

Table 7: GLUT4 expression measured by MFI post-treatment

Test	GLUT4 Mean Fluorescence intensity
Cell control	7.04
STD control	37.42333
Aqueous-alcoholic extract of Grewia multiflora juss (S1)	21.81667

Table 8: Results of comparative body weight variations among groups

Group No.	Treatment	Initially (0 week)	First week	Third week	Fifth week	Seven week
I	Normal Control (Vehicle treated)	198.98±4.183	201.03±4.127	203.12±4.488	204.59±4.408	206.42±4.423
II	HFD+ STZ induced only	193.85±2.471	191.65±2.457	188.70±3.072	185.31±3.104	182.16±3.285
III	HFD+STZ+ Standard Metformin (250mg/kg)	196.43±3.33^{NSP}	194.82±2.915 ^{NSP}	194.20±3.761^{NSP}	193.65±4.587**	194.17±4.137**
IV	HFD+STZ+D1GM (200mg/kg)	198.23±4.672**	199.92±3.789*	197.86±4.625**	201.38±3.645*	200.27±5.106**
V	HFD+STZ+D2GM (400mg/kg)	196.12±1.682^{NSP}	188.50±1.718 ^{NSP}	190.88±1.456^{NSP}	192.14±2.045^{NSP}	195.38±1.567**

*The data (n = 6) were presented as mean ± SD. One-way ANOVA and Bonferroni's post hoc test were used for statistical analysis. *P < 0.05, **P < 0.001, and not significant (NS) at P > 0.05 were the thresholds for comparisons with the HFD/STZ-treated group.*

Table 9. Impact of metformin and *Grewia multiflora* extract on fasting blood glucose levels in rats with stz-induced diabetes

Group No.	Treatment	Initially (0 week)	First (1st) week	Third (3rd) week	Fifth (5th) week	Seven (7th) week
I	Normal Control (Vehicle treated)	87.66±3.077	89.66±3.077	90.16±2.639	91.16±2.483	93.00±1.789
II	HFD+ STZ induced only	270.166±2.401	275.5±2.345	284.33±3.502	302.00±7.874	309.16±7.360
III	HFD+ STZ+ Standard etformin (250mg/kg)	264.66±7.118^{NS}	213.16±7.167**	155.33±7.257**	105.16±3.656**	95.833±3.656**
IV	HFD+STZ+ D1GM 200mg/kg)	269.43±5.194^{NS}	257.30±5.164**	201.60±5.801**	182.54±6.564**	145.50±5.214**
V	HFD+STZ+ D2GM (400mg/kg)	265.61±4.187^{NS}	219.45±6.675**	156.75±4.675**	169±5.675**	167.74±4.023**

*The data are presented as MEAN±SD with n=6, one-way ANOVA followed by the Bonferroni test, with *P<0.050, **P<0.001, ^{NS}P>0.001 and NSP>0.001 compared to the HBG HFD/STZ treated group.*

Table 10: **Test sample-induced changes in oral glucose tolerance**

Group No.	Treatment	Initial 0 min	Thirty minutes	Sixty minutes	Ninety minutes	One twenty minutes
I	Normal Control	93±1.64	185.66±4.95	145.5±3.27	107.5±5.14	101.66±2.16
II	HFD+ STZ induced only	310.5±6.29	420.16±4.45	397.66±6.95	366.00±11.26	339.66±11.25
III	HFD+ STZ+ Standard Metformin (250mg/kg)	95.83±3.66**	226.00±4.60**	169.00±5.25**	128.66±3.67**	109.00±6.13**
IV	HFD+STZ+ D1GM (200mg/kg)	160.23±4.06**	356.61±8.56**	234.30±6.85**	206.45±4.66**	170.52±6.70**
V	HFD+STZ+ D2GM (400mg/kg)	145.60±3.31**	294.87±8.17**	245.60±6.75**	145.80±5.98**	136.62±8.57**

*The data are presented as MEAN±SD with n=6 in the OGTT Level, one-way ANOVA followed by the Bonferroni test, with *P<0.050, **P<0.001 and NSP>0.001 compared to the HBG control group treated with HFD/STZ.*

Table 11: Effect of treatments on liver function parameters

Group No.	Treatment	AST(IU/dl)	ALT(IU/dl)	ALP(IU/dl)	Total bilirubin (mg/dl)
I	Normal Control(Vehicle treated)	61.92±9.563	48.72±6.198	116.45±11.258	0.50±0.094
II	HFD+ STZ induced only	192.53±7.631	121.07±5.364	220.28±11.31	2.04±0.276
III	HFD+ STZ+ Standard Metformin (250mg/kg)	69.19±11.362**	54.34±5.816**	120.83±6.388**	0.55±0.082**
IV	HFD+STZ+D1GM (200mg/kg)	156.12±5.745**	82.67±4.042**	156.50±8.912**	1.29±0.067**
V	HFD+STZ+D2GM(400mg/kg)	106.01±3.564**	73.56±3.354**	146.25±8.345**	0.91±0.023**

*The results of the one-way ANOVA followed by the Bonferroni test are presented as MEAN±SD at n=6 for the liver function parameters, with *P<0.050, **P<0.001 and NSP>0.001 contrasted to the diabetic regulate group treated with HFD/STZ.*